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# Aims and Scope

The primary aim of *World Journal of Clinical Cases* (WJCC, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

# Indexing/Abstracting

The WJCC is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Scopus, PubMed, and PubMed Central. The 2021 Edition of Journal Citation Reports® cites the 2020 impact factor (IF) for WJCC as 1.337; IF without journal self cites: 1.301; 5-year IF: 1.742; Journal Citation Indicator: 0.33; Ranking: 119 among 169 journals in medicine, general and internal; and Quartile category: Q3. The WJCC’s CiteScore for 2020 is 0.8 and Scopus CiteScore rank 2020: General Medicine is 493/793.

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Lung injury after cardiopulmonary bypass: Alternative treatment prospects

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Abstract

Although the lung injury caused by cardiopulmonary bypass (CPB) has been extensively investigated, the incidence and mortality of lung injury after CPB remain a prominent clinical problem. The poor outcome has been attributed to multifactorial etiology, including the systemic inflammatory response and ischemia reperfusion (I/R) injury during CPB. Lung injury after CPB is a complex pathophysiological process and has many clinical manifestations of mild to severe disease. Which is associated with prognosis. To alleviate this lung injury, interventions that address the pathogenesis are particularly important. This review summarizes the pathogenesis, mechanism and treatment options of lung injury after CPB, such as lung protection with intralipid.
INFORMATION

More than 2 million patients worldwide undergo cardiac surgery annually, and most procedures use cardiopulmonary bypass (CPB) [6]. With the advent of extracorporeal circulation and improvement in surgical techniques, the incidence of postoperative complications after cardiac surgery should have been minimal. However, the incidence of pulmonary complications is 20%–35%, which is significant compared with complications after other types of surgery, partly due to systemic inflammatory response syndrome (SIRS) and ischemia reperfusion (I/R) injury during CPB [7-9]. Postoperative pulmonary complications after cardiac surgery with CPB, such as hypoxemia and acute respiratory distress syndrome, are thought to be significant, with poor prognosis and mortality up to 37.5% [10,11]. Survivors may have persistent physical, neuropsychiatric and neurocognitive disorders, which seriously affects quality of life and increases the medical burden.

In an attempt to minimize the deleterious effects of CPB, investigators have explored various strategies including improved CPB devices and methods [12-15] and pharmacological agents to reduce the systemic response. None of these interventions is, however, known to improve clinical outcomes. Steroids have been used for nearly 30 years as a basic treatment strategy for postoperative lung protection after CPB. However, there is conflicting evidence that steroids improve postoperative complications or reduce postoperative mortality in CPB patients [16,17].

Intralipid is a safe emulsion for intravenous application and is widely used in clinical settings [18]. Byrne et al. [19] demonstrated that pretreatment with intralipid attenuated intestinal I/R injury in rats. It is not known whether intralipid has a protective effect in the prevention and treatment of lung injury after CPB. Review discusses the pathogenesis and treatment of lung injury caused by CPB, and explores the effects of intralipid on mitochondrial function of pulmonary vascular endothelial cells from three aspects: Mitochondrial respiratory chain, mitochondrial permeability transition pore (mPTP), and mitochondrial membrane potential (ΔΨm). This provides an additional view of the pulmonary protective mechanism of intralipid.

LITERATURE SEARCH

We conducted a narrative review of the mechanism and treatment of lung injury after CPB. PubMed was searched for articles published from December 1983 to July 2021. We carried out the search with the following MeSH or free-text terms: CPB, lung injury, fat emulsion, intralipid, lipid emulsion, coronary heart disease, ischemia reperfusion, on-pump coronary artery bypass graft (CABG), SIRS, reactive oxygen species (ROS), mPTP, and mitochondrial membrane potential. The search was limited to papers written in English, with no restrictions on the type of article. Two independent reviewers (XM and YZ) evaluated the articles for potential inclusion by

Key Words: Cardiopulmonary bypass; Lung injury; Pathogenesis; Treatment; Intralipid; Systemic inflammatory response syndrome

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Core Tip: Respiratory dysfunction is a well-recognized side effect of cardiac surgery combined with cardiopulmonary bypass (CPB). The mechanism of lung injury after CPB is unclear, and the lack of effective treatment results in poor prognosis. This review summarizes the mechanisms of lung injury and proposes a new treatment option.


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INTRODUCTION

The introduction explains the background and purpose of the study. It provides an overview of the current understanding of lung injury after CPB and outlines the rationale for addressing this topic in the review. The introduction typically sets the stage for the subsequent sections by highlighting the significance of the topic, the gap in the existing literature, and the potential impact of the proposed treatment.

LITERATURE SEARCH

The literature search section describes the methods used to identify relevant articles. It includes details about the databases searched, the search terms used, and any additional criteria applied to filter the results. This section is crucial for ensuring that the review is comprehensive and unbiased.

INFORMATION

The information section provides additional context and details that may not fit neatly into the main text. This section can include methodological considerations, data analysis, or supplementary data that support the main findings of the study.

LITERATURE SEARCH

The literature search section may include a detailed description of the search strategy, including the databases searched, the search terms used, and any inclusion or exclusion criteria applied to the results. This section is essential for reproducibility and transparency in scientific research.
screening titles and abstracts. The senior author (MJ) further evaluated the full text of articles with any disagreement.

MECHANISM OF LUNG INJURY AFTER CPB

SIRS

Our understanding of the SIRS to CPB began with the study of Kirklin et al[16] in the 1980s. SIRS is characterized by activation of platelets, neutrophils and macrophages, and cascades (coagulation, fibrinolytic, and kallikrein), which result in increased endothelial permeability and vascular and parenchymal damage[17,18]. These inflammatory responses are associated with the development of lung injury after CPB (Figure 1).

The contact of blood elements with the artificial surfaces of the CPB machine primes, heparin administration, and damage to the hematologic system from incisional, anesthesia and surgery can activate the complement system. C3a expression is activated mainly by CPB via the alternative pathway, and C4a expression is activated by the heparin protamine complex via the classical pathway. Complement (especially C3, C4a and C5a) promotes the release of mast cells and basophils in response to inflammatory mediators such as histamine, resulting in increased permeability of pulmonary epithelial cells and vasodilation[19].

There is increased expression of CD18 and CD11b adhesion molecules on the surface of neutrophils after chemotaxis of interleukin (IL)-8 and induction of C5a[20]. Activated neutrophils release proteolytic enzymes and oxygen-free radicals (O2 and HO), which directly or indirectly damage pulmonary vascular endothelial cells and promote their apoptosis, leading to increased intrapulmonary shunt fraction and pulmonary vascular resistance, and increased lung permeability with interstitial edema[21].

C3a is a powerful platelet aggregation agent. Heparin, hypothermia and duct disruption in CPB release platelets, and platelet activation leads to platelet aggregation, adhesion, consumption and thrombi formation by adsorption with fibrin[22]. Moreover, in late CPB, neutrophils adhere and transmigrate into the lung parenchyma and platelets are retained to block the pulmonary microcirculation. Activated platelets release 5-hydroxytryptamine, prostaglandin, thromboxane A2 and platelet factor 4, which damage pulmonary vascular endothelial cells and mediate lung parenchymal damage locally through cellular and tissue injury. Xiao et al[23] found that peripheral circulating platelets in CPB lung injury were significantly decreased. In addition, Kunitomo et al[24] reported that isolating 20% of platelets from blood before CPB and returning them after surgery significantly improved postoperative cardiopulmonary function, which may be related to the reduced effect of CPB on platelet number and function.

Complement activation, ischemia reperfusion and cytokine interactions can lead to release of cytokines, with tumor necrosis factor (TNF)-α[25] and IL-1, IL-6, IL-8 and IL-10 being the main ones clearly associated with lung injury[26]. TNF-α and IL-1 synergistically activate nuclear factor-κB to promote generation of cytokines and polymorphonuclear cells (PMNs), exacerbating the cascade of cell death signals. These, in turn, lead to endothelial cell swelling, plasma and protein extravasation into the interstitial tissue, aggregation of PMNs and macrophages at the injury site, and, finally, impedance of intra-alveolar cellular perfusion and oxygen exchange, causing lung injury.

Factor XII is activated by blood contact with CPB ducts and by vascular endothelial cell injury resulting in subendothelial collagen exposure, then factor XIIa activates the endogenous coagulation system and kallikrein, bradykinin production, vasodilation and increased permeability. Meanwhile, enhanced fibrinolysis and fibrin degradation products during CPB can lead to lung injury.

Lung I/R injury

The lungs have a dual blood supply from the bronchial artery and pulmonary artery. It has been shown that, under normal physiological conditions, the bronchial artery blood flow is 3%–5% of the total blood flow to the lungs[27]. The vena cava is cut off during CPB, and the metabolic demands of the lungs are totally dependent on oxygen supply from the bronchial arteries. Therefore, the lungs are excluded from the systemic circulation ischemia and hypoxia. Subsequently, ATP and lung surfactant are affected after CPB. Finally, lung permeability increases along with protein exudation. At this time, the lungs are in a hypoxic and relatively hypermetabolic state and are
susceptible to endothelial cell injury. Vascular endothelial cells produce a large number of cytotoxic enzymes such as myeloperoxidase (MPO), leading to I/R injury. It has been shown that I/R injury leading to Na$^+$ pump inactivation and Ca$^{2+}$ overload is an important factor triggering lung tissue injury $^{[28,29]}$. In addition, mitochondria are important targets of intracellular Ca$^{2+}$ overload attack, and intracellular Ca$^{2+}$-dependent proteases are activated, causing impaired energy metabolism and release of cytochrome C (CytC) and apoptosis-inducing factor from the inner and outer mitochondrial membrane gap. Meanwhile, reduced synthesis of endothelium-derived relaxing factor (NO) due to I/R injury can also mediate lung parenchymal injury $^{[30]}$.

**ROS**

Systemic inflammation, surgical trauma, and reperfusion after ischemia play a pivotal role in oxidative stress by initiating a series of biochemical events that result in the generation of excessive amount of ROS $^{[31,32]}$. Lipid peroxidation is closely related to apoptosis $^{[33]}$. During CPB, ischemic injury occurs when the blood supply to tissue is suboptimal and accompanied by cellular ATP depletion due to its degradation by hypoxanthine. During periods of stress, cell membrane surface NADH/NADPH oxidase is activated. Meanwhile, ROS levels can increase drastically, leading to substantial damage to many cellular molecules such as lipids, proteins and DNA $^{[34]}$. Furthermore, ROS results in the production of CytC and damaged mitochondrial membranes with disruption of the alveolar barrier and apoptosis of alveolar epithelial cells $^{[35]}$.

**ALTERNATIVE TREATMENT PROSPECT: INTRALIPID**

Intralipid, is a safe lipid emulsion for intravenous application, which has been widely utilized as a vehicle for different drugs like propofol and etomidate $^{[14]}$. It is also used for parenteral nutrition to supplement the body with energy and essential fatty acids. In addition, intralipid has been used in the treatment of cardiotoxicity caused by overdose of local anesthetics such as bupivacaine $^{[36]}$. Recent animal studies have shown that postischemic administration of lipid emulsion protects the heart against I/R injury $^{[14,37,38]}$. Meanwhile, clinical studies have also demonstrated that intralipid postconditioning reduces the release of markers of myocardial injury after heart valve replacement and has a cardioprotective effect $^{[39]}$. In addition, animal studies also suggest that intralipid mitigates impaired pulmonary function induced by I/R through attenuation of local cellular injury and the subsequent SIRS $^{[40]}$. Therefore, we hypothesize that there is a pulmonary protective function of intralipid, and it is necessary to explore its potential mechanism of action.
The lungs are especially susceptible to the inflammatory attack and I/R injury ascribed to the use of CPB[4]. Oxidative stress and massive release of ROS caused by I/R-induced lung injury, which can lead to functional changes and apoptosis of pulmonary microvascular endothelial cells, result in increased capillary permeability, impaired pulmonary diffusion function, and accumulation of fluid in the interstitial space[41,42]. In solid organs, electron conduction defects occur in ischemic/hypoxic cells, leading to irreparable mitochondrial damage, which is a key mechanism of I/R-induced lung injury[43]. Therefore, maintaining mitochondrial functional homeostasis makes it possible to mitigate I/R damage. Mitochondria store the energy generated as electrochemical potential energy in the inner membrane, resulting in an asymmetric distribution of H+ and other ion concentrations on both sides of the inner membrane to form the ΔΨm[44], which is reflective of metabolic function[45]. ΔΨm is essential for maintaining mitochondria for oxidative phosphorylation and production of ATP, and stability of ΔΨm depends on normal respiratory chain complex activity, proton flow and ATP synthesis[46]. Alteration in the activity of mitochondrial respiratory enzyme complexes during reperfusion of various tissues, which results in an excess of free radicals derived from oxygen and cellular ATP imbalance, has been reported in organs such as the heart, liver and brain[47,48]. Moreover, Sommer et al[49] found that the lungs suffer from the same mitochondrial damage as other solid organs in the pathological situation of I/R, and that changes in the degree of ΔΨm polarization are critical for the development of lung mitochondrial dysfunction[49]. In the postischemic reperfusion phase in the lungs, respiratory chain complex dysfunction, lipid membrane oxidation, and ATP reduction impair the stability of ΔΨm. The above studies suggest that maintaining the stability of ΔΨm is important to reduce lung I/R injury during the early postischemic reperfusion phase.

There is abundant evidence that intralipid can exert myocardial protective effects by inhibiting the opening of mPTP[14]. I/R injury leads to mitochondrial respiratory chain damage and impaired oxidative phosphorylation[50,51]. Mitochondrial damage generates reactive oxygen clusters through complexes I and III and ROS promote oxidative stress. Meanwhile, ROS act as signaling molecules for apoptosis by decreasing mitochondrial ΔΨm and increasing mPTP opening. In the early phase of reperfusion, Ca2+ overload and oxidative stress due to ischemia cause the opening of mPTP[52]. mPTP opening is a key event in cell death after I/R because it causes an abrupt increase in the permeability of the inner mitochondrial membrane to solutes with molecular weights up to 1500 Da, which further causes a decrease in mitochondrial membrane polarization, leading to a decrease in CytC release and matrix swelling, activating caspase 3- and 9-dependent apoptotic cascade responses[52,53]. As mentioned earlier, numerous studies have suggested that intralipid can inhibit the opening of mPTP in cardiomyocytes, therefore, the three aspects of mitochondrial respiratory chain, mPTP and ΔΨm make it possible for intralipid to improve mitochondrial function in pulmonary vascular endothelial cells for lung protection.

CONCLUSION

Despite the advances in extracorporeal circulation and surgical techniques of recent years, a significant proportion of patients still have a poor outcome. Ischemic and pharmacological preconditioning and postconditioning has been reported to ease I/R injury[38,54]. However, the lack conditions in the clinical setting are not as precisely defined as in the laboratory; therefore, ischemic modulation has limited clinical application[55]. Besides, it is reported that remote ischemic preconditioning does not reduce morbidity or mortality in patients undergoing cardiac surgery with CPB[36]. Therefore, from the view of clinical practice, pharmacological preconditioning or postconditioning is especially promising. Intralipid is a necessary fatty acid carrier and may be a promising approach to improve outcomes after CPB.

Intralipid can be used to treat the cardiotoxicity caused by local overdose of anesthetics and has been clinically proven[57]. The most supported dosing regimen is an intravenous bolus of 1–1.5 mL/kg 20% intralipid. Approximately 12 mL/kg intralipid is the upper limit for initial dosing in adults and 15 mL/kg in children[57-59]. Previous research has provided evidence that intralipid reduces myocardial infarct size and improves cardiac function by pre-reperfusion infusions[38,60,61]. Yuan et al[62] recently conducted a randomized controlled trial using intralipid to assess prognosis and cardiac function in adult cardiac surgery patients after extracorporeal circulation. A single intravenous bolus of intralipid (2 mL/kg, 20%) did not cause
abnormal lipid metabolism, with no perioperative hepatic or renal dysfunction, or other related complications[39,62,63]. The duration of intravenous infusion of intralipid should be > 10 min as rapid administration of large amounts increases the risk of fat embolism. The dose of intralipid is selected on the basis of the pill dose when it is used in resusciting the cardiac arrest caused by local anesthetic toxicity, so it leads to a difference in the drug dose. Notably, some studies have found that short infusions of intralipid can cause elevated free fatty acids, induce insulin resistance and increase risk of hyperglycemic events[64], while hyperglycemia is an independent risk factor for mortality and postoperative complications in coronary heart disease surgery [65]. These may limit the use of intralipid on-pump CABG. However, Javaherforoosh Zadeh et al.[66] showed that using adjusted tight glycemic control to a level that is near to normal during cardiac surgery may reduce hyperglycemic complications. Due to the lack of research on proper intravenous dosage of intralipid in lung injury after CPB, more data are needed to confirm the specific dose to be used.

The prevention and treatment of lung injury after CPB face many challenges. First, with the advent of extracorporeal circulation and improvement in surgical techniques, the incidence of pulmonary complications and mortality rates are high. Second, the mechanism of lung injury caused by CPB is not clear and needs to be studied in depth. Third, there is a lack of effective treatment methods. The development and use of therapeutic agents against the lung injury after CPB are important. If intralipid demonstrates benefit, it would have rapid uptake globally and have tremendous impact.

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Acute myocardial injury in patients with COVID-19: Possible mechanisms and clinical implications

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Abstract
Severe acute respiratory syndrome coronavirus 2 infection affects not only the lungs, but also the cardiovascular system, having a major impact on patients' outcomes. Myocardial injury (MI) occurs in the context of coronavirus infectious disease 2019 (COVID-19) and is associated with a higher risk of severe clinical outcome and mortality. COVID-19-related MI can have various clinical manifestations, of which the main ones are myocarditis, stress cardiomyopathy, acute coronary syndrome, and pulmonary embolism. The exact mechanisms of how MI occurs in these patients are not yet fully known. Direct injury, through direct viral myocardial invasion, and indirect injury, through interaction with angiotensin I converting enzyme 2, increased inflammation, and thrombocyte and endothelial dysfunction, could be involved in acute MI in patients with COVID-19. A better understanding of these multiple potential mechanisms may help to develop new targeted therapeutic strategies. The purpose of this review is to provide the current understanding of the potential mechanisms involved in MI induced by COVID-19 and to discuss the current progress in the therapeutic strategies.

Key Words: Myocardial injury; Myocarditis; Stress cardiomyopathy; Acute coronary syndrome; Pulmonary embolism; Coronavirus infectious disease; SARS-CoV-2

Core Tip: Myocardial injury (MI) has been described in coronavirus infectious disease 2019 patients and is associated with a higher risk of severe clinical outcome and mortality, but the exact mechanisms involved are not completely elucidated. Multiple potential mechanisms have been proposed, such as direct viral infection and indirect injury through inflammation, angiotensin I converting enzyme 2 interaction and hemostatic anomalies. Understanding the mechanisms underlying MI is needed to...
The COVID-19 virus was detected in the interstitial and vascular endothelium, and other tissues, mechanisms and clinical implications. World J Clin Cases 2022; 10(3): 762-776
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INTRODUCTION
Since December 2019, coronavirus infectious disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) has quickly become a global health issue that is having a major impact on the healthcare system worldwide. High infectivity and rapid transmission of the virus have led to an international public health crisis. A wide range of symptoms had been reported, with most infected patients developing respiratory tract disease with different severity level. Not only the lungs are affected, and other organs are involved, with COVID-19 affecting multiple organs and systems, with different cardiovascular implications. Also, cardiovascular comorbidities have an important impact on the severity of COVID-19 and they seem to be linked with severe clinical outcomes and higher risk of death. Clinical studies have reported that COVID-19 can significantly affect the heart, causing acute myocardial injury (MI)[1-3], in patients with and without pre-existing cardiovascular disease[4]. MI is defined as an elevation of at least one cardiac troponin (cTn) concentration above the 99th percentile upper reference limit[5]. COVID-19-related MI can have various clinical manifestations, of which the main ones are myocarditis and pulmonary edema, acute coronary syndrome, acute myocardial infarction, and pulmonary embolism[14-16]. In this review, we aim to provide an overview of the potential mechanism involved in MI induced by COVID-19, and the progress in the therapeutic strategies addressing it.

PUTATIVE MECHANISMS OF MI
COVID-19 may cause MI via various mechanisms, either directly, or indirectly. The first mechanism might be a direct injury to myocardial cells due to a viral invasion of endothelial cells and cardiomyocytes via angiotensin I converting enzyme (ACE)2. Other possible mechanisms are: downregulation of ACE2, cytokine storm/cytokine releasing syndrome, and hypercoagulation (Figure 1).

Direct injury
SARS-CoV-2 is an RNA virus with a high affinity for ACE2. For virus attachment to the receptor, SARS-CoV-2 uses the S protein and the transmembrane protease serine 2 (TMPRSS2) to cleave the S protein and facilitate infection[18,19]. The receptors of ACE2 are located in the lung, heart, endothelial cells and immune cells[20]. These locations could explain intracellular viral replication in the myocardium and other tissues, resulting in degeneration, necrosis and dysfunction. Recently, it has been showed that ACE2 and other mediators of SARS-CoV-2 entry (such as cathepsin B and cathepsin L) are preferentially enriched in cardiomyocytes, explaining at least in part the cardiac susceptibility to COVID-19[21].

Only a few case reports have demonstrated the presence of the genome of SARS-CoV-2 in cardiac samples[4,5]. The COVID-19 virus was detected in the interstitial and endothelial cells and not necessarily in the myocytes, which emphasized the presence of lymphocyte and monocyte infiltration, and a particularly high level of monocytes causes myocardial ischemia[24,25]. Varga et al[27] have suggested that viral attack determines endothelium injury. This issue causes endotheliitis with the recruitment of inflammatory cells, apoptosis and pyroptosis, and subsequent microcirculatory distress[26,27]. Hence, the latest Position Statement[28] issued by the Working Group on Atherosclerosis and Vascular Biology, together with the Council of Basic Cardiovascular Science of the European Society of Cardiology acknowledges the key role of the endothelium in COVID-19-associated cardiovascular pathophysiology, and recommend that endothelial biomarkers and tests of function to be considered for early detection of cardiovascular complications.
Downregulation of ACE2

Recognition of ACE2 as the primary human receptor for the SARS-CoV-2 was the first step to identify the virus tropism and pathogenicity\[29,30\]. The literature shows that ACE2 is expressed in type II alveolar epithelial cells, myocardial cells, vascular endothelium, esophageal and bladder epithelium cells, and renal cells\[31,32\]. The virus uses S protein for binding to the ACE2 receptor of target cells, and the cellular serine protease TMPRSS2 cleaves the S protein into two functional domains, S1 that binds to ACE2 and S2 designed for membrane fusion\[30,33-37\]. The cleavage can be produced near a fusion peptide located within the S2 domain\[33,38\]. This mechanism helps the virus priming and entry into the cells and promotes virus infectivity\[30,33,39\]. Lai et al\[39\] have demonstrated that SARS-CoV fusion depends on calcium level, so a low level of calcium decreases infectivity. It is known that ACE2 and ACE are linked to the renin–angiotensin–aldosterone system, which promotes angiotensin I maturation, and has a crucial effect on the cardiovascular system\[41\].

Angiotensin I hydrolyzation produced by ACE2 yields angiotensin 1-9 peptide, on which ACE acts to produce angiotensin 1-7 (Ang 1-7)\[17\]. Ang 1-7 is the ligand for the G-protein Mas receptor that provides cardioprotective effects as vasodilatory, antiproliferative and antioxidative effects\[17\]. ACE2 has a direct effect on angiotensin II, producing Ang 1-7, but also acts on bradykinin ligand receptor, Des-arg9-bradykinin, thereby inactivating an inflammatory response\[17,41,42\].

In SARS-CoV 2 infection, decreased ACE2 expression causes lower levels of Ang 1-7 and an increase in angiotensin II level\[17,41,43\]. This effect results in vasoconstriction, inflammation, proliferation, fibrosis, apoptosis, and de novo heart injury or aggravation of pre-existing cardiovascular problems\[43\]. Ang II activates both mitogen-activated protein kinase and ADAM-17 phosphorylation that generates reactive oxygen species (ROS), which promote endothelial dysfunction and thrombosis\[44,45\].

Downregulation of ACE2 causes an increased level of angiotensin II, which induces production of inflammatory cytokines such as interferon-γ, interleukin (IL)-6, and the chemokine monocyte chemoattractant protein (MCP)-1, promoting inflammation\[45-47\]. MCP-1 can be an ROS source, promoting negative remodeling after MI\[44,46\].

Cytokine storm/cytokine release syndrome

Many severe infectious and noninfectious diseases, including COVID-19, are associated with cytokine overproduction, activating lots of signals and communication pathways\[48,49\]. The inflammation starts in the lungs via ACE2 receptor, which is localized in the pneumocytes, local pulmonary macrophages, and dendritic cells, and it spreads through the circulation to organs expressing ACE2, with significant effects on the cardiovascular system\[50\].

Oudit et al\[51\] have shown that an increased level of Ang II determines infiltration and activation of neutrophils in the myocardium, which release inflammatory cytokines (IL-6, IL-1β) and MCP-1 and are a source of ROS, with a negative inotropic effect on murine myocardial contraction\[51\].
SARS-CoV-2 activates the innate immune system and triggers the JAK-STAT pathway via the pattern of recognition receptor, with overproduction of IFNs[38,52]. IFN type I increases the inflammatory factors and activates the cytokine storm[38,52,53]. Rapid replication of the virus determines the activation and differentiation of T helper (Th)1 cells, producing cytokines such as IL-6, granulocyte-macrophage colony-stimulating factor and IFN-γ, and increases the number of Th1 and Th2 cells, macrophages and natural killer cells[54]. The virus has developed new mechanisms through nonstructural protein to avoid the immune system, and suppresses the effects of IFNs, which lead to virus dissemination and promotion of cytokine realizing syndrome[50,54,56-57]. The first cytokines produced in the early phase of the infection are IL-6, tumor necrosis factor (TNF)-α, IL-1, IL-8 and MCP-1[56,58].

In the severe form of COVID-19, chemokines CCL3, CXCL8, CXCL9 and CXCL10 are released into the blood circulation, as well as proinflammatory cytokines TNF-α, IFN-γ, IFN-α, IL-12, IL-1β, IL-6, IL-33, IL-18 and transforming growth factor β, leading to an important inflammatory response[18,53,58,59]. Latest studies indicate that a higher level of inflammatory biomarkers such as IL-6, IL-8 and TNF-α determine MI and correlate with high mortality[60,61].

IL-6 plays the main role in inflammation. There are two mechanisms through the JAK-STAT3 signaling pathway for activating and promoting inflammation[59]. The first mechanism of action is the cis-signaling pathway that uses membrane IL-6 receptor, which activates the innate and acquired immune system[62,63]. The second mechanism is through the trans-signaling pathway, which uses soluble IL-6 receptors for activating cells without IL-6 membrane receptors, such as endothelial cells[62,64]. The IL-6 mechanism results in oversecretion of vascular endothelial growth factor, MCP-1, IL-8 and IL-6, and decrease of E-cadherin on endothelial cells, promoting apoptosis of cardiac cells and left ventricular remodeling[49,65]. Del Turco et al[62] have shown that hyperinflammation promotes vascular permeability, leakage, endothelial dysfunction, and hypercoagulation with a significant impact on the cardiovascular system. Also, the production of matrix metalloproteinase by the monocytes/macrophages increases the risk of atherosclerotic plaque rupture and the probability of MI[62].

**Hypercoagulability**

Endothelial dysfunction, hyperinflammation, and hypoxia induced by SARS-CoV-2 contribute to a procoagulant status with major effects on the cardiovascular system [66]. Inflammation and coagulation play a bidirectional role in vascular disease[67]. The inflammation causes endothelial dysfunction, which activates coagulation and together with the coagulation factors, increases cytokine production by the endothelial and mononuclear cells[67]. Endothelial dysfunction by activating the complement system, causes a hypercoagulant state and promotes inflammation[68]. The central role in thrombogenesis is played by tissue factor (TF)[50,68]. TF is a transmembrane protein expressed on the surface of macrophages, cardiomyocytes and smooth muscle cells[48,49]. The monocytes in atherosclerotic plaques tend to express more TF than the circulating ones, which stimulates cytokines such as IL-6, platelet-derived growth factor and MCP-1, and leads to thrombus formation. In severe infection, cytokines, especially IL-6, determine TF exposure and systemic activation of coagulation[68-70]. TF binds to factor VIIa, leading to thrombin formation, which converts fibrinogen into fibrin and determines coagulation[70]. Thrombin also binds to another class of specific receptors, protease-activating cell receptors (PARs), which are expressed in many cell types, including endothelial cells, monocytes, platelets, smooth muscle cells, and fibroblasts. Their activation is a key promoter of both coagulation and inflammation[70]. Four PAR types are identified; type 2 determines TF exposure and systemic activation of coagulation[68-70]. The second cytokines produced in the early phase of the infection lead to multiple effects such as endothelial inflammation with metabolic changes that affect ATP production and an increase in mitochondrial ROS, which causes platelet hyperactivation and apoptosis with release of proinflammatory and procoagulant factors[75,76].

Severe hypoxia activated in SARS-CoV-2 infection leads to multiple effects such as endothelial inflammation with metabolic changes that affect ATP production and an increase in mitochondrial ROS, which causes platelet hyperactivation and apoptosis with release of proinflammatory and procoagulant factors[75,76]. Hypoxia promotes thrombogenesis through a direct mechanism via early growth response factor 1 induction, but also through and an indirect mechanism mediated by inflammatory cytokines (TNF-α and IL-1)[75-78]. Hypoxia also activates hypoxia-inducible transcription factors that promote coagulation targeting factors such as...
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Platelets play a crucial role in coagulation and are the first blood cells that respond to endothelial damage. Coronavirus disease causes platelet hyperactivation due to P-selectin increased membrane expression, which interacts with its counter-receptors on neutrophils or other inflammatory cells, thereby activating thrombogenesis.[80] After autopsy of patients with acute MI, many megakaryocytes and inflammatory cells are found in the microvascular system, along with venous thrombosis and platelet-rich thrombi.[81] Recent studies have shown that the antiphospholipid antibodies interact with complement factors, platelets, and endothelial cells, promoting coagulation; ongoing and future research will validate the role of antiphospholipid syndrome in COVID-19.[82]

CLINICAL IMPLICATIONS

Various potential therapeutic strategies addressing specific pathophysiological mechanisms are currently used to prevent and/or alleviate the MI caused by COVID-19. Some pharmacological agents target mechanisms with definite evidence of causing cardiovascular damage, hence being part of standard of care therapy and recommended by existing guidelines, while others address hypothetical mechanisms, hence being under study.

Is it safe to continue using ACE inhibitors or angiotensin receptor blockers in COVID-19 patients?

Considering that one potential mechanism of acute MI is mediated by ACE2[83], the question arises whether therapy with ACE inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) should be continued or stopped. Existing evidence-based consensus and position statements[84-87] issued by prominent cardiovascular and hypertension societies recommend against modifying this therapy if it is already underway, and to prescribe it for newly diagnosed patients as usual, given the absence of consistent evidence regarding their potential risk[88,89]. A randomized clinical study of 659 patients hospitalized with mild to moderate COVID-19 and ACEIs or ARBs therapy prior to hospitalization has shown that there was no significant difference in the mean number of days alive and out of the hospital between the patients assigned to discontinue or continue this therapy[90]. Also, a large meta-analysis of > 28000 hypertensive patients with COVID-19 on ACEIs or ARBs has found a beneficial effect of using renin–angiotensin-aldosterone system inhibitors in these patients[91]. Nevertheless, additional studies are warranted to evaluate the role of ACE2 polymorphisms in conferring an increased risk of adverse outcomes, as recently disclosed by a systematic review and meta-analysis that evaluated the clinical outcomes in COVID-19 patients on ACEIs or ARBs[92].

Potential therapies for COVID-19 patients with cytokine storm mechanism

IL-6 receptor antagonists such as tocilizumab and sarilumab may represent an interesting alternative for patients with significantly elevated IL-6, ferritin, D-dimer and high-sensitivity troponin I (TnI) levels[89]. A Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) has investigated the effectiveness of tocilizumab and sarilumab on survival and organ support in critically ill COVID-19 patients and it has shown improved outcomes and survival[93]. Other clinical trials are underway[94-96].

Another potential therapeutic is colchicine, due to its anti-inflammatory effect through inhibition of cytokine production and neutrophil activity, and it does not have an immunosuppressive effect compared with tocilizumab and sarilumab[97]. Several small randomized controlled trials have already shown a positive impact of adding colchicine to the standard treatment in COVID-19 patients[98-100]. Randomized trials with larger populations are in progress[101-103]. Given the high prevalence of thromboembolic anomalies and coagulopathy in patients with COVID-19, use of thromboprophylaxis may be necessary.

What are the current recommendations for antithrombotic therapy in VTE prophylaxis in patients with COVID-19?

In nonhospitalized patients with mild COVID-19, anticoagulants and antiplatelet
therapy are not recommended routinely\textsuperscript{[104]}, but should be considered depending on risk assessment\textsuperscript{[105]}. For those with confirmed VTE, the CHEST guidelines recommend a direct oral anticoagulant (DOAC) with apixaban, rivaroxaban, dabigatran or edoxaban (before dabigatran and edoxaban an initial parenteral anticoagulation is needed). When a DOAC is not used, vitamin K antagonists are recommended over low-molecular-weight heparin (LMWH)\textsuperscript{[106]} (Table 1).

In acutely ill hospitalized patients with COVID-19, anticoagulant thromboprophylaxis is recommended. The CHEST guidelines are in favor of anticoagulation with LMWH or fondaparinux over unfractionated heparin (UFH) or DOAC\textsuperscript{[106]}. UFH is not preferred, in order to limit staff exposure, and DOAC is not recommended as a primary prevention strategy due to possible risk of interactions between therapies for COVID-19 and oral anticoagulants\textsuperscript{[106]}. The American Society of Hematology guidelines do not recommend any specific anticoagulant to be used as first-choice treatment\textsuperscript{[107]}. There is no recommendation to increase intensity of anticoagulation thromboprophylaxis, and the current standard dose should be used over intermediate or full treatment dosing\textsuperscript{[106-109]}. However, the Italian Society on Thrombosis and Haemostasis suggests that the use of intermediate dose of LMWH should be considered in patients with multiple risk factors for VTE\textsuperscript{[110]}. Also, the Royal College of Physicians suggests that a higher dose of LMWH may be considered in these patients\textsuperscript{[105]}.

In acutely ill hospitalized patients with COVID-19 with confirmed VTE, the CHEST guidelines recommend initial parenteral anticoagulation with LMWH or IV UFH or initial direct oral anticoagulation with apixaban or rivaroxaban (dabigatran and edoxaban can be used after initial parenteral anticoagulation)\textsuperscript{[106]}.

In critically ill patients with COVID-19 anticoagulant thromboprophylaxis is recommended. The CHEST guidelines are in favor of anticoagulation with LMWH or UFH over fondaparinux or a DOAC\textsuperscript{[106]}. If there is any contraindication to pharmacological thromboprophylaxis, mechanical thromboprophylaxis may be considered, but it is not recommended to add it to pharmacological treatment\textsuperscript{[106]}.

Most guidelines recommend the use of current standard dose over intermediate or full treatment dosing due to insufficient data regarding intensified treatment\textsuperscript{[106-108]}. Nevertheless, the Anticoagulation Forum suggests, based on expert opinion, that an increased dose of anticoagulant thromboprophylaxis such as enoxaparin 40 mg or 0.5 mg/kg subcutaneous twice daily, UFH 7500 U subcutaneous three times daily or low-intensity heparin infusion, should be considered for these patients\textsuperscript{[109]}. The Royal College of Physicians also suggests intermediate dose of LMWH\textsuperscript{[105]}. In critically ill COVID-19 patients with confirmed VTE, the CHEST guidelines recommend parenteral anticoagulation with LMWH or fondaparinux over UFH\textsuperscript{[106]}. If there is any contraindication to pharmacological thromboprophylaxis, mechanical thromboprophylaxis may be considered, but it is not recommended to add it to pharmacological treatment\textsuperscript{[106]}.

In COVID-19 patients discharged from hospital, we may consider extending thromboprophylaxis for those with increased postdischarge risk of VTE and low bleeding risk\textsuperscript{[105,106,109]}. The Royal College of Physicians recommends a duration of 14–28 d of thromboprophylaxis with LMWH\textsuperscript{[105]}. The Anticoagulation Forum suggests using anticoagulants such as betrixaban maximum 35–42 d, rivaroxaban maximum 31–39 d or enoxaparin maximum 6–14 d\textsuperscript{[109]}.

In patients with recurrent VTE and COVID-19 despite anticoagulation with DOAC or vitamin K antagonist therapy, the CHEST guidelines recommend switching treatment to LMWH. In patients with recurrent VTE despite anticoagulation with LMWH they suggest increasing the dose of LMWH by 25%–30%\textsuperscript{[106]}.

Regarding antiplatelet therapy for COVID-19 patients, there are no data that would suggest any benefit of using antiplatelet agents to prevent thrombosis and we should consider the risk associated with the use of them given that a thrombocytopenic status may exist in patients with COVID-19\textsuperscript{[66,67]}. Furthermore, the CHEST guidelines recommend against the use of antiplatelet agents for VTE prevention\textsuperscript{[106]}.

Many studies have shown that there is a high prevalence of arterial and venous thromboembolism in hospitalized patients with COVID-19 despite standard thromboprophylaxis\textsuperscript{[14]}. Hence, is it possible that a higher dose of anticoagulant might be necessary? The recommendation of the intensity of anticoagulant thromboprophylaxis is not based on direct evidence of the effects of intermediate or therapeutic dose in primary prevention because of the lack of well-designed randomized clinical studies. A collaboration between three randomized clinical trial platforms ATTACC (Antithrombotic Therapy to Ameliorate Complications of COVID-19), REMAP-CAP (Randomized Embedded Multi-factorial, Adaptive Platform Trial) and ACTIV-4a (Accelerating COVID-19 Therapeutic Interventions and Vaccines) is ongoing in order to clarify this issue\textsuperscript{[113]}.
**Table 1 Recommendations of thromboprophylaxis and treatment of VTE in patients with coronavirus disease 2019**

<table>
<thead>
<tr>
<th>COVID-19 patients</th>
<th>Prevention</th>
<th>Treatment</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outpatient</td>
<td>Thromboprophylaxis is not routinely recommended</td>
<td>DOAC (apixaban, rivaroxaban, dabigatran or edoxaban)</td>
<td>NIH COVID-19 Treatment Guidelines[104], CHEST Guideline[106]</td>
</tr>
<tr>
<td>Acutely ill hospitalized patient</td>
<td>LMWH or fondaparinux standard dose</td>
<td>Initial anticoagulation with LMWH or IV UFH or DOAC (apixaban, rivaroxaban)</td>
<td>CHEST Guideline[106]</td>
</tr>
<tr>
<td>Critically ill COVID-19 patient</td>
<td>LMWH or UFH standard or intermediate dose</td>
<td>LMWH or fondaparinux</td>
<td>The Royal College of Physicians[105], CHEST Guideline[108], ASH guidelines[107], ISTH interim guidance[109]</td>
</tr>
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**Potential therapies for COVID-19 patients with thrombocytopathy and endotheliopathy**

As mentioned before, currently there is no specific recommendation for using antiplatelet agents in COVID-19 patients. However, according to the present understanding of the mechanisms of thrombocytopathy and endotheliopathy, targeting therapeutics to both endothelium and platelets may be effective. Considering the effects of aspirin such as antithrombotic and anti-inflammatory actions and inhibition of virus replication[114], clinical trials on the protective effect of aspirin in COVID-19 patients are underway[115,116].

In addition to this, antithrombotic agents with vasodilatory action on vascular smooth muscle cells and anti-inflammatory action, such as prostacyclin and NO, may become a potential therapeutic alternative in patients with thrombocytopathy and endotheliopathy[80]. Clinical trials on administration of prostacyclin or NO in COVID-19 patients are in progress[117,118]. Similarly, dipyridamole, a phosphodiesterase 3 inhibitor with antiplatelet and anti-inflammatory action, could have beneficial effects in COVID-19 patients[119]. The potential therapeutic benefits are being investigated[120,121].

A recent systematic review and meta-analysis has shown that the use of statins in patients with COVID-19 has a beneficial effect on improving clinical outcomes[122]. However, we must consider that elevated liver enzymes are common in patients with moderate to severe COVID-19, even though its impacts is still unknown and statin therapy should be discontinued in these patients[123,124]. Multiple clinical trials on using statins in COVID-19 patients are ongoing[116,125-127].

**Experimental therapies**

Various pharmacological agents aiming to limit viral entry into cells are currently under study. Previous data[128-130] have endorsed recombinant human ACE2 as an attractive therapeutic target for the current COVID-19; the molecule acting as a decoy receptor, hence curbing viral entry[83]. The efficacy of recombinant ACE2 is being investigated in a small pilot trial including patients with severe COVID-19 (Clinical-trials.gov NCT04287686).

An alternative way of blocking SARS-CoV-2 cell invasion is inhibition of TMPRSS2 activity. Some potential therapeutic strategies targeting TMPRSS2 are already tackling COVID-19 clinically, while others are just being tested in the laboratory[131]. The former includes serine protease inhibitors such as camostat mesylate[19], which is presently considered for off-label treatment of SARS-CoV-2-infected patients (Clinical-trials.gov NCT04321096).

**CONCLUSION**

MI is an important cardiovascular manifestation in COVID-19 patients associated with increased severity and high risk of mortality. At this point, the pathophysiology underlying COVID-19-related MI is not fully understood, but clinical evidence has shown that not only a direct mechanism is involved, but also SARS-CoV-2 might affect the cardiovascular system in an indirect manner through interaction with ACE2, production of cytokines, thrombocyte and endothelium dysfunction, and hypercoagu-
lation. Elucidating the mechanisms underlying MI could help develop effective therapeutic strategies.

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Anemia in cirrhosis: An underestimated entity

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Abstract
Anemia in a patient with cirrhosis is a clinically pertinent but often overlooked clinical entity. Relevant guidelines highlight the algorithmic approach of managing a patient of cirrhosis presenting with acute variceal hemorrhage but day-to-day management in hospital and out-patient raises multiple dilemmas: Whether anemia is a disease complication or a part of the disease spectrum? Should iron, folic acid, and vitamin B complex supplementation and nutritional advice, suffice in those who can perform tasks of daily living but have persistently low hemoglobin. How does one investigate and manage anemia due to multifactorial etiologies in the same patient: Acute or chronic blood loss because of portal hypertension and bone marrow aplasia secondary to hepatitis B or C viremia? To add to the clinician’s woes the prevalence of anemia increases with increasing disease severity. We thus aim to critically analyze the various pathophysiological mechanisms complicating anemia in a patient with cirrhosis with an emphasis on the diagnostic flowchart in such patients and proposed management protocols thereafter.

Key Words: Anemia; Cirrhosis; Iron deficiency anemia; Macrocytic anemia; Normocytic anemia

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Core Tip: Anemia in a patient with cirrhosis is an important but often neglected disease
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**INTRODUCTION**

Anemia is a fairly common clinical condition. Its prevalence in the general population varies from 10%-24%[1]. However, in critically ill patients, and those with underlying malignancies or autoimmune disorders, the prevalence increases to 95%[2]. Anemia may be seen in 66%-75% of patients with liver cirrhosis[1,3]. Iron deficiency, which is the commonest type of anemia, has been observed in 22% of patients with compensated cirrhosis and 78% in those with decompensated disease. Apart from anemia, thrombocytopenia and leucopenia are other abnormal hematological indices seen in patients with cirrhosis. Thrombocytopenia is by far the commonest hematological abnormality seen in patients with cirrhosis followed by leucopenia and anemia [4]. The pathophysiological sequelae of cirrhosis adversely affect the synthetic and immunological functions of the liver. This manifests as hematological dysfunctions including anemia.

**SIGNIFICANCE OF ANEMIA IN CIRRHOSIS**

The presence of anemia in a cirrhotic patient can be considered a ‘vicious cycle’. The following facts need consideration: The severity of iron deficiency anemia (IDA) increases with increasing Child’s Pugh Turcotte (CTP) score: CTP A (26.5%), CTP B (59.2%), and CTP C (69%). Higher the degree of portal hypertension, the higher is the risk of developing severe anemia (< 10 gm/dL)[1]. Acute gastrointestinal (GI) bleed can be potentially catastrophic sequelae of portal hypertension[5]. Anemia by itself has been found to have an increased risk of hepatic decompensation and liver-related mortality in patients with compensated cirrhosis[6]. Anemic patients with cirrhosis have been reported to have higher model for end-stage liver disease (MELD) scores and lower albumin levels. The latest research has highlighted that hemoglobin (Hb) can be considered as a marker for worse disease. Conversely, the higher the MELD score, more likely is the possibility of having hematological complications[7]. Anemia has been postulated to have a pathophysiological role in the development of hepato-renal syndrome[8]. Besides increasing the risk of mortality, anemia is associated with a higher incidence of acute on chronic liver failure (ACLF) and increased risk of hospitalization[1]. The vicious cycle of portal hypertension, worsening disease severity, and anemia in cirrhosis are depicted in Figure 1. Moreover, blood transfusion in anemia can itself precipitate secondary iron (Fe) overload thereby increasing the risk of hepatocellular carcinoma (HCC) and mortality. Thus, it may be noteworthy to understand anemia as a part of the disease process rather than just a disease complication.

**ETIOPATHOGENESIS OF ANEMIA IN CIRRHOSIS**

The liver, owing to its unique portal circulation, synthetic and immunological functions can give rise to multiple hematological manifestations, and anemia in cirrhosis is often multifactorial (Table 1).
### Table 1 Etiopathogenesis and prevalence of anemia in cirrhosis

<table>
<thead>
<tr>
<th>Type of anemia</th>
<th>Etiology</th>
<th>Prevalence (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocytic</td>
<td>Anemia of chronic disease</td>
<td>40-51.4</td>
<td>Singh et al[7], Özatlı et al[56]</td>
</tr>
<tr>
<td></td>
<td>Acute blood loss (variceal hemorrhage)</td>
<td>5-15/yr; Increasing risk with severity of liver dysfunction and red wale marks on varices</td>
<td>Singh et al[7], European Association for the Study of the Liver[45]</td>
</tr>
<tr>
<td></td>
<td>Portal hypertensive gastropathy</td>
<td>20-80</td>
<td>Gkamprela et al[37]</td>
</tr>
<tr>
<td></td>
<td>Gastric antral vascular ectasia</td>
<td>4</td>
<td>Selinger and Ang[57]</td>
</tr>
<tr>
<td></td>
<td>Peptic ulcer</td>
<td>35-53</td>
<td>Singh et al[7], Loperfido et al[58]</td>
</tr>
<tr>
<td></td>
<td>Hemolytic anemia in patients on interferon and ribavirin</td>
<td>9-13</td>
<td>Gonzalez-Casas et al[3]</td>
</tr>
<tr>
<td>Microcytic anemia</td>
<td>Hemolytic anemia due to hypersplenism</td>
<td>24</td>
<td>Özatlı et al[56]</td>
</tr>
<tr>
<td></td>
<td>Folic acid (Vit B9) deficiency</td>
<td>44</td>
<td>Herbert et al[59]</td>
</tr>
<tr>
<td></td>
<td>Vit B12 (cyanocobalamin) deficiency</td>
<td>31.8 in PBC; 43 in NAFLD</td>
<td>Singh et al[7], Sharma and Jahnavi[60], Shizuma[61]</td>
</tr>
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<td>Macrocytic anemia</td>
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<td>Singh et al[7], Sharma and Jahnavi[60], Shizuma[61]</td>
</tr>
</tbody>
</table>

Vit: Vitamin; NAFLD: Nonalcoholic fatty liver disease.

**Figure 1 Vicious cycle of portal hypertension and anemia in chronic liver disease.** HVPG: Hepatic venous pressure gradient; MELD: Model foe end stage liver disease; CTP: Child Turcotte Pugh score.

To understand the development of anemia, it is imperative to understand the critical role played by this oxygen-carrying micronutrient: Iron. 80% of the body’s total iron stores (0.2-0.4 g) are stored as Hb in red blood cells (RBC). Ferric iron (Fe$^{3+}$) following its absorption in the duodenum is converted to ferrous iron (Fe$^{2+}$) by the action of ferric reductase duodenal cytochrome b. This iron is transported into the cytoplasm of the enterocyte and is either stored or exported by the iron exporter enzyme ferroportin. The next step involves oxidization of Fe$^{2+}$ to Fe$^{3+}$ form to ferroxidase hephaestin and ceruloplasmin (Cp). Fe$^{3+}$ in combination with enzyme transferrin (Tf) undergoes circulation in the body. Erythrocyte precursors, known as erythroblasts, utilize a principal portion of Tf bound iron (Figure 2). A highly efficient recycling system in the spleen and hepatic macrophages ensures optimal utilization of iron stores. Thus, the human liver is an important component of this highly efficient iron homeostasis. The liver synthesizes proteins involved in iron homeostasis: Tf (80 kDa glycoprotein), Cp (copper linked serum ferroxidase), multi-subunit protein ferritin, and a 25 amino acid peptide, hepcidin.

IDA may occur secondary to acute or chronic blood loss. The various causes are variceal hemorrhage which usually presents with an overt GI bleed, and portal hypertensive gastropathy (may present with either overt or obscure GI bleed). The incidence of duodenal ulcer and ulcer-related bleed is more common in patients with cirrhosis. Besides, nutritional deficiencies including those of iron, Vitamin (Vit) B12, B6, and folate are common in patients suffering from cirrhosis. In addition, hypersplenism secondary to portal hypertension may contribute to iron deficiency. Amongst
the etiological agents leading to the development of cirrhosis, a few, in particular, have been found to have a predominant role in the pathogenesis of anemia: alcohol may cause blood loss because of alcohol-induced gastritis. It also has a direct toxic effect on erythroid precursors and may cause Vit B12, folic acid deficiency. Besides, alcohol-related malnutrition may lead to reduced iron absorption. Hepatitis B and C may cause bone marrow aplasia, Wilson’s disease may be associated with hemolytic anemia in around 1%-12% of cases as copper released following hepatocyte necrosis causes oxidative dysfunction of phospholipids lining the RBC membrane leading to hemolysis and worsening of liver dysfunction[9]. Another form of acquired hemolytic anemia: Spur cell anemia may occur in alcohol-related cirrhosis wherein abnormal lipid metabolism leads to altered RBC membrane causing reduced deformability of RBC. A triad consisting of cholestatic jaundice, transient hyperlipidemia and hemolytic anemia — Zieve’s syndrome has been rarely reported in alcohol-related cirrhosis[10]. Although now of historical interest, Ribavirin, a nucleoside anti-metabolite, used in the treatment of chronic hepatitis C (CHC), causes dose-related hemolytic anemia in around 10% of patients[11]. Autoimmune hemolytic anemia may also be seen in patients with autoimmune hepatitis. D-Penicillamine, a copper chelating agent, used in the treatment of Wilson’s disease, in turn, may cause iron chelation manifesting as IDA. Another drug used in Wilson’s disease, Trientine, may cause sideroblastic anemia[9]. However, the most common cause of anemia in cirrhosis is anemia of chronic disease, which develops secondary to an underlying chronic inflammatory state. Before we proceed further to understand the pathophysiology of anemia of chronic disease in cirrhosis, it is worthwhile to mention the proposed hypothesis of ‘Eryptosis’: Programmed cell death of erythrocytes which may contribute to anemia. This is akin to apoptosis of nucleated cells despite the absence of organelles involved in apoptosis. In a murine model, a high bilirubin level has been shown to increases Ca²⁺ influx, sphingomyelinase activation within erythrocytes, thereby triggering eryptosis[12].

**Anemia of chronic disease and the critical role of hepcidin**

It is worthwhile to emphasize the role of ‘Hepcidin’ (hepatic bactericidal protein), an iron regulatory hormone, produced in excess by the liver, in maintaining iron homeostasis. Increased iron levels in plasma and increased iron storage stimulates hepcidin production which further blocks dietary iron absorption and storage. In iron deficiency states hepcidin production is suppressed, which ensures increased dietary iron absorption. Erythropoietic processes cause high iron consumption to suppress hepcidin production. This ensures that stored iron is released by hepatocytes and macrophages and intestinal absorption of dietary iron increases. In chronic inflammatory states like cirrhosis hepcidin production is mediated by two distinct mechanisms: Interleukin-6 (IL-6) (a pro-inflammatory cytokine), mediated as well as IL-6 independent pathways. It is especially pertinent to understand that in inflammatory states like cirrhosis, increased body iron stores no longer suppress hepcidin...
production *i.e.*, even if plasma iron level is low, hepcidin production is increased by IL-6 mediated pathway (while under normal circumstances, hepcidin production should have been downregulated in iron deficiency). Moreover, in cirrhosis, hepcidin is produced by myeloid cells by activating toll-like receptor-4, a receptor present on surface membranes of neutrophils and macrophages. This excess of iron gets trapped within the cells causing reduced availability of iron for forming Hb and thereby manifesting as anemia of chronic disease[2,13]. Three major pathways control stimuli related and basal hepcidin expression. The best-studied is the SMAD/bone morphogenetic signaling pathway which explains the evolution of anemia in inflammatory disorders. IL-6 is an inflammatory mediator that activates the Janus kinases-Signal transducer and activator of transcription proteins (JAK-STAT-3) pathway by binding to IL-6 receptor (IL6-R). STAT-3, in turn, causes increased hepcidin expression. The other important pathway involves binding of TF to transferrin receptor-1 (TfR-1) which in turn causes dissociation of transferrin receptor-1-human homeostatic iron regulator (TfR-1 HFE complex). The available HFE interacts with TfR-2 to increase BMP6 mediated phosphorylation of SMAD1/5/8. SMAD 1/5/8 further recruits SMAD4 to increase hepcidin expression. The least understood pathway is the one wherein hypoxia and erythropoiesis inhibit hepcidin expression by direct binding of hypoxia-inducible factor (HIF) to the promoter region of the hepcidin receptor. Figure 2 explains the pathophysiological role of Hepcidin in the development of anemia[13-15].

HEPCIDIN AS A BIOMARKER IN LIVER DISEASE

Hepcidin has been postulated as a potential biomarker in liver fibrosis and cirrhosis. Alcohol, a well-established cause of liver cirrhosis has been associated with low hepcidin levels. Low hepcidin levels have been documented in individuals with chronic alcohol consumption and preserved liver functions. Low hepcidin levels have been shown to worsen liver fibrosis in patients with CHC and CHB infection as well as autoimmune liver disease. Diagnostic use however is limited by lack of standardization especially in patients with liver disease[16].

While the pathophysiological mechanisms involved in iron deficiency and chronic inflammation leading to the development of anemia in cirrhosis have been described in the literature, the pathophysiological changes leading to macrocytic anemia have been poorly understood. It has been postulated that splenomegaly secondary to portal hypertension causes secondary hemolysis which in turn causes increased plasma volume and macrocytosis. Alcohol per se can cause secondary malnutrition and folic acid deficiency besides adversely affecting erythropoiesis in the bone marrow.

TYPES OF ANEMIA IN CIRRHOSIS

To co-relate with the underlying pathogenesis and to evaluate a patient with anemia with underlying cirrhosis, anemia may be classified as per the RBC indices, as follows: Normocytic: Anemia of chronic disease; Microcytic: Acute variceal hemorrhage, chronic blood loss due to portal gastropathy, alcohol-related gastritis or intestinal malabsorption, treatment-related (D-Penicillamine); Macrocytic: Vit B12, B6, Folate deficiency; Hemolytic: Wilson’s disease, Spur cell anemia, Autoimmune hemolytic anemia, treatment-related (Ribavirin); Aplastic: Hepatitis B, hepatitis C related; and Sideroblastic: Drug-induced (Trientene in Wilson’s disease).

DIAGNOSTIC EVALUATION IN A CASE OF ANEMIA IN CIRRHOSIS

Anemia in cirrhosis is independently associated with increased mortality and morbidity. Moreover, there could be an interplay involving multiple etiologies. Therefore, it becomes imperative to have a simple, easily available, yet informative diagnostic algorithm to aid in the diagnosis and thereafter management of the predominant etiology of anemia in cirrhosis. The following parameters may be considered as baseline laboratory investigations for the evaluation of anemia in cirrhosis. Since there are multiple pathogenic mechanisms into play, none of these parameters are specific to diagnose the cause of anemia in cirrhosis. However, each of these investigations is an important tool for initial screening as well as prognosis for the severity of the underlying liver disease.
These parameters include: Hb level; White blood count (WBC) and differential cell count (DLC); Platelet count; RBC indices; Mean corpuscular volume (MCV); Absolute reticulocyte count; Serum iron studies; Serum ferritin; Transferrin saturation (TSAT); and Hepcidin.

**Hb level**

Estimation of Hb is the initial screening method for the diagnosis of anemia. World Health Organization (WHO) classifies anemia as < 13 g/dL for men, < 12 g/dL for non-pregnant females and < 11 g/dL for pregnant females[17]. Hb, being an easily reproducible test across different laboratories and with a lower coefficient of variance vs. hematocrit, is the preferred investigation. Moreover, variables like patients’ serum glucose and storage time of samples do not affect the measurement of Hb[18]. Complete cell count including WBC, DLC, and platelet count, estimate the bone marrow function. Hypersplenism, Vit B12 deficiency, aplasia secondary to hepatitis B or C may cause pancytopenia in patients with cirrhosis.

**Absolute reticulocyte count and reticulocyte index**

Absolute reticulocyte count and reticulocyte index (reticulocyte count which has been adjusted to the degree of anemia) is a useful screening test to ascertain the appropriate bone marrow response to anemia. An abnormal reticulocyte count along with low Hb concentration is associated with increased mortality in liver transplantation (LT) patients[19].

**RBC indices**

**Red cell distribution width:** Red cell distribution width (RDW) has been suggested as a potential marker of inflammatory diseases. Studies on this aspect have shown conflicting results. Some researchers have postulated that RDW increases with worsening liver disease. They have shown that increased RDW is associated with increased 3-mo mortality in decompensated cirrhosis[20,21]. Other researchers, however, have provided evidence to the contrary and failed to prove any statistical significance with worsening liver disease or any significance in differentiating the type of anemia in cirrhosis[22].

**MCV**

Changes in erythrocyte membrane morphology and erythrocyte volume have been documented in patients with cirrhosis irrespective of the presence of anemia. Macrocytosis and normocytosis are the most frequently observed changes in cirrhosis [23]. MCV is an important investigation in the diagnosis of anemia with a high predictive value in diagnosing alcohol-related liver diseases as well as alcohol abuse. Studies have demonstrated that macrocytosis (MCV > 100 fL) was seen in 64%-84.5% of patients with alcohol consumption > 80 g/d even in the absence of anemia[24,25]. Vit B 12 and folate deficiency, increased deposition of cholesterol in RBC membrane, presence of immature RBCs’ (20% larger than mature erythrocytes), may all contribute to macrocytosis in cirrhosis. In patients with hepatitis B-related decompensated cirrhosis, macrocytosis is associated with severe disease (determined by higher MELD scores) and a higher risk of death secondary to HCC[26,27].

**Serum iron studies**

**Serum ferritin:** The hepatocyte is the principal site of production of ferritin, a marker of iron homeostasis and an acute phase reactant. Serum ferritin level < 30 μg/dL has a sensitivity of 92% to diagnose IDA in the general population[28,29]. However, in patients with underlying inflammatory disorders and cirrhosis a value < 100 μg/dL has a better predictive value to diagnose IDA[30]. Systemic analysis on the utility of measuring ferritin in patients with cirrhosis revealed that values, 15 g/dL were highly specific to establish a diagnosis of IDA in cirrhosis while values > 100 g/dL virtually ruled out IDA[31].

Another important fact worth considering is that 10%-30% of patients with cirrhosis have iron overload. This is particularly significant in individuals with nonalcoholic fatty liver disease, alcoholic liver diseases, CHC, and primary biliary cholangitis. Excess iron has been demonstrated in 8% of patients with an advanced liver disease akin to hemochromatosis even in the absence of specific genetic mutations. Iron excess may initiate the second process of liver injury and increases the risk of HCC[32]. Besides diagnosing IDA and predicting increased risk of HCC, elevated levels of serum ferritin have also been shown to independently predict mortality similar to MELD score in patients of end-stage liver disease[33].
**TSAT**

High serum ferritin and low transferrin are oft-reported findings in cirrhosis. Available literature suggests that transferrin and TSAT are independent predictors of mortality in ACLF and decompensated Cirrhosis[34]. TSAT value < 20% may be considered the level to initiate treatment for IDA[35]. EASL recommends TSAT > 45% in females and > 50% in males as a screening biochemical test for hereditary hemochromatosis[36]. However, the TSAT value needs to be read in the clinical context as TSAT has acute phase reactivity is affected by diurnal and dietary fluctuations of serum iron[37].

**Serum transferrin receptor**

The level of transferrin receptors in the serum can be used to ascertain the iron stores. It can be used to differentiate IDA (levels raised in IDA) from anemia of chronic disease[38]. In patients with cirrhosis, serum transferrin receptor is 91.6% sensitive and 84.6% specific to diagnose IDA in the absence of hemolysis and acute blood loss[39]. Lack of standardized tests, availability, and cost remain the limitations to its widespread use in clinical setting[37].

**Folic acid, Vit B12, Vit B6**

Deficiency of Vit B12, B6, and folic acid may be a contributing factor to the development of anemia in cirrhosis. European Society for Clinical Nutrition and Metabolism guidelines recommend baseline screening for Vit and micronutrients in all patients with cirrhosis[40]. However, the laboratory assays for detecting micronutrient deficiencies are not standardized for patients with cirrhosis. The various assays are Erythrocyte folate level < 140 ng/mL, plasma pyridoxal 5’ phosphate level < 20 nmol/mL, and methylmalonic acid level > 0.4 μmol/L qualify as folic acid, Vit B6 and Vit B12 deficiency respectively[41].

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**MANAGEMENT**

The management of anemia in a patient with cirrhosis may be considered under the following subheads: Patients with ongoing/acute bleeding; Patient without active/acute bleeding.

**MANAGEMENT OF A PATIENT OF ANEMIA IN CIRRHOSIS WITH ONGOING/ACUTE BLEEDING**

GI bleed is the second most common cause of decompensation in a patient with cirrhosis[38]. Bleeding esophageal varices, which constitute the predominant source of the variceal bleed, is associated with 10%-20% mortality over 6 wk. Initial management focuses on maintenance of intravascular volume and ‘restrictive transfusion strategy’ *i.e.*, initiating transfusion at Hb level < 7 g/dL to maintain Hb between 7-9 g/dL. This has been found to have a survival benefit in patients with Child’s A and B cirrhosis and it also decreases the risk of rebleeding in all patients with cirrhosis[42,43,44]. Intravenous splanchnic vasoconstrictors (terlipressin, somatostatin, octreotide), antibiotic prophylaxis, and intravenous proton pump inhibitors are recommended in the initial management of all cases of acute variceal bleed[45]. Combination treatment of endoscopic variceal ligation (EVL) and intravenous vasoconstrictors is the ‘standard of care’. Early rebleeding or failure of endoscopic therapy has been reported in 10%-15% of all patients with acute variceal bleed[5]. Rescue transjugular intrahepatic portosystemic shunt (TIPS) may be considered in such patients in addition to EVL and intravenous vasoconstrictors. Those patients with increased risk of re-bleeding (Child C status, score < 14 and no contraindications for TIPS), may be considered for preemptive TIPS and as a bridge to eventual LT[45]. Nonselective beta-blockers in addition to EVL form the cornerstone of management strategy to prevent a rebleed.
MANAGEMENT OF A PATIENT OF ANEMIA IN CIRRHOSIS WITH NO EVIDENCE OF ACUTE/ONGOING GI BLEED

Once the predominant cause has been identified, treatment should be initiated to provide symptomatic relief besides addressing the underlying disease, reduction of portal pressure, prevention of progressive fibrosis besides management of complications, and screening for HCC. The definite treatment, however, remains as LT.

MANAGEMENT OF IDA IN CIRRHOSIS

Treatment aims to provide symptomatic relief, replace iron stores and normalize RBC indices. Management of IDA secondary to acute blood loss has already been discussed in the section on ‘Management of anemia in cirrhosis with acute/ongoing GI bleed’. The efficacy of the available tests to diagnose IDA and to assess the adequacy of treatment has inherent drawbacks in patients with cirrhosis. These have been highlighted in the discussion on ‘specific investigations’ mentioned above. There are no available guidelines to manage a case of anemia in cirrhosis. Experience gained from the management of anemia in patients with chronic kidney disease (CKD), another progressive inflammatory disorder, may be utilized in patients with cirrhosis. Iron can be given as oral iron preparations (indicated for mild anemia > 11 g/dL or < 10.9 g/dL but > 8 g/dL): Divalent iron salts, ferrous sulphate, ferrous gluconate and ferrous fumarate. Ferrous sulphate is the universally available form. Traditionally the recommended dose in IDA is 100 -200 mg of elemental iron, preferably empty stomach, of which 10%-20% of elemental iron is absorbed. Although evidence is scarce, ascorbic acid 250-500 mg/d may be prescribed along with oral iron preparations. Treatment may be prescribed for a minimum of 3 mo to achieve adequate replacement for iron stores. Oral iron preparations are associated with considerable side effects: altered metallic taste, nausea, occasional vomiting, epigastric burning sensation, constipation, or diarrhea. Patients with cirrhosis may have a suboptimal response (< 1g/dL increase in Hb after 3 wk of therapy) owing to malabsorption or disease complications like hepatic encephalopathy (HE) because of constipation. Recent evidence suggests that altering the dosing schedule i.e., the alternate-day schedule may be as effective as the traditional daily dose regimen. Newer oral preparations like sucrosomial iron (SI) have been tried with efficacy similar to injectable iron in non-dialysis dependent CKD patients but not in patients with cirrhosis.

Failure of oral iron therapy, malabsorption, severe IDA are some of the universally accepted indications of intravenous (IV) iron therapy. Some or all of these may be present in patients of cirrhosis with IDA. IV iron preparations have different pharmacokinetics vs. oral iron preparations. Once into the bloodstream, elemental iron is taken up by macrophages and released via ferroportin thus circumventing, intestinal absorption. Adverse effects can vary from minor infusion reactions (rash, palpitation, myalgia, chest discomfort) to serious anaphylactoid reactions causing respiratory or hemodynamic changes. Among the available IV iron preparations, maximum single dose that can be administered and minimum administration time are as follows: Fe gluconate: 125 mg (30-60 min), Fe-succrose: 200 mg (30 min), Fe-carboxymaltose: 1000 mg (15 min), Fe-isomaltoside: 20 mg Fe/kg (15 min), Ferumoxytol: 510 mg (15 min) respectively. Blood transfusion (packed RBC transfusion) is generally reserved for patients who remain symptomatic despite IV iron therapy or are hemodynamically unstable. The key concept is not to target a normal Hb level but one at which iron supplementation can be safely initiated.

MANAGEMENT OF ANEMIA OF CHRONIC DISEASE IN PATIENTS WITH CIRRHOSIS

Two distinct yet complementary treatment strategies may be adopted while managing a patient with cirrhosis with anemia of chronic disease. The first caters to ‘silencing’ the underlying disease and managing its complications. Although, LT is the only definite cure, prevention of fibrosis, cessation of alcohol, treatment for HBV and HCV, all have a role to play. The second strategy involves managing nutritional deficiencies. Thus, it is imperative to treat co-existing IDA, Vit B12, B6, or folate deficiencies. This also brings to light the often ignored or neglected dietary prescription in patients
Table 2 Ongoing trials on evaluation and management of anemia in cirrhosis

<table>
<thead>
<tr>
<th>S No</th>
<th>Trial name</th>
<th>Clinical Trials.gov identifier</th>
<th>Aim</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Etiopathogenesis of anemia in chronic liver disease</td>
<td>NCT04622449</td>
<td>(1) To determine the prevalence of various etiologies of anemia in patients with liver disease; and (2) Association of liver disease severity as measured by MELD, MELD Na and CTP scores with severity of anemia</td>
<td>Premkumar [62]</td>
</tr>
<tr>
<td>2</td>
<td>Iron deficiency anemia in children with liver cirrhosis</td>
<td>NCT03482076</td>
<td>To determine prevalence of IDA in liver cirrhosis</td>
<td>Mohamed [63]</td>
</tr>
<tr>
<td>3</td>
<td>Lactoferrin in treatment of Fe deficiency anemia in cirrhosis</td>
<td>NCT04335058</td>
<td>(1) Correction of anemia (time frame: 1 mo): Number of participants achieving hemoglobin level &gt; 12 g/dL; (2) Correction of anemia (time frame: 3 mo): Number of participants achieving hemoglobin level &gt; 12 g/dL</td>
<td>Premkumar [64]</td>
</tr>
</tbody>
</table>

MELD: Model for end-stage liver disease; CTP: Child Turcotte Pugh score; IDA: Iron deficiency anemia.

Anemia is present in 60%-75% of all patients with cirrhosis [3]. The presence of anemia and various RBC indices, serum ferritin, TSAT, have all been independently associated with worsening disease severity and poor prognosis. However, to date, there are no universally available guidelines that dwell on this common but rather difficult to treat disease manifestation. There are lacunae in our understanding of disease and its management. Furthermore, the available parameters for the diagnosis and evaluation of anemia, and the laboratory assays have not been standardized or validated in patients with cirrhosis.

IDA is a potentially treatable condition and unlike other chronic inflammatory conditions like CKD and inflammatory bowel disease, cut-off values of serum ferritin and TSAT have not been validated in cirrhosis. How do we diagnose IDA in cirrhosis? What is the best method of replacement of elemental iron in cirrhosis? Define randomized controlled trials (RCTs) are lacking on this management aspect. The ongoing RCTs have been highlighted in Table 2. What is the protocol for diagnosing and supplementing other micronutrient deficiencies e.g., Folic acid, Vit B12, B6 which contribute to the development of anemia in cirrhosis? When and how often to supplement and reevaluate for assessment of body stores for these micronutrients? How does one prevent or screen for iron overload, which by itself is associated with increased mortality in cirrhosis? Last but not the least, should the presence of anemia or indices like serum ferritin be incorporated into existing severity scores like MELD.

VIT B12, B6, AND FOLIC ACID SUPPLEMENTATION

Micronutrient deficiencies are common in patients with cirrhosis. For patients with suspected or proven folate acid deficiency IV supplementation with 0.4-4 mg of folic acid daily for 3 d followed by recommended daily allowance (RDA) of 400 μg/d is advisable. In case of suspected intestinal malabsorption IV dosage may be prolonged. Folic acid supplementation > 1 mg/d may mask Vit B12 deficiency. The RDA for Vit B6 is 1.3 mg/d for men and women 19-50 years of age, 1.5 mg and 1.7 mg for women and men > 51 years of age respectively. The recommended dosage for Vit B12 in patients with concomitant neuropsychiatric signs is 1000 μg intramuscular every alternate day for 3 wk followed by monthly 1000 μg intramuscular injections or 1000-2000 μg oral supplementation [41].

MANAGEMENT OF ANEMIA IN CIRRHOSIS: WORK IN PROGRESS

Anemia is present in 60%-75% of all patients with cirrhosis [3]. The presence of anemia and various RBC indices, serum ferritin, TSAT, have all been independently associated with worsening disease severity and poor prognosis. However, to date, there are no universally available guidelines that dwell on this common but rather difficult to treat disease manifestation. There are lacunae in our understanding of disease and its management. Furthermore, the available parameters for the diagnosis and evaluation of anemia, and the laboratory assays have not been standardized or validated in patients with cirrhosis.

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score to improve their prognostic accuracy? All these are areas of potential research and may help us decipher this enigma and its potential contribution to the outcome of cirrhosis!

We have proposed an algorithm for evaluation and management of anemia in cirrhosis as per available evidence (Figure 3); it will require validation and subsequent modification prospectively. More so when more research is carried out to fill the lacunae in the existing understanding of the subject.

CONCLUSION

The evaluation and management of anemia in cirrhosis is an important aspect of disease management. IDA is a potentially treatable cause of anemia wherein RBC indices and serum iron studies have prognostic significance. Patients should be screened for deficiency of micronutrients like Folic acid, Vit B12, Vit B6 at baseline and supplementation should be initiated. Future research into various aspects dealing with diagnosis, management of anemia, and newer therapeutic modalities is the need of the hour. In addition, the role of anemia in the prognostication of cirrhosis is an area that needs further research in prospective trials.

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Retrospective Cohort Study

High tumor mutation burden indicates a poor prognosis in patients with intrahepatic cholangiocarcinoma

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Institutional review board statement: Ethics approval and patient consent were waived by the MSKCC Institutional Review Board.

Informed consent statement: Informed consent from patients was waived by the MSKCC IRB per 45 CFR 46.116 and 45 CFR 164.512, since our data were retrieved from a public database.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: The data that support the findings of this study are available in MSKCC (MSKCC cohort: http://www.cbioportal.org/study/summary?id=ihc_msk_2021)

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE

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Abstract

BACKGROUND
Intrahepatic cholangiocarcinoma (ICC) is malignancies of the biliary duct system and constitutes approximately 10%-20% of all primary liver cancers. Tumor mutation burden (TMB) is a useful biomarker across many cancer types for the identification of patients who will benefit from immunotherapy. Despite the role of TMB in calculating the effectiveness and prognosis of immune checkpoint inhibitors has been confirmed in multiple human cancer types, the prognostic value of TMB in ICC patients is rare investigated.

AIM
To investigate the prognostic value of TMB in patients with ICC.

METHODS
Data of 412 patients with ICC were included in the study. TMB was calculated as the total number of somatic non-silent protein-coding mutations divided by the coding region. The Kaplan-Meier method was used to analyze overall survival (OS), and relapse free survival (RFS). The cut-off value of TMB was determined by time-dependent receiver operating characteristic (ROC) curve. Cox regression was performed for multivariable analysis of OS. The nomogram and calibration curve were analyzed to construct and evaluate the prognostic model.
Tumor mutation burden; Intrahepatic cholangiocarcinoma; Prognosis

RESULTS
In the analysis of the time-dependent ROC curve, we defined 3.1 mut/Mb as the cut-off value of TMB. The Kaplan-Meier plot revealed that patients with high TMB had poor OS (HR = 1.47, P = 0.002) and RFS (HR = 1.42, P = 0.035). Cox regression analysis also demonstrated that TMB was an independent risk predictor for ICC (HR = 1.43, P = 0.0240). Furthermore, independent prognostic factors of ICC included CA19-9 (HR = 1.78, P = 0.0005), chronic viral hepatitis (HR = 1.72, P = 0.0468), tumor resection (HR = 2.58, P < 0.0001) and disease progression (metastatic disease vs. solitary liver tumor; HR = 2.55, P = 0.0002). The nomogram and calibration curve also indicated the effectiveness of the constructed prognostic model.

CONCLUSION
TMB was an independent prognostic biomarker in patients with ICC. Moreover, patients with ICC with high TMB had poor OS and RFS as compared to those with low TMB.

Key Words: Tumor mutation burden; Intrahepatic cholangiocarcinoma; Prognosis; Nomogram

INTRODUCTION
Cholangiocarcinomas are malignancies of the biliary duct system, classified as being either intrahepatic or extrahepatic in origin. Particularly, intrahepatic cholangiocarcinoma (ICC) constitutes approximately 10%-20% of all primary liver cancers[1]. Despite its increasing incidence rate worldwide, the etiology of ICC remains unclear [2]. Moreover, although surgery is the only potentially curative treatment for ICC, more than two-thirds of patients have been found to be unsuitable for surgery at the time of diagnosis, and more than 60% of patients who underwent surgery reported relapse of disease[3]. A previous study also showed that the 5-year survival rate and median survival time of patients with ICC (hereinafter, ICC patients) who underwent curative resection was approximately 30% and 28 mo, respectively[4]. Besides surgical resection, the standard treatment for ICC includes gemcitabine-based chemotherapy, liver transplantation, and local treatment, such as transarterial chemoembolization[5].

Of the several prognostic factors of ICC, radical resection (R0), number of tumors (single or multiple), vascular invasion, and lymph node metastasis have all been recognized as the most important independent prognostic predictors for ICC patients [6].

Multiple studies have also demonstrated that tumor mutation burden (TMB), defined as the total number of somatic coding errors, base substitutions, and indel mutations per million bases[7], can effectively estimate both overall mutational and neoantigen load[8]. Recent studies have shown that TMB is associated with immunotherapy response, since it reflects the overall neoantigen load[9-11]. Moreover, TMB
can be used to predict immune checkpoint inhibitor (ICI) therapy, acting as a useful biomarker across many cancer types for the identification of patients who will benefit from immunotherapy, [12, 13]. In addition to the identification of patients viable for immunotherapy, TMB has also been shown to be an indicator of immunotherapy efficacy. Specifically, high TMB is associated with higher rates of treatment response and longer survival among patients who received treatment with ICIs [14-16]. However, among patients who did not receive ICI treatment, high TMB was generally associated with poorer overall survival in many cancer types [17]. Furthermore, despite the role of TMB in calculating the effectiveness and prognosis of ICIs has been confirmed in multiple human cancer types, the prognostic value of TMB in ICC patients is rare investigated.

Therefore, in this study, we used the ICC database from the Memorial Sloan Kettering (MSK) Cancer Center to investigate the impact of TMB on the prognosis of ICC patients in combination with other clinical features, confirming that TMB was an independent prognostic factor for ICC patients.

### MATERIALS AND METHODS

**Data collection and processing**

Data of 412 ICC patients from the MSK Cancer Center cohort (MSK cohort: http://www.cbioportal.org/study/summary?id=ihch_msk_2021) were included [18]. TMB was calculated as the total number of somatic, non-silent, protein-coding mutations divided by the coding region captured in each MSK-IMPACT panel (341 genes, 0.98 Mb; 410 genes, 1.06 Mb; 468 genes, 1.22 Mb). Ethics approval and patient consent were waived by the MSKCC Institutional Review Board and the need for informed consent has been waived by the MSKCC IRB per 45 CFR 46.116 and 45 CFR 164.512, since our data were retrieved from a public database. Clinicopathological information, including age, gender, BMI, TMB, CA19-9, chronic viral hepatitis, tumor resection, tumor grade, disease progression and smoking status, were all reviewed retrospectively.

**Cox regression analysis and survival analysis**

Cox regression analysis was performed to examine the correlation between TMB and patient’s overall survival (OS). According to the time-dependent receiver operating characteristic (ROC) curve, patients were divided into either the high (TMB > 3.1 mut/Mb) or low TMB (TMB ≤ 3.1 mut/Mb) group. Kaplan-Meier method was used to construct the survival curves of patients. The time dependent specificity and sensitivity of survival were analyzed by deploying timeROC and survival in the R package. The log-rank test was used to examine the differences between the curves, and a P value < 0.05 was considered to be statistically significant. The nomogram model and calibration curve were also analyzed using the rms package in R.

**Statistical analysis**

Statistical analyses were performed using the SPSS version 25.0 (IBM Corp.) software. The Kaplan-Meier curve was analyzed using the survival package in R version 3.6.3, and the time dependent ROC curve was analyzed using the timeROC package, wherein the picture was generated by the ggplot2 package in R version 3.6.3. All reported P values were two-tailed, and P ≤ 0.05 was considered statistically significant for all analyses in this study.

### RESULTS

**Overview of the MSK-IMPACT cohort**

In this study, the MSK-IMPACT cohort included a total of 412 ICC patients who were mainly compared using TMB as an independent prognostic factor. Most patients in this cohort were examined using the 341- (IMPACT341) and 410-gene (IMPACT410) panels. In comparison to the latest 468-gene panel (IMPACT468), the unsequenced genes in the earlier versions were assumed to be wild-type or non-mutated. Clinical data in this study included age (< 65, ≥ 65), gender (male, female), BMI (< 28, ≥ 28), TMB (≤ 3.1, > 3.1), CA19-9 (< 40 U/mL, ≥ 40 U/mL), chronic viral diseases (negative, positive), tumor resection (resected, unresected), tumor grade (well differentiated, moderately differentiated, poorly differentiated), disease progression (solitary liver...
tumor, multifocal liver disease, metastatic disease), and smoking status (never smoked, former smoker, current smoker). Baseline clinicopathological features of the study cohort are summarized in Table 1 (median age: 63 years, range: 18-88; 46.1% of patients were females; median: TMB 2.5 mut/Mb, range: 0.51.6).

**Prognostic impact of TMB in ICC patients**

First, we analyzed the utility of TMB in prognosis, calculating a median TMB of 2.5 mut/Mb (range: 0-51.6 mut/Mb). To analyzed the predictive performance of TMB relating to OS, we generated a time-dependent ROC curve which showed the area under the curve (AUC) for TMB involving 1-, 3-, and 5-year survival was 0.545, 0.592, and 0.605, respectively (Figure 1A). Afterwards, we used the 1-, 3-, and 5-year ROC curve analysis with the corresponding maximum Youden index to calculate the TMB threshold values. As a result, when the TMB cut-off value was 3.1, the maximal AUC value was achieved (1-year sensitivity: 0.448, specificity: 0.656; 3-year sensitivity: 0.430, specificity: 0.742; 5-year sensitivity: 0.402, specificity: 0.767). Therefore, we defined 3.1 mut/Mb as the cut-off value. Patients with a TMB > 3.1 mut/Mb were clarified as the high group (n = 140), and patients with a TMB ≤ 3.1 mut/Mb were clarified as the low group (n = 239).

Following TMB classification, the Kaplan-Meier plotter of survival analysis showed that high TMB patients had a poor OS (HR = 1.47, P = 0.002; Figure 1B) and RFS (HR = 1.42, P = 0.035; Figure 1C), as compared to low TMB patients. We then performed subgroup analysis of prognosis to assess the impact of TMB in different clinical subsets (Table 2). For tumor grade, high TMB patients had poor OS in moderately differentiated (HR = 1.46, P = 0.026; Figure 1E) and poorly differentiated subsets (HR = 1.72, P = 0.007; Figure 1F). In contrast, no definite results can be obtained in well differentiated subsets due to the small sample size (HR = 0.64, P = 0.582; Figure 1D).

For disease progression, high TMB indicated poor OS in patients with multifocal liver disease (HR = 1.85, P = 0.026; Figure 1H). However, no significant differences in survival between the high TMB and low TMB groups were found in patients with solitary liver tumor (HR = 1.42, P = 0.140; Figure 1G) and metastatic disease (HR = 1.17, P = 0.357; Figure 1I).

For tumor resection, high TMB indicated a shorter OS in patients who underwent tumor resection (HR = 1.77, P = 0.002; Figure 1J). Conversely, no differences in prognosis were observed between the high TMB and low TMB groups in patients without tumor resection (HR = 1.13, P = 0.461; Figure 1K).

**Construction of multivariate survival model**

Finally, we would like to screen the independent prognostic factors and establish a prognostic model of ICC patients. Multivariate Cox regression analysis to was used to analyze the associations between OS and specific factors, including age, sex, and TMB. As a result, TMB was identified as an independent risk predictor for ICC patients [HR = 1.43 (1.05-1.96), P = 0.0240]. Additionally, independent prognostic factors of ICC included CA19-9 [HR = 1.78 (1.28-2.46), P = 0.0005], chronic viral hepatitis [HR = 1.72 (1.01-2.95), P = 0.0468], tumor resection [HR = 2.58 (1.72-3.88), P < 0.0001], and disease progression [metastatic disease vs solitary liver tumor HR = 2.55 (1.55-4.20), P = 0.0002] (Table 3). Following this, we constructed a predictive nomogram based on the Cox regression coefficients of selected variables, and the predictive accuracy of every nomogram was evaluated using calibration plots (Figure 2A). The total score for ICC patients can be calculated to predict the 1-, 3-, and 5-year survival rates, which would help clinicians assess the risk level of ICC patients in clinical practice. Notably, the calibration curve indicated that the observed and predicted values were consistent in predicting OS (Figure 2B).

**DISCUSSION**

In this study, we investigated the role of TMB in predicting survival among patients with ICC. First, the clinical and mutation data of the 412 ICC patients were obtained from the MSK public database. Next, the best cut-off TMB value was determined using time-dependent ROC curve. Combined with other clinical features, univariate and multivariate Cox regression analyses were used to establish a risk model for prognosis prediction, showing that elevated TMB was associated with poor OS and RFS. In addition to TMB, CA19-9, chronic viral hepatitis, tumor resection, and disease progression (metastatic disease vs solitary liver tumor) were also found to be independent predictors of OS in ICC patients. Based on these risk factors, a reliable
## Table 1 Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients (n = 412)</th>
</tr>
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<td>Range</td>
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<td>≥ 40 U/mL</td>
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<td><strong>Chronic viral hepatitis</strong></td>
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<td>Positive</td>
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<td><strong>Tumor resection</strong></td>
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</tr>
<tr>
<td>Unresected</td>
<td>209</td>
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<tr>
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<tr>
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<td>231</td>
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<tr>
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<tr>
<td><strong>Disease progression</strong></td>
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<td>Multifocal liver disease</td>
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<td>Metastatic disease</td>
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<td>Former smoker</td>
<td>166</td>
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<tr>
<td>Current smoker</td>
<td>41</td>
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</tbody>
</table>
BMI: Body mass index; TMB: Tumor mutation burden.

### Table 2 Grouping analysis of the relationship between tumor mutation burden and overall survival

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Median survival (mo)</th>
<th>Log-rank test</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMB-high</td>
<td>TMB-low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>- ((n = 5))</td>
<td>56.1 ((n = 10))</td>
<td>0.64 (0.15-2.27)</td>
<td>0.582</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>26.5 ((n = 79))</td>
<td>42.5 ((n = 134))</td>
<td>1.46 (1.02-2.08)</td>
<td>0.026</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>20.2 ((n = 52))</td>
<td>29.8 ((n = 82))</td>
<td>1.72 (1.11-2.66)</td>
<td>0.007</td>
</tr>
<tr>
<td>Disease progression</td>
<td></td>
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<tr>
<td>Solitary liver tumor</td>
<td>55.1 ((n = 44))</td>
<td>69.4 ((n = 93))</td>
<td>1.42 (0.85-2.38)</td>
<td>0.140</td>
</tr>
<tr>
<td>Multifocal liver disease</td>
<td>24.4 ((n = 31))</td>
<td>40.6 ((n = 46))</td>
<td>1.85 (1.00-3.43)</td>
<td>0.026</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>15.5 ((n = 65))</td>
<td>15.8 ((n = 100))</td>
<td>1.17 (0.83-1.66)</td>
<td>0.357</td>
</tr>
<tr>
<td>Tumor resection</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Resected</td>
<td>36.6 ((n = 67))</td>
<td>61.5 ((n = 127))</td>
<td>1.77 (1.17-2.66)</td>
<td>0.002</td>
</tr>
<tr>
<td>Unresected</td>
<td>17.5 ((n = 73))</td>
<td>17.7 ((n = 112))</td>
<td>1.13 (0.81-1.59)</td>
<td>0.461</td>
</tr>
</tbody>
</table>

TMB: Tumor mutation burden.

A nomogram model was then constructed, demonstrating a satisfactory performance in predicting OS in ICC patients. Therefore, this study provided an effective indicator for the clinical prognostic evaluation of ICC patients, as well as contributed to the screening of high-risk ICC patients and the provision of individualized treatment.

Recently, TMB has become a novel predictive biomarker with the potential to predict the therapeutic effect of ICIs and screen suitable patients for immunotherapy [19]. At present, the research on TMB has mainly focused on its ability to predict the efficacy of ICIs, with numerous studies showing its association with the survival rate of cancer patients. In particular, Xie et al. [20] found that papillary thyroid carcinoma patients with high TMB reported a worse prognosis. A study by Zhang et al. [21] also indicated that low TMB resulted in a better prognosis in patients with head and neck squamous cell carcinoma. Similarly, a study of 318 ICC patients showed that high TMB indicated a worse prognosis \([HR = 1.500 (1.085-2.073)]\) [22]. In the present study, the data of 412 ICC patients published by the MSK Cancer Center in March 2021 were used to determine the utility of TMB in prognosis prediction. Notably, the original researchers investigated the relationship between the mutation gene, clinical characteristics, and the prognosis of ICC patients; however, they did not explore the role of TMB in prognosis. Analyzing the aforementioned data, we found that ICC patients with high TMB had a poor OS and RFS, which was consistent with the findings of previous studies.

Clinically, CEA and CA19-9 levels are commonly used prognostic indicators in ICC [23, 24]. However, their prognostic thresholds vary widely across different reports, with a lack of a large meta-analysis to consolidate these values [25]. Moreover, some studies have reported on other prognostic indicators associated with poor prognosis in ICC patients, including elevated C-reactive protein, circulating osteopontin, as well as KRAS and TP53 mutations in tumor tissues [26-29]. With the wide application of immunotherapy, TMB has also become a common clinical index. In order to detect TMB, common mutations in ICC patients were detected, which reflected the overall mutation of tumor tissue. Therefore, TMB is a convenient and crucial prognostic value in clinical practice.

Medical nomograms use biologic and clinical variables, including tumor grade and patient age, to graphically depict a statistical prognostic model that generates a probability of a clinical event for a given individual, such as cancer recurrence or death. Furthermore, nomograms are user-friendly, can incorporate continuous variables and relevant disease determinants into prognosis, and are superior to clinician judgment in estimating disease course [30, 31]. In this study, we constructed a...
### Table 3 Univariable and multivariable analysis of overall survival

<table>
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<tr>
<th></th>
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<td>&lt; 65</td>
<td>0.96</td>
<td>0.76-1.21</td>
<td>0.7286</td>
<td>1.16</td>
<td>0.84-1.61</td>
<td>0.3662</td>
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<tr>
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<td>1.27</td>
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<td>0.94-1.72</td>
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<td>&lt; 28</td>
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<td>0.62-1.132</td>
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<td>≥ 28</td>
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<tr>
<td><strong>TMB, mut/Mb</strong></td>
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<td>Low (≤ 3.1)</td>
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<td>1.13-1.91</td>
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<tr>
<td>&lt; 40 U/mL</td>
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<td><strong>Tumor grade</strong></td>
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<tr>
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<td>0.49-2.38</td>
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<td>0.0211</td>
<td>1.15</td>
<td>0.52-2.37</td>
<td>0.7298</td>
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<tr>
<td><strong>Disease progression</strong></td>
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<tr>
<td>Solitary liver tumor</td>
<td>2.26</td>
<td>1.53-3.36</td>
<td>&lt; 0.0001</td>
<td>1.47</td>
<td>0.86-2.52</td>
<td>0.1587</td>
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<tr>
<td>Multifocal liver disease</td>
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<tr>
<td>Metastatic disease</td>
<td>3.79</td>
<td>2.87-5.01</td>
<td>&lt; 0.0001</td>
<td>2.55</td>
<td>1.55-4.20</td>
<td>0.0002</td>
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<tr>
<td><strong>Smoking status</strong></td>
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<td></td>
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<tr>
<td>Never smoked</td>
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<td>0.80-1.31</td>
<td>0.8591</td>
<td>0.94</td>
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<td>0.7077</td>
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<td>Current smoker</td>
<td>1.03</td>
<td>0.69-1.52</td>
<td>0.8971</td>
<td>1.64</td>
<td>0.98-2.73</td>
<td>0.0587</td>
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</tbody>
</table>

BMI: Body mass index; TMB: Tumor mutation burden; CI: Confidence interval.

predictive nomogram according to the Cox regression coefficients of selected variables to help clinicians evaluate the prognostic risk of ICC patients, calculate their survival rate, and make correct clinical decisions. Particularly, TMB and CA19-9 were combined to construct a nomogram model to predict the prognosis of ICC patients, which was helpful for its clinical application. To ensure the accuracy of this nomogram model, we used a calibration plot, as it allowed us to determine how close the nomogram estimated risk was to the observed risk.
Figure 1 Prognostic ability of tumor mutation burden in predicting the prognosis of intrahepatic cholangiocarcinoma patients. A: Time-dependent receiver operating characteristic curve analysis of tumor mutation burden (TMB) shows the area under the curve (AUC) for 1-, 3-, and 5-year survival were 0.545, 0.592, and 0.605 respectively. The best cut-off value of TMB for all three was 3.1; B and C: Kaplan-Meier plot shows that the intrahepatic cholangiocarcinoma (ICC) patients with high-TMB had poor overall survival (OS) (HR = 1.47, \( P = 0.002 \)); B) and relapse free survival (HR = 1.42, \( P = 0.035 \)); D-F: Kaplan-Meier analysis shows the impact of TMB on the OS of ICC patients with different tumor grades, including (D) well differentiated (HR = 0.64, \( P = 0.582 \)), (E) moderately differentiated (HR = 1.46, \( P = 0.026 \)), and (F) poorly differentiated subsets (HR = 1.72, \( P = 0.007 \)); G-I: Kaplan-Meier analysis shows the impact of TMB on the OS of ICC patients with different disease progressions, including (G) solitary liver tumor (HR = 1.42, \( P = 0.140 \)), (H) multifocal liver disease (HR = 1.85, \( P = 0.026 \)), and (I) metastatic disease (HR = 1.7, \( P = 0.357 \)); J-K: Kaplan-Meier analysis shows the impact of TMB on the OS of ICC patients with respect to tumor resection, including patients who were (J) resected (HR = 1.77, \( P = 0.002 \)) and (K) unresected (HR = 1.13, \( P = 0.461 \)).
CONCLUSION

In conclusion, we explored the prognostic role of TMB in ICC patients. Multivariate analysis indicated that TMB and CA19-9 were among the identified independent prognostic factors in ICC. Although our study confirmed the prognostic value of TMB, our study had several limitations. First, the clinical characteristics and TMB data of the cases analyzed in this study were all extracted from the MSK Cancer Center, of which some cases had missing data. As a result, this increased the analysis error in our study. Second, using a single data source also increases statistical error. Thus, further larger cohort studies are necessary to confirm the predictive value of TMB in the prognosis of ICC patients. For the benefit of future studies, we will continue to collect the clinical data of ICC patients and consolidate our conclusions by expanding the present study’s sample size.

ARTICLE HIGHLIGHTS

Research background
Intrahepatic cholangiocarcinoma (ICC) is malignancies of the biliary duct system and constitutes approximately 10%-20% of all primary liver cancers. Tumor mutation burden (TMB) is a useful biomarker across many cancer types for the identification of patients who will benefit from immunotherapy. This study collected the ICC database from the Memorial Sloan Kettering Cancer Center to investigate the impact of TMB on the prognosis of ICC patients.

Research motivation
The prognosis of ICC patients is very poor. Previous studies suggest that TMB can used to be a prognostic factor in many types of cancer. It is critical to analyze the
The prognostic value of TMB in ICC to help individual clinical treatment.

**Research objectives**
This study aims to investigate the prognostic value of TMB in patients with intrahepatic cholangiocarcinoma ICC. In particular, we sought to confirm that TMB is an independent prognostic factor of ICC and construct a nomogram model to predict the prognosis of ICC patients, which was helpful for its clinical application.

**Research methods**
This study is a retrospective cohort study of ICC patients. This is a study of large sample to investigate the prognostic value of TMB and other clinical characters in ICC.

**Research results**
TMB was an independent risk predictor for ICC. Furthermore, independent prognostic factors of ICC included CA19-9, chronic viral hepatitis, tumor resection and disease progression (metastatic disease vs solitary liver tumor). The clinical characteristics and TMB data of some cases had missing, which increased the analysis error in our study. Using a single data source also increases statistical error. Further larger-cohort studies are necessary to confirm the predictive value of TMB in the prognosis of ICC patients.

**Research conclusions**
These findings suggest that TMB was an independent prognostic biomarker in patients with ICC. Moreover, patients with ICC with high TMB had poor overall survival and relapse free survival as compared to those with low TMB.

**Research perspectives**
We will continue to collect the clinical data of ICC patients and consolidate our conclusions by expanding the present study’s sample size.

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Zhang L

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Kondo N, Murakami Y, Uemura K, Sudo T, Hashimoto Y, Sasaki H, Sueda T. Elevated perioperative


Retrospective Study

Does delaying ureteral stent placement lead to higher rates of preoperative acute pyelonephritis during pregnancy?

Mao-Mao He, Xiao-Ting Lin, Ming Lei, Xiao-Lan Xu, Zhi-Hui He

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Author contributions: He MM wrote the manuscript; He MM and Lin XT analyzed the data; Xu XL collected the data; Lei M and He ZH contributed to the protocol/project development; He ZH contributed to analysis, manuscript editing.

Institutional review board statement: The study was reviewed and approved by the First Affiliated Hospital of Guangzhou Medical University Institutional Review Board (Approval No. V1.0).

Informed consent statement: All study participants provided informed written consent prior to study enrolment.

Conflict-of-interest statement: No other sources of funding or conflicts of interest to disclose.

Data sharing statement: The dataset is available from the corresponding author at hemaomao1982@126.com. Participants gave informed consent for data sharing.

Abstract

BACKGROUND
Pregnancy with renal colic may cause pyelonephritis, decreased renal function, systemic infection and even shock in pregnant women, and cause premature birth and other adverse pregnancy outcomes. When surgery is necessary, the relationship between timing of the operation and the outcome of the mother and child are not known.

AIM
To investigate the association between time to ureteral stent placement and clinical outcomes of patients with renal colic during pregnancy.

METHODS
In this retrospective study, pregnant women with renal colic who underwent surgery were studied. Maternal preoperative acute pyelonephritis (PANP), pregnancy outcome, and length of hospital stay (LOS) were compared between the two groups.

RESULTS
100 patients were included in the analysis, median age was 30 years. Median time to ureteral stent placement was 48 h (interquartile range, 25-96 h), and 32 patients (32%) were diagnosed with PANP. PANP was closely related to hospitalization costs, re-admission to the hospital due to urinary tract infection after surgery and premature delivery. Multivariate analysis found that stone location and time from pain to admission were related to PANP.
CONCLUSION
Both early and delayed surgery are safe and effective for the treatment of renal colic during pregnancy. Early surgery may be superior to a delayed procedure due to shorter LOS. For pregnant patients with renal colic, delayed surgery within 48 h is not related to the clinical outcome of the mother and child. However, the time from pain to hospital admission was related to PANP.

Key Words: Renal colic; Ureteral stent placement; Acute pyelonephritis; Pregnancy

Core Tip: Acute renal colic is one of the most common reasons for pregnant women to be hospitalized for non-obstetric reasons. Renal colic in most patients is resolved after conservative treatment. However, when conservative treatment fails, active surgical treatment is necessary, thus the choice of the timing of the operation is very important. In this study, we examined the relationship between the timing of the operation and the outcome of the mother and child.

INTRODUCTION
Acute renal colic is one of the most common reasons for pregnant women to be hospitalized for non-obstetric reasons. The incidence of renal colic during pregnancy is about 1 in 1500[1]. Renal colic may cause adverse maternal and fetal outcomes, such as premature delivery, premature rupture of membranes, urinary tract infection and sepsis, pregnancy loss and preclampsia[2-4]. The main causes of renal colic in pregnancy are urinary stones and hydronephrosis. Several anatomical and physiological changes occur during pregnancy and may affect the entire urinary system. Antenatal hydronephrosis and hydroureret are the result of compression of the ureter at the pelvic brim due to the growing uterus and smooth muscle relaxation induced by elevated progesterone levels[5,6]. Moreover, the secretion of placental 1, 25-dihydroxycholecalciferol and parathyroid hormone are reduced, resulting in transient hypercalciuria during pregnancy. These substances in the urine combine with each other and obstruction of the urinary tract leads to the deposition of crystals in the urine in the poorly drained area, thereby forming stones[7]. The above may cause acute pyelonephritis during pregnancy. Acute pyelonephritis is a manifestation of infection of the upper urinary tract and kidneys. Most cases of pyelonephritis occur during the second and third trimesters. Pregnant women are at risk for both medical and obstetric complications resulting from pyelonephritis.

The clinical features of acute pyelonephritis during pregnancy include fever (> 38 °C), chills, low back pain, nausea, vomiting, or costal and spinal angle pain, with or without typical symptoms of cystitis. Pregnant women require special attention when they develop acute pyelonephritis. Acute pyelonephritis not only adversely affects pregnant women, but also causes anemia, renal insufficiency or respiratory insufficiency; It also affects the fetus[8].

Conservative treatment is effective in 70%-80% of patients with renal colic during pregnancy[9]. Pregnant women who develop a stone may need three types of medication: painkillers, antibiotics and anesthetic drugs. Patients with simple renal colic without other complications should be given antispasmodic, analgesic and anti-inflammatory treatment, and if necessary, uterine contraction suppression treatment should be given[10]. However, when conservative treatment is ineffective, active surgical intervention is necessary[3]. Surgical methods include ureteroscopy, ureteroscopic lithotripsy, surgery, and nephrostomy[1].
At present, few studies have investigated the relationship between operation time and the clinical outcome of the mother and child. Therefore, the purpose of this study is to compare the effects of early surgery (less than 48 h from onset of renal colic to surgery) or delayed surgery (more than 48 h from onset of renal colic to surgery) in patients diagnosed with renal colic during pregnancy.

**MATERIALS AND METHODS**

**Study design**

A retrospective study of all pregnant women with the diagnosis of renal colic admitted to The First Affiliated Hospital of Guangzhou Medical University from January 1, 2009 to December 31, 2019 was performed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was reviewed and approved by the First Affiliated Hospital of Guangzhou Medical University Institutional Review Board (Approval No. V1.0). Diagnosis of renal colic was as follows: left or right low back pain, with or without fever, frequent urination, urgency, hematuria, obvious percussive pain in the kidney area, and B-ultrasound confirmed stones or hydronephrosis on the affected side. The patients diagnosed with renal colic met the following criteria: percussion pain in the renal area, and B-ultrasound revealed hydronephrosis. Two hundred and twelve patients were diagnosed with renal colic. Patients with complications (such as diabetes, hypertension, immune system diseases, etc.) were excluded. All patients initially underwent conservative treatment, including hydration, pain relief and antibiotic treatment if necessary. If conservative treatment failed, patients with persistent renal colic, febrile urinary tract infection, sepsis, acute renal failure, or single kidney with obstruction, surgical intervention was necessary. The surgical method evaluated in this study was ureteral stent placement. Ureteroscopy was generally performed before ureteral stent placement. Among the 102 eligible patients who underwent surgery, two patients did not have follow-up data. Therefore a total of 100 patients were included in the study.

Time to ureteral stent placement (TTU), was defined as the period from diagnosis of renal colic to surgery. The median TTU in our hospital was 48 h. The patients were divided into two groups according to the TTU, the early TTU (< 48 h, n = 42) and delayed TTU (≥ 48 h, n = 58) groups. The demographic (age, BMI [body mass index], gestation) and clinical characteristics including history of stones, laboratory examination such as white blood cell (WBC) count and C-reactive protein (CRP), imaging data (stone size, stone location, and hydronephrosis), clinical outcome (PANP, preoperative fetal obstetric complications, UTI after surgery, newborn weight, cesarean section rate and preterm delivery), length of hospital stay (LOS) and total charges were compared between the two groups. Acute pyelonephritis was suggested by the presence of flank pain, nausea/vomiting, fever (> 38 °C or 100.4 °F), and/or costovertebral angle tenderness, with or without typical symptoms of cystitis, or was confirmed by the presence of bacteriuria in the setting of these symptoms. The diagnosis was confirmed if the patient met the following three criteria: renal colic, fever, and positive urine culture. Once acute pyelonephritis was diagnosed, broad spectrum intravenous antibiotics (Cephalosporin-based therapy) were administered immediately for about 7-10 d after surgery. If a susceptibility test was carried out before treatment, we used antibiotics sensitive to bacteria according to the susceptibility test results. Fetal obstetric complications were defined as premature delivery, threatened premature delivery, premature rupture of membranes, or fetal loss. UTI after surgery was defined as patients who underwent surgery for renal colic and were re-admitted to the hospital for UTI after surgery.

Parametric distributed numerical data are presented as mean ± standard deviation. Non-parametric distributed continuous variables are presented as interquartile ranges (Q1, Q3). Categorical data are presented as numbers and percentages. T tests and Mann-Whitney U tests were used to evaluate the difference between quantitative measurements that had non-parametric distribution. Chi-squared tests were used for categorical data. The associations of preoperative and operative characteristics with the TTU and with acute pyelonephritis were evaluated using Pearson χ² tests.

Statistical analysis was performed with SPSS 26.0 software (SPSS, Mac). The α value was set at 0.05, and all statistical tests were 2-tailed. A logistic regression model was used to test whether the risk factors were related to the outcome variables.
RESULTS

Among 212 patients with renal colic in pregnancy, 102 patients underwent surgery. Due to missing data in 2 women, 100 pregnant women with renal colic were included in this study. If conservative treatment failed or the patient developed any of the following conditions, surgical intervention was required: clinical indications included all situations that require emergency intervention for patients with non-pregnant stones, such as isolated renal obstruction, bilateral obstruction, deterioration of renal function, intractable symptoms and related urosepsis.

The characteristics of patients in the early and delayed surgery groups are listed in Table 1. The median age was 30 years and median gestation was 22 wk. The median surgery time was 48 h. Forty-two patients (42%) underwent early surgery and 58 patients (58%) underwent delayed surgery. There were no differences in basic information such as age, BMI, gestation, and history of stones between the two groups. There was no significant difference with regard to WBC count and CRP when laboratory examination data were compared. In addition, there was no significant difference in stone size, stone location, and hydronephrosis between the groups when the imaging data were compared. In terms of clinical outcome, there was a significant difference in the length of hospital stay between the two groups (7 d vs 9 d), but there was no difference in preoperative-fetal complications, PANP, UTI, total cost, newborn weight, cesarean section rate, and preterm delivery between the two groups. We found that the timeliness of consultation was related to the time of surgical intervention. The consultation time in early surgery group was 19 h, which was significantly earlier than that in the delayed surgery group (48 h) (Table 1).

The incidence of PANP was 32%. PANP was closely related to hospitalization costs, LOS, postoperative infection, re-admission to hospital due to UTI after surgery and premature delivery (Table 2).

In univariate analyses, increased risk of PANP was associated with BMI, gestation, time from pain to admission, time from pain to surgery, hydronephrosis and stone location (Table 2). Multivariate analysis showed that stone location and time from pain to admission were closely related to PANP (Table 3).

Renal colic symptoms were eliminated after surgery in all patients. Laboratory data were also improved (Table 4). As the patients’ creatinine levels were normal before and after surgery, creatinine levels were not compared.

DISCUSSION

In this study, we assessed the relationship between the timing of surgery and clinical outcomes of the mother and child in pregnant patients with renal colic. The results showed that longer TTU was not associated with an increased risk of complications or adverse outcomes when surgery was performed within 48 h of presentation. The timeliness of surgery was closely related to urology consultation. Nevertheless, the length of hospital stay in patients with early surgical intervention was significantly shortened. Furthermore, we analyzed the relationship between acute pyelonephritis and the timing of surgical intervention and found time from pain to admission and the location of stones were risk factors for acute pyelonephritis caused by renal colic during pregnancy. Taken together, these results suggest that it is unlikely that the timing of surgery affected the risk of complications and adverse outcomes if performed within a reasonable time frame.

The timing of surgery is very important due to the impact of surgical emergencies and their complications. Previous studies showed that a delay in appendectomy within 24 h of presentation was not associated with increased risk of complicated appendicitis or surgical site infections[11,12]. Both early and delayed laparoscopic common bile duct exploration are safe and effective for the treatment of common bile duct stone-related non-severe acute cholangitis during emergent admissions[13]. Renal colic during pregnancy is an acute abdomen caused by non-obstetric reasons, and there are few reports on the timing of surgery.

The median time from admission to surgical intervention in pregnant patients with renal colic was 48 h. Based on this, we divided the patients into the early and delayed intervention groups, with 42% in the early intervention group and 58% in the delayed intervention group. The results showed that the 48-h delay from admission to surgery was not associated with an increased risk of poor clinical outcome in the mother and child. There was no difference in the effect of early and delayed surgery [see Table 4]. Management of renal colic as an urgent rather than emergency procedure was
Table 1 Patient characteristics associated with early and delayed time to ureteral stent placement

<table>
<thead>
<tr>
<th>Demographic data/clinical parameters</th>
<th>Total (n = 100)</th>
<th>Early TTU (n = 42)</th>
<th>Delayed TTU (n = 58)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr), mean ± SD</td>
<td>30 ± 4.55</td>
<td>31 ± 4.87</td>
<td>29 ± 4.19</td>
<td>0.06</td>
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<td>BMI (kg/m²), mean ± SD</td>
<td>23.05 ± 3.06</td>
<td>23.38 ± 3.28</td>
<td>22.81 ± 2.89</td>
<td>0.36</td>
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<tr>
<td>Gestation (wk), mean ± SD</td>
<td>22 (18, 27)</td>
<td>22 ± 7.83</td>
<td>22 ± 5.02</td>
<td>0.99</td>
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<td>History of stones, n (%)</td>
<td>26 (76)</td>
<td>14 (53.8)</td>
<td>12 (46.2)</td>
<td>0.16</td>
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<tr>
<td>WBC count (× 10⁹/L), mean ± SD</td>
<td>13.48 ± 3.49</td>
<td>14.19 ± 4.12</td>
<td>12.97 ± 2.87</td>
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<td>CRP (mg/dL), median (Q1, Q3)</td>
<td>2.64 (1.04, 4.35)</td>
<td>4.25 (1.9, 5.89)</td>
<td>1.77 (0.98, 4.0)</td>
<td>0.12</td>
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<tr>
<td>Stone size (mm), median (Q1, Q3)</td>
<td>8.25 (4.28, 14)</td>
<td>9.98 (4.5, 12)</td>
<td>9.07 (0.15)</td>
<td>0.59</td>
</tr>
<tr>
<td>&lt; 10 mm, n (%)</td>
<td>55 (55)</td>
<td>21 (38.2)</td>
<td>34 (61.8)</td>
<td>0.39</td>
</tr>
<tr>
<td>≥ 10 mm, n (%)</td>
<td>45 (45)</td>
<td>21 (46.7)</td>
<td>24 (53.3)</td>
<td></td>
</tr>
<tr>
<td>Hydronephrosis (mm), median (Q1, Q3)</td>
<td>26.5 (15, 62)</td>
<td>47 (15, 80)</td>
<td>34 (15, 42)</td>
<td>0.30</td>
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<tr>
<td>None/light, n (%)</td>
<td>62 (62)</td>
<td>21 (40.4)</td>
<td>31 (59.6)</td>
<td>0.73</td>
</tr>
<tr>
<td>Moderate/severe, n (%)</td>
<td>38 (38)</td>
<td>21 (43.8)</td>
<td>27 (56.3)</td>
<td></td>
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<tr>
<td>Stone location, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>24 (24)</td>
<td>11 (45.8)</td>
<td>13 (54.2)</td>
<td></td>
</tr>
<tr>
<td>Ureter</td>
<td>48 (48)</td>
<td>24 (50)</td>
<td>24 (50)</td>
<td></td>
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<tr>
<td>Kidney</td>
<td>28 (28)</td>
<td>7 (25)</td>
<td>21 (75)</td>
<td></td>
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<tr>
<td>Urology consultation time (h), median (Q1, Q3)</td>
<td>27 (19, 52)</td>
<td>19 (13, 23)</td>
<td>48 (24, 90)</td>
<td>0.001</td>
</tr>
<tr>
<td>≤ 24 h, n (%)</td>
<td>53 (53)</td>
<td>34 (64.2)</td>
<td>19 (35.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt; 24 h, n (%)</td>
<td>47 (47)</td>
<td>8 (17)</td>
<td>39 (83)</td>
<td></td>
</tr>
<tr>
<td>Preoperative fetal complications, n (%)</td>
<td>12 (12)</td>
<td>6 (50)</td>
<td>6 (50)</td>
<td>0.55</td>
</tr>
<tr>
<td>PANP, n (%)</td>
<td>32 (32)</td>
<td>15 (46.9)</td>
<td>17 (53.1)</td>
<td>0.50</td>
</tr>
<tr>
<td>UTI after surgery, n (%)</td>
<td>22 (22)</td>
<td>11 (50)</td>
<td>11 (50)</td>
<td>0.39</td>
</tr>
<tr>
<td>LOS (d), mean ± SD</td>
<td>7 ± 3.56</td>
<td>6 ± 3.03</td>
<td>8 ± 3.60</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cost (Yuan in RMB), mean ± SD</td>
<td>12382.79 ± 5665.26</td>
<td>10448.95 ± 2412.28</td>
<td>13783.16 ± 6841.61</td>
<td>0.001</td>
</tr>
<tr>
<td>Newborn weight (g), mean ± SD</td>
<td>2893.30 ± 542.10</td>
<td>2891.67 ± 509.18</td>
<td>2894.48 ± 569.15</td>
<td>0.98</td>
</tr>
<tr>
<td>Cesarean section rate, n (%)</td>
<td>38 (38)</td>
<td>17 (44.7)</td>
<td>21 (55.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>Preterm delivery, n (%)</td>
<td>8 (8)</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

TTU: Time to ureteral stent placement; PANP: Preoperation acute pyelonephritis; UTI: Urinary tract infection; LOS: Length of stay.

reasonable during pregnancy. We found that timeliness of intervention was related to the urology consultation. This is consistent with previous research that the availability of specialists to perform the necessary procedures has been implicated in delays in acute stone intervention[14,15]. Faw et al.[16] reported that patients who were stented within 6, 10, and 14 h of admission had more expeditious urologic consults compared with their counterparts, indicating that early urologic consultation is vital to ensure prompt intervention.

Pyelonephritis is a severe complication of pregnancy. It has been estimated that as many as 20% of women with severe pyelonephritis develop complications that include septic shock syndrome or its variants, such as acute respiratory distress syndrome (ARDS)[17-19]. We further analyzed the risk factors for PANP, time from pain to admission and stone location, and we found that stone location was closely related to PANP. According to our data analysis, most of the patients with delayed visits were transferred to our hospital due to poor results after treatment in another hospital, which may be the reason for pyelonephritis. Therefore, we should strengthen the management of patients referred from other hospitals, and active intervention is necessary. When patients with suspected acute pyelonephritis are admitted to our hospital, empirical antibiotic use is very important to control the disease.
Table 2 Associations between preoperative characteristics and preoperative acute pyelonephritis

<table>
<thead>
<tr>
<th>Demographic data/clinical parameters</th>
<th>PANP (n = 32)</th>
<th>No-PANP (n = 68)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr), mean ± SD</td>
<td>29 ± 5.01</td>
<td>30 ± 4.04</td>
<td>0.53</td>
</tr>
<tr>
<td>BMI (kg/cm²), mean ± SD</td>
<td>21.66 ± 2.44</td>
<td>23.30 ± 2.97</td>
<td>0.007</td>
</tr>
<tr>
<td>Gestation (wk), median (Q1, Q3)</td>
<td>20 (19, 25)</td>
<td>23 (20, 28)</td>
<td>0.04</td>
</tr>
<tr>
<td>History of stones, n (%)</td>
<td>12 (46.2)</td>
<td>14 (53.8)</td>
<td>0.07</td>
</tr>
<tr>
<td>Pain to surgery (h), median (Q1, Q3)</td>
<td>114.5 (70, 140)</td>
<td>84.5 (61, 120)</td>
<td>0.00</td>
</tr>
<tr>
<td>&lt; 96 h, n (%)</td>
<td>9 (17.6)</td>
<td>42 (82.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>≥ 96 h, n (%)</td>
<td>23 (46.9)</td>
<td>26 (53.1)</td>
<td>0.81</td>
</tr>
<tr>
<td>Admission to surgery (h), median (Q1, Q3)</td>
<td>53 (24, 160)</td>
<td>50 (36, 90)</td>
<td>0.81</td>
</tr>
<tr>
<td>&lt; 48 h, n (%)</td>
<td>15 (35.7)</td>
<td>27 (64.3)</td>
<td>0.81</td>
</tr>
<tr>
<td>≥ 48 h, n (%)</td>
<td>17 (29.3)</td>
<td>41 (70.7)</td>
<td>0.00</td>
</tr>
<tr>
<td>Pain to admission (h), median (Q1, Q3)</td>
<td>90 (50, 120)</td>
<td>24 (12, 48)</td>
<td>0.00</td>
</tr>
<tr>
<td>&lt; 48 h, n (%)</td>
<td>3 (6.1)</td>
<td>46 (93.6)</td>
<td>0.00</td>
</tr>
<tr>
<td>≥ 48 h, n (%)</td>
<td>29 (56.9)</td>
<td>22 (43.1)</td>
<td>0.00</td>
</tr>
<tr>
<td>WBC count (× 10⁹/L), mean ± SD</td>
<td>14.59 ± 4.40</td>
<td>12.96 ± 2.84</td>
<td>0.06</td>
</tr>
<tr>
<td>CRP (mg/dL), median (Q1, Q3)</td>
<td>3.20 (1.47, 6.32)</td>
<td>2.23 (0.97, 3.86)</td>
<td>0.01</td>
</tr>
<tr>
<td>Stone location, n (%)</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6 (25)</td>
<td>18 (75)</td>
<td></td>
</tr>
<tr>
<td>Ureter</td>
<td>8 (16.7)</td>
<td>40 (83.3)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>18 (64.3)</td>
<td>10 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Stone size (mm), median (Q1, Q3)</td>
<td>8.25 (4.5, 17)</td>
<td>8 (0, 12)</td>
<td>0.25</td>
</tr>
<tr>
<td>&lt; 10 mm, n (%)</td>
<td>18 (32.7)</td>
<td>37 (67.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>≥ 10 mm, n (%)</td>
<td>14 (31.1)</td>
<td>31 (68.9)</td>
<td></td>
</tr>
<tr>
<td>Hydronephrosis (mm), median (Q1, Q3)</td>
<td>31 (20, 60)</td>
<td>19.5 (14, 62)</td>
<td>0.05</td>
</tr>
<tr>
<td>None/light, n (%)</td>
<td>12 (23.1)</td>
<td>40 (76.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Moderate/severe, n (%)</td>
<td>20 (41.7)</td>
<td>28 (58.3)</td>
<td></td>
</tr>
<tr>
<td>Urology consultation time (h), median (Q1, Q3)</td>
<td>23 (13, 68)</td>
<td>34 (20, 53)</td>
<td>0.23</td>
</tr>
<tr>
<td>≤ 24 h, n (%)</td>
<td>16 (30.2)</td>
<td>37 (69.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>&gt; 24 h, n (%)</td>
<td>16 (34)</td>
<td>31 (66)</td>
<td></td>
</tr>
<tr>
<td>Total cost (Yuan in RMB), mean ± SD</td>
<td>16522.59 ± 8871.61</td>
<td>10236.07 ± 3281.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Preoperative fetal complications, n (%)</td>
<td>6 (50)</td>
<td>6 (50)</td>
<td>0.27</td>
</tr>
<tr>
<td>UTI after surgery, n (%)</td>
<td>12 (54.5)</td>
<td>10 (45.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>LOS (d), mean ± SD</td>
<td>11 ± 5.34</td>
<td>7 ± 2.98</td>
<td>0.001</td>
</tr>
<tr>
<td>Newborn weight (g), mean ± SD</td>
<td>2706.56 ± 649.47</td>
<td>2978.97 ± 460.58</td>
<td>0.02</td>
</tr>
<tr>
<td>Cesarean section rate, n (%)</td>
<td>14 (36.8)</td>
<td>24 (63.2)</td>
<td>0.42</td>
</tr>
<tr>
<td>Preterm delivery, n (%)</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

PANP: Preoperation acute pyelonephritis, UTI: Urinary tract infection, LOS: Length of stay.

Ultrasonography is a commonly used examination in obstetric patients with renal colic. The main objective of imaging is to evaluate any processes that may delay response to therapy or warrant intervention, such as a calculus or obstruction[20]. For patients with stones before pregnancy, the risk of pyelonephritis was higher than patients without stones. Pyelonephritis caused by kidney stones had a higher risk than pyelonephritis caused by ureteral stones, which deserves attention. Blackwell et al[21]
showed a benefit with timely decompressive intervention for obstructing urinary stones and sepsis leading to improved health outcomes.

PANP was closely related to re-admission to hospital due to UTI after surgery and premature delivery in our study. This was consistent with previous research[22,23]. Chen et al.[24] found ureteral stent placement was a risk factor for PANP. Patients with PANP developed UTI after surgery (OR 3.48, 95%CI: 1.31-9.28), which was reported in our previous studies[25]. Therefore, active anti-infection treatment is required during the perioperative period to avoid adverse outcomes in such patients.

It is known that pyelonephritis is associated with adverse pregnancy outcomes. An 18-year retrospective study included more than 500000 singleton pregnancies in a large health care system in the United States. The results showed that among 2894 women with pyelonephritis during pregnancy, the preterm birth rate (mainly at 33-36 wk) was higher than those without pyelonephritis (10.3% vs 7.9%, OR 1.3, 95%CI: 1.2-1.5)[26]. The incidence of preterm birth was 8%, and 75% of preterm pregnant women suffered preoperative acute pyelonephritis (OR 7.62, 95%CI: 1.44-40.19).

In addition to the implications for patient health outcomes, our data also suggest an economic benefit with timely intervention. Delayed surgery (≥ 48 h) can lead to longer hospital stay, but did not increase hospitalization costs. The increase in hospitalization costs was mainly related to preoperative acute pyelonephritis. In conclusion, both early and delayed surgery are safe and effective for the treatment of renal colic during pregnancy. Early surgery is recommended for patients with pyelonephritis as it tends to decrease costs and reduce mother and child complications.

The limitation of the current study is its relatively small sample size and lack of patients with very severe complications. Therefore, a large cohort study and randomized controlled trials are needed to validate our findings. We also did not evaluate the degree of pain, which may be an important factor leading to timely intervention of surgery. Despite these limitations, we believe that our findings can still help obstetricians and urologists provide patient consultation.

CONCLUSION

Using local data, we have identified the association between time to ureteral stent placement and clinical outcomes, and analyzed the risk factors for preoperative acute pyelonephritis in pregnant women with renal colic during pregnancy. Delayed surgery does not affect clinical outcomes, but leads to longer hospital stay. Time from pain to hospitalization and the location of the stones are risk factors for preoperative acute pyelonephritis. Our research will have important significance in the clinic.
ARTICLE HIGHLIGHTS

Research background
Pregnancy with renal colic may cause pyelonephritis, decreased renal function, systemic infection and even shock in pregnant women, and cause premature birth and other adverse pregnancy outcomes.

Research motivation
When surgery is necessary, the relationship between timing of the operation and the outcome of the mother and child are not known.

Research objectives
To investigate the association between time to ureteral stent placement and clinical outcomes of patients with renal colic during pregnancy.

Research methods
In this retrospective study, pregnant women with renal colic who underwent surgery were studied. Maternal preoperative acute pyelonephritis (PANP), pregnancy outcome, and length of hospital stay (LOS) were compared between the two groups.

Research results
PANP was closely related to hospitalization costs, re-admission to hospital due to urinary tract infection after surgery and premature delivery. Multivariate analysis showed that stone location and time from pain to admission were related to PANP.

Research conclusions
Both early and delayed surgery are safe and effective for the treatment of renal colic during pregnancy. Early surgery may be superior to a delayed procedure due to shorter LOS. For pregnant patients with renal colic, delayed surgery within 48 h is not related to the clinical outcome of the mother and child. However, the time from pain to hospital admission was related to PANP.

Research perspectives
Delayed surgery does not affect clinical outcomes, but leads to longer hospital stay. Time from pain to hospitalization and location of the stones are risk factors for preoperative acute pyelonephritis. Our research will have important significance in the clinic.

ACKNOWLEDGEMENTS
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REFERENCES
He MM et al. Time to ureteral stent placement and acute pyelonephritis


Retrospective Study

Management of retroperitoneal sarcoma involving the iliac artery:
Single-center surgical experience

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Author contributions: Li WX and Tong HX collected the data and performed statistical analyses; Lv CT and Yang H prepared the figures and tables; Li WX wrote the paper; Zhang Y, Zhao G, and Lu WQ were responsible for designing the study, critically reviewed the article, and approved the final version of the article to be published.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Zhongshan Hospital, Fudan University.

Informed consent statement: Written informed consent for inclusion in our database was obtained from all patients.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Abstract

BACKGROUND
Management of retroperitoneal sarcoma (RPS) involving the iliac artery is challenging and requires the concerted efforts of multidisciplinary team (MDT) members during surgical treatment.

AIM
To summarize the clinicopathologic features of RPS involving the iliac artery and our retroperitoneal soft tissue tumor MDT surgical experience.

METHODS
In this retrospective study, 15 patients with RPS involving the iliac artery who underwent surgery at our retroperitoneal soft tissue tumor center from July 2004 to June 2020 were analyzed. Statistical analyses were performed by Student’s t-test with SPSS 16.0.

RESULTS
Complete tumor resection (R0/R1) and iliac artery reconstruction were achieved in all 15 patients. All the operations were successful, with no serious complications or perioperative death. Resection with bilateral iliac artery reconstruction required a higher intraoperative blood transfusion volume than resection with unilateral iliac artery reconstruction. Recurrent cases were more likely to bleed and required a higher blood transfusion volume than primary cases. As of January 2021, 11 patients were alive, and 4 had died. Local recurrence occurred in two patients, one of whom developed liver metastasis.

CONCLUSION
Resection of RPS involving iliac vessels is feasible and effective when performed
by MDT members. Iliac artery oncovascular resection and reconstruction are key to a successful operation. Adequate blood preparation is important for successful completion of surgery.

**Key Words:** Retroperitoneal sarcoma; Vascular reconstruction; Multidisciplinary team; Iliac artery; Blood transfusion

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**INTRODUCTION**

Retroperitoneal sarcomas (RPSs) are malignant tumors originating from mesenchymal tissue and are exceedingly rare entities, among which liposarcoma is the most common type, followed by leiomyosarcoma[1]. Solitary fibroma and invasive fibroma are uncommon RPS subtypes that may arise in any location, including the retroperitoneum. Surgical excision remains the main treatment for primary tumors and is also recommended for recurrent tumors[2]. Specifically, tumors in the unique anatomic location of the retroperitoneum, which involves important blood vessels and organs, require the concerted efforts of a multidisciplinary team (MDT) during surgical treatment. Studies have shown that the success and radical resection rates were higher and the local relapse rate was lower for patients treated by professional MDTs than for those treated in nonprofessional centers[3]. High-throughput MDT centers are key to ensuring the effectiveness of medical services and the desired patient outcomes.

RPSs, especially recurrent lesions, are more likely to invade blood vessels, and resection of these major vessels is necessary for complete oncological clearance. The most frequently involved vessels for RPS are iliac vessels. Dissociation and reconstruction of iliac vessels are critical for the success of surgery. Vascular surgeons play an indispensable role in surgery in cases of aggressive tumors and in treating complications during surgery[4]. Artificial vascular reconstruction is most often performed between the common or external iliac artery and the femoral artery. The majority of artificial vessels used in the clinic are composed of polytetrafluoroethylene (PTFE), and satisfactory clinical results have been obtained[5,6]. Recanalization and monitoring of reconstructed blood vessels and anticoagulation after operation are indispensable during the postoperative recovery period[7].

Since its establishment in 2009, our retroperitoneal soft tissue tumor MDT has been committed to the standardized treatment of RPSs and has accumulated considerable experience in the surgical treatment of major involved vessels. This paper summarizes the treatment of RPSs involving the iliac artery from July 2004 to June 2020 and illustrates the key steps in vascular reconstruction.
MATERIALS AND METHODS

Clinical information
From July 2004 to June 2020, a total of 15 patients aged 12 to 73 years underwent RPS resection combined with iliac artery reconstruction. Patient data were retrospectively gathered in a retroperitoneal tumor database and analyzed. Clinical manifestations included abdominal pain (3 cases) and abdominal mass (5 cases). The other seven tumors were found during follow-up examination. Six cases were primary, and nine were recurrent (Table 1).

All patients underwent preoperative computed tomography (CT), CT angiography (CTA), or magnetic resonance imaging (MRI) to clarify the relationship between the tumor and important vessels in the abdominal or pelvic cavity. Most patients with recurrence underwent PET examination.

Surgery and vascular reconstruction
The incisions varied according to the location, size, and extent of the tumor. A median abdominal incision was usually adopted to facilitate exposure and incision extension, which could extend from the xiphoid process to the pubic symphysis. A lateral incision was added when necessary. In cases in which the mass encompassed the unilateral iliac artery and vein (Figure 1A and B), one side of the inguinal ligament was cut off, and the femoral artery was dissociated with a thin catheter to control bleeding during mass exposure (Figure 1C). The femoral and common iliac veins were ligated. The common iliac artery was isolated. Heparin was injected at 0.5 mg/kg body weight before vascular occlusion. After circulatory block, the femoral and common iliac arteries were cut off approximately 1 cm from the edge of the tumor, and the tumor was removed "en bloc" together with involved blood vessels and organs. Vessel reconstruction was completed between the common iliac artery and femoral artery (Figure 1D). In some cases, the mass encompassed the abdominal aorta and bilateral iliac arteries and adhered to the inferior vena cava (IVC) (Figure 2A and B). The abdominal aorta and left external iliac artery were fully exposed during mass exposure (Figure 2C). The right common iliac artery was further dissociated, and the left internal iliac artery was ligated. Resection was completed along with partial resection of the abdominal aorta and right common iliac artery and whole resection of the left common iliac artery. "Y-type" artificial vascular reconstruction was established among the abdominal aorta, right common iliac artery, and left external iliac artery (Figure 2D).

Statistical analysis
Statistical analyses were performed using SPSS software (v16.0, SPSS Inc., Chicago, IL, United States) and GraphPad Prism software (v5, GraphPad Software, San Diego, CA, United States). The mean and range were used for the analysis of variables. Differences between groups were analyzed using Student’s t-test for comparing means. P values < 0.05 were considered statistically significant.

RESULTS
All the surgeries were "en bloc" compartmental resections (R0/R1). Ten patients received unilateral iliac artery reconstruction, and five received bilateral iliac artery reconstruction (Table 1). A mean of 1.3 organs were excised, and the most commonly excised organ was the kidney. All the patients had good dorsalis pedis pulses postoperatively.

There were no perioperative deaths or infections of artificial vessels. One patient developed postoperative deep vein thrombosis in one leg after IVC transection during surgery, and no invasive treatment was performed. One patient developed external iliac artery occlusion 3 d after reconstruction. The condition improved after enhanced anticoagulant therapy. All the patients were discharged as scheduled. Pathology confirmed that there were eight cases of liposarcoma (LPS), two cases of leiomyosarcoma (LMS), two cases of undifferentiated pleomorphic sarcoma (UPS), and three cases of other types of tumors (solitary fibroma, invasive fibroma, and myxofibrosarcoma) (Table 1).

The differences in intraoperative blood loss and transfusion volume were further analyzed. For patients who underwent unilateral or bilateral iliac artery reconstruction, no significant difference in blood loss was found ($P = 0.06$) (Figure 3A). However, the volume of transfused blood was significantly higher for patients who received bilateral arterial reconstruction than for those who received unilateral arterial reconstruction.
Table 1 Clinicopathological data of patients who underwent tumor resection combined with vascular reconstruction

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of cases</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female vs male)</td>
<td>8/7</td>
<td></td>
</tr>
<tr>
<td>Age (&lt; 60 yr vs ≥ 60 yr)</td>
<td>11/4</td>
<td>52 (12-73)</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>LMS</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>UPS</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Primary/recurrent</td>
<td>9/6</td>
<td></td>
</tr>
<tr>
<td>Organs resected</td>
<td></td>
<td>1.3 (0-4)</td>
</tr>
<tr>
<td>Artery reconstruction type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral iliac artery</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Bilateral iliac artery</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IVC partial resection/transection</td>
<td>2/1</td>
<td></td>
</tr>
<tr>
<td>Common/internal iliac vein ligation</td>
<td>3/1</td>
<td></td>
</tr>
<tr>
<td>Volume of blood loss (mL)</td>
<td></td>
<td>1700 (500-5000)</td>
</tr>
<tr>
<td>Volume of transfused blood (mL)</td>
<td></td>
<td>743 (0-3150)</td>
</tr>
<tr>
<td>Postoperative vascular complications</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

IVC: Inferior vena cava; UPS: Undifferentiated pleomorphic sarcoma; LPS: Liposarcoma; LMS: Leiomyosarcoma.

For patients with recurrence, the volume of blood loss and transfusion volume were significantly improved compared with primary cases (Figure 4A and B).

All 15 patients were followed routinely, and 11 of them remained alive at the last follow-up. The follow-up duration ranged from 4 mo to 6.4 years, with an average of 20.8 mo. Two patients relapsed during the follow-up (1 case of recurrent UPS and 1 case of recurrent LMS). Four patients died, including two patients with UPS, one with primary LMS, and one with recurrent LPS (Table 2).

**DISCUSSION**

Retroperitoneal tumors, especially RPSs, frequently invade major vessels due to their unique anatomical location and growth characteristics[8]. The most commonly involved vessels are iliac vessels, followed by the abdominal aorta, IVC, and renal veins. The most common type of RPS requiring vascular reconstruction is LPS, particularly for multiple recurrent lesions. Retroperitoneal neoplasms involving major blood vessels are not a contraindication for surgical resection. Management of RPS is technically feasible with appropriate planning and requires interdisciplinary cooperation among surgeons in professional MDTs led by sarcoma surgeons, including specialists in general surgery, urology surgery, vascular surgery, and interventional therapy. Multiple imaging modalities, such as CT, CTA, MRI, or 3D imaging, are recommended when necessary to clarify the relationship between the tumor and adjacent vessels. Intraoperative arteriography can not only block the blood flow of the artery to be ligated but also further specify the blood supply of the tumor. Complete surgical resection of RPSs invading vital organs and major vessels requires intraoperative cooperation, especially between vascular and urological surgeons[9].

More often, RPSs invade or surround the unilateral common iliac artery and the internal and external iliac arteries. In most cases, the internal iliac artery is ligated, and part of or all the external iliac arteries are resected. The common iliac artery or external iliac artery is reconstructed with the femoral artery. In our group, one patient underwent artery reconstruction between the external iliac artery and ipsilateral reconstruction (P < 0.001) (Figure 3B). For patients with recurrence, the volume of blood loss and transfusion volume were significantly improved compared with primary cases (Figure 4A and B).
### Table 2 Clinicopathological data of the four patients who died

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Tumor type</th>
<th>Pathology</th>
<th>DFS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>Female</td>
<td>50</td>
<td>Primary</td>
<td>UPS</td>
</tr>
<tr>
<td>Case 2</td>
<td>Male</td>
<td>51</td>
<td>Recurrent</td>
<td>LPS</td>
</tr>
<tr>
<td>Case 3</td>
<td>Male</td>
<td>71</td>
<td>Primary</td>
<td>LMS</td>
</tr>
<tr>
<td>Case 4</td>
<td>Male</td>
<td>54</td>
<td>Recurrent</td>
<td>UPS</td>
</tr>
</tbody>
</table>

DFS: Disease free survival; UPS: Undifferentiated pleomorphic sarcoma; LPS: Liposarcoma; LMS: Leiomyosarcoma.

---

Figure 1 Computed tomography images from a case of retroperitoneal invasive fibroma and key steps in surgery. A: The arterial phase of the computed tomography (CT) scan indicated a massive pelvic mass with a diameter of approximately 20 cm surrounding the right internal iliac artery (white arrow) and external iliac artery (black arrow); B: The venous phase of the CT scan indicated that the mass surrounded the right common iliac vein and caused compression occlusion (arrow); C: During surgery, the right femoral artery (white arrow) was dissociated; the blue arrow indicates the mass; D: Artificial vessel reconstruction between the right common iliac artery (blue arrow) and femoral artery (black arrow) was performed after tumor removal.

---

superficial femoral artery because of superficial invasion. Contralateral iliac artery transposition for reconstruction of the common iliac artery has also been studied in other centers and has achieved good clinical results[10]. Resection of major involved vessels such as the IVC or abdominal aorta is sometimes necessary for complete oncological clearance[11]. If the abdominal aorta below the level of the renal artery is involved, the tumor can be removed together with the abdominal aorta and iliac artery. Nephrectomy can be performed in the context of normal renal function of the other kidney. However, when the tumor involves the abdominal aorta above the renal artery level, complete resection is often difficult to achieve because of the affected celiac trunk or superior mesenteric artery. For the treatment of invaded veins, partial resection and angioplasty of the renal vein should be adopted as often as possible. If they are invaded, the common iliac vein and internal iliac vein can be resected, which will not cause severe postoperative lower limb edema due to the presence of an adequate collateral vessel network. In the case of iliac vessel involvement, the close proximity of the vein and artery often requires both venous and arterial resection to gain local control. Only three patients in our group underwent IVC partial resection or transection because most tumors were still arterially invasive. The IVC can be partially excised if it has been invaded or ligated directly if the invasion is below the renal vein. The need for IVC reconstruction should be assessed according to
Figure 2 Computed tomography images of a case of myxofibrosarcoma and key steps in the surgery. A: A layer of the arterial phase of the computed tomography scan indicated a retroperitoneal mass with a diameter of approximately 9 cm surrounding the abdominal aorta (white arrow) and adherent to the inferior vena cava (red arrow); B: Another layer indicated that the mass surrounded the left common iliac artery (white arrow); C: During surgery, the left external iliac artery (lower left arrow) and abdominal aorta (upper right arrow) were dissociated; the blue arrow indicates the mass; D: “Y-type” artificial vascular reconstruction of the abdominal aorta (white arrow), right common iliac artery (black arrow), and left external iliac artery (blue arrow) was performed after tumor removal.

Figure 3 Differences in blood loss and transfusion volume based on the pattern of iliac artery reconstruction. A: No statistically significant difference was found in the volume of blood loss between the unilateral and bilateral iliac artery reconstruction groups ($P = 0.06$); B: The volume of transfused blood was significantly higher for patients who received bilateral iliac artery reconstruction than for those who received unilateral iliac artery reconstruction ($P < 0.001$).

The most common intraoperative complication of resection of RPS involving iliac vessels is hemorrhage. Increased intraoperative bleeding is associated with a poor prognosis[2]. Herein, no statistically significant difference in blood loss was found between the unilateral and bilateral iliac arterial reconstruction groups. The volume of blood transfusion, however, was significantly higher for cases with bilateral arterial reconstruction than for those with unilateral arterial reconstruction. This result was mainly due to accurate preoperative evaluation of tumor arterial blood supply, individualized selection of surgical approach, and effective blood occlusion. Regarding bilateral artery reconstruction cases, patients with larger tumors were more often affected by severe anemia than those with smaller tumors, and occlusion of the preoperative imaging, intraoperative findings, and the extent of surgery[12].
abdominal aorta was more likely to cause unstable blood pressure than occlusion of other vessels, so a higher volume of intraoperative blood transfusion was needed.

The difficulty of resecting recurrent RPS increases over time, especially for lesions involving major blood vessels and organs. Recurrent RPS is usually more aggressive and less differentiated than primary RPS, which results in a worse prognosis. Well-differentiated liposarcoma (WDLPS) may undergo subtype transition and become dedifferentiated liposarcoma (DDLPS). It was found that 47.8% (11/23) of patients with initial WDLPS experienced pathological progression such that their recurrent tumors were of the DDLPS subtype[13]. Recurrent tumors adhere to blood vessels in a dense manner, and the original anatomic location is usually slightly different. Therefore, recurrent tumors are more likely to bleed during surgery than are primary tumors. Here, we found that the amount of bleeding and the transfusion volume were significantly increased for patients with recurrence.

Complete or "en bloc" compartmental resection of RPS with involvement of blood vessels may offer the only chance for cure for those patients who are eligible for surgery. However, for some highly malignant and recurrent RPSs, such as UPS and LMS, survival remains poor despite vascular reconstruction. In this group, four patients died, including one who suffered recurrence, one with primary UPS, and one with primary LMS (Table 2). Therefore, adjuvant therapies that include radiation and immunotherapy need to be explored to further improve patient survival.

Combined excision of the organs or structures involved requires a balance between the expected morbidity and the oncologic benefit. It is debatable whether excision should be extended to include uninvolved organs adjacent to the primary tumor. Challenging structures such as the pancreas or the spine, if clinically involved, may also be removed, but this practice increases morbidity and mortality[14]. If the tumor invades the iliac vessel and penetrates into pelvic muscle or even bone, an overly cautious resection may increase the clinical risk and probability of positive microscopic margins. In such circumstances, a second operation after neoadjuvant chemoradiotherapy is highly recommended[15].

However, there are some limitations to this retrospective clinical analysis. In large-sample studies, arterial reconstruction was found to be accompanied by high postoperative morbidity, such as patency of arteries[16]. Due to the limited sample size, we only reported one patient with artificial vessel occlusion. Given the short mean follow-up time and limited sample size, it was impossible to calculate the overall survival or the local recurrence rate at 3 or 5 years; thus, future studies need to consider these clinical outcomes.

CONCLUSION

Resection for RPS involving the iliac artery is safe, effective, and practical in a specialized MDT center that is highly experienced in this complex field of surgery. Despite the recurrence rate of RPS remaining high, resection combined with vascular
reconstruction improves the R0 or R1 resection rate and results in encouraging survival for patients who were otherwise considered unresectable. Bleeding control and adequate blood transfusion affect patient recovery and surgical outcomes to a certain extent. Members from general surgery, vascular surgery, anesthesiology, blood transfusion, and intensive care teams should cooperate closely for the treatment of RPS.

**ARTICLE HIGHLIGHTS**

**Research background**
Management of retroperitoneal sarcoma (RPS) involving the iliac artery is challenging and requires the concerted efforts of MDT members. Complete tumor resection together with iliac artery reconstruction can confer an outcome advantage for patients.

**Research motivation**
The retroperitoneal soft tissue tumor MDT of our hospital focuses on the standardized treatment of retroperitoneal sarcoma and has accumulated considerable experience in the surgical treatment of major involved vessels. Therefore, many typical cases and surgeries need to be further summarized and shared to improve the standardized treatment of RPS.

**Research objectives**
To summarize the clinicopathological features of patients who received RPS excision and iliac artery reconstruction and share our surgical experience in the management of the involved iliac artery.

**Research methods**
A retrospective analysis of a maintained database consisting of 15 consecutive patients with RPS invading the iliac artery in our center from July 2004 to June 2020 was conducted. Information on baseline characteristics, type of vascular reconstruction, combined number of excised organs, volume of intraoperative blood loss and transfusion, postoperative pathology, and complications was retrieved.

**Research results**
Fifteen patients were enrolled in this study and received complete tumor resection (R0/R1) with iliac artery reconstruction. No serious complications occurred, and there were no perioperative deaths. Resection with bilateral iliac artery reconstruction required more intraoperative blood transfusions than resection with unilateral iliac artery reconstruction. Recurrent cases were more likely to bleed and required more blood transfusion volume than primary cases. As of January 2021, 11 patients were alive, and 4 patients had died. Local recurrence occurred in two patients.

**Research conclusions**
Resection of RPS involving iliac vessels is feasible and effective when performed in collaboration with members of a multidisciplinary team (MDT) to ensure the best survival outcomes. Iliac artery oncovascular resection and reconstruction are key to a successful operation. Adequate blood preparation is important for the successful completion of surgery. However, for some highly malignant RPSs, survival remains poor despite vascular reconstruction.

**Research perspectives**
Additional studies with large samples are needed to calculate survival and recurrence rates and to analyze related risk factors for patients who undergo tumor resection with iliac artery reconstruction.

**ACKNOWLEDGEMENTS**
The authors thank Ma LJ at the Department of General Surgery, Shanghai Public Health Clinical Center, Fudan University for helping with image editing.
REFERENCES


Retrospective Study

COVID-19 pandemic changed the management and outcomes of acute appendicitis in northern Beijing: A single-center study

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Author contributions: Zhang P and Zhang Q contributed equally to this work; Zhang P and Zhang Q designed the research study; Zhang P, Zhang Q and Zhao HW performed the research; Zhang Q and Zhao HW contributed new reagents and analytic tools; Zhang P and Zhang Q analyzed the data and wrote the manuscript; all authors have read and approve the final manuscript.

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Country/Territory of origin: China

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Abstract

BACKGROUND

Since the outbreak of the coronavirus disease 2019 (COVID-19) pandemic, outcomes and management of many diseases have been affected. Acute appendicitis is a common acute abdomen. The incidence rate is 0.05%-0.5%. Studies reported that the number of patients with appendicitis admitted to emergency department significantly decreased since the pandemic. People avoided going to the hospital for fear of being infected. Different countries have different epidemic prevention measures that result in different treatment outcomes. The Chinese government also published some temporary measures in order to prevent the outbreak.

AIM

To explore the changes in management and outcomes of acute appendicitis during the COVID-19 pandemic in the North of Beijing.

METHODS

Patients with acute appendicitis admitted to Beijing Tsinghua Changgung Hospital between February and June 2019 and February and June 2020 were retrospectively reviewed. Cases were grouped according to admission year. The demographic characteristics, present illnesses, medical history, symptoms and signs, comorbidities, blood test results, imaging data, appendix pathology, and treatment details were compared.

RESULTS

Overall, 74 patients received nonsurgical treatment and 113 patients underwent surgical treatment in group 2019, whereas 159 patients received nonsurgical treatment and 26 patients received surgical treatment in group 2020. Fever, thick appendix, nonsurgical management, and uncomplicated appendicitis (simple or
Since the outbreak of the COVID-19 pandemic, the outcomes and management of many diseases have been affected. Acute appendicitis is a common acute abdomen. The incidence rate is 0.05%-0.5% [1,2]. A study conducted in Italy reported that the number of patients with appendicitis admitted to the emergency department (ER) has significantly increased. Currently, over 100 million patients have been infected worldwide. The first COVID-19 case in Beijing was confirmed in January, after which the pandemic reached its peak by the end of June 2020, with 922 confirmed cases. Our paper showed that patients suffering from acute appendicitis in the northern part of Beijing tended to present with severe symptoms and opt for nonsurgical treatment during the coronavirus disease 2019 pandemic. For patients who underwent surgical treatment, the operation was delayed and was more difficult during the pandemic. Nevertheless, the hospital stay and the incidence of postsurgical complications did not change. We also found laparoscopic appendectomy was safe for patients followed by Chinese government temporary measures.

**Key Words:** COVID-19; Acute appendicitis; Case management; Treatment outcome; Antibiotic treatment; Laparoscopic appendectomy

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**Core Tip:** Our paper showed that patients suffering from acute appendicitis in the northern part of Beijing tended to present with severe symptoms and opt for nonsurgical treatment during the coronavirus disease 2019 pandemic. For patients who underwent surgical treatment, the operation was delayed and was more difficult during the pandemic. Nevertheless, the hospital stay and the incidence of post-surgical complications did not change. We also found laparoscopic appendectomy was safe for patients followed by Chinese government temporary measures.

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**DOI:** https://dx.doi.org/10.12998/wjcc.v10.i3.820

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**INTRODUCTION**

The World Health Organization declared a global coronavirus disease 2019 (COVID-19) pandemic on March, 2020. Then, the number of infected patients significantly increased. Currently, over 100 million patients have been infected worldwide. The first COVID-19 case in Beijing was confirmed in January, after which the pandemic reached its peak by the end of June 2020, with 922 confirmed cases.

Since the outbreak of the COVID-19 pandemic, the outcomes and management of many diseases have been affected. Acute appendicitis is a common acute abdomen. The incidence rate is 0.05%-0.5% [1,2]. A study conducted in Italy reported that the number of patients with appendicitis admitted to the emergency department (ER) has significantly decreased since the pandemic [3]. Another study conducted in Israel showed that weekly admissions decreased by 40.7% [4]. People were avoiding going to the hospital for fear of being infected. Consequently, the United Kingdom published new guidelines and changed the management of acute appendicitis [5]. The Chinese government also published several temporary measures to prevent the outbreak. All patients requiring admission were asked to perform blood tests for complete blood count (CBC), immunoglobulin (Ig) G, and IgM antibody. Chest computed tomography (CT) and swabs were also ordered.

Different countries have different epidemic prevention measures that result in different treatment outcomes. The aim of this research was to explore whether the COVID-19 pandemic changed the management and outcomes of acute appendicitis in Beijing.
MATERIALS AND METHODS

Case selection
Patients with acute appendicitis treated in Beijing Tsinghua Changgung Hospital from February to June 2019 and February to June 2020 were reviewed retrospectively. Cases treated in 2020 were categorized as group 2020, and cases treated in 2019 were categorized as group 2019. The diagnostic criteria for acute appendicitis including: The right lower quadrant abdominal pain; tenderness in the right lower quadrant, especially at the McBurney point; increased white blood cell count (WBC), c-reactive protein (CRP) level, or neutrophil percentage (N%); a swelling appendix was found by ultrasound or CT. The inclusion criteria were as follows: (1) Admitted with acute appendicitis; and (2) Older than 16 years and younger than 80 years. The exclusion criterion was that the patient was admitted with a periappendicular abscess. This paper was approved by the Beijing Tsinghua Changgung Hospital ethics committee (ID: 21039-6-01).

Management
All patients were treated surgically or nonsurgically according to their decisions. Some patients who needed surgical management selected nonsurgical management due to the fear of being infected during the pandemic.

IV antibiotics, which were given as nonsurgical management in the ER, included ertapenem 1 g + 0.9% normal saline (NS) 100 mL qd, ceftriaxone 2 g + 0.9% NS 100 mL qd, ornidazole 0.5 g q12 h, levofloxacin 0.5 g qd, and ornidazole 0.5 g q12 h. CBC and CRP were performed 3 d later. The patients were discharged with oral antibiotics for 3 d if their blood tests were near normal (WBC < 10 × 10⁹/L, N% < 85%, and CRP < 50 mg/L). Otherwise, IV antibiotics were continued until the blood test reached the listed criteria above. Abdominal ultrasound or CT scans were performed if the CBC was elevated or the abdominal pain was more severe than before. Ultrasound- or CT-guided percutaneous puncture was performed if the imaging test suggested a periappendicular abscess.

Laparoscopic appendectomy was performed as surgical management. The patients were placed in the supine position and received general anesthesia. A 1 cm incision was made on the umbilicus. A 12-14 mmHg pneumoperitoneum was formed by inflation with carbon dioxide through a pneumoperitoneum needle. A 10-mm trocar was used to puncture the abdominal cavity, and the laparoscope came through it. Under laparoscopy, 1-cm and 0.5-cm small incisions were made at the anti-McBurney point and 3 cm on the pubic symphysis, and 10-mm and 5-mm trocars were placed, respectively. Laparoscopic instruments were used to find and resect the appendix. Hem-o-lok (Teleflex Medical, United States) occlusion was performed to close the root mesentery of the appendix. The appendix root was ligated with a 7# silk thread (Mersilk, Ethicon) or occluded with a Hem-o-lok at 0.5 cm from the root of the appendix. The appendix was removed by a fetching bag from the trocar in the left lower abdomen. The incision was sutured after the abdominal pelvic fluid was suctioned.

Ertapenem 1 g once daily or ceftriaxone 2 g once + metronidazole 0.5 g every 8 h daily were used as intravenous antibiotic treatment. The patients were discharged if the blood test results were near normal, the patient tolerated semiliquid food, had no fever or wound infection, and the pain was controlled.

Data collection and statistical analysis
Data collection: The following indicators were collected for patients receiving nonsurgical treatment: Age, sex, disease onset time, gastrointestinal symptoms, comorbidities, history of appendicitis, fever, peritonitis, WBC, CRP, N%, neutrophil-to-lymphocyte ratio (NLR), appendix diameter, appendicolith, ascites in imaging, uncomplicated appendicitis (simple or suppurative appendicitis) ratio, IV antibiotic types, antibiotic treatment days, conversion to operation rate and recurrence.

The following indicators of surgical cases were collected: Age, sex, disease onset time, gastrointestinal symptoms, comorbidities, history of appendicitis, fever, peritonitis, WBC, CRP, N%, NLR, appendix diameter, appendicolith, ascites in imaging, time from diagnosis to surgery, surgical time (defined as the time from skin incision to anesthesia intubation removed), intraoperative blood loss, intraoperative adhesions or ascites, appendix pathology, hospital stay, and postoperative complications.

Of 6 mo’ followed up was performed via telephone call or in the outpatient department in the 2020 group, while cases in the 2019 group were followed up for 18
mo in the outpatient department or via telephone call after discharge.

**Statistical analysis**

SPSS 16.0 (IBM, United States) was used to analyze all results. T-test was used for continuous variables, while the chi-square test was used for the frequency data. A $P < 0.05$ indicated a statistically significant difference.

**RESULTS**

**All case results**

Overall, 159 patients received nonsurgical treatment and 26 patients received surgical treatment in 2020, whereas 74 patients received nonsurgical treatment and 113 patients received surgical treatment in 2019. Group 2020 comprised 95 male and 90 female patients aged 40.40 ± 14.90 years, while group 2019 comprised 83 male and 104 female patients aged 40.45 ± 15.66 years. A higher fever rate (64.5% vs 52.9%, $P = 0.02$), thicker appendix diameter (9.31 ± 4.05 mm vs 4.78 ± 4.20 mm, $P < 0.01$), higher rate of nonsurgical management (85.9% vs 39.6%, $P < 0.01$), and higher rate of uncomplicated appendicitis were observed (52.4% vs 64.2%, $P = 0.02$) in group 2020 than in group 2019. As shown in Table 1, no deaths were reported after follow-up. No operation team member was infected after follow-up.

**Nonsurgical management case results**

N% (80.49 ± 12.31% vs 76.63 ± 12.88%, $P = 0.01$), NLR (10.51 ± 9.95 vs 7.22 ± 6.33, $P = 0.02$), and the rate of recurrence were higher (1.3% vs 21.6%, $P < 0.001$) in group 2020 than in group 2019 (Table 2).

**Surgical management case results**

There were more cases with gastrointestinal symptoms (80.8% vs 58.4%, $P = 0.03$) and peritonitis (96.2% vs 67.3%, $P < 0.01$) in group 2020 than in group 2019. Higher WBC (14.92 ± 4.39 vs 13.22 ± 3.72, $P = 0.04$), a higher rate of ascites in the image (50% vs 25.7%, $P = 0.02$), longer time from diagnosis to surgery (32.44 ± 47.95 h vs 10.70 ± 8.77 h, $P < 0.01$), longer surgical time (87.35 ± 51.68 min vs 72.75 ± 38.25 min, $P = 0.02$), higher intraoperative blood loss (14.23 ± 14.74 mL vs 11.30 ± 6.83 mL, $P = 0.03$) and a higher rate of intraoperative adhesion or ascites (92.3% vs 67.3%, $P = 0.01$) were observed in group 2020 compared to group 2019, as shown in Table 3.

**DISCUSSION**

Beijing Tsinghua Changgung Hospital is one of the only two large hospitals in the northern part of Beijing, serving 700000 residents. Accordingly, our data represent the real-life situation in northern Beijing. Our study suggested that patients with acute appendicitis presented with more severe conditions at admission during the pandemic, and they preferred nonsurgical management. For patients who underwent surgical management, the operation was delayed and was more difficult during the pandemic. However, the hospital stay and the incidence of postsurgical complications did not change.

In the present study, the number of admitted patients did not decrease, which was inconsistent with previous studies [3,4]. Nevertheless, we found that the proportion of uncomplicated appendicitis was lower than that in the same period the previous year (52.4% vs 64.2%), which suggested that the morbidity of acute appendicitis did not change. A previous study reported that the appendix was thicker and that the inflammation around the appendix was more severe during the pandemic based on CT scans [6], which is consistent with our study results (9.31 ± 4.05 mm vs 4.78 ± 4.20 mm, $P < 0.01$). These findings suggested that patients feared becoming infected while in the hospital and that they preferred to stay at home until their symptoms became too serious to manage at home.

The 2020 WSES guidelines for acute appendicitis recommend that nonsurgical treatment should be the first choice for uncomplicated appendicitis [7], while surgery should be the first choice for complicated appendicitis (gangrene or perforated appendicitis). As patients did not want to spend a long time in the hospital because of the fear of becoming infected during the pandemic, many selected nonsurgical treatment [8]. Some doctors in other countries selected nonsurgical management for
most patients with acute appendicitis because they could not determine whether the patient was infected with COVID-19 before treatment[9]. A previous study showed that 74% of surgeons modified their practice to predominantly nonsurgical management, while 61% of patients selected nonsurgical management to decrease their time spent in the hospital[10]. A global survey also suggested that doctors preferred nonoperative management during the pandemic[11]. This study found that, after the pandemic, the proportion of nonsurgically managed cases of appendicitis in our hospital increased from 39.6% the previous year to 85.9%, which is consistent with the situation abroad. Further research on the outcome of nonsurgical treatment with complicated appendicitis should be performed in the future.

The NLR has been widely used to evaluate various malignant tumors as an indicator of immune status[12,13]. NLR has also been used as an indicator for the diagnosis and severity evaluation of acute appendicitis. Previous studies reported that the NLR could be used as an important parameter in the diagnosis of appendicitis[14,15], while there was also a substantial correlation between the NLR and disease severity. This study demonstrated that patients who selected nonsurgical management during the pandemic presented with higher N% and NLR. We also detected some severe patients whose condition was more appropriate for surgical management but who underwent nonsurgical management during the nonpandemic period. This was consistent with the increased proportion of nonsurgical management during the pandemic observed in the present study. Nevertheless, there was no significant difference in the rates of conversion to surgery between groups, which indicated that the outcomes of IV antibiotic treatment were the same as those during the nonpandemic period.

Recurrence is an important problem of nonsurgical management. The APPAC study reported that the 1-year, 3-year, and 5-year recurrence rates of nonsurgical treatment were 27.3%, 35.2%, and 39.1%, respectively[16]. Our findings demonstrated a significant decrease in recurrence during the pandemic (1.3% vs 21.6%); however, bias was possible due to the short follow-up in group 2020.

Among surgically managed cases, our study demonstrated that patients presented with more gastrointestinal symptoms (80.8% vs 58.4%) and more severe physical signs during the pandemic period, especially peritonitis (96.2% vs 67.3%). Peritonitis emerges when periappendiceal exudation stimulates the parietal peritoneum, thus

### Table 1 Comparison of characteristics between the two groups (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Group 2020 (n = 185)</th>
<th>Group 2019 (n = 187)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male, n (%)</td>
<td>95, 51.4%</td>
<td>83, 44.4%</td>
<td>0.18</td>
</tr>
<tr>
<td>Age (year)</td>
<td>40.40 ± 14.90</td>
<td>40.45 ± 15.66</td>
<td>0.70</td>
</tr>
<tr>
<td>Disease onset time (h)</td>
<td>36.78 ± 57.05</td>
<td>33.27 ± 34.92</td>
<td>0.18</td>
</tr>
<tr>
<td>Nausea, vomiting or diarrhea, n (%)</td>
<td>94, 50.8%</td>
<td>104, 55.6%</td>
<td>0.35</td>
</tr>
<tr>
<td>Peritonitis, n (%)</td>
<td>75, 40.5%</td>
<td>93, 49.7%</td>
<td>0.08</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td>24, 13%</td>
<td>17, 9.1%</td>
<td>0.23</td>
</tr>
<tr>
<td>History of appendicitis, n (%)</td>
<td>30, 16.2%</td>
<td>28, 15%</td>
<td>0.74</td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>120, 64.5%</td>
<td>99, 52.9%</td>
<td>0.02</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>12.94 ± 4.42</td>
<td>12.73 ± 4.11</td>
<td>0.41</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>33.40 ± 51.64</td>
<td>36.00 ± 56.17</td>
<td>0.78</td>
</tr>
<tr>
<td>N%</td>
<td>80.92 ± 11.91</td>
<td>80.72 ± 10.69</td>
<td>0.58</td>
</tr>
<tr>
<td>NLR</td>
<td>10.56 ± 9.58</td>
<td>9.60 ± 8.17</td>
<td>0.32</td>
</tr>
<tr>
<td>Appendix diameter (mm)</td>
<td>9.31 ± 4.05</td>
<td>4.78 ± 4.20</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Appendicolith, n (%)</td>
<td>65, 35.1%</td>
<td>68, 36.4%</td>
<td>0.81</td>
</tr>
<tr>
<td>Ascites in image, n (%)</td>
<td>43, 23.2%</td>
<td>45, 24.1%</td>
<td>0.85</td>
</tr>
<tr>
<td>Treatment, non-surgical treatment, n (%)</td>
<td>159, 85.9%</td>
<td>74, 39.6%</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Uncomplicated appendicitis, n (%)</td>
<td>97, 52.4%</td>
<td>120, 64.2%</td>
<td>0.02</td>
</tr>
</tbody>
</table>

WBC: White blood cell count; CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio.
Zhang P et al. COVID-19 pandemic changed the acute appendicitis

Table 2 Comparison of characteristics and outcomes between the two groups with non-surgical management (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Group 2020 (n = 159)</th>
<th>Group 2019 (n = 74)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Sex, male, n (%)</td>
<td>85, 53.5%</td>
<td>36, 48.6%</td>
<td>0.49</td>
</tr>
<tr>
<td>Age (year)</td>
<td>40.03 ± 15.12</td>
<td>40.50 ± 17.10</td>
<td>0.77</td>
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<tr>
<td>Disease onset time (h)</td>
<td>35.24 ± 54.86</td>
<td>39.89 ± 48.44</td>
<td>0.16</td>
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<tr>
<td>Nausea, vomiting or diarrhea, n (%)</td>
<td>84, 52.8%</td>
<td>29, 39.2%</td>
<td>0.05</td>
</tr>
<tr>
<td>Peritonitis, n (%)</td>
<td>50, 31.4%</td>
<td>17, 23.0%</td>
<td>0.18</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td>18, 11.3%</td>
<td>3, 4.1%</td>
<td>0.07</td>
</tr>
<tr>
<td>History of appendicitis, n (%)</td>
<td>26, 16.4%</td>
<td>11, 14.9%</td>
<td>0.77</td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>107, 67.3%</td>
<td>53, 71.6%</td>
<td>0.51</td>
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<td>WBC (10⁹/L)</td>
<td>12.67 ± 4.37</td>
<td>11.85 ± 4.48</td>
<td>0.35</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>30.16 ± 48.19</td>
<td>28.58 ± 45.21</td>
<td>0.92</td>
</tr>
<tr>
<td>N%</td>
<td>80.49 ± 12.31</td>
<td>76.63 ± 12.88</td>
<td>0.01</td>
</tr>
<tr>
<td>NLR</td>
<td>10.51 ± 9.95</td>
<td>7.22 ± 6.33</td>
<td>0.02</td>
</tr>
<tr>
<td>Appendicitis diameter (mm)</td>
<td>10.13 ± 3.34</td>
<td>9.84 ± 2.24</td>
<td>0.10</td>
</tr>
<tr>
<td>Appendicolith, n (%)</td>
<td>53, 33.3%</td>
<td>21, 28.4%</td>
<td>0.45</td>
</tr>
<tr>
<td>Ascites in image, n (%)</td>
<td>30, 18.9%</td>
<td>16, 21.6%</td>
<td>0.62</td>
</tr>
<tr>
<td>Uncomplicated appendicitis, n (%)</td>
<td>84, 52.8%</td>
<td>42, 56.8%</td>
<td>0.58</td>
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<tr>
<td>IV antibiotics</td>
<td></td>
<td></td>
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<tr>
<td>Cephalosporin, n (%)</td>
<td>137, 86.2%</td>
<td>65, 87.8%</td>
<td></td>
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<tr>
<td>Others, n (%)</td>
<td>9, 5.7%</td>
<td>6, 8.1%</td>
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<tr>
<td>None, n (%)</td>
<td>13, 8.2%</td>
<td>3, 4.1%</td>
<td></td>
</tr>
<tr>
<td>Antibiotic treatment days (d)</td>
<td>4.08 ± 3.28</td>
<td>4.03 ± 2.63</td>
<td>0.85</td>
</tr>
<tr>
<td>Convert to operation, n (%)</td>
<td>16, 10.1%</td>
<td>3, 4.1%</td>
<td>0.12</td>
</tr>
<tr>
<td>Recurrence, n (%)</td>
<td>2, 1.3%</td>
<td>16, 21.6%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

WBC: White blood cell count; CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio.

representing severe abdominal infection. Patients also presented with higher WBCs (14.92 ± 4.39 vs 13.22 ± 3.72), which was consistent with a previous study[8]. A global survey revealed that 56.1% of the study cases had more severe septic abdominal diseases during the pandemic, especially appendicitis and cholecystitis (41.8% and 40.2% of the study cases, respectively)[17].

To prevent COVID-19 infection among medical teams, the Chinese government ordered all patients to take a blood test for CBC, IgG, and IgM antibodies; chest CT; and swabs before admission. Although some foreign countries increased the CT scan rates[18], they did not require every patient to undergo all the tests before admission [19]. It took nearly 12 h to obtain the results of all of these tests and examinations in our hospital, which was why patients experienced a longer time from diagnosis to operation during the pandemic. A longer waiting time might lead to more severe ischemia of the appendix wall and an increased possibility of gangrene or perforation. A previous study reported that a time from onset to operation > 48 h, the rate of perforated appendicitis was 3.58 times that within 24 h[20]. Severe infection can lead to more severe intraoperative abdominal adhesions and ascites, thus increasing the difficulty of operation, prolonging the surgical time, and increasing the intraoperative blood loss, all of which were found in the present study. Some doctors are concerned that pneumoperitoneum may leak virus-contaminated gas from the trocar during laparoscopic surgery[5], while others are worried that electronic devices might aerosolize COVID-19, although there is no evidence for this. British guidelines recommended open surgery as the predominant procedure for acute appendicitis. The proportion of open surgeries significantly increased in the United Kingdom[19]. For the same reason, Italian doctors prefer open appendectomy without electronic devices.
Zhang P et al. COVID-19 pandemic changed the acute appendicitis

<table>
<thead>
<tr>
<th></th>
<th>Group 2020 (n = 26)</th>
<th>Group 2019 (n = 113)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male, n (%)</td>
<td>10, 38.5%</td>
<td>47, 41.6%</td>
<td>0.77</td>
</tr>
<tr>
<td>Age (year)</td>
<td>42.08 ± 12.69</td>
<td>40.56 ± 14.97</td>
<td>0.23</td>
</tr>
<tr>
<td>Disease onset time (h)</td>
<td>50.73 ± 62.32</td>
<td>27.63 ± 21.22</td>
<td>0.38</td>
</tr>
<tr>
<td>Nausea, vomiting or diarrhea, n (%)</td>
<td>21, 80.8%</td>
<td>66, 58.4%</td>
<td>0.03</td>
</tr>
<tr>
<td>Peritonitis, n (%)</td>
<td>25, 96.2%</td>
<td>76, 67.3%</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td>6, 23.1%</td>
<td>14, 12.4%</td>
<td>0.29</td>
</tr>
<tr>
<td>History of appendicitis, n (%)</td>
<td>4, 15.4%</td>
<td>17, 15.0%</td>
<td>1.00</td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>13, 50%</td>
<td>46, 40.7%</td>
<td>0.39</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>14.92 ± 4.39</td>
<td>13.22 ± 3.72</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>63.35 ± 74.51</td>
<td>38.72 ± 58.94</td>
<td>0.11</td>
</tr>
<tr>
<td>N%</td>
<td>85.15 ± 7.28</td>
<td>83.01 ± 8.11</td>
<td>0.98</td>
</tr>
<tr>
<td>NLR (%)</td>
<td>12.30 ± 7.46</td>
<td>10.75 ± 8.66</td>
<td>0.58</td>
</tr>
<tr>
<td>Appendice diameter (mm)</td>
<td>12.29 ± 4.94</td>
<td>10.75 ± 2.89</td>
<td>0.18</td>
</tr>
<tr>
<td>Appendicolith, n (%)</td>
<td>12, 46.2%</td>
<td>47, 41.6%</td>
<td>0.67</td>
</tr>
<tr>
<td>Ascites in image, n (%)</td>
<td>13, 50%</td>
<td>29, 25.7%</td>
<td>0.02</td>
</tr>
<tr>
<td>Time from diagnosis to surgery (h)</td>
<td>32.44 ± 47.95</td>
<td>10.70 ± 8.77</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Surgical time (min)</td>
<td>87.35 ± 51.68</td>
<td>72.75 ± 38.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Blood loss (mL)</td>
<td>14.23 ± 14.74</td>
<td>11.30 ± 6.83</td>
<td>0.03</td>
</tr>
<tr>
<td>Intraoperative adhesion or ascites</td>
<td>24, 92.3%</td>
<td>76, 67.3%</td>
<td>0.01</td>
</tr>
<tr>
<td>Uncomplicated appendicitis, n (%)</td>
<td>13, 50%</td>
<td>78, 69%</td>
<td>0.07</td>
</tr>
<tr>
<td>Hospital stay (d)</td>
<td>5.31 ± 3.53</td>
<td>4.37 ± 2.19</td>
<td>0.31</td>
</tr>
<tr>
<td>Complications, n (%)</td>
<td>2, 7.7%</td>
<td>2, 1.8%</td>
<td>0.16</td>
</tr>
</tbody>
</table>

WBC: White blood cell count; CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio.

However, the 2020 WSES guidelines recommended laparoscopic appendectomy as the first choice for complicated appendicitis[7]. Laparoscopic surgery leads to a shorter hospital stay and a lower rate of wound infection. In our study, laparoscopic appendectomy was the only operation used for surgical management of these patients. As all patients underwent a blood test for CBC, IgG and IgM antibodies and swabs before admission, the medical team did not perform the operation until negative results were obtained, thus putting at ease the medical team who did not have to worry about the possibility of COVID-19 infection during the operation. Some countries have used smoke evacuation systems with filters to evacuate surgical smoke during laparoscopic appendectomy[11], which will increase the ratio of minimally invasive surgery and bring the best benefits to patients.

According to a previous study, the incidence of postoperative complications during the pandemic was twice as high as that before the pandemic[22]. The authors suggested that the increased severity of appendicitis might be caused by a fear of admission. There was no significant difference in postoperative complication rates between groups in the present study, which might be related to a lower number of operation cases and short follow-up time during the pandemic or indirectly related to the proper government orders.

This study has several limitations. As this was a retrospective study, it was inevitably biased. This was a single-center study with a small sample size. The follow-up time during the pandemic was short. Therefore, the results need to be further confirmed by large case studies.

In summary, the proportion of cases using nonsurgical management for appendicitis in northern Beijing increased during the COVID-19 pandemic. The patients presented with more serious conditions. To prevent COVID-19 infection, a
more complex preoperative test and examination were adopted, which resulted in a longer preoperative waiting time and surgical time. Intraoperative blood loss increased. However, the complex preoperative examination was useful, as it screened the patients in need of laparoscopic appendectomy, ensured better postoperative outcomes, and did not significantly increase the postoperative complication rate.

**CONCLUSION**

During the COVID-19 pandemic, patients suffering from acute appendicitis in Beijing tended to present with severe symptoms and opt for non-surgical treatment. For patients who underwent surgical management, the operation was delayed and more difficult during the pandemic. The hospital stay and the incidence of post-surgical complications did not change. The complex preoperative examination can ensure the safety of laparoscopic appendectomy, which leads to a better postoperative outcomes.

**ARTICLE HIGHLIGHTS**

**Research background**

Since the outbreak of the coronavirus disease 2019 (COVID-19) pandemic, the outcomes and management of acute appendicitis have been affected. Different countries have different epidemic prevention measures that result in different treatment outcomes. The aim of this research was to explore whether the COVID-19 pandemic changed the management and outcomes of acute appendicitis in Beijing.

**Research motivation**

How did the COVID-19 pandemic change the management and outcomes of acute appendicitis in Beijing?

**Research objectives**

Explore the changes in management and outcomes of acute appendicitis during the COVID-19 pandemic in Beijing.

**Research methods**

Patients with acute appendicitis treated in Beijing Tsinghua Changgung Hospital from February to June 2019 and February to June 2020 were reviewed retrospectively. All patients were treated surgically or non-surgically according to their decisions. The demographics, symptoms, signs, laboratory parameters, imaging results, operation details, uncomplicated appendicitis rate, complications rate and recurrence rate were collected. SPSS 16.0 (IBM, United States) was used to analyze all results. T-test was used for continuous variables, while the chi-square test was used for the frequency data. A $P < 0.05$ indicated a statistically significant difference.

**Research results**

There were 74 patients who received non-surgical treatment and 113 patients who underwent surgical treatment in group 2019 vs 159 patients with non-surgical treatment and 26 patients with surgical treatment in group 2020. Higher fever rate, thicker appendix diameter, a higher rate of non-surgical management, and a higher rate of uncomplicated appendicitis were observed in group 2020 than in group 2019. Among the non-surgical management cases, the neutrophil percentage, neutrophil-to-lymphocyte ratio, and the recurrence rate were higher in group 2020. There were more cases with gastrointestinal symptoms and peritonitis in group 2020. Higher white blood cell count, a higher rate of ascites in the image, longer time from diagnosis to surgery, longer surgical time, higher intraoperative blood loss and a higher rate of intraoperative adhesion or ascites were observed in group 2020 compared to group 2019.

**Research conclusions**

During the COVID-19 pandemic, patients suffering from acute appendicitis in Beijing tended to present with severe symptoms and opt for non-surgical treatment. For patients who underwent surgical management, the operation was delayed and more difficult during the pandemic. The hospital stay and the incidence of post-surgical
complications did not change. The complex preoperative examination can ensure the safety of laparoscopic appendectomy, which leads to a better postoperative outcomes.

**Research perspectives**

This study has some limitations. As this was a retrospective study, it was inevitably biased. This was a single-center study with small sample size. The follow-up time during the pandemic was short. Therefore, the results need to be further confirmed by large case studies or RCT studies.

**REFERENCES**

Zhang P et al. COVID-19 pandemic changed the acute appendicitis


Retrospective Study

Laparoscopic approach for managing intussusception in children: Analysis of 65 cases

Sheng-Miao Li, Xiao-Ying Wu, Chun-Fen Luo, Lin-Jun Yu

Abstract

BACKGROUND
Intussusception can be managed by pneumatic reduction, ultrasound-guided hydrostatic reduction, open or laparoscopic surgery, but laparoscopy in such cases remains controversial.

AIM
To explore the clinical characteristics, effectiveness, and complications of surgical reduction for intussusception using laparoscopy in children.

METHODS
This study was a retrospective case series of pediatric patients with intussusception who underwent surgical reduction by laparoscopy from May 2011 to April 2016 at Taizhou Hospital of Zhejiang Province. Clinical characteristics (operation time, intraoperative blood loss, conversion rate of laparotomy, reasons for conversion, postoperative hospital stay, and adverse events) were described.

RESULTS
The 65 patients included 45 boys and 20 girls. The average age was 2.3 years (27.5 ± 24.5 mo). Of the 65 patients, 61 underwent surgical reduction by laparoscopy after a failed enema reduction of intussusception, and four underwent the procedure directly. All patients were treated successfully and 57 (87.7%) patients underwent successful laparoscopic surgery, two of which had a spontaneous reduction. Among the remaining cases, one was converted to open surgery via right upper quadrant incision, and seven required enlarged umbilical incisions. Intestinal resection was performed in 5 patients because of abnormal bowel lesions. There were no complications (intestinal perforations, wound infections, or intestinal adhesions) during the follow-up of 3 years to 8 years. Two patients experienced a recurrence of intussusception; one was resolved with pneumatic reduction, and the other underwent a second laparoscopic surgery.
CONCLUSION
Laparoscopic approach for pediatric intussusception is feasible and safe. Bowel resection if required can be performed by extending umbilical incision without the conventional laparotomy.

Key Words: Laparoscopy; Intussusception; Air reduction; Benefits; Complications

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Core Tip: This study aimed to explore the clinical characteristics, effectiveness, and complications of surgical reduction for intussusception using laparoscopy in children. Laparoscopic intussusception reduction can be beneficial and without significant complications if well indicated, and if an extension of the umbilical incision is used if needed. Laparoscopic assistance can also yield good treatment outcomes in some complicated cases requiring intestinal resection.

INTRODUCTION
For intussusception, the laparoscopic approach is a feasible alternative when pneumatic reduction or ultrasound-guided hydrostatic reduction failure. Laparoscopic intussusception reduction can be beneficial and without significant complications if well indicated, and an extension of the umbilical incision is used if needed. Laparoscopic assistance can also yield good treatment outcomes in some complicated cases requiring intestinal resection. Next, we can take the results of this study as a starting point for further prospective multicenter cohort studies.

MATERIALS AND METHODS
Intussusception can be managed by laparoscopic surgery, but it remains controversial. The results of this study strongly suggest that intussusception reduction using the laparoscopic approach is effective and without complications in patients with complex conditions or those requiring bowel resection.

Laparoscopy has been reported to be effective and without major complications for the management of intussusception[1,2], supporting the present study. In the present study, the success rate of laparoscopy was 88%, which is within the 79%-90% range reported by many case series[1-3]. Nevertheless, the use of laparoscopy remains controversial because laparoscopic intussusception reduction has a high rate of conversion to laparotomy, as high as 70% in some reports[4]. Nevertheless, with the improvement of laparoscopic techniques in recent years, the conversion rate of laparoscopic intussusception reduction to laparotomy has decreased from 14.3% to 5.4%[5,6]. In this study, the overall conversion rate to open laparotomy was 12%, including seven patients with extension of the umbilical incision. Considering that the small transverse umbilical incision is concealed, it is more aesthetically appealing than traditional open surgery. Besides, laparoscopically-assisted surgery can be considered a form of minimally invasive surgery with laparoscopic assistance; thus, there was only one case of true conversion to open surgery, resulting in a rate of conversion to open surgery of only 1.5%.

It has been reported that patients over 3 years of age have a higher probability of intestinal resection[7,8]; for these patients, laparoscopic intussusception reduction surgery is not recommended[9]. In this study, two patients were > 3 years of age, in whom intussusception was diagnosed using preoperative B-mode ultrasound. Considering the patients’ age, there was a possibility of intestinal lesions, and laparoscopic surgery was performed directly. After reduction was completed, erosion was...
found in the terminal ileum in one patient, and the tissue was frozen and rapidly sent for pathological examination, revealing lymphoma. The umbilical incision was extended to remove the diseased tissue and the ileocecal segment. The patient recovered after chemotherapy, and the incision site maintained a good aesthetic appearance. It was also reported that laparoscopic reduction is not recommended for patients aged < 3 mo[7,8]. In this study, one 1-month-old patient underwent difficult laparoscopic reduction, and the umbilical incision had to be extended. It was found that the terminal ileum had duplication malformations that were managed with intestinal resection and anastomosis. With the improvement of laparoscopic techniques and instrumentation, we believe that laparoscopic surgery will not be contraindicated by age or intestinal resection because minimally invasive surgery through a small umbilical incision can be achieved even for intestinal resection[1-3].

Another major controversy involves the fact that laparoscopic intussusception reduction is associated with a higher recurrence rate than is conventional open surgery. Nevertheless, it was recently reported that the recurrence rate after laparoscopic intussusception was 3.4%[6]. In this study, there were two recurrences (3.1%), nearly the same as traditional open surgery[10]. The ileum's seromuscular layers and the ascending colon were repaired with two sutures, and the terminal ileum and the ascending colon were juxtaposed. If the ileocecal segment was relatively isolated, it was fixed to the lower right abdominal wall with two sutures to prevent relapse. The recurrence rate in our cohort of only 3.1% suggests good effectiveness. One patient experienced recurrence and required re-operation. The original fixation suture was found to be loose and was re-fixed, without subsequent recurrence. In our opinion, intestine fixation to the abdominal wall may be an effective method for intussusception. Although this method of intestine fixation to the abdominal wall has not occurred intestinal torsion in this study, but we do not know whether it is a risk factor for intestinal torsion. Therefore, we need longer follow-up time and more cases.

It has been reported that the appendix is also a site susceptible to intussusception[4]. The appendix is prone to becoming a lead point for intussusception because of viral or bacterial infection or abnormal anatomical position. Therefore, when intussusception is found in the appendix during operation, the appendix exhibiting congestion and swelling is resected. There were no recurrences after appendicular resection in this study. Besides, there were no complications in our cohort, such as intestinal adhesions, wound infections, and intestinal perforations, as supported by the low rates of complications observed in previous studies[2,4].

The short operative time and hospital stay reflect the advantages of laparoscopic techniques[5,6]. Besides, laparoscopic techniques have obvious advantages for intussusception reduction. First, owing to muscle relaxation after anesthesia, some of the intussuscepted intestines might resolve spontaneously. Second, laparoscopic surgery can more intuitively locate organic lesions such as lymphomas of the terminal ileum and intestinal polyps. With open surgery, only the intussusception at the diverticulum's distal end might have been discovered, and the intussusception of the diverticulum itself might have been missed. Nevertheless, laparoscopy might also have some disadvantages, mainly related to the small abdominal cavity in children and the significant amount of edema in intussusception. First, if the intra-abdominal pressure is too high, the cardiopulmonary function of the patient will be affected. If it is too low, the operating field will be too small. Second, given the abdominal cavity's limited space, a gastric tube could be placed before surgery. Third, intussusception masses are often found in the upper right abdomen because the appendix is sometimes involved. Therefore, searching along the terminal ileum and the appendix is more difficult. Fourth, during reduction, the right-hand grasping forceps pulls the neck of the intussuscepted ileum along the fan-shaped curvature of the mesentery, while the left-hand grasping forceps pulls the neck of the intussuscepted sheath to the opposite direction; in this way, the two forces are maintained in the fan-shaped curvature of the mesentery. If the directions of the two forces are along a straight line, the traction on the mesentery tends to be insufficient. In addition, in the reduced intestine, it is important to change the two forceps' positions at an appropriate time to maintain some tension between the two forces. Finally, if it is found that the intussusception is challenging to reduce by laparoscopy, the umbilical incision should be extended, and the reduction should be performed through the umbilical incision. If necessary, bowel resection should be performed. Although laparoscopic intussusception reduction has many advantages, there are specific contraindications. First, laparoscopic surgery should not be performed in cases with longer times of onset (especially > 48 h), instability of the respiratory or circulatory systems, intestinal perforation, peritonitis, severe abdominal distension, or other manifestations. Second, the intussusceptum ideally should not extend past the middle segment of the transverse colon.
advantages and disadvantages have been highlighted in previous studies [2,4-6], but this study innovates by indicating that the umbilical excision can be enlarged when laparoscopy is difficult, without the need to convert the surgery to a true open surgery.

This study has limitations. The sample size was relatively small and from a single center. A treatment bias might be observed due to local practices. The retrospective nature of the study prevented the observation of some variables. Prospective multicenter cohort studies should be performed in the future.

**RESULTS**

**Characteristics of the patients**

During the study period, laparoscopic intussusception reduction was performed in 65 patients (Table 1). There were 45 (69.2%) boys and 20 (30.8%) girls, of whom the youngest was 1 mo old, and the oldest was 13 years old, and the average age was 2.3 years (27.5 ± 24.5 mo). The clinical presentations included paroxysmal crying or abdominal pain in 60 cases. There was an abdominal mass in 45 patients, and jam-colored bowel movements in 48 patients. The time of onset was 26.3 ± 7.8 h and was within 48 h in 64 cases and up to 52 h in one patient. Sixty (92.3%) had primary intussusception. Among all patients, three (4.6%), 40 (61.5%), and 22 (33.9) had ileum, ascending colon, and transverse colon involvement, respectively. Nine (13.8%) patients had acute gastroenteritis, seven (10.8%) had respiratory infection, and one (1.5%) had urinary tract infection. Six (9.2%) were taking probiotics, eight (12.3%) proton pump inhibitors, 13 (20.0%) antibiotics, and four (6.2%) gastrointestinal motility drugs.

**Surgical outcomes**

Of the 65 patients, laparoscopic reduction surgery was completed in 57 (87.7%) (Table 2). For two (3.1%) patients, laparoscopy revealed that the intussusception had spontaneously resolved. Among the other 8 patients, one patient's intussusceptum was found to have extended past the middle segment of the transverse colon. The mass was large and challenging to reduce under laparoscopy, and the procedure was directly converted to a transverse incision in the right upper abdomen to complete the operation; the operation of the other seven patients was completed with an extension of the umbilical incision. Among them, five (7.7%) patients had abnormal bowel lesions, one (1.5%) had intestinal necrosis, and one (1.5%) had severe bowel nesting. Postoperative pathological findings indicated two patients with lymphoma of the terminal ileum, one patient with Meckel’s diverticulum, one patient with small intestinal duplication, and one patient with small intestine polyps. There were 15 with appendicular intussusception among all patients, in which the appendixes exhibited congestion and swelling and were resected. The average operative time was 42.2 ± 12.2 min. All patients were discharged uneventfully, and the hospital stays were 3-12 d, with an average of 4.5 d (4.5 ± 1.3). The intraoperative blood loss was 2.2 ± 1.6 mL.

**Complications**

The follow-up period ranged from 3 years to 8 years. There were no complications, such as intestinal adhesions, wound infections, or intestinal perforations. There were two cases of recurrent intussusception, one of which was resolved with pneumatic reduction, and one underwent a second laparoscopic surgery.

**DISCUSSION**

**Study design and patients**

This study was a retrospective case series of pediatric patients with intussusception who underwent surgical reduction by laparoscopy from May 2011 to April 2016 at the Department of Pediatric Surgery of Taizhou Hospital of Zhejiang Province. The cases in this study were consecutive cases and were operated by the same surgical team. The study was approved by the ethics committee of Taizhou Hospital. The committee waived the requirement for informed consent because of the study’s retrospective nature. The indications for laparoscopic surgery: (1) Diagnosed with intussusception by B-mode ultrasound[11]; (2) Unsuccessful gaseous enema reduction; (3) Organic lesions are highly suspected; and (4) Repeated intussusception times were more than three times within 1 wk.
Table 1 Characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, means ± SD (range)</td>
<td>27.5 ± 24.5 mo (1 mo-13 yr)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45 (69.2)</td>
</tr>
<tr>
<td>Female</td>
<td>20 (30.8)</td>
</tr>
<tr>
<td>Time of onset, h, means ± SD</td>
<td>26.3 ± 7.8</td>
</tr>
<tr>
<td>Clinical presentations, n (%)</td>
<td></td>
</tr>
<tr>
<td>Paroxysmal crying or abdominal pain</td>
<td>60 (92.3)</td>
</tr>
<tr>
<td>Abdominal mass</td>
<td>45 (69.2)</td>
</tr>
<tr>
<td>Jam-colored bowel movements</td>
<td>48 (73.8)</td>
</tr>
<tr>
<td>Type of intussusception, n (%)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>60 (92.3)</td>
</tr>
<tr>
<td>Secondary</td>
<td>5 (7.7)</td>
</tr>
<tr>
<td>Level of intussusceptum, n (%)</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>3 (4.6)</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>40 (61.5)</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>22 (33.9)</td>
</tr>
<tr>
<td>Descending colon</td>
<td>0</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td></td>
</tr>
<tr>
<td>Acute gastroenteritis</td>
<td>9 (13.8)</td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>7 (10.8)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Medication, n (%)</td>
<td></td>
</tr>
<tr>
<td>Probiotics</td>
<td>6 (9.2)</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>8 (12.3)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>13 (20.0)</td>
</tr>
<tr>
<td>Gastrointestinal motility</td>
<td>4 (6.2)</td>
</tr>
</tbody>
</table>

SD: Standard deviation.

The contra-indications for laparoscopic surgery: (1) Children with high abdominal distension and pneumoperitoneum could not be established; (2) Patients with abnormal cardiopulmonary functions and unable to tolerate pneumoperitoneum; or (3) The intussusception was large, and its head exceeded the middle section of the transverse colon. The cases in this study were consecutive cases and were operated by the same surgical team.

**Surgical method**

Routine blood tests, electrolyte tests, and blood gas analyses were performed before surgery. Before the operation, a gastric tube and an indwelling catheter were placed when it was considered necessary. General anesthesia by tracheal intubation was used in all cases. The patient was positioned with the head downward and tilted to the left to expose the ileocecal segment of the intestine fully. The operator was positioned on the left side of the patient. An incision of 0.5-1.0-cm in length was made at the lower margin of the umbilicus. A trocar was placed under direct vision. CO₂ was slowly injected, and the pressure was maintained at 8-12 mmHg. A 0° laparoscope was placed, 5-mm trocars were inserted in the lower right abdomen and the lower left abdomen under laparoscopic monitoring, and minimally invasive grasping forceps were placed. First, we find the hepatic segment of the transverse colon below the liver,
Table 2 Clinical characteristics and prognosis related to surgery

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation time, min, means ± SD</td>
<td>42.2 ± 12.2</td>
</tr>
<tr>
<td>Intraoperative blood loss, mL, means ± SD</td>
<td>2.2 ± 1.6</td>
</tr>
<tr>
<td>Conversion rate of laparotomy, n (%)</td>
<td>8 (12.3)</td>
</tr>
<tr>
<td>With a transverse incision in the right upper abdomen</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>With an extension of the umbilical incision</td>
<td>7 (10.8)</td>
</tr>
<tr>
<td>Reasons for conversion, n (%)</td>
<td></td>
</tr>
<tr>
<td>Abnormal bowel lesions</td>
<td>5 (7.7)</td>
</tr>
<tr>
<td>Intestinal necrosis</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Severe bowel nesting</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Postoperative pathology, n (%)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma of the terminal ileum</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Meckel’s diverticulum</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Small intestinal duplication</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Small intestine polyps</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Intraoperative appendectomy</td>
<td>15 (23.1)</td>
</tr>
<tr>
<td>Postoperative hospital stay, d, means ± SD</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>Postoperative complications, n (%)</td>
<td></td>
</tr>
<tr>
<td>Intestinal adhesion, intestinal obstruction</td>
<td>0</td>
</tr>
<tr>
<td>Infection of incision</td>
<td>0</td>
</tr>
<tr>
<td>Intussusception recurred</td>
<td>2 (3.0)</td>
</tr>
</tbody>
</table>

SD: Standard deviation.

and then find the intussusception along its proximal direction. With the right hand, the ileum’s neck was grasped with the grasping forceps and pulled outward, paying attention to pull along the fan-shaped curvature of the mesentery. With the left hand, the intussuscepted sheath’s neck was pulled in the opposite direction (Figure 1). Based on the length of the reduced intestine, the positions of the two grasping forceps were changed appropriately so that the two forces maintained some tension and were in the mesentery’s curvature. The neck of the intussuscepted mass was frequently tight, and the left-hand forceps were used to open the neck of the outer sheath, while the right-hand forceps were used to pull the neck of the intussuscepted ileum so that some of the fluid in the neck could be drained. Simultaneously, part of the mesenteric tissue was pulled outward so that the neck was relatively loose and easy to reduce. After reduction, the intestines were examined for necrosis and other organic lesions. If none were found, the ileum’s seromuscular layer and the ascending colon were repaired with two sutures. If the ileocecal segment was relatively isolated, it was fixed to the lower right abdominal wall with two sutures. Appendectomy was performed depending on the condition of the appendicular blood supply and swelling. When there was a reduction with a single laparoscope were found to be complicated or organic lesions were present, the neck of the intussusception mass or the intestine with organic lesions was fixed with grasping forceps under laparoscopic monitoring, and then the umbilical incision was extended transversely. The intussusception mass was then pulled out of the umbilical incision for manual reduction or intestinal resection (Figure 2). After anesthesia and return to consciousness, the patients were transferred to the general pediatric surgical ward. They received conventional second-generation cephalosporin antibiotics (50-100 mg/kg/d), omeprazole injection (0.7 mg/kg/d), and intravenous fluids for 3-7 d. The patients began to eat and gradually returned to normal diet 1-5 d after the operation according to their condition.
Figure 1 Laparoscopic approach for managing intussusception. The neck of the ileum was grasped with grasping forceps and pulled outward. Other grasping forceps were used to pull the neck of the intussusceptum sheath to the opposite direction.

Figure 2 Conversion because of complicated or organic lesions. The umbilical incision was extended, and grasping forceps were used to grasp the intussusception mass out of the incision for manual reduction.

Data collection and follow-up
The data of all patients were collected from the paper medical records of the Department of Pediatric Surgery of Taizhou Hospital of Zhejiang Province, including basic characteristics (age, sex, time of onset, type of intussusception, level of intussusceptum, comorbidities, and medication) and clinical characteristics (operation time, intraoperative blood loss, conversion rate of laparotomy, reasons for conversion, postoperative hospital stay, and adverse events).

The family members of all patients were followed until June 2019 by outpatient reexamination, telephone or Wechat contact at 1 wk, 1 mo, half a year and 1 year, respectively, to collect data including wound healing, diet, bowel movements, abdominal pain and vomiting, sleep, growth and development, etc. Follow-up was conducted by 3 practicing physicians with more than 5 years of clinical experience in pediatric surgery.

Statistics
All data were analyzed using SPSS 19.0 for Windows (IBM, Armonk, NY, United States). The continuous data were tested for normal distribution using the Kolmogorov-Smirnov test; those meeting the normal distribution are presented as means ± SD; otherwise, they are presented as medians (ranges). The categorical data are presented as n (%). Descriptive statistics were used.
**CONCLUSION**

Intussusception is an invagination of a proximal segment of the intestine into a distal part of the intestine that may result in bowel obstruction, venous congestion, and bowel wall edema[12-15]. It is a common cause of acute abdominal emergency in infants and children. It is most common in infants and children aged 3 mo to 3 years, with a peak incidence between 5 and 9 mo of age[12,13,16]. The vast majority of cases are ileocolic, but small bowel to small bowel and colocolonic intussusceptions may also occur[12,13]. The incidence is 3-40 cases per 10000 live births in the United States of America, Europe, and Australia[7,14,17,18]. Typical clinical presentation of intussusception includes paroxysmal crying, vomiting, an abdominal sausage-like mass, and jam-like, bloody stool[8,12,13]. It is primarily diagnosed through medical history taking, physical examination, ultrasound, and computed tomography [8,12,13]. Pneumatic reduction and ultrasound-guided hydrostatic reduction are the most widely used methods for treating intussusception in children[12,13,16,19-21]. They have a success rate as high as 95%, but pneumatic reduction and ultrasound-guided hydrostatic reduction still fails in some intussusception cases[7,8], and such children will require surgical treatment.

In the past, conventional open surgery was the mainstream surgical treatment for intussusception[12,13,20,21]. However, conventional surgery is associated with significant trauma in children[12,13]. With the development of laparoscopic techniques, there have been increasingly more reports of successful laparoscopic treatment of intussusception, limiting the surgical trauma and allowing faster recovery [1,21]. Nevertheless, laparoscopic treatment for intussusception remains controversial because the port setting is challenging because of the small working space in infants and small children and because the affected segment may vary among children. Besides, the reported series are small.

Therefore, this retrospective study aimed to explore the clinical characteristics, effectiveness, and complications of surgical reduction for intussusception using laparoscopy in children. The results could provide additional data for the management of this condition.

**ARTICLE HIGHLIGHTS**

**Research background**

Intussusception can be managed by pneumatic reduction, ultrasound-guided hydrostatic reduction, open or laparoscopic surgery. On the other hand, the use of laparoscopy in such cases remains controversial.

**Research motivation**

The use of laparoscopy in infants with intussusception could be less morbid for the patients.

**Research objectives**

To explore the clinical characteristics, effectiveness, and complications of surgical reduction for intussusception using laparoscopy in children.

**Research methods**

This retrospective case series included pediatric patients with intussusception who underwent surgical reduction by laparoscopy from May 2011 to April 2016 at Taizhou Hospital of Zhejiang Province. The clinical characteristics (operation time, intraoperative blood loss, conversion rate of laparotomy, reasons for conversion, postoperative hospital stay, and adverse events) of the patients were described.

**Research results**

The study could include 65 patients (45 boys and 20 girls). They were 2.3 years (27.5 ± 24.5 mo). Of the 65 patients, 61 underwent surgical reduction by laparoscopy after a failed enema reduction of intussusception, and four underwent the procedure directly. All patients were treated successfully. Fifty-seven (87.7%) patients underwent successful laparoscopic surgery, two of which had a spontaneous reduction. Among the remaining cases, one was converted to open surgery via right upper quadrant incision, and seven required enlarged umbilical incisions. Intestinal resection was performed in five patients because of abnormal bowel lesions. There were no complications (intesti-
nal perforations, wound infections, or intestinal adhesions) during the follow-up of 3 years to 8 years. Two patients experienced a recurrence of intussusception; one was resolved with pneumatic reduction, and the other underwent a second laparoscopic surgery.

Research conclusions
Laparoscopic approach for pediatric intussusception is feasible and safe. Bowel resection if required can be performed by extending umbilical incision without the conventional laparotomy.

Research perspectives
This study provides useful data for the management of infants with intussusception.

REFERENCES


Retrospective Study

Clinical features and risk factors of severely and critically ill patients with COVID-19

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xih@bjhmoh.cn
Chu X et al. Clinical features of severe COVID-19 patients

Abstract

BACKGROUND
As of June 1, 2020, over 370000 coronavirus disease 2019 (COVID-19) deaths have been reported to the World Health Organization. However, the risk factors for patients with moderate-to-severe or severe-to-critical COVID-19 remain unclear.

AIM
To explore the characteristics and predictive markers of severely and critically ill patients with COVID-19.

METHODS
A retrospective study was conducted at the B11 Zhongfaxincheng campus and E1-3 Guanggu campus of Tongji Hospital affiliated with Huazhong University of Science and Technology in Wuhan. Patients with COVID-19 admitted from 1st February 2020 to 8th March 2020 were enrolled and categorized into 3 groups: The moderate group, severe group and critically ill group. Epidemiological data, demographic data, clinical symptoms and outcomes, complications, laboratory tests and radiographic examinations were collected retrospectively from the hospital information system and then compared between groups.

RESULTS
A total of 126 patients were enrolled. There were 59 in the moderate group, 49 in the severe group, and 18 in the critically ill group. Multivariate logistic regression analysis showed that age [odd ratio (OR) = 1.055, 95% (confidence interval) CI: 1.099-1.104], elevated neutrophil-to-lymphocyte ratios (OR = 4.019, 95%CI: 1.045-15.467) and elevated high-sensitivity cardiac troponin I (OR = 10.126, 95%CI: 1.088-94.247) were high-risk factors.

CONCLUSION
The following indicators can help clinicians identify patients with severe COVID-19 at an early stage: age, an elevated neutrophil-to-lymphocyte ratio and high sensitivity cardiac troponin I.

Key Words: COVID-19; SARS-CoV-2; Critically ill; Risk factors; Aspartate transaminase; Amino-terminal pro-brain natriuretic peptide; Creatinine; Calcium

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Core Tip: Here, we conducted a case-control study and found out that early drug treatment is an important measure in the treatment of patients with coronavirus disease 2019 (COVID-19). And the following indicators can help clinicians identify patients with severe COVID-19 at an early stage: an elevated neutrophil-to-lymphocyte ratio; elevated aspartate transaminase, N-terminal pro b-type natriuretic peptide, and creatinine levels; as well as decreased serum calcium level.


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INTRODUCTION

Since the first report of coronavirus disease 2019 (COVID-19) in Wuhan, China, in December 2019, this highly infectious respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly throughout the world, posing a serious threat to global health. Similar to SARS-CoV, the novel SARS-CoV-2 uses angiotensin converting enzyme II (ACE2) receptors to invade not only type
MATERIALS AND METHODS

Study design and participants

In this retrospective study, we enrolled all inpatients who were hospitalized for COVID-19 from 1st February to 8th March 2020, at the B11 Zhongfaxincheng campus and E1-3 Guanggu campus of Tongji Hospital affiliated with Huazhong University of Science and Technology in Wuhan, China. These two campuses were designated hospitals treating mainly severely and critically ill patients, and according to the arrangement of the Chinese government, patients with mild to moderate COVID-19 were isolated from their families and communities and then transferred and admitted to Fangcang shelter hospitals[12]. All patients in our study were confirmed with throat swab specimens to extract viral RNA for laboratory confirmation of SARS-CoV-2 infection. The study was approved by the Research Ethics Commission of Beijing Hospital (2020BJYYEC-047-01), and the requirement for written informed consent was waived by the Ethics Commission for emerging infectious diseases.

Procedures

Epidemiological data, demographic data, clinical symptoms and outcomes, complications, laboratory examinations and imaging test information were extracted from electronic medical records, and clinical outcomes were followed until March 26, 2020. If data were missing from the medical records or clarification was needed, we obtained data by direct communication with attending doctors and other health-care providers. All data were checked by two researchers and two physicians from each campus.

According to COVID-19 severity defined by the Chinese management guideline for COVID-19 (version 7.0)[5], we categorized the patients into 3 groups, namely, the moderate group (level 2), severe group (level 3) and critically ill (level 4) group, to analyze the clinical features and high-risk factors of severe and critical COVID-19.

Definitions

The severity of COVID-19 (according to the Chinese management guideline for COVID-19 (version 7.0) was classified as follows[8].
Chu X et al. Clinical features of severe COVID-19 patients

Mild (level 1): The patient had light clinical symptoms but no evidence of pneumonia on X-ray or computed tomography (CT) examination.

Moderate (level 2): The patient had fever and respiratory symptoms, and the X-ray or CT examination showed evidence of pneumonia.

Severe (level 3): Patients aged over 18 years who met the following conditions: (1) Shortness of breath, with a respiratory rate ≥ 30; (2) Resting-state oxygen saturation values from one finger of one arm of ≤ 93%; (3) Arterial partial pressure of oxygen (PaO₂)/fraction of inspired oxygen (FiO₂) ≤ 300 mmHg; and (4) Rapid progression of lesions over 50% within 24-48 h.

Critically ill (level 4): Patients aged over 18 years who met the following conditions: (1) Acute respiratory failure requiring mechanical ventilation support; (2) Shock; and (3) COVID-19 complicated by other organ failure and the need for critical care in the Intensive care unit.

Statistical analysis
EpiData 3.1 was used for the data collection and SPSS (version 22.0) for the analyses. Continuous variables are presented as the mean ± SD if they were normally distributed or the median (interquartile range, IQR) if they were not, and variables were compared by one-way ANOVA, the Mann-Whitney U test or Kruskal-Wallis test. Categorical variables are presented as n (%) and were compared by the χ² test or Fisher’s exact test. A two-sided α of less than 0.05 was considered statistically significant. The high-risk factors for severe and critically ill COVID-19 were analyzed by logistic regression analysis. An ordinal logistic regression model was adopted and used with JMP15.0 software to explore potential risk factors associated with the severity of COVID-19. According to clinical significance, which was the most important measure, data completeness and the single-factor screening results of the χ² analysis were considered. Under clinician guidance, potential collinear variables were categorized to process the collinearity diagnosis by SPSS 22.0 (IBM Corp., Armonk, NY, United States). Timeline charts of laboratory parameters were plotted using GraphPad Prism version 8.0.

RESULTS
As of March 8, 126 patients with COVID-19 were included in this study, 67 from the BI1 Zhongfaxincheng campus and 59 from the E1-3 Guanggu campus. There were 59 in the moderate group, 49 in the severe group, and 18 in the critically ill group. Although more than half of the infected patients in the severe and critically ill groups were men, there was no significant difference in sex between groups; however, compared with the moderate group, the difference became significant when we merged the severe and critically ill together (P = 0.008). Men were more vulnerable to COVID-19.

The median age of the patients was 61.00 years (IQR 48.00-68.00), ranging from 24 years to 91 years. Patients in the critically ill group were significantly older than those in the moderate group (65.44 vs 54.76 years, P = 0.019). The median time from onset of symptoms to first hospital admission was 8 d (IQR 3.00-14.00), and among the three groups, this duration was longest in the moderate group. The median time from onset of symptoms to COVID-19 Laboratory confirmation (via throat swab samples) was 7.5 d (IQR 3.35-13.75), and there were no differences between groups (Table 1).

Of the 126 patients, 104 (82.5%) had 1 or more coexisting medical conditions. Hypertension [46 (36.5%)], diabetes [22 (17.5%)], cancer [11 (8.7%)], and coronary heart disease [7 (5.6%)] were the most common coexisting conditions. Compared with the critically ill group, the moderate group had fewer patients with cerebrovascular diseases or cancer (P < 0.05). Compared with the severe group, the moderate group had fewer patients with chronic liver disease (P < 0.05). There were more patients in the critically ill group with 1 or more underlying diseases than in the other groups. Before admission, 33 (33/67, 49.3%) patients reported having taken antibiotics, and third-generation cephalosporins, and quinolone antibiotics were the most common. Twenty-eight (28/67, 41.8%) patients reported taking oseltamivir. Two (2/126, 1.6%) patient reported taking lopinavir/ritonavir. Seventy (70/126, 55.6%) patients reported having taken albendol. In addition, 83 patients reported having taken traditional Chinese medicine, mainly Lianhua Qingwen capsules. Regarding patients who had
Table 1 Baseline and clinical characteristics of patients with coronavirus disease 2019

<table>
<thead>
<tr>
<th>Demographics and clinical characteristics</th>
<th>Total</th>
<th>Moderate</th>
<th>Severe</th>
<th>Critically ill</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td>67/126 (53.2)</td>
<td>24/59 (40.7)</td>
<td>30/49 (61.2)</td>
<td>13/18 (72.2)</td>
<td>0.022</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>54.76 ± 1.73</td>
<td>59.22 ± 2.10</td>
<td>65.44 ± 3.17</td>
<td>P (Critically ill vs moderate) = 0.019</td>
<td></td>
</tr>
<tr>
<td><strong>Exposure history</strong></td>
<td>28/67 (41.8)</td>
<td>10/19 (52.6)</td>
<td>13/36 (36.1)</td>
<td>5/12 (41.7)</td>
<td>0.498</td>
</tr>
<tr>
<td><strong>Time from illness onset to hospital admission (d)</strong></td>
<td>16.00 (11.00-18.00)</td>
<td>7.00 (3.75-11.25)</td>
<td>6.00 (4.00-7.00)</td>
<td>P (Critically ill vs moderate) = 0.000; P (Severe vs moderate) = 0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Time from illness onset to laboratory confirmation (d)</strong></td>
<td>10.00 (5.00-13.00)</td>
<td>7.50 (3.75-18.00)</td>
<td>2.00 (0.0-4.50)</td>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td><strong>Comorbidity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>104/126 (82.5)</td>
<td>51/59 (86.4)</td>
<td>35/49 (71.4)</td>
<td>18/18 (100)</td>
<td>P (Critically ill vs severe) &lt; 0.05</td>
</tr>
<tr>
<td><strong>COPD/CB</strong></td>
<td>6/126 (4.8)</td>
<td>1/59 (1.7)</td>
<td>3/49 (6.1)</td>
<td>2/18 (11.1)</td>
<td>0.152</td>
</tr>
<tr>
<td><strong>Cerebrovascular disease</strong></td>
<td>3/126 (2.4)</td>
<td>0/59 (0)</td>
<td>1/49 (2.0)</td>
<td>2/18 (11.1)</td>
<td>P (Critically ill vs moderate) &lt; 0.05</td>
</tr>
<tr>
<td><strong>Coronary heart disease</strong></td>
<td>7/126 (5.6)</td>
<td>4/59 (6.8)</td>
<td>3/49 (6.1)</td>
<td>0/18 (0)</td>
<td>0.325</td>
</tr>
<tr>
<td><strong>Chronic liver disease</strong></td>
<td>6/126 (4.8)</td>
<td>0/59 (0)</td>
<td>5/49 (10.2)</td>
<td>1/18 (5.6)</td>
<td>P (Severe vs moderate) &lt; 0.05</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>46/126 (36.5)</td>
<td>21/59 (35.6)</td>
<td>16/49 (32.7)</td>
<td>9/18 (50.0)</td>
<td>0.417</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>22/126 (17.5)</td>
<td>7/59 (11.9)</td>
<td>10/49 (20.4)</td>
<td>5/18 (27.8)</td>
<td>0.238</td>
</tr>
<tr>
<td><strong>Hyperlipidemia</strong></td>
<td>1/126 (0.8)</td>
<td>0/59 (0)</td>
<td>1/49 (2.0)</td>
<td>0/18 (0)</td>
<td>0.532</td>
</tr>
<tr>
<td><strong>Drug history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>108/126 (85.7)</td>
<td>55/59 (93.2)</td>
<td>40/49 (81.6)</td>
<td>13/18 (72.2)</td>
<td>P (Critically ill vs moderate) &lt; 0.05</td>
</tr>
<tr>
<td><strong>Antibiotics</strong></td>
<td>33/67 (49.3)</td>
<td>7/19 (36.8)</td>
<td>19/36 (53.7)</td>
<td>7/12 (58.3)</td>
<td>0.418</td>
</tr>
<tr>
<td><strong>Traditional Chinese medicine</strong></td>
<td>83/126 (65.9)</td>
<td>49/59 (83.1)</td>
<td>28/49 (57.1)</td>
<td>6/18 (33.3)</td>
<td>P (Critically ill vs moderate) &lt; 0.05; P (Severe vs moderate) &lt; 0.05</td>
</tr>
<tr>
<td><strong>Arbidol</strong></td>
<td>70/126 (55.6)</td>
<td>43/59 (72.9)</td>
<td>20/49 (40.8)</td>
<td>7/18 (38.9)</td>
<td>P (Critically ill vs moderate) &lt; 0.05; P (Severe vs moderate) &lt; 0.05</td>
</tr>
<tr>
<td><strong>Oseltamivir</strong></td>
<td>26/67 (41.8)</td>
<td>10/19 (52.6)</td>
<td>16/36 (44.4)</td>
<td>2/12 (16.7)</td>
<td>0.126</td>
</tr>
<tr>
<td><strong>Lopinavir/ritonavir</strong></td>
<td>2/126 (1.6)</td>
<td>1/59 (1.7)</td>
<td>0/49 (0)</td>
<td>1/18 (5.6)</td>
<td>0.266</td>
</tr>
<tr>
<td><strong>Fever</strong></td>
<td>98/126 (77.8)</td>
<td>44/59 (46.8)</td>
<td>42/49 (85.7)</td>
<td>12/18 (66.7)</td>
<td>0.175</td>
</tr>
<tr>
<td><strong>Maximum body temperature</strong></td>
<td>38.90 (38.00-39.40)</td>
<td>38.70 (38.00-39.00)</td>
<td>39.00 (38.92-39.25)</td>
<td>0.440</td>
<td></td>
</tr>
<tr>
<td><strong>Rigor</strong></td>
<td>32/67 (47.8)</td>
<td>7/19 (36.8)</td>
<td>20/36 (55.6)</td>
<td>5/12 (42.7)</td>
<td>0.375</td>
</tr>
<tr>
<td><strong>Fatigue</strong></td>
<td>43/67 (64.2)</td>
<td>10/19 (52.6)</td>
<td>24/36 (66.7)</td>
<td>9/12 (75.0)</td>
<td>0.405</td>
</tr>
<tr>
<td><strong>Sore throat</strong></td>
<td>25/67 (37.3)</td>
<td>6/19 (31.6)</td>
<td>14/36 (38.9)</td>
<td>5/12 (41.7)</td>
<td>0.816</td>
</tr>
</tbody>
</table>
Running nose 1/67 (1.5) 0/19 (0.0) 1/36 (2.8) 0/12 (0.0) 1.000  
Stuffy nose 6/67 (9.0) 1/19 (5.3) 5/36 (13.9) 0/12 (0.0) 0.169  
Cough 98/126 (77.8) 46/59 (78.0) 40/49 (81.6) 12/18 (66.7) 0.426  
Expectoration 39/67 (58.2) 11/19 (57.9) 22/36 (61.1) 6/12 (50.0) 0.571  
White sputum 14/67 (20.9) 1/19 (5.3) 11/36 (30.6) 2/12 (16.7) 0.083  
Blood-stained sputum 15/67 (22.4) 5/19 (26.3) 8/36 (22.2) 2/12 (16.7) 0.083  
Shortness of breath 49/126 (38.9) 19/59 (32.2) 22/49 (44.9) 8/18 (44.4) 0.352  
Exertional dyspnea 24/67 (35.8) 6/19 (31.6) 14/36 (38.9) 4/12 (33.3) 0.848  
Headache 22/67 (32.8) 7/19 (36.8) 11/36 (30.6) 4/12 (33.3) 0.895  
Myalgia 29/67 (43.3) 5/19 (26.3) 18/36 (50.0) 6/12 (50.0) 0.211  
Abdominal pain 21/67 (31.3) 4/19 (21.1) 13/36 (36.1) 4/12 (33.3) 0.497  
Diarrhea 40/126 (31.7) 16/59 (27.1) 20/49 (40.8) 4/18 (22.2) 0.202  
Nausea 29/67 (43.3) 7/19 (36.8) 17/36 (47.2) 5/12 (41.7) 0.755  
Anorexia 32/67 (47.8) 6/19 (31.6) 20/36 (55.6) 6/12 (50.0) 0.235  
Vomiting 22/67 (32.8) 4/19 (21.1) 13/36 (36.1) 5/12 (41.7) 0.392  
Conjunctivitis 0/67 (0.0) 0/19 (0.0) 0/36 (0.0) 0/12 (0.0) -  

Data are presented as n/n (%) or mean ± SD or median (interquartile range). P values were calculated by Mann-Whitney U test, χ² test, or Fisher’s exact test, as appropriate.

taken traditional Chinese medicine and arbidol before admission, they were more likely to be in the moderate group (P < 0.05) (Table 1).

Among the 126 patients in the study, the most common symptoms at disease onset were fever [98 (77.8%)], cough [98 (77.8%)], shortness of breath [49 (38.9%)] and fatigue (43/67, 64.2%). Less common symptoms included expectoration, rigor, anorexia, myalgia, and nausea (Table 1). No significant differences in the symptoms at disease onset were found between groups (Table 1).

All patients had received radiographic examination in our study. Among 86 imaging diagnostic reports, patchy shadows were found in 45.3% and 44% of patients’ early chest CT and X-ray reports, respectively, and 47.7% of the patients’ reports showed multiple area involvement. A total of 18.6% of images showed pleural adhesions, 15.1% showed emphysema, and 14.0% showed enlarged mediastinal lymph nodes. 35 patients who were admitted to the B11 Zhongfaxincheng campus of Tongji Hospital had ground-glass opacity.

All the laboratory data were collected through patients’ electronic medical records. Patient’s first laboratory results are shown in Table 2. Of all the patients, 58 (58/123, 47.2%) had hemoglobin levels below normal. In addition, 44 (44/121, 36.4%) patients had lymphocyte counts below normal, and 35 (35/125, 28.0%) patients had lower lymphocyte ratios. Seventy-eight (78/123, 63.4%) patients had decreased hematocrit levels. 107/122 (87.7%) patients had higher-than-normal levels of C-reactive protein. Regarding coagulation tests, 8 (8/66, 12.1%), 11 (11/66, 16.7%) and 39 (39/66, 59.1%) patients had elevated thrombin times, prothrombin times and fibrinogen levels, respectively; notably, 80 (80/124, 64.5%) patients showed significantly increased D-dimer levels. Additionally, we found that 71 (71/120, 59.2%) patients had increased lactate dehydrogenase, 45 (45/62, 72.6%) patients had higher ferritin levels, and 68 (68/120, 56.7%) patients had decreased calcium levels (Table 2).

Neutrophil ratio, eosinophil ratio, lymphocyte count and amnio-terminal pro-brain natriuretic peptide are significantly different between each group in the two-group comparisons. Additionally, compared with the moderate group, there are 21 more laboratory terms are significantly different (details of the between-group comparisons are shown in Table 2).
<table>
<thead>
<tr>
<th>Laboratory findings</th>
<th>Total</th>
<th>Moderate</th>
<th>Severe</th>
<th>Critically ill</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count, × 10^9/L</td>
<td>6.02 ± 2.07</td>
<td>5.79 ± 0.21</td>
<td>6.09 ± 0.32</td>
<td>6.66 ± 0.71</td>
<td>0.26</td>
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<tr>
<td>Total</td>
<td>125/125 (100)</td>
<td>59/125 (47.2)</td>
<td>49/125 (39.2)</td>
<td>17/125 (13.6)</td>
<td></td>
</tr>
<tr>
<td>Lower (&lt; 3.5)</td>
<td>13/125 (10.4)</td>
<td>4/59 (6.8)</td>
<td>6/49 (12.2)</td>
<td>3/17 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Normal (3.5-9.5)</td>
<td>104/125 (83.2)</td>
<td>53/59 (89.8)</td>
<td>39/49 (79.6)</td>
<td>12/17 (70.6)</td>
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</tr>
<tr>
<td>Higher (&gt; 9.5)</td>
<td>8/125 (6.4)</td>
<td>2/59 (3.4)</td>
<td>4/49 (8.2)</td>
<td>2/17 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Neutrophil count, × 10^9/L</td>
<td>3.78 (2.40-5.15)</td>
<td>3.37 (2.40-4.19)</td>
<td>4.05 (2.34-5.17)</td>
<td>5.71 (2.82-7.45)</td>
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<tr>
<td>Total</td>
<td>121/121 (100)</td>
<td>56/121 (46.3)</td>
<td>47/121 (38.7)</td>
<td>18/121 (14.5)</td>
<td>P (Moderate vs critically ill) &lt; 0.05</td>
</tr>
<tr>
<td>Lower (&lt; 1.8)</td>
<td>13/121 (10.8)</td>
<td>3/56 (5.4)</td>
<td>7/47 (14.9)</td>
<td>3/18 (16.7)</td>
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<tr>
<td>Normal (1.8-6.3)</td>
<td>91/121 (75.2)</td>
<td>50/56 (89.2)</td>
<td>33/47 (70.2)</td>
<td>8 (44.4)</td>
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<tr>
<td>Higher (&gt; 6.3)</td>
<td>17/121 (14.0)</td>
<td>3/56 (5.4)</td>
<td>7/47 (14.9)</td>
<td>7 (38.9)</td>
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<tr>
<td>Neutrophil ratio</td>
<td>63.70 (53.75-73.80)</td>
<td>57.85 (52.80-57.85)</td>
<td>68.55 (52.53-75.78)</td>
<td>81.05 (72.90-87.25)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>124/124 (100)</td>
<td>58/124 (46.8)</td>
<td>48/124 (38.7)</td>
<td>18/124 (14.5)</td>
<td>P (Moderate vs severe) &lt; 0.05; P (Moderate vs critically ill) &lt; 0.05; P (Severe vs critically ill) &lt; 0.05</td>
</tr>
<tr>
<td>Lower (&lt; 40)</td>
<td>4/124 (3.2)</td>
<td>0/58 (0)</td>
<td>4/48 (8.5)</td>
<td>0/18 (0)</td>
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<tr>
<td>Normal (40-75)</td>
<td>89/124 (71.8)</td>
<td>53/56 (94.1)</td>
<td>31/47 (66.0)</td>
<td>5/18 (27.8)</td>
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<tr>
<td>Higher (&gt; 75)</td>
<td>31/124 (25.0)</td>
<td>5/56 (8.6)</td>
<td>13/47 (27.1)</td>
<td>13/18 (72.2)</td>
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</tr>
<tr>
<td>Eosinophil count, × 10^9/L</td>
<td>0.08 (0.03-0.15)</td>
<td>0.13 (0.06-0.20)</td>
<td>0.07 (0.02-0.14)</td>
<td>0.00 (0.00-0.06)</td>
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</tr>
<tr>
<td>Total</td>
<td>121/121 (100)</td>
<td>56/121 (46.3)</td>
<td>47/121 (38.7)</td>
<td>18/121 (14.5)</td>
<td>P (Moderate vs severe) &lt; 0.05; P (Moderate vs critically ill) &lt; 0.05; P (Severe vs critically ill) &lt; 0.05</td>
</tr>
<tr>
<td>Lower (&lt; 0.02)</td>
<td>25/121 (20.7)</td>
<td>3/56 (5.4)</td>
<td>11/47 (23.4)</td>
<td>11/18 (61.1)</td>
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<tr>
<td>Normal (0.02-0.52)</td>
<td>95/121 (78.5)</td>
<td>52/56 (92.9)</td>
<td>36/47 (76.6)</td>
<td>7/18 (38.9)</td>
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</tr>
<tr>
<td>Higher (&gt; 0.52)</td>
<td>1/121 (0.8)</td>
<td>1/56 (1.8)</td>
<td>0/56 (0)</td>
<td>0/56 (0)</td>
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<tr>
<td>Eosinophil ratio</td>
<td>1.35 (0.40-2.83)</td>
<td>2.20 (1.10-3.40)</td>
<td>1.25 (0.20-2.18)</td>
<td>0 (0-0.65)</td>
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<tr>
<td>Total</td>
<td>122/122 (100)</td>
<td>56/122 (45.9)</td>
<td>48/122 (39.3)</td>
<td>18/122 (14.8)</td>
<td>P (Moderate vs severe) &lt; 0.05; P (Moderate vs critically ill) &lt; 0.05; P (Severe vs critically ill) &lt; 0.05</td>
</tr>
<tr>
<td>Lower (&lt; 0.4)</td>
<td>29/122 (23.8)</td>
<td>3/56 (5.4)</td>
<td>14/48 (29.2)</td>
<td>12/18 (67.8)</td>
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</tr>
<tr>
<td>Normal (0.4-8)</td>
<td>91/122 (74.6)</td>
<td>51/56 (91.1)</td>
<td>34/48 (70.8)</td>
<td>6/18 (74.6)</td>
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<tr>
<td>Higher (&gt; 8)</td>
<td>2/122 (1.6)</td>
<td>2/56 (3.6)</td>
<td>0/48 (0)</td>
<td>0/18 (0)</td>
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<tr>
<td>Monocyte count, × 10^9/L</td>
<td>0.53 (0.40-0.66)</td>
<td>0.55 (0.46-0.66)</td>
<td>0.55 (0.38-0.67)</td>
<td>0.39 (0.20-0.56)</td>
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<tr>
<td>Total</td>
<td>123/123 (100)</td>
<td>57/123 (46.3)</td>
<td>48/123 (39.0)</td>
<td>18/123 (14.7)</td>
<td>0.266</td>
</tr>
<tr>
<td>Lower (&lt; 0.1)</td>
<td>1/123 (0.8)</td>
<td>0/57 (0)</td>
<td>0/48 (0)</td>
<td>1/18 (5.6)</td>
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</tr>
<tr>
<td>Normal (0.1-0.6)</td>
<td>80/123 (65.0)</td>
<td>37/57 (64.9)</td>
<td>30/48 (62.5)</td>
<td>13/18 (72.2)</td>
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<tr>
<td>Higher (&gt; 0.6)</td>
<td>42/123 (34.1)</td>
<td>20/57 (35.1)</td>
<td>18/48 (37.5)</td>
<td>4/18 (22.2)</td>
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<tr>
<td>Monocyte ratio</td>
<td>9.05 (7.90-10.33)</td>
<td>9.15 (8.23-11.03)</td>
<td>9.3 (8.03-10.28)</td>
<td>5.90 (3.75-8.80)</td>
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<tr>
<td>Total</td>
<td>122/122 (100)</td>
<td>56/122 (45.9)</td>
<td>48/122 (39.3)</td>
<td>18/122 (14.8)</td>
<td>P (Moderate vs critically ill) &lt; 0.05</td>
</tr>
<tr>
<td>Lower (&lt; 3)</td>
<td>3/122 (2.5)</td>
<td>0/56 (0)</td>
<td>1/48 (2.1)</td>
<td>2/18 (11.1)</td>
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</tr>
<tr>
<td>Normal (3-10)</td>
<td>82/122 (67.2)</td>
<td>35/56 (62.5)</td>
<td>33/48 (68.8)</td>
<td>14/18 (77.8)</td>
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<tr>
<td>Higher (&gt; 10)</td>
<td>37/122 (30.3)</td>
<td>21/56 (37.5)</td>
<td>14/48 (29.2)</td>
<td>2/18 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte count, × 10^9/L</td>
<td>1.25 (0.92-1.88)</td>
<td>1.79 (1.25-2.01)</td>
<td>1.19 (0.90-1.44)</td>
<td>0.70 (0.52-0.87)</td>
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<tr>
<td>Total</td>
<td>121/121 (100)</td>
<td>56/121 (46.3)</td>
<td>47/121 (38.8)</td>
<td>18/121 (14.9)</td>
<td>P (Moderate vs severe) &lt; 0.05; P (Moderate vs critically ill) &lt; 0.05; P (Severe vs critically ill) &lt; 0.05</td>
</tr>
<tr>
<td>Measure</td>
<td>Lower (&lt; 1.1)</td>
<td>Normal (1.1-3.2)</td>
<td>Higher (&gt; 3.2)</td>
<td>Total</td>
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<tr>
<td>----------------------------------------------</td>
<td>---------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>44/121 (36.4)</td>
<td>47/56 (83.9)</td>
<td>2/121 (1.7)</td>
<td>125/125 (100)</td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>20/47 (42.6)</td>
<td>26/47 (55.3)</td>
<td>1/47 (2.1)</td>
<td>48/125 (38.4)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>35/125 (28.0)</td>
<td>50/59 (84.7)</td>
<td>3/125 (2.4)</td>
<td>127.00 (114.00-136.00)</td>
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</tr>
<tr>
<td>Lymphocyte ratio, %</td>
<td>18/123 (14.4)</td>
<td>22/48 (45.8)</td>
<td>0/18 (0)</td>
<td>123.00 (114.75-136.75)</td>
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</tr>
<tr>
<td>Hematocrit</td>
<td>44/121 (35.8)</td>
<td>27/57 (47.4)</td>
<td>1/123 (0.8)</td>
<td>268.00 (212.50-336.75)</td>
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<tr>
<td>Neutrophil to lymphocyte ratio</td>
<td>10/122 (8.2)</td>
<td>21/57 (36.6)</td>
<td>0/18 (0)</td>
<td>267.00 (193.00-368.00)</td>
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<tr>
<td>Lactate dehydrogenase, U/L</td>
<td>89/123 (73.4)</td>
<td>41/56 (73.2)</td>
<td>1/122 (0.8)</td>
<td>55/121 (45.4)</td>
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<tr>
<td>Alanine aminotransferase, U/L</td>
<td>43/57 (74.4)</td>
<td>32/48 (66.7)</td>
<td>1/55 (16.4)</td>
<td>48/121 (39.7)</td>
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</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>34/123 (27.6)</td>
<td>14/57 (24.6)</td>
<td>9/55 (16.4)</td>
<td>55/121 (45.4)</td>
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</tr>
<tr>
<td>Lactate dehydrogenase, U/L</td>
<td>14/56 (24.6)</td>
<td>15/49 (30.6)</td>
<td>5/57 (16.4)</td>
<td>4/55 (16.4)</td>
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</tr>
<tr>
<td>Total</td>
<td>14/120 (4.2)</td>
<td>1/57 (16.4)</td>
<td>0/57 (16.4)</td>
<td>1/55 (16.4)</td>
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<tr>
<td>Normal (≥ 33), %</td>
<td>44/120 (36.7)</td>
<td>24/55 (43.6)</td>
<td>1/57 (16.4)</td>
<td>4/55 (16.4)</td>
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</tr>
<tr>
<td>Lactate dehydrogenase, U/L</td>
<td>27/55 (49.1)</td>
<td>28/57 (59.6)</td>
<td>28/57 (59.6)</td>
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<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>24.00 (17.00-37.00)</td>
<td>18.00 (12.00-41.00)</td>
<td>14/120 (4.2)</td>
<td>28.00 (13.50-45.50)</td>
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<tr>
<td>Total</td>
<td>123/123 (100)</td>
<td>57/123 (46.4)</td>
<td>17/123 (13.8)</td>
<td>49/123 (39.8)</td>
<td></td>
</tr>
<tr>
<td>Normal (≥ 32), %</td>
<td>94/123 (76.4)</td>
<td>48/57 (84.2)</td>
<td>10/49 (20.4)</td>
<td>9/57 (15.8)</td>
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<tr>
<td>Lactate dehydrogenase, U/L</td>
<td>29/123 (23.6)</td>
<td>9/57 (15.8)</td>
<td>9/142 (6.3)</td>
<td>10/17 (58.8)</td>
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</tbody>
</table>

**Note:** Values are given as median (P25-P75).
<table>
<thead>
<tr>
<th>Tests</th>
<th>Moderate (n=77)</th>
<th>Severe (n=35)</th>
<th>Critically ill (n=29)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum amylase, U/L</td>
<td>69.33 ± 2.65</td>
<td>66.51 ± 3.55</td>
<td>71.59 ± 5.17</td>
<td>0.13</td>
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<tr>
<td>Lower (&lt; 28)</td>
<td>77/77 (100)</td>
<td>35/77 (45.4)</td>
<td>29/77 (39.7)</td>
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</tr>
<tr>
<td>Normal (28-100)</td>
<td>1/77 (1.3)</td>
<td>1/35 (2.9)</td>
<td>0/29 (0)</td>
<td></td>
</tr>
<tr>
<td>Higher (&gt; 100)</td>
<td>10/77 (13.0)</td>
<td>2/35 (5.7)</td>
<td>7/29 (24.1)</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin, μmol/L</td>
<td>8.00 (5.90-12.00)</td>
<td>7.40 (4.30-10.60)</td>
<td>8.65 (5.98-12.43)</td>
<td>0.18</td>
</tr>
<tr>
<td>Lower (&lt; 28)</td>
<td>1/77 (1.3)</td>
<td>1/35 (2.9)</td>
<td>0/29 (0)</td>
<td></td>
</tr>
<tr>
<td>Normal (28-100)</td>
<td>66/77 (85.7)</td>
<td>32/35 (91.4)</td>
<td>22/29 (75.9)</td>
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</tr>
<tr>
<td>Higher (&gt; 100)</td>
<td>10/77 (13.0)</td>
<td>2/35 (5.7)</td>
<td>7/29 (24.1)</td>
<td></td>
</tr>
<tr>
<td>Total albumin, g/L</td>
<td>36.19 ± 4.87</td>
<td>39.25 ± 0.51</td>
<td>34.44 ± 0.56</td>
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</tr>
<tr>
<td>Lower (&lt; 35)</td>
<td>46/121 (38.0)</td>
<td>7/55 (12.7)</td>
<td>27/48 (56.3)</td>
<td>12/18 (66.7)</td>
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<tr>
<td>Normal (35-52)</td>
<td>75/121 (62.0)</td>
<td>48/55 (87.3)</td>
<td>21/48 (43.8)</td>
<td>6/18 (33.3)</td>
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<tr>
<td>Creatinine, μmol/L</td>
<td>69.00 (59.00-78.00)</td>
<td>67.00 (48.00-80.00)</td>
<td>69.00 (60.00-76.00)</td>
<td>73.00 (54.75-87.50)</td>
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<tr>
<td>Lower (&lt; 45)</td>
<td>13/121 (10.7)</td>
<td>2/55 (3.6)</td>
<td>6/48 (12.5)</td>
<td>5/18 (27.8)</td>
</tr>
<tr>
<td>Normal (45-84)</td>
<td>101/121 (83.5)</td>
<td>51/55 (92.7)</td>
<td>41/48 (85.4)</td>
<td>9/18 (50)</td>
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<tr>
<td>Higher (&gt; 84)</td>
<td>7/121 (5.8)</td>
<td>2/55 (3.6)</td>
<td>1/48 (2.1)</td>
<td>4/18 (22.2)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>6.55 (1.60-38.50)</td>
<td>2.30 (1.20-6.70)</td>
<td>12.40 (2.68-48.58)</td>
<td>91.00 (37.3-131.25)</td>
</tr>
<tr>
<td>Total</td>
<td>122/122 (100)</td>
<td>57/122 (46.7)</td>
<td>48/122 (39.4)</td>
<td>17/122 (13.9)</td>
</tr>
<tr>
<td>Creatine kinase, U/L</td>
<td>46.00 (33.50-66.50)</td>
<td>41.00 (34.50-58.50)</td>
<td>52.00 (33.00-77.00)</td>
<td>55.00 (40.00-148.00)</td>
</tr>
<tr>
<td>Lower (&lt; 45)</td>
<td>15/121 (12.3)</td>
<td>12/55 (21.1)</td>
<td>2/48 (4.2)</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Normal (45-84)</td>
<td>107/122 (87.7)</td>
<td>45/57 (78.9)</td>
<td>46/48 (95.8)</td>
<td>16/17 (94.1)</td>
</tr>
<tr>
<td>Creatine kinase, U/L</td>
<td>94/101 (93.1)</td>
<td>46/49 (93.9)</td>
<td>36/37 (97.3)</td>
<td>12/15 (80)</td>
</tr>
<tr>
<td>Higher (&gt; 100)</td>
<td>7/101 (6.9)</td>
<td>3/49 (6.1)</td>
<td>1/37 (2.7)</td>
<td>3/15 (20)</td>
</tr>
<tr>
<td>Procalcitonin3, ng/mL</td>
<td>37/91 (40.7)</td>
<td>37/91 (40.7)</td>
<td>17/91 (18.6)</td>
<td>0.09</td>
</tr>
<tr>
<td>Total</td>
<td>91/91 (100)</td>
<td>37/91 (40.7)</td>
<td>37/91 (40.7)</td>
<td></td>
</tr>
<tr>
<td>Lower (&lt; 0.02)</td>
<td>6/91 (6.6)</td>
<td>0/37 (0)</td>
<td>5/37 (13.5)</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Normal (0.02-0.05)</td>
<td>44/91 (48.4)</td>
<td>25/37 (67.6)</td>
<td>17/37 (45.9)</td>
<td>2/17 (11.8)</td>
</tr>
<tr>
<td>Higher (&gt; 0.05)</td>
<td>41/91 (45.0)</td>
<td>12/37 (32.4)</td>
<td>15/37 (40.5)</td>
<td>14/17 (82.4)</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.24 (3.96-4.64)</td>
<td>4.24 (3.97-4.40)</td>
<td>4.30 (3.92-4.75)</td>
<td>4.15 (3.85-4.59)</td>
</tr>
<tr>
<td>Total</td>
<td>64/64 (100)</td>
<td>16/64 (25.0)</td>
<td>36/64 (56.3)</td>
<td>12/64 (18.7)</td>
</tr>
<tr>
<td>Lower (&lt; 3.5)</td>
<td>1/64 (1.6)</td>
<td>0/16 (0)</td>
<td>0/36 (0)</td>
<td>1/12 (8.3)</td>
</tr>
<tr>
<td>Normal (3.5-5.1)</td>
<td>58/64 (90.6)</td>
<td>14/16 (87.5)</td>
<td>34/36 (94.4)</td>
<td>10/12 (83.3)</td>
</tr>
<tr>
<td>Higher (&gt; 5.1)</td>
<td>5/64 (7.8)</td>
<td>2/16 (12.5)</td>
<td>2/36 (5.6)</td>
<td>1/12 (8.3)</td>
</tr>
<tr>
<td>Corrected calcium, mmol/L</td>
<td>2.41 ± 0.10</td>
<td>2.41 ± 0.02</td>
<td>2.40 ± 0.02</td>
<td>2.45 ± 0.04</td>
</tr>
<tr>
<td>Total (&lt; 2.15)</td>
<td>63/63 (100)</td>
<td>16/63 (25.4)</td>
<td>35/63 (55.6)</td>
<td>12/63 (19.0)</td>
</tr>
<tr>
<td>Normal (2.15-2.57)</td>
<td>59/63 (93.7)</td>
<td>15/16 (93.8)</td>
<td>33/35 (94.3)</td>
<td>11/12 (91.7)</td>
</tr>
<tr>
<td>Higher (&gt; 2.57)</td>
<td>4/63 (6.3)</td>
<td>1/26 (6.3)</td>
<td>2/35 (5.7)</td>
<td>1/12 (8.3)</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.16 ± 0.11</td>
<td>2.20 ± 0.01</td>
<td>2.14 ± 0.01</td>
<td>2.10 ± 0.03</td>
</tr>
<tr>
<td>Parameter</td>
<td>Values</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------</td>
<td>----------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>120/120 (100)</td>
<td>54/120 (45.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lower (&lt; 2.15, &lt; 2.20)</strong></td>
<td>68/120 (56.7)</td>
<td>17/54 (31.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (2.15-2.5, 2.2-2.55)</strong></td>
<td>52/120 (43.3)</td>
<td>37/54 (68.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thrombin time, s</strong></td>
<td>16.25 (15.28-16.93)</td>
<td>15.15 (14.53-16.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>66/66 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lower (&lt; 14)</strong></td>
<td>1/66 (1.5)</td>
<td>0/18 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (14-19)</strong></td>
<td>57/66 (86.4)</td>
<td>18/18 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Higher (&gt; 19)</strong></td>
<td>8/66 (12.1)</td>
<td>0/18 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prothrombin time, s</strong></td>
<td>13.65 (13.20-14.23)</td>
<td>13.25 (13.18-13.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>66/66 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (11.5-14.5)</strong></td>
<td>55/66 (83.3)</td>
<td>18/18 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Higher (&gt; 14.5)</strong></td>
<td>11/66 (16.7)</td>
<td>0/18 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prothrombin activity</strong></td>
<td>94.00 (86.00-100.00)</td>
<td>99.50 (95.50-102.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fibrinogen, g/L</strong></td>
<td>4.46 ± 0.18</td>
<td>3.72 ± 0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>66/66 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lower (&lt; 2)</strong></td>
<td>3/66 (4.5)</td>
<td>0/18 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (2-4)</strong></td>
<td>24/66 (36.4)</td>
<td>11/18 (61.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Higher (&gt; 4)</strong></td>
<td>39/66 (59.1)</td>
<td>7/18 (38.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D-dimer, pg/mL</strong></td>
<td>124/124 (100)</td>
<td>57/124 (46.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (&lt; 0.5)</strong></td>
<td>44/124 (35.5)</td>
<td>34/57 (60.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Higher (≥ 0.5)</strong></td>
<td>80/124 (64.5)</td>
<td>23/57 (40.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High-sensitivity cardiac troponin, pg/mL</strong></td>
<td>123/123 (100)</td>
<td>56/123 (46.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (&lt;15.6, ≤34.2)</strong></td>
<td>117/123 (95.1)</td>
<td>54/56 (96.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Higher (&gt;15.6, &gt;34.2)</strong></td>
<td>6/123 (4.9)</td>
<td>2/56 (3.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amino-terminal pro-brainnatriuretic peptide, pg/mL</strong></td>
<td>123/123 (100)</td>
<td>57/123 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (&lt; 285, &lt; 486)</strong></td>
<td>83/123 (67.5)</td>
<td>50/57 (87.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Higher (≥ 285, ≥ 486)</strong></td>
<td>40/123 (32.5)</td>
<td>7/57 (12.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ferritin, μg/L</strong></td>
<td>468.25 (292.18-1022.08)</td>
<td>9.80 (4.30-18.68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>62/62 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lower (&lt; 15, &lt; 30)</strong></td>
<td>2/62 (3.2)</td>
<td>1/18 (5.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal (15-150, 30-400)</strong></td>
<td>15/62 (24.2)</td>
<td>8/18 (44.4)</td>
<td></td>
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</tr>
</tbody>
</table>
Major laboratory markers were tracked in 67 patients on the B11 Zhongfaxincheng campus beginning at admission (Figure 1). During hospitalization, most patients had marked lymphopenia, and patients in the critically ill group developed more severe lymphopenia over time. The level of D-dimer was significantly higher in the critically ill group than in the other groups and rapidly increased from day 11 after admission. Levels of high-sensitivity cardiac troponin I, amino-terminal pro-brain natriuretic peptide (NT-pro BNP), and lactate dehydrogenase were clearly elevated in the critically ill group compared with the other groups throughout the early clinical course but decreased from day 11. The level of albumin was lowest in the critically ill group and decreased with illness deterioration.

An ordinal logistic regression model was adopted and conducted with JMP15.0 software to explore potential risk factors associated with the severity of COVID-19. Based on data completeness, the clinical significance and the single-factor screening results in Table 1 and Table 2, we first took all the variables proven to be significant in the $\chi^2$ tests as candidate variables while excluding the time from disease onset to hospital admission, thrombin time, prothrombin time, prothrombin activity and fibrinogen for incomplete data sets, as only one of our research centers had collected these data. Second, we categorized the remaining candidate variables as comorbidity, drug treatment before admission, coagulation system, inflammation markers and liver functional system to the process collinearity diagnosis. Thus, we excluded hemoglobin, chronic liver disease, lactate dehydrogenase and C-reactive protein. Remaining candidate variables included age, sex, cerebrovascular disease, cancer, neutrophil-to-lymphocyte ratio, monocyte ratio, creatinine, aspartate transaminase (AST), drug treatment before admission, albumin, calcium, hematocrit, procalcitonin, NT-pro BNP, platelet count, eosinophil ratio and high-sensitivity cardiac troponin I. Then, a step-by-step regression method ($P$ for inclusion = 0.05, $P$ for exclusion = 0.05) was applied, and 3 variables were included in the final model: age, neutrophil-to-lymphocyte ratio and high-sensitivity cardiac troponin I (Table 3).

The results showed that the total model test $\chi^2$=17.380, $P$ < 0.001, which meant at least one $\beta$ in the equation did not equal 0, and the final model was preceded to a constant value. The goodness-of-fit test showed $\chi^2$=59.137, $P$ = 0.968 > 0.05, meaning that the final model fit well. We found that neutrophil-to-lymphocyte ratio ($P$ = 0.042) and high-sensitivity cardiac troponin I ($P$ = 0.043) were statistically significant independent risk factors (details in Table 3). Compared with patients who had normal neutrophil-to-lymphocyte ratios and high-sensitivity cardiac troponin I, the odd ratio (OR) for severe COVID-19 in patients with elevated neutrophil-to-lymphocyte ratios were 4.019 times higher [95% confidence interval (CI): 1.045-15.467] and elevated high-sensitivity cardiac troponin I was 10.126 times higher [95%CI: 1.088-94.247].

As of 22nd March, 2020, 114 (90.5%) of 126 patients were discharged, 8 (6.3%) patients died, and 4 (3.2%) remained hospitalized. Fitness for discharge was based on abatement of fever for at least 3 d, with the disappearance of respiratory symptoms, improvement based on chest radiographic evidence, and two successive indications (interval not less than 24 h) of viral clearance in respiratory samples obtained from the upper respiratory tract. For those who were discharged, the length of hospital stay was 18 d (IQR, 10.0-26.0). For those who died, the time from admission to death was 5.00 d (IQR 1.75-16.50).

**DISCUSSION**

This retrospective study discovered several clinical features and risk factors for critical illness in patients who were hospitalized with COVID-19 in Tongji Hospital. As of March 8, 2020, 126 patients with COVID-19 were included in this study: 67 from the B11 Zhongfaxincheng campus and 59 from the E1-3 Guanggu campus. Of the 59
Good-to-fitness test showed that total model test was 59.137, which means at least one levels of D-dimer, lactate dehydrogenase, and creatine kinase and the neutrophil-to-lymphocyte ratio were consistent with those of several other studies, which also confirmed that high-sensitivity cardiac troponin I, NT-pro BNP and lactate dehydrogenase levels were increased with disease progression, and lymphopenia markedly decreased. Our results were consistent with those of several other studies, which also confirmed that high levels of D-dimer, lactate dehydrogenase, and creatine kinase and the neutrophil-to-

**Table 3 Logistic regression results of potential risk factors for severity of coronavirus disease 2019**

<table>
<thead>
<tr>
<th></th>
<th>Wald χ²</th>
<th>P value</th>
<th>OR</th>
<th>95%CI Upper bound</th>
<th>95%CI Lower bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (Moderate)</td>
<td>1.800</td>
<td>0.180</td>
<td>9.816</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intercept (Severe)</td>
<td>1.122</td>
<td>0.460</td>
<td>3.13</td>
<td>1.800</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>5.477</td>
<td>0.019</td>
<td>1.055</td>
<td>1.009</td>
<td>1.104</td>
</tr>
<tr>
<td>High-sensitivity cardiac troponin I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≤ 15.6, ≤ 34.2)</td>
<td>4.094</td>
<td>0.043</td>
<td>4.019</td>
<td>1.045</td>
<td>15.467</td>
</tr>
<tr>
<td>Neutrophil to lymphocyte ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Higher (&gt; 3.13)</td>
<td>5.477</td>
<td>0.042</td>
<td>10.126</td>
<td>1.088</td>
<td>94.247</td>
</tr>
<tr>
<td>Normal (≤ 3.13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 The normal and abnormal range applied to female patients.
2 The normal and abnormal range applied to male patients.

Ordinal logistic regression model was adopted in JMP15.0 software to explore the risk factors associated with severity of coronavirus disease 2019 patients. Step-by-step regression method (P for inclusion = 0.05, P for exclusion = 0.05) was applied and 3 variables were included in the final model. Results showed that total model test χ² = 17.380, P < 0.001, which means at least one β in the equation did not equal to 0 and this model was preceded to constant 1. Good-to-fitness test showed χ² = 59.137, P = 0.968 > 0.05, which means this model fit well. OR: Odds ratio.

(46.8%) patients in the moderate group, 24 were male. Of the 49 (38.8%) patients in the severe group, 30 were male. Of the 18 (14.3%) patients in the critically ill group, 13 were male. By 26th March, according to National Health Commission statistics, there were 3460 confirmed cases (2880 cases in Wuhan) and 1034 severe cases (995 cases in Wuhan) in China, and to date, 81340 cumulative cases have been confirmed, 3292 have died and 74588 have been discharged[13]. The focus of medical services has now changed to treat patients with severe disease. In the latest Chinese CDC report, 31.1% of confirmed patients were aged over 60, and the crude mortality was highest among patients ≥ 80-years-old (14.8%) and among patients with chronic underlying diseases (5.6%-10.5%)[9]. Similar results were reported in several studies in which increased age and comorbidity were associated with death among patients with COVID-19[5,6, 11,14]. Older COVID-19 patients with chronic comorbidities such as hypertension, diabetes, cardiovascular disease, cancer or other coexisting medical conditions were more likely to develop disease involving multiple systems and organs and rapidly progress to poor outcomes[5]. In our study, the median age of the patients was 61.00 years (IQR 48.00-68.00). Patients in the critically ill group were significantly older than those in the moderate group (65.44 years vs 54.76 years, P = 0.019). Patients with cerebrovascular disease, chronic liver disease, and cancer also presented with more severe disease; however, we did not find a significant difference in sex between the 3 groups. When we merged the severe and critically ill group together, the men in the merged group appeared to be more vulnerable to the COVID-19 than those in the moderate group. Recently, several studies have noted that more men than women were diagnosed with severe disease and that men had a higher case fatality rate[15-17]. Channappanavar et al[18] and Ling Ma et al[19] indicated that SARS-CoV-2 may affect male gonadal function via ACE2 receptors and that estrogen receptor signaling may provide a protective effect during coronavirus infection.

In terms of laboratory tests, the most common laboratory abnormalities observed in the severe and critically ill groups were decreased lymphocytes and albumin, as well as elevated lactate dehydrogenase, C-reactive protein, fibrinogen and D-dimer. In our study, compared with the moderate group, patients in the severe and critically ill groups had numerous laboratory abnormalities, which suggests that COVID-19 may be associated with coagulation activation, liver dysfunction, acute kidney injury, cardiac injury, and immune deficiency. The dynamic change in laboratory findings was tracked in 67 patients with COVID-19. In the critically ill group, the D-dimer, high-sensitivity cardiac troponin I, NT-pro BNP and lactate dehydrogenase levels increased with disease progression, and lymphopenia markedly decreased. Our results were consistent with those of several other studies, which also confirmed that high levels of D-dimer, lactate dehydrogenase, and creatine kinase and the neutrophil-to-

**Clinical features of severe COVID-19 patients**

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lymphocyte ratio were independent risk factors for mortality among hospitalized patients with COVID-19 [5,11,20].

Chai et al [21] indicated that liver dysfunction in patients with COVID-19 might be induced by cholangiocyte damage rather than hepatocyte damage, which is consistent with our finding that elevated AST and decreased albumin were associated with progression to more severe disease. However, we did not find significant difference between groups in ALT and total bilirubin results. Further studies could investigate whether other causes might participate in liver injury, such as systemic inflammatory response or hypoxemia.

Compared to the moderate group, the severe and critically ill groups had more patients with abnormal myocardial zymograms and patients who presented with elevated levels of lactate dehydrogenase (59.6% and 88.9%), creatine kinase (2.1% and 22.2%), high-sensitivity cardiac troponin I (0% and 22.2%), and NT-pro BNP (39.6% and 77.8%). Myocardial injury associated with COVID-19 may be due to hypoxemia and systemic pro-inflammatory cytokine responses. In a fatal case of COVID-19 in
China, interstitial mononuclear inflammatory infiltrates in heart tissue were confirmed, but parenchymal damage and viral detection were not evident [22].

Regarding the coagulation indicators, D-dimer was above the normal range in 79.6% of the patients in the severe group and in 100% of the patients in the critically ill group; the thrombin time was longer than normal in 11.1% of the patients in the severe group and 33.3% of the patients in the critically ill group; and the prothrombin time was longer than normal in 16.7% of the patients in the severe group and 41.7% of the patients in the critically ill group, indicating the profound influence of COVID-19 on the coagulation system. Possible reasons for coagulation activity may be direct injury to endothelial cells by SARS-CoV-2 [23,24], which is also related to atherosclerotic plaque rupture induced by inflammation and the release of procoagulant factors released [11]. Basic studies also confirmed that an inflammatory cytokine storm induced by the virus could lead to lymphocyte apoptosis and that lymphocytes express ACE2 receptors, which make them direct targets of SARS-CoV-2 [25]. The elevation in proinflammatory factors may increase fibrin deposition in the pulmonary microvasculature, contributing to acute respiratory distress syndrome and disseminated intravascular coagulation and significantly increasing blood lactic acid and D-dimer levels [26,27]. Acute kidney injury is directly related to viral attack and cytokine storms, causing metabolic acidosis with elevated creatine and decreased serum calcium levels [1,2,11,17].

However, there are still some limitations to our study. First, the majority of our patients were transferred from local hospitals during the late phases of their illnesses, which caused the collection of medical history to be limited. Second, in this retrospective study, some of the laboratory tests were not routinely performed for all patients or were periodically conducted during the progression of the disease. Third, our study was conducted only on two campuses in one nation (China); not all the analyses were available simultaneously at both centers, and some comparisons were performed only on one campus.

**CONCLUSION**

In conclusion, people of all ages, both male and female, are susceptible to COVID-19. Early drug treatment is an important measure in the treatment of patients with COVID-19, and the following indicators can help clinicians identify patients with severe COVID-19 at an early stage: age, an elevated neutrophil-to-lymphocyte ratio and high sensitivity cardiac troponin I.

**ARTICLE HIGHLIGHTS**

**Research background**

Since it was first reported, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly throughout the world, posing a serious threat to global health. However, the risk factors for patients with moderate-to-severe or severe-to-critical coronavirus disease 2019 (COVID-19) remain unclear.

**Research motivation**

A comprehensive description of the clinical characteristics, laboratory changes, in addition to oxygen levels and radiographic examinations enable clinicians to provide more accurate prognoses and specific care which vary according to subclinical or latent severe cases.

**Research objectives**

This study aimed to explore the characteristics and predictive markers of severe COVID-19.

**Research methods**

Patients with COVID-19 admitted from 1st February 2020 to 8th March 2020 were enrolled and categorized into 3 groups: the moderate group, severe group and critically ill group. Information was extracted from hospital information systems. Epidemiological and demographic, clinical symptoms and outcomes, complications, laboratory and radiographic examinations were collected retrospectively and then
Research results
A total of 126 patients were enrolled. There were 59 in the moderate group, 49 in the severe group, and 18 in the critically ill group. Over 50% patients have increased levels of lactate dehydrogenase, aspartate transaminase (AST), C-reactive protein, fibrinogen, D-dimer, tumor necrosis factor-α, ferritin, as well as decreased levels of hematocrit and calcium. Compared with the moderate group, the severe and critically ill group has significant higher rates of abnormality in levels of neutrophil ratio, eosinophil ratio, lymphocyte ratio, platelets count, neutrophil to lymphocyte ratio, AST, albumin, procalcitonin, calcium, D-dimer, interleukin-6, high-sensitivity cardiac troponin, amino-terminal pro-brain natriuretic peptide (NT-pro BNP), and ferritin. Multivariate logistic regression analysis showed that no drug treatment before admission, a higher neutrophil-to-lymphocyte ratio, a higher AST level, a higher NT-pro BNP level, a higher creatinine level, and serum calcium below the normal range were high-risk factors.

Research conclusions
People of all ages, both male and female, are susceptible to COVID-19. Early drug treatment is an important measure in the treatment of patients with COVID-19, and the following indicators can help clinicians identify patients with severe COVID-19 at an early stage: an elevated neutrophil-to-lymphocyte ratio; elevated AST, NT-pro BNP, and ferritin. Multicenter Study of Clinical Features.

Research perspectives
A large sample size with long-term survival data is needed in future studies.

REFERENCES


Retrospective Study

Evaluating tumor-infiltrating lymphocytes in hepatocellular carcinoma using hematoxylin and eosin-stained tumor sections

Min Du, Yu-Meng Cai, Yu-Lei Yin, Li Xiao, Yuan Ji

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Author contributions: Du M performed the research; Cai YM contributed to data collection and analysis; Yin YL and Xiao L helped in data analysis and modification of the manuscript; Yuan J contributed to the conception and design of the study; and all authors have read and approved the final manuscript.

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Informed consent statement: Informed consent was waived by the Review Board because of the nature of retrospective study.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

Abstract

BACKGROUND
Tumor-infiltrating lymphocytes (TILs) constitute a prognostic factor in hepatocellular carcinoma (HCC). However, different methods of assessing TILs have various pre-analytical, analytical, and post-analytical challenges. The evaluation of TILs in hematoxylin and eosin (H&E)-stained tumor sections proposed by the International Immuno-Oncology Biomarker Working Group was demonstrated to be a reproducible, affordable and easily applied method in many tumors.

AIM
To evaluate the prognostic significance of TILs in H&E-stained slides of HCCs.

METHODS
This was a retrospective study performed in the hospital. HCC patients who underwent liver resection between 2015 and 2017 in Zhongshan Hospital were enrolled in this study. Patients who experienced recurrence or received therapy in addition to antiviral therapy before surgery at this time were excluded. A total of 204 patients were enrolled in the study. The ILS were counted manually in tumor sections stained with H&E under an optical microscope at 400 ×. The ILs were assessed separately in the center of the tumor (TILs<sub>CT</sub>), the invasive front (TILs<sub>IF</sub>), and peritumor (PILs) areas. Univariate and multivariate survival analyses were performed using a Cox regression model. P < 0.05 was considered statistically significant and all P-values were two-sided.

RESULTS
Among the 204 patients, univariate analysis indicated that macrovascular invasion (MaVI) (P = 0.001), microvascular invasion (MVI) (P = 0.012), multiple tumors (P = 0.008), large tumors (> 10 cm) (P = 0.001), absence of a tumor capsule...
Cancer incidence and mortality are rapidly growing globally. Hepatocellular carcinoma (HCC) is one of the most common primary malignancies of the liver, representing the third leading cause of cancer-related deaths worldwide[1]. HCC is associated with chronic inflammation and fibrosis arising from different etiologies, including hepatitis B and C and alcoholic and non-alcoholic fatty liver diseases[2]. The stromal component of tumors consists of fibroblasts, endothelial cells, and various immune cells. Together, these cells play a critical role in tumor development and response to treatment.

Many different methods have demonstrated the prognostic effect of tumor infiltrating lymphocytes (TILs) in HCC[3]. For instance, the densities of tumor-infiltrating T cells and B cells are correlated with superior survival in HCC patients[4], and patients with high-grade HCC of the predominant immune-high subtype had significantly better prognosis[5]. Different methods of assessing TILs have various pre-analytical, analytical, and post-analytical challenges. For example, semi-quantitative hematoxylin and eosin (H&E)-based scores suffer from low precision and poor interobserver reproducibility due to lack of guidance, while digital quantification of immunohistochemical (IHC)-stained sections may have varied results due to inaccurate measurement of the test variable without controlled calibration.
Furthermore, the immuno-score proposed by Jerome Galon showed great prognostic power and outperformed the tumor node metastasis classification for disease-free survival, disease-specific survival and overall survival\[^6, 7\]. However, the immuno-score requires rigorous pathology and experimental practice for the staining, and deviation from the predefined standardized operating procedure might result in improper quantification\[^8\].

Accumulating evidence suggests that lymphocytic infiltration in tumor tissues can be assessed as a significant parameter by evaluating H&E-stained tumor sections\[^9\], which achieved good consistency and reproducibility in pathologists, including pathology resident trainees\[^10\]. The criteria have been assessed in many different solid tumors, including lung, colon, upper gastrointestinal tract, head and neck, genitourinary tract, gynecological organs, mesothelioma, melanoma, and primary brain tumors\[^11\]. However, evaluating of infiltrating lymphocytes in H&E slides of HCC has rarely been studied.

The present study aimed to assess the prognostic effect and the clinicopathological correlation of TILs evaluated in H&E sections of HCC patients.

**MATERIALS AND METHODS**

**Patients and samples**

HCC samples that met the following criteria were enrolled in the present study: (1) Patients who underwent liver resection for the first time from January 2015 to December 2017 in the Department of Liver Surgery, Zhong Shan Hospital, Fudan University, China; (2) Liver resection samples diagnosed as HCC by a pathologist; and (3) Complete clinicopathological data and disease-progression information. Patients who received therapy in addition to antiviruses were excluded, e.g., transarterial chemoembolization, ablation, bland embolization, radioembolization, chemotherapy, and immunotherapy.

The study was approved by the Human Ethics Institutional Review Board of Huadong Hospital, Fudan University (approval number 2019K119), and informed consent was waived by the Review Board because of the retrospective nature of the study.

**H&E staining of tumor tissue**

H&E staining was performed on a high-throughput fast automatic platform (Dako coverstainer, United States) according to standard protocols.

According to the architectural growth patterns\[^12\], distinctive and easily recognizable histological features were defined with a predominant (> 50%) architectural pattern. HCC was divided into microtrabecular/pseudoglandular, macrotrabecular, compact, and lymphoepithelioma-like subtypes\[^13\]. The macrotrabecular subtype is classified as a predominant trabecular architectural pattern which is more than six cells thick\[^14\].

**Density of infiltrating lymphocytes**

Two general pathologists and one senior pathologist were involved in this study. The density of ILs was determined based on the recommendation by the International Immuno-Oncology Biomarker Working Group\[^15\]: (1) The number of ILs on full sections was scanned at low magnification and evaluated at higher magnification (400 ×) manually under an optical microscope; (2) ILs were assessed in the areas of the tumor center (TILs\[^CT\]), the invasive front (TILs\[^IF\]) and on the portal areas of the peritumour 1 cm away from the border (PILs). The “invasive front” (IF) is defined as the region centered on the border separating the host tissue from the malignant nests by 1 mm. Areas with crush artifacts, necrosis, and previous biopsy sites were excluded; and (3) All mononuclear cells, including lymphocytes and plasma cells, were counted (polymorphonuclear leukocytes were excluded from the count of ILs, and neutrophils were recorded separately from the count of ILs).

**Immunohistochemistry staining**

Programmed cell death-ligand 1 (PD-L1) (SP142) rabbit monoclonal primary antibody (Ventana Medical Systems Inc, Tucson, AZ, United States) was optimized for a fully automated IHC assay on the BenchMark ULTRA (Ventana Medical Systems Inc) staining platform using the OptiView DAB IHC Detection Kit and OptiView Amplification Kit (Ventana Medical Systems Inc)\[^16\]. All the tissues were subjected to
PD-L1 (SP142) IHC staining. The expression of PD-L1 on tumor cells (TCs) was assessed as the proportion of TCs showing membrane staining of any intensity. The expression on TILs was assessed as the proportion of stromal areas occupied by PD-L1-positive TILs of any intensity (approved by the US Food and Drug Administration).

Follow-up
Patients were followed up by ultrasound, computed tomography (CT), or magnetic resonance imaging every 3-6 mo after the resection, with a maximum period of 1063 d. The primary study endpoint was progression-free survival (PFS), which refers to the duration of patient survival without any evidence of the tumor.

Statistical analyses
Univariate and multivariate survival analyses were performed using Cox regression model. A non-paired t-test was conducted to compare the clinicopathological parameters of the immune subtypes. All statistical analyses were performed using GraphPad Prism 7 software. P < 0.05 was considered statistically significant and all P-values were two-sided. The statistical methods of this study were reviewed by Xin-xin Xu from Huadong Hospital.

RESULTS

Clinical and pathological factors
A total of 204 patients were included in the present study, 91.67% of the patients were hepatitis B virus infected. Macrovascular invasion (MaVI) was presented in 21 (10.29%) tumors, while microvascular invasion (MVI) was observed in 110 (53.92%) tumors. A total of 156 patients had a single tumor and 117 tumors were capsulated. Cirrhosis was observed in 171 (83.82%) tumors (Table 1). Areas with microtrabecular/pseudo-glandular, macrotrabecular, compact, and lymphoepithelioma-like histological architectural patterns were identified in 42.64%, 52.94%, 2.45%, and 1.96% of the tumors, respectively (Table 1). A total of 42/204 (20.6%) patients experienced tumor recurrence. The univariate analysis indicated that MaVI (P = 0.001), MVI (P = 0.012), multiple tumors (P = 0.008), large tumors (> 10 cm) (P = 0.001), absence of a tumor capsule (P = 0.026), and the macrotrabecular histological subtype (P = 0.001) were independent predictors of PFS (Supplementary Figure 1 and Table 2). MaVI (P = 0.009) and absence of a capsule (P = 0.031) were multivariate analysis predictors of PFS (Table 2).

Immune microenvironment was heterogeneous
In the current study cohort, the number of TILs CT, TILs IF, and PILs was 10-1200/high power field (HPF). The ILs showed a great diversity among TILs CT, TILs IF, and PILs. Compared to the adjacent non-tumor liver tissues, the tumor microenvironment was found to be relatively inert due to a lower number of immune cell densities in the tumor center, invasive front, and peritumor regions were converted into percentiles: 0%-25% was scored as low, and 25%-100% was scored as high. Patients with high TILs CT, TILs IF, and PILs had better PFS than those with low TILs CT, TILs IF, and PILs (Figure 1). Multivariate analysis, including those variables that appeared statistically significant in the univariable analysis, showed that low TILs IF (P = 0.0495) and PILs (P = 0.047) were independent risk factors for PFS in patients with HCC.

Immune high patients had better PFS and a lower rate of MVI
Immune cell densities in the tumor center, invasive front, and peritumor regions were converted into percentiles: 0%-25% was scored as low, and 25%-100% was scored as high. Patients with high TILs CT, TILs IF, and PILs had better PFS than those with low TILs CT, TILs IF, and PILs (Figure 1). Multivariate analysis, including those variables that appeared statistically significant in the univariable analysis, showed that low TILs IF (P = 0.0495) and PILs (P = 0.047) were independent risk factors for PFS in patients with HCC.

After integrating TILs CT, TILs IF, and PILs, we divided HCCs into three-category analysis: (1) Immunehigh subtype [(TILs CT) high, (TILs IF) high, and PILs high, 83 cases]; (2) Immune intermediate subtype (tumours other than Immunehigh and Immune low subtypes, 94 cases); (3) Immune low subtype [(TILs CT) low, (TILs IF) low, and PILs low, 27 cases]. The H&E images of the three immune subtypes are illustrated in Figure 2.

A higher number of the immune high subtype (46.1%) HCCs was noted compared to the immune high subtype (40.7%), while 13.2% of the HCCs were immune low subtype. Recurrent disease was identified in 10.8% of the immune high patients compared to the 25.5% of the immune low patients and 33.3% of the immune low patients (P = 0.0153). The...
Du M et al. TILs in H&E sections of HCC

<table>
<thead>
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<td>&gt; 10 cm</td>
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<td>TILs\IF</td>
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<tr>
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<td>PILs</td>
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<td>113</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>89</td>
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MaVI: Macrovascular invasion; MVI: Microvascular invasion; TILs\CT: Tumor infiltrating lymphocytes in the tumor center; TILs\IF: Tumor infiltrating lymphocytes in the invasive front 1 mm spacing from the malignant nests, two cases cannot assess infiltrating lymphocytes in the invasive front; PILs: Infiltrating lymphocytes in the peritumor, two cases cannot assess infiltrating lymphocytes in peritumor areas; HBV: Hepatitis B virus.

immune\mod subtype had a lower rate of MVI (40.96%) than the immune\mod (61.70%; P = 0.017) and immune\low (66.67%; P = 0.020) subtypes. A large number of patients had neutrophils in the microenvironment of the immune\mod and immune\mod subtypes compared with the immune\mod subtype (Figure 3).

Regarding other parameters, including MaVI, multiple tumors, tumor diameter, capsule, differentiation, histological subtype, and lymphoid follicle, PD-L1 (SP142) expression did not exhibit a significant difference between the three groups (Table 3).
**Table 2 Results of univariate and multivariate analysis**

<table>
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<th>Variable</th>
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<td></td>
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<td>95% CI</td>
<td>P value</td>
<td>HR</td>
<td>95% CI</td>
<td>P value</td>
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<td>MaVI</td>
<td>3.09</td>
<td>1.02-9.34</td>
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<td>3.77</td>
<td>1.63-7.40</td>
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<td>MVI</td>
<td>2.80</td>
<td>1.51-5.16</td>
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<td>1.19</td>
<td>0.64-2.23</td>
<td>0.693</td>
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<td>Tumor number</td>
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<td>1.95</td>
<td>1.04-3.77</td>
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<td>Largest tumor diameter</td>
<td>3.31</td>
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<td>0.001</td>
<td>1.76</td>
<td>0.95-3.45</td>
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<tr>
<td>Capsule</td>
<td>1.99</td>
<td>1.07-3.70</td>
<td>0.026</td>
<td>0.42</td>
<td>0.20-0.83</td>
<td>0.031</td>
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<tr>
<td>Macrotubular histological subtype</td>
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<td>1.77-5.86</td>
<td>0.001</td>
<td>1.89</td>
<td>1.03-3.67</td>
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<td><strong>TILs</strong>&lt;sup&gt;CT&lt;/sup&gt; (≤ 30)</td>
<td>0.49</td>
<td>0.22-0.92</td>
<td>0.039</td>
<td>0.85</td>
<td>0.41-1.63</td>
<td>0.734</td>
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<tr>
<td><strong>TILs</strong>&lt;sup&gt;IF&lt;/sup&gt; (≤ 200)</td>
<td>0.37</td>
<td>0.14-0.98</td>
<td>0.014</td>
<td>0.46</td>
<td>0.25-0.86</td>
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<td><strong>PILs</strong> (≤ 200)</td>
<td>0.40</td>
<td>0.22-0.75</td>
<td>0.010</td>
<td>0.37</td>
<td>0.19-0.77</td>
<td>0.0495</td>
<td></td>
</tr>
</tbody>
</table>

MaVI: Macrovascular invasion; MVI: Microvascular invasion; TILs<sup>CT</sup>: Tumor infiltrating lymphocytes in the tumor center; TILs<sup>IF</sup>: Tumor infiltrating lymphocytes in the invasive front 1 mm spacing from the malignant nests; PILs: Infiltrating lymphocytes in the peritumor; HR: Hazard ratio; CI: Confidence interval.

**Patients with neutrophils or tertiary lymphoid structures among the TILs had a low recurrence rate**

Neutrophils and tertiary lymphoid structures (TLSs) were distinguished in the tumor microenvironment on H&E-stained slides. Therefore, we recorded the presence and density of these inflammatory cells. Patients with neutrophils among the TILs exhibited a tendency for decreased recurrence, albeit without a significant difference. The patients with TLSs in the microenvironment did not show any recurrence after a follow-up of 37-791 d.

**High PD-L1 (SP142) expression on TILs was associated with better PFS**

PD-L1 (SP142) was expressed on TCs in 80 patients and TILs in 200 patients. Patients with a higher expression of PD-L1 (SP142) on TILs (> 5%) had a lower recurrence rate than those with lower expression (Figure 4). The greater the number of TILs, the higher the level of PD-L1 (SP142) expression on the TILs. However, the expression of PD-L1 (SP142) on TCs was not associated with PFS or TILs in our cohort. Additionally, we observed the expression of PD-L1 (SP142) on neutrophils; however, the proportion of neutrophils in TILs was not significantly associated with the expression of PD-L1 (SP142).

We performed the IHC assay of (SP142), (28-8), and (E1L3N) in the other cohort of HCC patients; (SP142) is a more robust PD-L1 staining reagent than (28-8) and (E1L3N) in both tumors and immune cells of HCC, while (28-8) and (E1L3N) have similar staining effect in tumor cells. Therefore, we chose (SP142) as the major reagent analyzed in this study (Supplementary Figure 2).

**DISCUSSION**

This study revealed that the density of infiltrating lymphocytes in H&E-stained tissues can predict the recurrence of HCC. The International Immuno-Oncology Biomarker working Group proposed that TILs should be reported separately for the stromal compartment (= % stromal TILs) and the tumor cell compartment (= % intra-tumoral TILs). The stroma of classical HCC is composed of sinusoid-like blood spaces lined by a single layer of endothelial cells, which sometimes show varying degrees of dilatation or may be difficult to recognize owing to compression by tumor cells[17]. Most classical HCCs do not induce a desmoplastic stroma, therefore the method of stromal TILs is not suitable for HCC assessment. The method of intra-tumoral TILs with tumor cell area for the denominator is hard to accomplish manually, as visual estimation is subjective and TILs are manifested as infiltrating nests in tumor area in our study; meanwhile in daily practice most pathologists will report discrete estimates, for
example 13.5% will be rounded to 15%, which will result in underestimation of the difference. Therefore, we tried to distinguish the immune subtypes of HCC by recording the densities of infiltrating lymphocytes in the tumor center, invasive front and peritumor. However, this method is admittedly challenging, and inter-observer reproducibility requires particular attention. The method showed a prognostic effect for HCC recurrence and might be helpful to select patients with the highest likelihood of responding to immunotherapeutic agents.

HCC is characterized by immune tolerance and comprises numerous infiltrated immune cells, a large number of suppressive molecules, complex proinflammatory/immunoregulatory signaling and intricate interactions between different components. The immune microenvironment in HCC plays a key role in HCC progression and recurrence[18]. The immune system plays a dual role in cancer: It can not only suppress tumor growth by destroying cancer cells or inhibiting their outgrowth but also promote tumor progression either by selecting tumor cells that are more fit to survive in an immunocompetent host or by establishing conditions within the tumor microenvironment that facilitate tumor outgrowth[19]. Regulatory T cells and myeloid-derived suppressor cells are two major types of immunosuppressive

### Table 3 Clinicopathological data between the three immune subtypes

<table>
<thead>
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<th>Variable</th>
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<th>Immune&lt;sup&gt;mod&lt;/sup&gt;</th>
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<tr>
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<td></td>
<td>No</td>
<td>74</td>
<td>70</td>
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</table>

MaVI: Macrovascular invasion; MVI: Microvascular invasion; PD-L1: Programmed cell death-ligand 1; TIL: Tumor infiltrating lymphocyte; HBV: Hepatitis B virus.
Figure 1 The distribution and recurrence association of tumor infiltrating lymphocytes in the tumor center, invasive front, and peritumor.
A: The spectrum of tumor infiltrating lymphocytes in the tumor center (TILs\textsuperscript{CT}), TILs in the invasive front (TILs\textsuperscript{IF}), and TILs in the peritumor (PILs) of 204 cases. TILs\textsuperscript{CT} were lower than TILs\textsuperscript{IF} and PILs. TILs\textsuperscript{IF} was the most prominent area among the three areas; B: Dot map of TILs\textsuperscript{CT}, TILs\textsuperscript{IF}, and PILs, indicating a heterogeneous distribution of inflammation; C: Comparison of the mean of TILs\textsuperscript{CT}, TILs\textsuperscript{IF} and PILs from patients with tumor recurrence (black bars) or without tumor recurrence (white bars); D: Median survival time for all patients, with high densities (red bars) or low densities (black bars) of TILs\textsuperscript{CT}, TILs\textsuperscript{IF} and PILs; E, F and G: Patients with high TILs\textsuperscript{CT}, TILs\textsuperscript{IF}, and PILs had a lower recurrence rate. TILs\textsuperscript{CT}: Tumor infiltrating lymphocytes in the tumor center; TILs\textsuperscript{IF}: Tumor infiltrating lymphocytes in the invasive front 1 mm spacing from the malignant nests; PILs: infiltration lymphocytes in the peritumor.
Du M et al. TILs in H&E sections of HCC

Figure 2 Representative hematoxylin and eosin images of the three immune subtypes (200 ×). A and B: Tumors with high infiltrating lymphocytes in the tumor center, invasive front and peritumor (Immune$^{\text{high}}$) subtype; C and D: Tumors other than immune$^{\text{high}}$ and immune$^{\text{low}}$ (Immune$^{\text{mod}}$) subtype; E and F: Tumors with low infiltrating lymphocytes in the tumor center, invasive front and peritumor (Immune$^{\text{low}}$) subtype; A, C, and E: Immune cells in tumor center; B, D, and F: Immune cells in the peritumor region.

Figure 3 The comparison of immune$^{\text{high}}, \text{immune}^{\text{mod}}$ and immune$^{\text{low}}$ subtypes. A: Immune subtypes can predict patients' progression-free survival. Immune$^{\text{high}}$ patients had a low recurrence rate, and immune$^{\text{low}}$ patients experienced a high recurrence rate; B: The median survival time for all patients divided into three categories: Immune$^{\text{high}}$ (red bars), immune$^{\text{mod}}$ (green bars), and immune low (purple bars); C: The incidence rate of microvascular invasion (MVI) in three immune subtypes, immune$^{\text{high}}$ subtype had a lower rate of MVI compared to immune$^{\text{mod}}$ and immune$^{\text{low}}$ subtypes; D: The presence of neutrophils in the three immune subtypes, a high incidence of neutrophils was detected in immune$^{\text{high}}$ and immune$^{\text{mod}}$ subtypes. Immune$^{\text{low}}$: Tumors with high infiltrating lymphocytes in the tumor center, invasive front and peritumor; Immune$^{\text{mod}}$: Tumors other than immune$^{\text{high}}$ and immune$^{\text{low}}$; Immune$^{\text{low}}$: Tumors with low infiltrating lymphocytes in the tumor center, invasive front and peritumor; PFS: Progression-free survival; MVI: Microvascular invasion.
Du M et al. TILs in H&E sections of HCC

Figure 4  The pathological picture and recurrence association of programmed cell death-ligand 1 SP142 expression in tumor cells and immune cells (200 ×). A and B: Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) picture of programmed cell death-ligand 1 (PD-L1) SP142 in tumor cells of one case; C and D: H&E and IHC picture of PD-L1 SP142 in immune cells of another case; E: Patients with high expression of PD-L1 (SP142) on tumor infiltrating lymphocytes tend to have less recurrence; F: Expression of PD-L1 (SP142) on tumor cells was not statistically significant. PD-L1: Programmed cell death-ligand 1; IHC: TILs: Tumor infiltrating lymphocytes; TCs: Tumor cells; PFS: Progression-free survival.

Neutrophils and TLSs were associated with lower recurrence in the present study. The bulk of the clinical evidence assessing neutrophil to lymphocyte ratios (NLRs) mostly supports the notion that neutrophils promote, rather than inhibit, cancer progression\cite{25}. In comparison with NLR, the prognostic and predictive power of intratumoral neutrophils is murkier and more variable, and positive (gastric cancer), negative (renal cancer and melanoma) or no (lung cancer) correlation with patient outcome has been observed in different studies. However, experimental studies have highlighted multifaceted and sometimes opposing roles of neutrophils in cancer\cite{26}. Analysis of the current literature shows that the presence of TLSs is associated with a favorable clinical outcome for cancer patients, regardless of the approach used to quantify TLSs and the stage of the disease\cite{27}. Researchers have indicated that TLSs represent a privileged area for the recruitment of lymphocytes into tumors and the generation of central memory T and B cells that circulate and limit cancer progression\cite{28}.

Different immunotherapeutic modalities have been used to treat HCC, including diverse vaccine platforms, adoptive T-cell therapy, cytokines, gene therapy and monoclonal antibodies that target immune checkpoint molecules\cite{29}. The importance of lymphocytes has been highlighted in many studies, wherein increasing infiltration of tumors with lymphocytes has been associated with enhanced response to cytotoxic treatment and prognosis in cancer patients\cite{30}. HCC immunogenicity is indicated by the presence of tumor-infiltrating lymphocytes and an evident reduction in relapse rates after resection and transplantation in patients with dense lymphocytic infiltration.
Nevertheless, the present study had some limitations. This was a retrospective, single-center study with a small number of patients. Additionally, this method is more challenging to implement in daily practice and has lower inter-observer reproducibility than stromal TILs. The method should be improved upon with further study undertaken and as evidence becomes available. The study lacked immune cell characterization. Understanding the types and function of immune cells as well as different cytokines will provide more insight into tumor immunology and immunotherapy.

**CONCLUSION**

HCC patients with high infiltrating lymphocytes tend to have a lower recurrence rate and less microvascular invasion. The evaluation of TILs in H&E-stained specimens could be a prognostic parameter for HCC.

**ARTICLE HIGHLIGHTS**

**Research background**
As successful use of immune checkpoint inhibitors and other forms of immunotherapy has become a clinical reality, the need for widely applicable, accessible and reliable biomarkers is clear. Different methods of assessing tumor infiltrating lymphocytes (TILs) have various pre-analytical, analytical, and post-analytical challenges. The evaluation of TILs in hematoxylin and eosin (H&E) stained tumor sections proposed by the International Immuno-Oncology Biomarker Working Group was demonstrated to be a reproducible, affordable and easily applied method in many tumors. However, this method has barely been conducted in hepatocellular carcinoma (HCC). The exploration of TILs in H&E sections of HCC could provide a detailed information for the selection of patients who receive the immunotherapy and evaluation of the prognostic effect of immunotherapy.

**Research motivation**
There have been few suggestions to evaluate HCC by examining TILs in H&E sections. The key problem is to build a method suitable for the tissue specificity of HCC. Once a consensus of the method is established, it will be helpful to manifest the inflammatory condition of the tumor and help to select patients that will experience the greatest benefit of immunotherapy as well as to gain deep insight into immunotherapy.

**Research objectives**
The main objective of this study was to explore whether evaluating TILs in H&E-stained sections has a prognostic effect in HCC. Based on this study, evaluating TILs in H&E-stained sections could be a prognostic method for HCC. Increasing multicenter research to validate and improve this method should be implemented in the future.

**Research methods**
H&E staining was performed on a high-throughput fast automatic platform (Dako coverstainer, United States) according to standard protocols. Programmed cell death-ligand 1 (PD-L1) (SP142) rabbit monoclonal primary antibody (Ventana Medical Systems Inc, Tucson, AZ, United States) was optimized for a fully automated immunohistochemical (IHC) assay on the BenchMark ULTRA (Ventana Medical Systems Inc) staining platform using the OptiView DAB IHC Detection Kit and OptiView Amplification Kit (Ventana Medical Systems Inc). The method to record TILs was described as follows: (1) The number of ILs on full sections was scanned at low magnification and evaluated manually at higher magnification (400 ×) under an optical microscope; (2) ILs were assessed in the areas of the tumor center (TILs<sub>CT</sub>), the invasive front (TILs<sub>IF</sub>) and on the portal areas of the peritumor 1 cm away from the border (PILs). The “invasive front” (IF) is defined as the region centered on the border separating the host tissue from the malignant nests by 1 mm. Areas with crush artifacts, necrosis, and previous biopsy sites were excluded; and (3) All mononuclear cells, including lymphocytes and plasma cells, were counted. Kaplan-Meier univariate and multivariate survival analyses were performed using a Cox regression model. A nonpaired t-test was conducted to compare the clinicopathological parameters of the immune subtypes.
Research results

Based on this research, low density of TILs\textsuperscript{CT} (P = 0.039), TILs\textsuperscript{B} (P = 0.014), and PILs (P = 0.010) were independent predictors of progression-free survival (PFS). The immune\textsuperscript{mod} subtype [(TILs\textsuperscript{CT})\textsuperscript{high}, (TILs\textsuperscript{B})\textsuperscript{high}, and PILs\textsuperscript{mod}, 83 cases] had a lower rate of microvascular invasion (MVI) (40.96\%) than the immune\textsuperscript{mod} (tumors other than immune\textsuperscript{mod} and immune\textsuperscript{low} subtypes, 94 cases) (61.70\%, P = 0.017) and immune\textsuperscript{low} [(TILs\textsuperscript{CT})\textsuperscript{low}, (TILs\textsuperscript{B})\textsuperscript{low}, and PILs\textsuperscript{low}, 27 cases] (66.67\%, P = 0.020) subtypes. The recurrence rates of the immune\textsuperscript{mod} and immune\textsuperscript{low} subtypes were 10.8\%, 25.5\% and 33.3\%, respectively.

Research conclusions

This study proposed that the density of TILs in HCC tissues can predict the recurrence of the patient. The method of evaluating TILs in H&E-stained specimens may also be meaningful in HCC.

Research perspectives

Increasing multicenter research to validate and improve this method should be implemented in the future.

ACKNOWLEDGEMENTS

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suppression and promotion. Science 2011; 331: 1565-1570 [PMID: 21436444 DOI: 10.1126/science.1203486]


Clinical Trials Study

Role of carbon nanotracers in lymph node dissection of advanced gastric cancer and the selection of preoperative labeling time

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Author contributions: Zhao K, Shan BQ and Gao YP designed the research study; Zhao K, Shan BQ performed the research; Zhao K, Shan BQ and Xu JY analyzed the data and wrote the manuscript; all authors have read and approve the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Weifang People’s Hospital Institutional Review Board (Approval No. 2021-021).

Clinical trial registration statement: This study is registered at chinese clinical trial registry: https://www.chictr.org.cn, registration No. ChiCTR2100050003.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors of this manuscript have

Abstract

BACKGROUND

The incidence of gastric cancer is high. The number of dissected lymph nodes was an independent factor affecting prognosis. Although preoperative labeling is helpful in lymph nodes resection, there are no guidelines for when to perform preoperative labeling.

AIM

To investigate the role of nanocarbon in lymph node dissection during gastrectomy, and to discuss the relationship between the timing of preoperative injection of carbon nanoparticles and the extent of lymph node dissection.

METHODS

A prospective analysis was performed on the clinical data of 307 patients with advanced gastric cancer who underwent laparoscopic surgery in the General Surgery Department of Weifang People’s Hospital between June 2018 and February 2021. The patients were randomly divided into experimental group and control group based on whether they received preoperative nanocarbon injection or not. The experimental group was divided into different groups according to the preoperative labeling time. The number of dissected lymph nodes and the number of lymph nodes with black staining were compared in each group after surgery, and the number of nanocarbon in the number of dissected lymph nodes, pathological staging, and the relationship with prognosis were discussed.

RESULTS

The average number of dissected lymph nodes in the experimental group was higher than that in the control group. In the experimental group, the number of lymph node dissections and number of black-staining lymph nodes in the nanocarbon-labeling group at 2 d and 1 d before surgery were higher than in the labeling group on the day before surgery ($P < 0.05$).

CONCLUSION
Preoperative nanocarbon labeling can safely and effectively guide lymph node dissection. To improve the detection rate of lymph nodes, it is conducive to subsequent comprehensive anti-tumor therapy.

**Key Words:** Gastric cancer; Carbon nanotracers; Lymph node dissection; Preoperative labeling time

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Core Tip: This study investigated the role of carbon nanoparticles in lymph node dissection during gastrectomy, and discussed the relationship between the timing for preoperative labeling and the number of lymph nodes dissected. It was found that carbon nanoparticle labeling has a role in guiding laparoscopic lymph node dissection of gastric cancer. Preoperative submucosal injection of carbon nanoparticles could significantly improve the detection rate of lymph nodes, which is conducive to pathological staging and subsequent comprehensive anti-tumor therapy.

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**INTRODUCTION**

Stomach cancer is the fifth most common cancer worldwide and the third leading cause of cancer mortality. There are significant regional differences in the incidence of gastric cancer. The incidence in Asia is significantly higher than that in Europe and the United States. Gastric cancer deaths in China account for > 40% of the global total in the same period[1]. Reducing cancer-related mortality and improving quality of life is one of the current research focuses.

Radical surgery is still the preferred treatment method for advanced gastric cancer. Improvements in technology have resulted in laparoscopy becoming the preferred surgical method at present, which has obvious advantages over open surgery in terms of surgical field and number of lymph nodes dissected. Previous studies[2-4] have shown that there is a correlation between the number of dissected lymph nodes and prognosis. The staging method based on the number of lymph node metastases in gastric cancer (pN staging) is currently recognized as the best staging method for lymph node metastasis in gastric cancer. Postoperative lymph node detection rate is one of the major factors affecting pN staging of lymph node metastasis after radical gastrectomy[5]. To help surgeons correctly distinguish the normal tissue from the lymph nodes and dissect lymph nodes as much as possible[6], markers can be injected around the tumor to stain the lymph nodes. Currently, the most commonly used lymphatic tracer is indocyanine green (ICG), whose mechanism of action is as follows: (1) After injection around the tumor, some ICG binds to albumin in the tissue and remains locally, therefore the tumor is colored under fluorescent laparoscopy, some of the ICG gradually combines with albumin in lymphatic vessels and drains to the lymph nodes, finally returning to the blood circulation, and metabolizes in the liver [6]. The slow flow rate of lymphatic fluid and the presence of lymph nodes make the lymphatic system slow to transport ICG, so ICG can persist in the lymphatic system for a long time, and this is how ICG is applied to lymph node dissection, but the application of ICG requires special fluorescence laparoscopy; (2) Methylene blue is a lymphatic contrast agent that is easy to prepare and inject. After injection, methylene blue enters the lymphatic and blood capillaries, quickly turning the surrounding tissues blue, but its diffusion is too fast, so lymph nodes must be identified and removed quickly[7]. Therefore, due to its rapid diffusion, some lymph nodes may be missed; and (3) Nanocarbon suspension injection, as a new type of tracer, has stable physical and chemical properties and a strong affinity for lymphatic tissue. The nanometer carbon lymph tracer tag is injected near the tumor tissue. Within a short
time, it is taken up by the macrophages, accumulated in the lymph vessels, and remains in the lymph nodes due to its high lymphoid affinity. The lymph nodes are stained black, which serves the purpose of staining the draining lymph nodes near the tumor tissue[8].

In this study, preoperative endoscopic injection of carbon nanoparticles was selected due to the obvious contrast after the labeling of carbon nanoparticles, longer tissue fixation time, high lymph node affinity, and lack of need for special instruments and other characteristics. In gastric cancer and other gastrointestinal malignancies, there are still no specific guidelines for when preoperative labeling should be performed. On the basis of previous research, we extended the time of preoperative submucosal tracer injection, and the experimental group was divided into three: operation day (2-6 h) labeling group, preoperative 1-d (18-24 h) labeling group and preoperative 2-d (42-48 h) labeling group. The differences in the number of lymph nodes detected and the number of black-staining lymph nodes between the three groups and the control group were compared. The results provide direction for further research.

MATERIALS AND METHODS

Clinical data
We prospectively analyzed 307 patients with advanced gastric cancer who were hospitalized for surgery in the Department of General Surgery of Weifang People’s Hospital between June 2018 and February 2021. According to the different primary location of the tumor in the stomach, laparoscopic subtotal gastrectomy (LSG) or laparoscopic total gastrectomy (LTG) was selected, and D2 Lymph node dissection was performed for all patients. The lymph nodes that needed to be dissected for different surgical procedures are shown in Table 1. There were 180 patients in the experimental group and 127 in the control group. To determine the preoperative labeling time, patients were randomly allocated to receive endoscopic labeling with nanocarbon suspension in the experimental group on the day of surgery, 1 d before surgery, and 2 d before surgery. The control group was not labeled with nanocarbon and the other treatment measures were the same.

Inclusion criteria
The inclusion criteria were: (1) Signed informed consent was given for surgery; (2) Gastroscopic examination and pathological biopsy confirmed malignant tumor of the stomach; (3) Abdominal enhanced computed tomography or magnetic resonance imaging and other ancillary examinations showed no distant metastasis; (4) No important organ dysfunction and could tolerate surgery; and (5) Clinical stage advanced gastric cancer.

Exclusion criteria
The exclusion criteria were: (1) Secondary examination suggested multiple metastases without surgical indications; (2) Patients with important organ dysfunction or other major diseases and could not tolerate surgery; and (3) Other contraindications and could not undergo D2 radical surgery.

Nanocarbon labeling method
When performing the preoperative nanocarbon labeling, if the submucosal injection level is too shallow, the nanocarbon could not enter the lymphatic flow to mark cancer tissue, and if the injection level is too deep, the nanocarbon would penetrate the serosal membrane and pollute the surgical field of view. Therefore, the sandwich labeling method was adopted: and 2-4 points were selected at 0.5-1.0 cm from the tumor edge. Normal saline was first injected to raise the submucosa, nanocarbon was injected into the submucosal surface, and subsequently, normal saline was injected again to increase the pressure of submucosal carbon nanoparticles suspension, so that the carbon nanoparticles could easily penetrate into the lymphatic tissues. A total of 2.0 mL nanocarbon suspension was injected. Radical proximal gastric cancer resection was not considered, and all patients were labeled at the oral but not at the anal end. Figure 1 shows preoperative carbon nanoparticles injection, and Figure 2 shows intraoperative black staining of lymph nodes and surrounding tissues.

Surgical method
All 307 patients received standard gastric cancer resection and D2 Lymph node
Table 1 Lymphnodes dissection extent in laparoscopic total gastrectomy

<table>
<thead>
<tr>
<th>Lymphnodes dissection extent</th>
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<td>D2</td>
<td>D1 + No. 8a, 9, 10, 11p, 11d, 12a</td>
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<tr>
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<tr>
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<td>No. 1, 3, 4sb, 4d, 5, 6, 7</td>
</tr>
<tr>
<td>D2</td>
<td>D1 + No. 8a, 9, 11p, 12a</td>
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</table>

For tumors invading the esophagus, D2 includes No. 19, 20, 110 and 111. LTG: Laparoscopic total gastrectomy; LSG: Laparoscopic subtotal gastrectomy.

Figure 1 The figure shows preoperative carbon nanoscale markers.

Figure 2 The figure shows intraoperative carbon nanoparticles and black-stained lymph nodes.

dissection. According to different gastric cancer locations, 176 patients underwent LTG and 131 patients underwent LSG. LTG lymph node dissection and anastomosis procedures were as follows: The gastrocolonic ligament was dissected along the transverse colon, the right gastroomental vessel was ligated at the root, and No. 6 Lymph nodes were dissected. The anterior lobe of the hepatoduodenal ligament was separated and resected, the right gastric vessel was severed and ligated, and No. 5 and 12 Lymph nodes were dissected. Duodenum was severed from the lower duodenal bulb of pylorus with a linear stapler. The stomach was turned to the left upper abdomen, the left gastric vessels were ligated at the root and cut off, and No. 7, 8, 9, and 11 Lymph nodes were dissected. The hepatogastric ligament was opened, and the No. 1 Lymph nodes were dissected along the right side of cardia. The greater curvature omentum was removed, the left gastro-omental artery was ligated at the root, the spleen and stomach ligaments and the posterior gastric artery were severed, and the No. 2, 4, and 10 Lymph nodes were dissected. The Esophagojejunal (π) anastomosis and Braun anastomosis were performed.
LSG lymph node(M1) dissection and anastomosis procedures were: The loose connective tissue between the anterior and posterior lobes of the right transverse mesocolon was extended gradually to the left until the first short gastrovesSEL behind the root of the left gastroomentum artery. The No. 6 Lymph nodes were dissected from the right omentum vessel. Hepatoduodenal ligament was opened, and No. 5, and 12a lymph nodes were dissected along the proper hepatic artery. The root of the right gastric artery was exposed and along the main trunk of the right gastric artery, the surrounding soft tissue was dissected, the left gastric artery and the beginning part of the coronary vein were exposed, the root of the vessel was ligated, and the surrounding No. 7, 8a, 9, and 11p lymph nodes were dissected. Mesangial tissue was isolated along the lesser curvature of the stomach till the right diaphragm, and No. 1, 3a, 3b and 5 Lymph nodes were dissected. Billroth II and Braun anastomosis was performed. All procedures were performed by the same group of surgeons.

**Postoperative lymph node sorting**

After the surgical specimen was isolated, the senior attending physician placed it according to its anatomical position and took photographs. The lymph nodes in each group around the stomach were cut and marked according to the blood vessels. The tissues in each group were finely separated and the surface tissues of lymph nodes were removed, and bagged separately. All lymph nodes were sent to the pathology department for postoperative analysis according to their corresponding perigastric lymph node groups. For the patients with total gastrectomy, the gastric peripheral lymph nodes on the lower cardia side were classified as No. 1, the gastric peripheral lymph nodes on the greater cardia side were classified as No. 2, and the peripheral tissues of short gastric vessels above the left arteriovenous Hemlok clip were classified as No. 4sa. The left arteriovenous clipped tissue along the gastric omentum was classified as No. 4sb, the right arteriovenous clipped tissue along the gastric omentum was classified as No. 4d, and the subpyloric region was classified as No. 6. Ligation of the right gastric arteriovenous Hemlok clipped to the upper part of the pylorus was classified as No. 5, from the ligation of the left gastric arteriovenous Hemlok clipped to its first branch was classified as No. 7, and the remaining perigastric tissue near the lesser curvature was classified as No. 3. Figures 3 and 4 shows lymph node sorting after gastric cancer.

**Observation target**

The number of dissected lymph nodes, black-stained lymph nodes were counted, and basic information, including gender, age, pathological types, postoperative complications such as intraoperative blood loss, and anastomotic fistula, were observed.

**Statistical analysis**

SPSS 25.0 was used for statistical analysis. Measurement data were expressed as mean ± SD, and a t test was used for comparison between the two groups. One-way analysis of variance and multiple comparisons were used for intragroup comparison, and P < 0.05 was considered statistically significant.

**RESULTS**

**Basic information**

A total of 307 patients were enrolled. Gender, age, pathological type, pathological stage, tumor markers, intraoperative blood loss (significant), postoperative complications and other indicators are shown in Table 2, and the differences were not significant.

**Comparison between groups**

There were 180 patients in the experimental group (99 treated with LTG and 81 with LSG), and a total of 6105 Lymph nodes (3460 LTG and 2645 LSG) were detected, with an average of 34.95 ± 4.81/case for LTG and 32.65 ± 3.82/case for LSG. There were 127 patients in the control group (77 treated with LTG and 50 with LSG), and a total of 4000 Lymph nodes (2456 LTG and 1544 LSG) were detected, with an average of 31.90 ± 4.47/case for LTG and 30.88 ± 2.69/case for LSG (Tables 3 and 4). The differences in the number of dissected lymph nodes, and number of black-stained lymph nodes at D1 and D2 stations under different surgical methods and different preoperative labeling times are shown in Tables 5 and 6.
Table 2 Basic information of 307 patients

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<td>Normal</td>
<td>43</td>
<td>24</td>
<td>50</td>
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<tr>
<td>Increase</td>
<td>34</td>
<td>26</td>
<td>49</td>
<td>47</td>
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<tr>
<td>Intraoperative blood loss (mL)</td>
<td>71.13 ± 21.33</td>
<td>90.70 ± 31.77</td>
<td>61.53 ± 20.38</td>
<td>75.69 ± 20.18</td>
<td>&lt; 0.05</td>
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<tr>
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<td>2</td>
<td>2</td>
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<tr>
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<td>0</td>
<td>0</td>
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<td>Anastomotic fistula</td>
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<td>0</td>
<td>1</td>
<td>2</td>
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<tr>
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<td>0</td>
<td>1</td>
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<tr>
<td>Postoperative hospital stay (d)</td>
<td>6.55 ± 4.63</td>
<td>7.23 ± 4.51</td>
<td>6.54 ± 4.16</td>
<td>7.21 ± 4.32</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

LTG: Laparoscopic total gastrectomy; LSG: Laparoscopic subtotal gastrectomy.

DISCUSSION

Over the past 20 years, with the progression of medical science, the comprehensive treatment of gastric cancer has made great strides. Surgical treatment is still the most used method, and lymph node dissection is one of the most important techniques in radical gastrectomy. How to remove a sufficient number of lymph nodes in gastric cancer surgery more safely and effectively to achieve the goal of radical resection has been one of the topics studied by gastrointestinal surgeons. For advanced gastric cancer, the surgical criteria were D2 or D2+ radical surgery: tumor resection and regional lymph node dissection. The metastasis of gastric cancer is mainly via the
Table 3 Difference in the number of dissected D1 lymph nodes in laparoscopic total gastrectomy and laparoscopic subtotal gastrectomy

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Dissected lymph nodes of D1 station</th>
<th>Average</th>
<th>Statistic difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>P value</td>
</tr>
<tr>
<td>LTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental group</td>
<td>99</td>
<td>2088</td>
<td>19.65 ± 3.08</td>
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<tr>
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<td>77</td>
<td>1590</td>
<td>21.09 ± 3.08</td>
<td>0.003</td>
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<tr>
<td>LSG</td>
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<td></td>
</tr>
<tr>
<td>Experimental group</td>
<td>81</td>
<td>1622</td>
<td>20.02 ± 2.69</td>
<td>1.700</td>
</tr>
<tr>
<td>Control group</td>
<td>50</td>
<td>965</td>
<td>19.30 ± 1.72</td>
<td>0.091</td>
</tr>
</tbody>
</table>

LTG: Laparoscopic total gastrectomy; LSG: Laparoscopic subtotal gastrectomy.

Table 4 Difference in the number of dissected D2 lymph nodes in laparoscopic total gastrectomy and laparoscopic subtotal gastrectomy

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Dissected lymph nodes of D2 station</th>
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<th>Statistic difference</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>P value</td>
</tr>
<tr>
<td>LTG</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental group</td>
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<td>1372</td>
<td>13.85 ± 2.26</td>
<td>5.059</td>
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<tr>
<td>Control group</td>
<td>77</td>
<td>943</td>
<td>12.25 ± 2.06</td>
<td>0.000</td>
</tr>
<tr>
<td>LSG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental group</td>
<td>81</td>
<td>1023</td>
<td>12.63 ± 2.22</td>
<td>2.855</td>
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<tr>
<td>Control group</td>
<td>50</td>
<td>579</td>
<td>11.58 ± 1.73</td>
<td>0.005</td>
</tr>
</tbody>
</table>

LTG: Laparoscopic total gastrectomy; LSG: Laparoscopic subtotal gastrectomy.

lymphatic pathway, so survival depends not only on the primary lesion but also on the presence of regional lymph node metastasis[9]. Baxter et al[10] found that a certain number of lymph nodes should be dissected during radical gastrectomy for gastric cancer, and there was a correlation between the number of lymph nodes dissected and prognosis. According to the clinical data of gastric cancer patients in the American Surveillance, Epidemiology, and End Results database[11,12], prognosis can be improved by the addition of 10 lymph nodes in postoperative specimens. Even for patients with negative lymph nodes after surgery, the number of detected lymph nodes is still an independent factor affecting prognosis[12]. Some researchers have reported that, in order to improve the accuracy of pathological lymph node staging of gastric cancer specimens, at least 10-15 lymph nodes should be detected in the N0 stage, and ≥ 20 should be detected in the N1-3 stage. If ≥ 30 lymph nodes are collected for examination, postoperative lymph node staging could be more accurate[13,14]. The number of dissected lymph nodes recommended by the 8th Edition of the International Union against Cancer/American Joint Committee on Cancer (UICC/AJCC) in TNM staging of gastric cancer should not be less than 16[15]. According to the Japanese Regulations on the Management of Gastric Cancer[16], the number of lymph nodes dissected during radical gastrectomy for gastric cancer should be ≥ 15, and an insufficient number of dissected lymph nodes will significantly affect the 5-year postoperative survival rate[17-19]. The more lymph nodes sent for examination, the greater the possibility of detection of metastatic lymph nodes[20]. The more reliable the accuracy of lymph node staging is, the more often the occurrence of lymph node staging migration can be avoided[21]. Therefore, effective lymph node dissection is indispensable for thorough radical treatment of gastric cancer. According to the multi-center analysis of postoperative gastric cancer survival data[22], there was an obvious postoperative lymph node stage migration phenomenon in Chinese patients with gastric cancer, especially in early-stage patients with < 15 lymph nodes dissected and
in advanced patients with < 35 Lymph nodes dissected. According to a study by Sano et al[17] in 2017, the average number of lymph nodes detected in each gastric cancer specimen in Japan reached 39.4/case, followed by 33.0/case in South Korea, while the figure for several major centers included in the survey in China was only 24.8/case—even lower than the 29.5/case in Europe and America[23]. In order to correctly distinguish between lymph node and normal tissue and to dissect the lymph nodes more thoroughly, we could selectively label the pericancerous lymph nodes. The existing lymphatic tracers can be divided into three generations: the first is represented by methylene blue and India ink, the second by iodine oil and activated carbon, and the third by nanocarbon. Nanocarbon lymph node tracers are essentially lymphatic tracers, and their physical and chemical properties have been described in the previous section. At the same time, due to the high contrast of the color, nanocarbon tracers can help surgeons to correctly distinguish the lymph nodes and normal tissues, reduce the damage to normal tissues and the time of surgical dissection, and increase the number of lymph node dissections. In recent years, nanocarbon tracers have gradually matured for malignant melanoma, breast cancer,
thyroid cancer, and some digestive malignant tumors[21]. Nanocarbon lymph node tracers can help surgeons to determine lymph node metastasis to a certain extent, and they can improve the effective removal of lymph nodes during surgery[8,24].

In this study, the injection dose of nanocarbon suspension was 2.0 mL at a total of four sites, with an average of 0.5 mL at each site. According to existing literature, the injection dose of nanocarbon was 0.4-0.6 mL in breast cancer patients[25] and 1.0 mL in thyroid cancer patients[26]. In patients with colorectal cancer, the injection dose of carbon nanoparticles was 1.0 mL[27]. Considering the deep infiltration of advanced gastric cancer and the thickness of gastric wall tissue compared with thyroid, breast and colorectal tissue, an injection dose < 2.0 mL may lead to unclear lymph node display in some patients. If the dose is > 2.0 mL, some patients may have excessively deep staining due to excessive dosing, which will affect the operation, and the sandwich injection method can be selected for preoperative labeling of carbon nanoparticles[28].

In the present study, the average number of dissected lymph nodes in the experimental group (LTG 34.95 ± 4.81/case; LSG 32.65 ± 3.82/case) was significantly higher than that in the control group (LTG 31.90 ± 4.47/case, LSG 30.88 ± 2.69/case). Under LTG operation, compared with the control group, the number of lymph nodes dissected at the D1 and D2 stations in the experimental group was significantly better than that in the control group. However, under LSG operation, the number of lymph nodes dissected at the D1 station showed no significant difference between the two groups, and the number of lymph nodes dissected at the D2 station was better than that in the control group. In a study by Cheng et al[29], the number of lymph nodes detected in the nanocarbon group and the non-nanocarbon group was 32.28 ± 4.10/case and 21.28 ± 2.74/case, respectively. In the study of Jia[30], 15484 Lymph nodes were detected in the nanocarbon group and 7963 in the non-nanocarbon group. The average number of lymph nodes detected in each patient in the nanocarbon group and non-nanocarbon group was 31.99 ± 8.99 and 19.81 ± 4.74, respectively. The results in our experimental group are consistent with the previous studies. In our study, there
were 180 patients in the experimental group, and no complications such as marker point bleeding or perforation occurred after endoscopic carbon nanolabeling. In addition, there was no significant difference in the operating time, postoperative hospital stay, and incidence of postoperative complications such as postoperative anastomotic fistula, anastomotic bleeding, and obstruction, which proved that the effectiveness and safety of nanocarbon tracers were similar to those in previous studies.

It is worth noting that our study showed that the intraoperative blood loss of the experimental group was less than that of the control group (under the same operation), which may have been due to clearly visualized lymph nodes after preoperative nanocarbon labeling, thus avoiding unnecessary tissue and vascular damage and reducing intraoperative blood loss.

Although preoperative injection of nanocarbon tracer is helpful for lymph node dissection, there is still no consensus or guidelines on the optimal time point for preoperative tracer labeling. By comparison among experimental groups in this study, we found that under the premise of the same operation and the same lymph node station, the results (number of lymph nodes detected and number stained black) of the nanocarbon labeling group 2 and 1 d before surgery were significantly better than those of the labeling group on the day before surgery. At the same time, there was no significant difference in the number of lymph nodes detected between the 2-d and 1-d preoperative labeling groups. This may be because the optimal time for imaging in tissues after injection of carbon nanoparticles is 2-12 h after injection and there was no significant difference compared with previous results.

Our study had some limitations. We only studied the influence of three different labeling time points on the results of lymph node dissection after radical resection of gastric cancer. Further determination of a more accurate labeling time of the tracer needs to be confirmed by more clinical trials. For example, whether better results can be achieved by changing the marker time to 3 or even 4 d before surgery or whether a better result can be achieved between 1 d before and on the day of surgery remains to be answered by more in-depth studies with larger sample sizes.

**CONCLUSION**

In conclusion, carbon nanoparticle labeling has a good guiding effect for laparoscopic lymph node dissection of gastric cancer, and is safe and effective. Compared with the control group, preoperative submucosal injection of carbon nanoparticles could significantly improve the detection rate of lymph nodes, which is conducive to pathological staging and subsequent comprehensive antitumor therapy.

**ARTICLE HIGHLIGHTS**

**Research background**
Gastric cancer deaths in China account for more than 40% of the global total of gastric cancer deaths in the same period. Reducing cancer-related mortality and improving quality of life is one of the current research focuses, and the number of lymph nodes dissected was an independent factor affecting postoperative staging of gastric cancer. There are still no specific guidelines for when preoperative labeling should be performed, based on many previous works, this work extended the time of preoperative submucosal tracer injection, and discuss whether it is effective.

**Research motivation**
In order to help surgeons to more lymph node dissection, and improve postoperative pathological staging; At the same time, whether the preoperative labeling time has a certain influence on the number of lymph node dissection was studied.

**Research objectives**
To study the influence of preoperative carbon nanoparticle labeling combined with radical gastrectomy on the number of dissected lymph nodes and postoperative anti-tumor treatment effect, and to study the influence of preoperative labeling time on the number of dissected lymph nodes.
Research methods
Retrospective analysis study was performed, all patients were randomly divided into experimental group (preoperative injection of carbon-nano group) and control group (preoperative injection of carbon-nano group) according to the principle of randomization; In the experimental group, according to the different groups of preoperative labeling time, the differences between the groups were studied.

Research results
The average number of dissected lymph nodes in the experimental group [34.95 ± 4.81/case in the laparoscopic total gastrectomy (LTG) group; 32.65 ± 3.82/case in the laparoscopic subtotal gastrectomy (LSG) group] was higher than that in the control group (31.90 ± 4.47/case in the LTG group; 30.88 ± 2.69/case in the LSG group, P < 0.05). In comparisons within the experimental group, the experimental results (number of lymph node dissections, number of black-staining lymph nodes) of the nano-carbon labeling group 2 and 1 d before surgery were better than those of the labeling group on the day before surgery (P < 0.05).

Research conclusions
(1) Nano-carbon labeling has a good guiding effect on lymph node dissection during laparoscopic gastric cancer, and it is safe and effective; and (2) Compared with the control group, submucosal injection of a carbon tracer in the experimental group at a certain time before surgery can significantly improve the lymph node detection rate (P < 0.05), which is conducive to pathological staging and follow-up anti-tumor comprehensive treatment.

Research perspectives
Gastric cancer is the fifth most common cancer in the world, radical operation is still the preferred treatment method for advanced gastric cancer, postoperative lymph node detection rate is one of the major factors affecting PN staging of lymph node metastasis after radical gastrectomy. In order to help the surgeon correctly distinguish the normal tissue from the lymph nodes and dissect lymph nodes as much as possible, endoscopic injection of carbon nanoparticles was selected.

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Craving variations in patients with substance use disorder and gambling during COVID-19 lockdown: The Italian experience

Maria Chiara Alessi, Giovanni Martinotti, Domenico De Berardis, Antonella Sociali, Chiara Di Natale, Gianna Sepede, Daniela Pia Rosaria Cheffo, Laura Monti, Pietro Casella, Mauro Pettorruso, Stefano Sensi, Massimo Di Giannantonio

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Author contributions: Martinotti G and Alessi MC designed the study and wrote the protocol; Sociali A and Di Natale C conducted literature searches and provided summaries of previous research studies; Sepede G conducted the statistical analysis; Casella P, Martinotti G, Sociali A, Cheffo DPR, Monti L, De Berardis D, and Sensi S recruited the patients with COVID; Martinotti G, Pettorruso M, and Di Giannantonio M wrote the first draft of the manuscript; and all authors contributed to and have approved the final manuscript.

Institutional review board statement: This study was approved by the Institutional Review Board.

Abstract

BACKGROUND
Following the development of the coronavirus disease-2019 (COVID-19) pandemic in Italy, a strict lockdown was imposed from March 9 to May 5, 2020. The risks of self-medication through alcohol or psychoactive substance abuse were increased, as well as the tendency to adopt pathological behaviors, such as gambling and internet addiction.

AIM
To evaluate the impact of the COVID-19 pandemic and associated containment measures on craving in a group of patients suffering from substance use disorder and/or gambling disorder who were in treatment in outpatient units or in residency programs as inpatients.

METHODS
One hundred and fifty-three patients completed a structured questionnaire evaluating craving and other behaviors using a visual analogue scale (VAS).
Forty-one subjects completed a pencil and paper questionnaire during the interview. The clinician provided an online questionnaire to 112 patients who had virtual assessments due to lockdown restrictions. Statistical analyses were performed using Statistica version 8.0. Quantitative parameters are presented as the mean ± SD and qualitative parameters as number and percentage per class. The Kolmogorov-Smirnov test was used to check for normality of distributions. Analysis of variance and Duncan post hoc test were employed to analyze differences among subgroup means. The associations between variables were measured using Pearson’s correlation. A P value of < 0.05 was considered significant.

**RESULTS**

The variation in craving between the present and the month before showed VAS-related reductions of craving in 57%, increases in 24%, and no significant change in 19% of the sample. The level of craving was significantly higher ($F = 4.36; P < 0.05$) in outpatients ($n = 97$; mean $= 3.8 ± 3.1$) living in their own home during the quarantine compared with inpatients ($n = 56$; mean $= 2.8 ± 2.8$) in residential programs. Craving for tetrahydrocannabinol was the greatest ($4.94, P < 0.001$) among various preferred substances.

**CONCLUSION**

The unexpected result of this study may be explained by a perceived lack of availability of substances and gambling areas and/or decreased social pressure on a subject usually excluded and stigmatized, or the acquisition of a new social identity based on feelings of a shared common danger and fate that overshadowed the sense of exclusion and rejection in the abuser.

**Key Words:** Substance use disorder; Addiction; COVID-19; Craving; Psychopathology

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**Core Tip:** Our data suggest that craving, regardless of whether determined by substances or behaviors, was globally reduced in a period that could be highly stressogenic such as the coronavirus disease-2019 lockdown.


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**DOI:** https://dx.doi.org/10.12998/wjcc.v10.i3.882

**INTRODUCTION**

Following the development of the coronavirus disease-2019 (COVID-19) pandemic in Italy, a strict lockdown was imposed from March 9 to May 5, 2020. In the general population, problems such as depression, anxiety, post-traumatic stress symptoms, insomnia, and adjustment disorder symptoms increased[1]. The risks of self-medication through alcohol or psychoactive substances abuse were also increased, as well as the tendency to adopt pathological behaviors, such as gambling and internet addiction[2,3]. Stressors are essential in the inception and protraction of substance use disorder (SUD). Many stressors are associated with lockdown conditions such as prolonged home confinement, depression and panic related to the disease’s uncertainties, working from home, and fear of job loss. People exposed to these stressors may take refuge in addictive substances, increasing SUD incidence among the general population[4] in a post-modern society that is increasingly oriented towards the use of substances, favoring the development of symptoms of psychopathological interest[5]. The COVID-19 pandemic and lockdown are risk conditions for developing internet, videogames, or other addiction, decreased physical activity and
Among 153 subjects that completed the questionnaire, the primary substances of abuse were measured using Pearson’s correlation. A VAS ranging from 0 (I do not use it/I do not do this anymore) to 10 (I use it/I do this much more than before) was employed. To assess changes in quality of life, we utilized a VAS ranging from 0 (my life is much worse than before) to 10 (my life is much better than before) (see Appendix D: Questionnaire).

In this study, we evaluated the impact of the COVID-19 pandemic and associated containment measures on craving, a prominent risk factor for relapse in a group of patients suffering from SUD and/or gambling disorder (GD) who were in treatment in outpatient units or in residency programs as inpatients.

MATERIALS AND METHODS

This study was commissioned by the Italian Society of Psychiatry and conducted at the University 'Gabriele d'Annunzio' of Chieti-Pescara during the Italian lockdown phase that lasted from March 3 to May 5, 2020. Recruitment centers were randomly chosen among all the structures providing services for SUD and GD patients in regions of Northern (Piemonte, Lombardia), Central (Lazio, Marche), and Southern Italy (Abruzzo, Calabria) (see Appendix A: List of recruitment centers). Randomization procedures were computerized (see Appendix B: Explanation of randomized procedures). Three online meetings were held to train clinicians to the administration of the questionnaire, before the study started. In each recruitment center, a clinician introduced the survey to all the eligible subjects. No compensation was provided for participation in the study. Of the 253 subjects recruited, 153 (mean age 39.8; 77.8% male) gave their consent and anonymously completed the questionnaire. Forty-one subjects completed a pencil and paper questionnaire during the interview. The clinician provided an online questionnaire to 112 patients who had virtual assessments due to lockdown restrictions. Questionnaires were anonymous and each subject was identified through a unique code with no other identifying data. Anonymity was maintained by placing the completed questionnaires in a box by the subject himself, so that the clinician could not associate the subject with his/her questionnaire. All participants provided informed consent. The inclusion criteria were: (1) Diagnosis of SUD or GD according to The Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders; and (2) being older than 18 years. The exclusion criteria were: (1) Diagnosis of dementia; and (2) refusal to give informed consent.

Our survey was organized into two sections. In the first section, we collected anamnestic and clinical variables (see Appendix C: List of anamnestic and clinical variables). In the second section, using a visual analogue scale (VAS), we asked the subjects to indicate the craving level for the primary substance of abuse and how much their craving and habits have changed from the beginning of lockdown. We chose to use the VAS because of its immediacy and extensive utilization to evaluate craving in addicted patients[13,14]. We investigated changes of: (1) Craving for substances and gambling; and (2) quality of life and life habits (Table 1). A VAS ranging from 0 (I do not use it/I do not do this anymore) to 10 (I use it/I do this much more than before) was employed. To assess changes in quality of life, we utilized a VAS ranging from 0 (my life is much worse than before) to 10 (my life is much better than before) (see Appendix D: Questionnaire).

Statistical analyses were performed using Statistica version 8.0. Quantitative parameters are presented as the mean ± SD and qualitative parameters as number and percentage per class. The Kolmogorov-Smirnov test was used to check for normality of distributions. Analysis of variance and Duncan post hoc test were employed to analyze differences among subgroup means. The associations between variables were measured using Pearson’s correlation. A P value of < 0.05 was considered significant.

RESULTS

Among 153 subjects that completed the questionnaire, the primary substances of abuse or pathological behavior are reported in Table 1.
Alessi MC et al. Craving variations in SUDs during COVID-19 lockdown

<table>
<thead>
<tr>
<th>Substance/pathological behavior</th>
<th>n</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Cocaine</td>
<td>66</td>
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</tr>
<tr>
<td>Alcohol</td>
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</tr>
<tr>
<td>THC</td>
<td>24</td>
<td>15.7</td>
</tr>
<tr>
<td>Gambling</td>
<td>12</td>
<td>7.9</td>
</tr>
<tr>
<td>Heroin</td>
<td>9</td>
<td>5.7</td>
</tr>
<tr>
<td>Ketamine</td>
<td>1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

THC: Tetrahydrocannabinol.

Sixty-seven (43.8%) of the participants reported a comorbid psychiatric condition, especially mood disorders (depression, bipolar disorder) and anxiety. In this subsample, a psychopharmacological treatment was reported by 94% of subjects. The variation in craving between the present and the month before showed VAS-related reductions of craving in 57%, increases in 24%, and no significant change in 19% of the sample (Figure 1).

The level of craving was significantly higher ($F = 4.36; P < 0.05$) in outpatients ($n = 97$; mean = 3.8 ± 3.1) living in their own home during the quarantine compared with inpatients ($n = 56$; mean = 2.8 ± 2.8) in residential programs. Craving for tetrahydrocannabinol was the greatest (4.94, $P < 0.001$) among various preferred substances (Figure 2).

Patients with a dual diagnosis ($n = 67$; mean craving VAS = 3.9) did not show a significant difference in the levels of craving [$F (1; 150) = 2.43, P > 0.121$] with respect to patients without psychiatric comorbidities ($n = 86$; mean craving VAS = 3.1).

Overall, we observed an increased consumption of coffee and cigarettes in about half of the sample. In contrast, symptoms indicative of behavioral addictions and other substances’ consumption remained almost stable (Table 2). Changes in life habits are shown in Table 2. Reduced quality of life due to COVID-19 driven by the lockdown was present in 51% of the patients; 25.5% declared no significant changes, and, surprisingly, 23.5% increased quality of life. Low levels of quality of life correlated with high craving scores ($r = -0.226, P = 0.005$).

**DISCUSSION**

In this study, the recruited group of patients with a diagnosis of SUD represents a real-life sample that reflects the Italian addiction scenario, including patients known by local services of seven different representative Italian regions. The evaluation of craving scores during the first phases of the COVID pandemic represents a relevant point, given the presence in that period of strict lockdown restrictions. Although other studies evaluated the psychopathological burden of alcohol and substance users during the strict lockdown[15,16], the specific evaluation of craving represents a novel and relevant aspect, given the crucial role of craving in treatment strategies. Different studies[17,18] have proposed that, together with negative affect states, cognitive factors, interpersonal problems, and lack of coping, craving is one of the leading risk factors for relapse[19]. Our data suggest that craving, regardless of whether determined by substances or behaviors, was globally reduced in a period that could be highly stressogenic. This data was unexpected, and is in contrast with other studies reporting increased levels of anxiety, depression, and psychotic symptoms during the early phase of the COVID-19 pandemic[20,21]. Moreover, in alcohol and substance users, other detrimental factors can act synergistically during the pandemic period: Psychological discomfort from social isolation, restricted freedom, and a quantitative and qualitative reduction in the addiction services’ assistance and in the stretching of their service.

In order to explain this controversial data, we propose the hypothesis of a perceived lack of availability of substances and gambling areas. Practical difficulties in sources of supply, such as the unavailability of the usual dealing spaces, may have interrupted the development of the craving priming. Craving is usually determined by the possibility to obtain a substance. When external measures limit this possibility, craving...
Table 2 Changes in consumption habits, behavioral addictions, and daily activities

<table>
<thead>
<tr>
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<th>Reduced</th>
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<td>n</td>
<td>n</td>
</tr>
<tr>
<td></td>
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<td><strong>Changes in consumption habits</strong></td>
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<td>14.4</td>
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<td>Coffee</td>
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<td>6.5</td>
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<td>Cigarettes</td>
<td>7</td>
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<td>Cocaine</td>
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<td>Opioids</td>
<td>7</td>
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<td>4.6</td>
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<td>Benzodiazepines and similar medical drugs</td>
<td>7</td>
<td>120</td>
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<td>14</td>
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<td>9.2</td>
<td>86.3</td>
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<td>Eating</td>
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<td>6.5</td>
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<td>Videogames</td>
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<td>115</td>
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<td></td>
<td>5.2</td>
<td>75.2</td>
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<td><strong>Changes in time spent for the following activities</strong></td>
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<td>Instant messaging with friends and relatives</td>
<td>6</td>
<td>66</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
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<td>51.6</td>
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<tr>
<td>Social network (for fun, reading)</td>
<td>7</td>
<td>72</td>
<td>73</td>
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<tr>
<td></td>
<td>4.6</td>
<td>47.1</td>
<td>47.7</td>
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<tr>
<td>Video calls with friends and relatives</td>
<td>8</td>
<td>60</td>
<td>84</td>
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<tr>
<td></td>
<td>5.2</td>
<td>39.2</td>
<td>54.9</td>
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<tr>
<td>Collecting online information about the current situation</td>
<td>9</td>
<td>81</td>
<td>62</td>
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<td>40.5</td>
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<td>Old and new hobbies</td>
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<td>Sports</td>
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<td>19.6</td>
<td>48.4</td>
<td>31.4</td>
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<td>Watching movies, TV shows</td>
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<td>5.9</td>
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<td>60.1</td>
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<tr>
<td>Watching pornographic material</td>
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<td>7.8</td>
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<tr>
<td>Hours of sleep each day</td>
<td>27</td>
<td>42</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>17.6</td>
<td>27.5</td>
<td>54.2</td>
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</tbody>
</table>

itself could be dramatically reduced, as the case of the strict lockdown. Second, we hypothesize the presence of decreased social pressure on a group of subjects who are usually excluded and stigmatized. Social exclusion is indeed a psychosocial stress factor[22] that can increase craving and drug use[23]. As social identification is the self-definition of a person in terms of group membership[10], the period of lockdown because of the COVID-19 pandemic can favor personal feelings of being part of a group facing a common danger and sharing a common fate. Therefore, this new social identity might overshadow the sense of exclusion and rejection in the abuser, ultimately with the positive outcome of reducing craving and substance abuse. This possibility is consistent with data from a survey released by the Israel Democracy Institute that showed how the sense of belonging and unity increased during the COVID-19 outbreak among groups usually sidelined[24]. In this direction, the use of a specific strategy such as telepsychiatry acquires great importance for careful monitoring of the patient’s clinical and psychopathological conditions, in order to prevent relapses and to promote social integration[25].

Our data also indicates that residential treatment in containment facilities during the quarantine is an effective procedure that positively impacts craving levels, probably reinforcing the first hypothesis regarding the unavailability of the substance as a means to reduce craving.

In line with other studies, our data showed an increase in the consumption of coffee and cigarettes. Increased cigarette use could be explained as a natural response to stressful events, especially as a consequence of depressive symptoms; the consumption of coffee could be determined by the tendency towards sugary foods and drinks, in order to find quick relief in stressful times[26,27].
Changes in craving levels

- Reduced: 51%
- Increased: 32%
- Unchanged: 17%

Figure 1 Changes in the levels of craving for the primary substance of abuse/gambling among 153 addicted subjects.

Figure 2 Craving visual analogue scale during quarantine period, with significantly higher craving for tetrahydrocannabinol vs other substances/gambling (ANOVA with Duncan post-hoc test; *P < 0.05; means and standard errors of means). VAS: Visual analogue scale; THC: Tetrahydrocannabinol.

It is also interesting to note that a relevant part of the sample reported reduced quality of life during the strict lockdown, with a negative correlation between craving and perceived quality of life. This data leads us to hypothesize that despite a substantial reduction in the perceived quality of life, the levels of craving have in any case been reduced, as a counter-proof of how much the unavailability of the substance and the increase in social integration may have had a direct positive effect on the reduction of craving.

**Limitations**

The main limitation of our study is the high prevalence of cocaine abusers. This demographic feature is different from other treatment-seeking cohorts where alcohol is generally the main substance of abuse. This discrepancy is probably because our recruitment centers are specialized in the treatment of cocaine use disorder. Another limitation of the study is the use of a VAS instead of validated scales. We chose to use VAS because of its immediacy to homogenize and accelerate the completion of the questionnaire, making it suitable also online during the virtual assessments due to lockdown restrictions. Our results are difficult to generalize because of the brief time of observation, and further studies are needed.
CONCLUSION

Our data suggest that craving was globally reduced in a period that could be highly stressogenic. This unexpected result may be explained by: (1) A perceived lack of availability of substances and gambling areas that interrupted the development of the craving priming; and (2) the presence of a decreased social pressure. Our results can lay the groundwork for future treatment policies in the direction of strategies that limit the availability of the substance and in parallel towards strategies that aim at greater social integration of subjects affected by addiction disorders.

ARTICLE HIGHLIGHTS

Research background
Following the development of the coronavirus disease-2019 (COVID-19) pandemic in Italy, a strict lockdown was imposed from March 9 to May 5, 2020. In the general population, problems such as depression, anxiety, post-traumatic stress symptoms, insomnia, and adjustment disorder symptoms increased. The risks of self-medication through alcohol or psychoactive substances abuse were also increased, as well as the tendency to adopt pathological behaviors, such as gambling and internet addiction.

Research motivation
Substance users and gamblers are groups at risk of developing psychopathological symptoms in a lockdown situation. The phenomenon is likely due to various reasons, including: (1) The limited availability of illegal substances on the black market; (2) the insufficient presence of active treatment programs and the low availability of substitute drugs; and (3) the greater psychopathological susceptibility and lower resilience in a period of reduced economic resources and financial hardship.

Research objectives
The objective of this study was to evaluate the impact of the COVID-19 pandemic and associated containment measures on craving in a group of patients suffering from SUD and/or gambling disorder (GD) who were in treatment in outpatient units or in residency programs as inpatients.

Research methods
In this cross-sectional study, 153 patients completed a structured questionnaire evaluating craving and other behaviors using a visual analogue scale (VAS). In each recruitment center, a clinician introduced the survey to all the eligible subjects. No compensation was provided for participation in the study. Of the 253 subjects recruited, 153 (mean age 39.8; 77.8% male) gave their consent and anonymously completed the questionnaire. Forty-one subjects completed a pencil and paper questionnaire during the interview. The clinician provided an online questionnaire to 112 patients who had virtual assessments due to lockdown restrictions. Questionnaires were anonymous and each subject was identified through a unique code with no other identifying data. Anonymity was maintained by placing the completed questionnaires in a box by the subject himself, so that the clinician could not associate the subject with his/her questionnaire. All participants provided informed consent. The inclusion criteria were: (1) Diagnosis of SUD or GD according to The Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders; and (2) being older than 18 years. The exclusion criteria were: (1) Diagnosis of dementia; and (2) refusal to give informed consent.

Research results
Sixty-seven (43.8%) of the participants reported a comorbid psychiatric condition, especially mood disorders (depression, bipolar disorder) and anxiety. In this subsample, a psychopharmacological treatment was reported by 94% of subjects. The variation in craving between the present and the month before showed VAS-related reductions of craving in 57%, increases in 24%, and no significant change in 19% of the sample. The level of craving was significantly higher \( (F = 4.36; P < 0.05) \) in outpatients \( (n = 97; \text{mean } = 3.8 \pm 3.1) \) living in their own home during the quarantine compared with inpatients \( (n = 56; \text{mean } = 2.8 \pm 2.8) \) in residential programs. Craving for tetrahydrocannabinol was the greatest \( (4.94, P < 0.001) \) among various preferred substances. Patients with a dual diagnosis did not show a significant difference in the levels of...
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craving [F (1; 150) = 2.43, P > 0.121] with respect to patients without psychiatric
comorbidities (n = 86; mean craving VAS = 3.1). Reduced quality of life due to COVID19 driven by the lockdown was present in 51% of the patients; 25.5% declared no
significant changes, and, surprisingly, 23.5% increased quality of life. Low levels of
quality of life correlated with high craving scores (r = -0.226, P = 0.005).

Research conclusions
Our data suggest that craving, regardless of whether determined by substances or
behaviors, was globally reduced in a period that could be highly stressogenic. This
data leads us to hypothesize that despite a substantial reduction in the perceived
quality of life, the levels of craving have in any case been reduced, as a counter-proof
of how much the unavailability of the substance and the increase in social integration
may have had a direct positive effect on the reduction of craving.

Research perspectives
Our results can lay the groundwork for future treatment policies in the direction of
strategies that limit the availability of the substance and in parallel towards strategies
that aim at greater social integration of subjects affected by addiction disorders.

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Concetta Incerti Chiara, Bartoletti Luigi, Barlati Stefano, Romeo Vincenzo Maria, and
Valchera Alessandro.

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Mesh safety in pelvic surgery: Our experience and outcome of biological mesh used in laparoscopic ventral mesh rectopexy

Anastasia Tsiaousidou, Linda MacDonald, Kawan Shalli

BACKGROUND
Laparoscopic ventral mesh rectopexy (LVMR) continues to be a popular treatment option for rectal prolapse, obstructive defecation/faecal incontinence and rectoceles. In recent years there have been concerns regarding the safety of mesh placements in the pelvis.

AIM
To assess the safety of the mesh and the outcome of the procedure.

METHODS
Eighty-six patients underwent LVMR with Permacol (Biological) mesh from 2012 to 2018 at University Hospital Wishaw. Forty were treated for obstructive defecation secondary to prolapse, rectocele or internal rectal intussusception, 38 for mixed symptoms obstructive defecation and incontinence, 5 for pain and bleeding secondary to full thickness prolapse and 3 with symptoms of incontinence. Questionnaires for the calculation of Wexner scores for constipation and incontinence were completed by the patients who were followed up in the clinic 12 wk after surgery and again in 6-12 mo. The average review of their notes was 18.3 ± 4.2 mo.

RESULTS
The median Wexner scores for constipation pre-operatively and post-operatively were 14.5 [Interquartile range (IQR): 10.5-18.5] and 3 (IQR: 1-6), respectively, while the median Wexner score for faecal incontinence was 11 (IQR: 7-15) and 2 (IQR: 0-5), respectively (P < 0.01). There were 4 (4.6%) recurrences, 2 cases that presented with erosion of a suture through the rectum and one with diskitis. No mesh complications or mortalities were recorded.

CONCLUSION
LVMR using a Permacol mesh is a safe and effective procedure for the treatment
of obstructive defecation/faecal incontinence, rectal prolapse, rectoceles and internal rectal prolapse/intussusception.

**Key Words:** Rectopexy; Prolapse surgery; Biological mesh; Pelvic floor disorders; Treatment

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**Core Tip:** Our study adds more evidence to support that laparoscopic mesh rectopexies using biological mesh is a safe and effective procedure and that it significantly improves bowel symptoms of obstructive defecation and faecal incontinence in patients. In our study, there were no mesh related complications, and the recurrence rates were in line with the ones reported in the literature. Although we acknowledge that the direct follow-up period was short, the absence of re-referral of those previously operated patients over the period of 5 years indirectly suggests the safety of the mesh over longer periods.

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**DOI:** https://dx.doi.org/10.12998/wjcc.v10.i3.891

**INTRODUCTION**

Laparoscopic ventral mesh rectopexy (LVMR) has recently become the preferred treatment for full thickness rectal prolapse, and it has been also widely used in the treatment of rectoceles, enteroceles and rectal intussusception with associated symptoms of obstructive defecation with or without faecal incontinence[1]. The procedure has good short term and long term results with minimum morbidity rates and low recurrence rates[2], particularly when compared to the perineal surgical approach used for treatment of rectal prolapse[2]. In addition, due to reduced postoperative complications, a shorter length of hospital stay is an advantage[1,2].

Over the last few years there have been concerns about the usage of meshes in pelvic surgery, especially since serious complications have been recorded in urogynaecology procedures where trans-vaginal placement of mesh in women was used to treat pelvic organ prolapse. This led many countries to scrutinise the use of mesh. This was particularly the case in the United Kingdom with the Scottish Government being the first to halt the use of trans-vaginal mesh in 2014[3]. However the incidence of mesh-related complications, and particularly mesh erosion, after LVMRs is low, especially when a biological mesh is used[4]. This was shown by Balla et al[4] in their review of literature where they demonstrated that the synthetic and the biological mesh-related erosion rates were 1.87% and 0.22%, respectively.

Furthermore, there is evidence that using biological mesh such as Permacol in LVMR results in significant improvement in function and quality of life outcomes, including improvement of urogynaecological symptoms[5]. In addition, latest results were comparable to synthetic mesh in terms of recurrence[5].

The aim of this study was to investigate the outcomes of LVMR using a biologic mesh in a district general hospital in an era where there is concern regarding the placement of pelvic mesh. We assessed the outcome of the procedure in relation to complications, bowel function and recurrences of symptoms following surgery.

**MATERIALS AND METHODS**

This is a retrospective study of 86 consecutive patients that underwent LVMR from June 2012 to August 2018 in University Hospital of Wishaw. For 40 of them obstructive defecation was the main symptom, for 38 it was both obstructive defecation and faecal incontinence, 5 (5.8%) presented with pain and bleeding related to full thickness rectal prolapse/intussusception, 5 (5.8%) presented with pain and bleeding related to full thickness rectal prolapse/intussusception.
prolapsed and 3 with mainly symptoms of faecal incontinence. All patients had a full history and physical examination, and a lower gastrointestinal endoscopic assessment. All, except those with obvious full thickness rectal prolapse, underwent a defecating proctogram, while 9 of them (10%) had anorectal physiology studies. Seven (0.08%) patients with not so clear symptoms and findings required an examination of the anorectum under general anaesthesia prior to the procedure. A detailed obstetric and pelvic surgery history was taken for women, and following formal development of Pelvic Floor multidisciplinary, all the patients were discussed on a monthly basis at the pelvic floor multidisciplinary team (Table 1).

The functional outcomes for these patients were calculated using the Wexner scoring system for constipation and incontinence before and after surgery. All patients had a follow-up appointment in the clinic 3 mo after surgery and further follow-up 6-12 mo later. We also reviewed the notes on average 18.3 ± 4.2 mo after the procedure. Clinical outcomes of surgery and any complications resulting from surgery were recorded in the Pelvic Floor Society hosted national database.

**Surgical technique**

At University Hospital Wishaw all LVMR procedures from June 2012 to August 2018 were performed by the same colorectal surgeon. After creating pneumoperitoneum and inserting the working ports (12 mm port on the right iliac fossa, 5 mm supra umbilical port and a 5 mm port in the right abdomen, the pelvic peritoneum at sacral promontory was opened using hook diathermy and continued distally and anteriorly down to the level of the levator muscles, while preserving the lateral ligaments and the hypogastric and sacral nerves. The biological porcine skin mesh that was used for all cases (permacol 4 × 18 cm long and 1 mm thick) was sutured as distally as possible onto the anterior rectal wall using interrupted seromuscular nonabsorbable sutures (2-0 Ethibond, Ethicon Endosurgery, Raritan, NJ, United States) and the upper part of the mesh was fixed to the sacral promontory using 4-5 spiral attachments (Pro-Tack™ Fixation Device, Medtronic, Dublin, Ireland). Also, the gap between vagina and mesh was closed in women using 2.0 PDS (Figure 1).

The peritoneum was closed over the mesh with a continuous suture (V-lock 180, 15 cm). Perioperative care was conducted per the enhanced recovery after surgery protocol. A urinary catheter was inserted after the patient was anesthetised and was removed on the first post-operative day.

**Statistical analysis**

Pre-operative and post-operative Wexner score values for constipation and incontinence were inserted in tables. The median and interquartile range (IQR) values were calculated, and comparison and analysis between pre-operative and post-operative values were performed using the Wilcoxon signed rank test. Complication and recurrence rates were evaluated and analysed using the Kaplan-Meier method. A \( P \) value < 0.05 was considered as significant. Libreoffice Calc 6.2.8 was used for the calculations (The Document Foundation).

**RESULTS**

A total of 86 patients underwent LVMR from June 2012 to August 2018. Eighty-two (95%) were female and 4 (5%) were male with a median age of 57 years (IQR: 47-70). The median hospital stay was 1 d (IQR: 1-2). The first follow-up of the patients was at 3 mo, and the second one was 6-12 mo after surgery.

The pre-operative Wexner scores were calculated during the first visit to the clinic, usually 6-9 mo prior to surgery, while the post operative Wexner scores for constipation and incontinence were calculated on forms filled in during the consecutive follow-up appointment with the patient and in some cases over a telephone conversation with the patient by one of the surgical team members. Out of the 86 patients, pre-operative data were obtained for 86 patients, while post-operative Wexner score was obtained for 80 patients, since 6 of them did not return the forms. For these 80 patients the median post-operative Wexner score for constipation was 3 (IQR: 1-6), which was significantly improved compared to the median pre-operative score for constipation which was 14.5 (IQR: 10.5-18.5) \( (P < 0.01) \). Again, comparing the median pre-operative Wexner score for incontinence, which was 11 (IQR: 7-15), to the median post-operative score for faecal incontinence, which was 2 (IQR: 0-5), there was also a significant improvement demonstrated \( (P < 0.01) \) (Table 2).
Table 1 Patient characteristics

<table>
<thead>
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</thead>
<tbody>
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<td>Number of patients</td>
<td>86</td>
</tr>
<tr>
<td>Mean age in years</td>
<td>57 yr (IQR: 47-70)</td>
</tr>
<tr>
<td>Sex</td>
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<tr>
<td>Female</td>
<td>82</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
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<tr>
<td>Indication for surgery</td>
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<td>Rectal prolapse</td>
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<tr>
<td>Obstructive Defecation/Faecal Incontinence</td>
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<td>Previous surgery for prolapse</td>
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<tr>
<td>Previous hysterectomy</td>
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<tr>
<td>Yes</td>
<td>24</td>
</tr>
<tr>
<td>No</td>
<td>58</td>
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<tr>
<td>Male</td>
<td>4</td>
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Table 2 Mean pre-operative and post-operative Wexner scores

<table>
<thead>
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<th>Mean Wexner scores</th>
<th>Pre-operative</th>
<th>Post-operative</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Constipation</td>
<td>14.5 (IQR: 10.5-18.5)</td>
<td>3 (IQR: 1-6)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Incontinence</td>
<td>11 (IQR: 7-15)</td>
<td>2 (IQR: 0-5)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

IQR: Interquartile range.

Figure 1 Intraoperative image of the fixated mesh.

All the procedures were completed laparoscopically, and there was no surgery related mortality recorded. No mesh related infection or erosion was recorded, although there was 1 case of diskitis that had to be treated with antibiotics after seen in the clinic for a follow-up. One of the patients developed an incarcerated femoral hernia post-surgery, which was seen intraoperatively but not repaired since the patient was not consented for that procedure, and it was repaired on day 2. Out of the 86 patients, 3 (3.4%) had issues with chronic pelvic pain after the procedure. Two of the patients complained of a foreign body sensation/irritation in rectum and were found to have a suture protruding through the rectum that was removed in clinic, which was followed by immediate relief of their symptoms. Out of the 86 patients, 4 (4.6%) of them came back with a recurrence of symptoms, 3 (2.3%) of which had a posterior
prolapse recurrence and 2 of which eventually underwent a modified Delorme’s procedure.
Overall recurrence at 12 mo was estimated with the Kaplan-Meier method as 1.4% (95%CI: 0.3%–4.0%), 7% (95%CI: 6.1%–15.5%) at 2 years and 11% (95%CI: 6.7%–16.8%) at 3 years (Figure 2).

DISCUSSION
Laparoscopic ventral mesh rectopexy is becoming one of the leading treatment options for the elective repair of rectal prolapse around the world[6,7]. Perineal procedures are still performed especially for elderly patients and those with associated significant comorbidity who are not candidates for transabdominal laparoscopic procedures[8,9]. However, there are recent studies that demonstrate that LVMRs would be safe for selected elderly patients as well[10]. In our series, there were 5 elderly patients over 80 that had a successful procedure with a good outcome.

When LVMRs are compared to resectional and posterior rectopexies, the functional results are better, especially since there is no interference with the sacral nerves and therefore fewer issues with slow transit constipation[11]. Other surgical procedures such as stapled transanal rectal resection can be used for rectal intussusception and obstructive defecation secondary to rectoceles as an alternative surgical approach to laparoscopic ventral mesh rectopexy[12]. However, this procedures has been associated with higher morbidity rates including pain, haemorrhage and sepsis[13].

Over the past years there has been a major concern over the use of mesh in pelvic surgery, but in our series of patients so far there were no mesh related complications, such as mesh erosion or infection. This is likely due to the consistent use of biological mesh in all of our cases, and our findings therefore come in agreement with previous studies’ findings that the mesh related complications are far less when using a biologic mesh instead of a synthetic one[4]. Although our directly obtained data of follow-up were for 1 year after surgery, the fact that there was only one colorectal surgeon that provides such surgery in Lanarkshire combined with the absence of re-referrals of previously operated patients for symptoms related to mesh complication, indirectly suggests that there was no mesh complication over a period of 5 years. Balla et al[4] have shown after reviewing the literature that using a biological mesh is a safer option than using a synthetic one, especially since the synthetic and the biological mesh-related erosion rates were 1.87% and 0.22%, respectively.

Although there was an initial concern that using biological mesh might be associated with higher recurrence rate, it has been demonstrated that there was no difference in recurrence when using a biological mesh compared to a synthetic one [11]. It has also been suggested that biological mesh should be preferred in patients with a high risk of fistula formation, such as those with diverticular disease, Crohn’s disease, previous pelvic irradiation and steroid use[12]. Additionally, in another study, Mercer-Jones et al[13] suggested it could be prudent to use a biological mesh in young adolescents or women of child-bearing age regardless of the higher cost.

Complications were observed in the current study. Lumbosacral discitis near the site of mesh fixation to the sacral promontory was observed in 1 patient. This is a rare but serious complication with patients typically presenting 1-3 mo after the initial operation with severe lower back pain, fever and malaise[14]. In this case, magnetic resonance imaging confirmed the diagnosis, and broad spectrum antibiotics were given as they are the treatment of choice[14,15]. Although an uncommon complication, it should always be considered for patients that present with lower back pain after an LVMR[14,15]. Two patients presented with rectal symptoms of discharge and discomfort and were found to have ethibond suture erosion into their rectum. This is likely related to the suturing technique or the material itself, although there is no report of this complication in the literature so far[16]. In both patients, symptoms improved dramatically after transanal removal of sutures at outpatient/endoscopy room.

In our study, we had 4 patients that had a recurrence of their symptoms (4.6%). A systematic review of the literature by Samaranayake et al[17] has demonstrated that across various studies with median follow-up ranging from 3 to 106 mo the recurrence rates varied from 0%–15.4%. Our Kaplan Meier analysis revealed a 2 year recurrence rate of 7%, which can be compared to other studies like McLean et al[5] who demonstrated a recurrence rate of 9.74% (95%CI: 6.1%–15.5%) at 2 years.

It is evident that our study demonstrates a significant improvement of patients’ symptoms of obstructive defecation. The median post-operative Wexner score for
constipation was 3 (IQR: 1-6) compared to the median pre-operative score which was 14.5 (IQR: 10.5-18.5), demonstrating a significant improvement ($P < 0.01$). These results are comparable to the results of Franceschilli et al\cite{18} who demonstrated that the mean Wexner score for constipation improved from 18.4 ± 11.6 to 5.4 ± 4.1 ($P = 0.04$). Comparing the average pre-operative Wexner score for incontinence (11, IQR: 7-15) to the median post-operative score for incontinence (2, IQR: 0-5), there was also a significant improvement demonstrated ($P < 0.01$).

There was an overall improvement of the daily life activity for the majority of patients, which correlates with the results of other studies\cite{4,17,18}. McLean et al\cite{5} demonstrated patient satisfaction levels of 93% at 5 years, Consten et al\cite{19} showed that both rates of faecal incontinence and obstructed defecation decreased significantly after LVR compared to the preoperative incidence.

**CONCLUSION**

In conclusion, our study adds more evidence to support that LVMR using biological mesh is a safe and effective procedure for the treatment of rectal prolapse and that it significantly improves bowel symptoms of obstructive defecation and faecal incontinence in patients with not only full thickness prolapse but also internal rectal prolapse and rectoceles\cite{6,7,17,19}. In our study there were no mesh related complications, and this result correlates with the low biological mesh complication rate reported in other studies\cite{4,13}. Our recurrence rates are in line with the ones reported in the literature\cite{16}, and although we acknowledge that the direct follow-up period was short, the absence of re-referral of those previously operated patients over the period of 5 years would indirectly suggest the safety of the mesh over longer periods. However, our continued effort is to follow this group of patients more directly and continue to assess formally their quality of life in the near future.

**ARTICLE HIGHLIGHTS**

**Research background**

Laparoscopic ventral mesh rectopexy (LVMR) has over the past years become the preferred treatment for full thickness rectal prolapse, rectoceles, enteroceles and
Tsiaousidou A et al. Safety of biological mesh in LVMRs

symptomatic rectal intussusception in many colorectal surgical centres around the world.

Research motivation
Over the last few years there have been concerns about the usage of meshes in pelvic surgery, especially since serious complications have been recorded in urogynaecology procedures.

Research objectives
To show that the incidence of mesh-related complications, and particularly mesh erosion, after LVMRs is low, especially when a biological mesh is used. We also wanted to investigate whether there is a significant improvement in function and quality of life outcomes.

Research methods
Questionnaires for the calculation of Wexner scores for constipation and incontinence were completed by 86 patients who underwent LVMR with Permacol (Biological) mesh from 2012 to 2018 at University Hospital Wishaw. The patients were followed up in the clinic 12 mo after surgery. Statistical analysis of the result included the calculation of median and interquartile range (IQR) values and comparison and analysis between pre-operative and post-operative values. Complication and recurrence rates were evaluated and analysed using the Kaplan-Meier method.

Research results
The median Wexner scores for constipation pre-operatively and post-operatively were 14.5 (IQR 10.5-18.5) and 3 (IQR: 1-6), respectively, while the median Wexner score for faecal incontinence was 11 (IQR: 7-15) and 2 (IQR: 0-5), respectively (P < 0.01). There were 4 (4.6%) recurrences, 2 cases with erosion of a suture through the rectum and 1 patient that returned with diskitis. There were no mesh complications or mortalities.

Research conclusions
In our results, it is demonstrated that LVMR using a biological mesh is both safe and effective for the treatment of rectal prolapse and that it fundamentally improves bowel symptoms of obstructive defecation and faecal incontinence in patients with internal rectal prolapse and symptomatic rectoceles.

Research perspectives
Since we acknowledge that the direct follow-up period was short, we will continue our efforts to follow up our patients and formally assess their quality of life again in the near future.

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Observational Study

Dynamic monitoring of carcinoembryonic antigen, CA19-9 and inflammation-based indices in patients with advanced colorectal cancer undergoing chemotherapy

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Author contributions: Manojlovic N was the guarantor and designed the study, participated in the acquisition, analysis and interpretation of the data, and drafted the initial manuscript; Savic G and Rancic N participated in the analysis, acquisition, interpretation of the data, and drafted the initial manuscript; Nikolic B participated in the acquisition, analysis, and interpretation of the data.

Institutional review board statement: The study protocol was reviewed and approved by the Ethics Committee of Military Medical Academy (approval number: No 8/2021), and the study was conducted in accordance with the Helsinki Declaration as revised in 2013.

Informed consent statement: All study participants, or their legal guardian, provided written consent prior to study enrollment.

Conflict-of-interest statement: All study participants, or their legal

Abstract

BACKGROUND
The roles of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA19-9) in monitoring the patient response to chemotherapy for metastatic colorectal cancer (mCRC) are not clearly defined, and inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII), have been sparsely investigated for this purpose.

AIM
To aim of this study was to evaluate the relationship between the kinetics of CEA, CA19-9, NLR, LMR, PLR and SII in serum and patient response to chemotherapy estimated by computed tomography (CT) in patients with unresectable mCRC.

METHODS
Patients with mCRC treated with a 1st-line and 2nd-line chemotherapy underwent at least 3 whole-body spiral CT scans during response monitoring according to the Response Evaluation Criteria in Solid Tumour 1.1 (RECIST 1.1), and
simultaneous determination of CEA, CA19-9, neutrophil, lymphocyte, platelet and monocyte levels was performed. The kinetics of changes in the tumour markers and inflammatory indices were calculated as the percentage change from baseline or nadir, while receiver operating characteristic curves were drawn to select the thresholds to define patients with progressive or responsive disease with the highest sensitivity (Se) and specificity (Sp). The correlation of tumour marker kinetics with inflammatory index changes and RECIST response was determined by univariate and multivariate logistic regression analysis and the clinical utility index (CUI).

**RESULTS**

A total of 102 patients with mCRC treated with chemotherapy were included. Progressive disease (PD), defined as a CEA increase of 25.52%, resulted in an Se of 80.3%, an Sp of 84%, a good CUI negative [CUI (Ve-)] value of 0.75 and a good fraction correct (FC) value of 81.2; at a CEA cut-off of -60.85% with an Se of 100% and an Sp of 35.7% for PD, CT could be avoided in 25.49% of patients. The 21.49% CA19-9 cut-off for PD had an Se of 66.5%, an Sp of 87.4%, an acceptable CUI (Ve-) value of 0.65 and an acceptable FC value of 75. An NLR increase of 11.5% for PD had an Se of 67% and an Sp of 66%; a PLR increase of 5.9% had an Se of 53% and an Sp of 69%; an SII increase above -6.04% had an Se of 72% and an Sp of 63%; and all had acceptable CUI (Ve-) values at 0.55. In the univariate logistic regression analysis, CEA (P < 0.001), CA19-9 (P < 0.05), NLR (P < 0.05), PLR (P < 0.05) and SII (P < 0.05) were important predictors of tumour progression, but in the multivariate logistic regression analysis, CEA was the only independent predictor of PD (P < 0.05).

**CONCLUSION**

CEA is a useful marker for monitoring the chemotherapy response of patients with unresectable mCRC and could replace a quarter of CT examinations. CA19-9 has poorer diagnostic characteristics than CEA but could be useful in some clinical circumstances, particularly when CEA is not increased. Dynamic changes in the inflammatory indices NLR, PLR and SII could be promising for further investigation as markers of the chemotherapy response.

**Key Words:** Tumour markers; Carcinoembryonic antigen; Carbohydrate antigen; Inflammatory -based indices; Chemotherapy response; Metastatic colorectal cancer

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**Core Tip:** A carcinoembryonic antigen increase of 24.5% discriminates progressive disease (PD) from disease control with 80.3% sensitivity (Se) and 84% specificity (Sp) and good clinical utility index negative [CUI (Ve-)] and fraction correct (FC) values, while a reduction of -60% exclude PD with 100% Se and 37.5% Sp allowing for a 25.49% reduction in control CT examinations of unresectable metastatic colorectal cancer patients. The carbohydrate antigen level cut-off for PD was 21.49% with 66.5% Se, 87.4% Sp and acceptable CUI (Ve-) and FC values. A neutrophile-to-lymphocyte ratio increase by 11.5%, a platelet-to-lymphocyte ratio increase by 5.9%, a systemic inflammatory-immune index increase above -6.04% had acceptable CUI (Ve-) values.

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**INTRODUCTION**

Colorectal cancer (CRC) is the second leading cause of cancer morbidity in men, and
The third leading cause in women[1]. Approximately 20%-30% of patients present with advanced cancer, and during the course of disease approximately 50% of patients develop metastases[2]. The goal of treatment for stage IV cancer is to control tumour growth, relieve symptoms caused by the tumour, and prolong patient survival times. Chemotherapy is the main-line treatment for patients with metastatic disease. Careful response evaluation during chemotherapy treatment is critical to prevent toxicity and the continuation of expensive treatments with ineffective regimens, and to save time for attempting therapies with other drugs that may be more effective. The guidelines for treatment monitoring are based on imaging evaluations conducted every 2 or 3 months using standardized criteria[3]. In general, treatment response is evaluated by imaging, and the Response Evaluation Criteria in Solid Tumors (RECIST) are based on the World Health Organization (WHO) criteria for evaluating tumour response[4]. The main challenge is to identify disease progression at an early stage using a simple method to allow for treatment modification for patients with unresectable metastatic colonic carcinoma (mCRC) treated with chemotherapy. Although the RECIST are the most widely accepted method for assessing tumour response in recent decades, limitations of the RECIST have become increasingly apparent, especially with recent advances in precision-medicine approaches to cancer therapy[5].

Carcinoembryonic antigen (CEA) is a complex glycoprotein of the membrane surface, that belongs to the immunoglobulin superfamily of cell adhesion proteins, and is the most commonly used tumour marker for the diagnosis of CRC and for the evaluation of patient prognosis or disease recurrence after treatment[6]. However, no consensus has yet been reached on the role of CEA in the assessment of tumour responses to chemotherapy, although some researchers have examined the efficacy of CEA monitoring for the evaluation of tumour response in palliative chemotherapy. CEA is recommended for monitoring advanced disease, especially if metastasis is difficult to measure by other means[7]. Currently, only limited data are available that indicate a correlation between CEA evolution and chemotherapy response on computed tomography (CT) imaging in patients with advanced CRC. CEA influences the biology of tumour cells through autocrine mechanisms, leading to an increase in cell survival and an inhibition of tumour cell differentiation, and by paracrine regulation, with activation of endothelial cells and tumour angiogenesis, inhibition of apoptosis[8-15], and promotion of tumour proliferation[16], eventually triggering or promoting a favourable state for tumour growth or immunosuppression[17,18].

Carbohydrate antigen (CA19-9) is a monoclonal antibody generated against a colon carcinoma cell line and is used to detect a monosialoganglioside found in patients with gastrointestinal adenocarcinoma. CA19-9 is elevated in 21%-42% of gastric cancer patients, 20%-40% of colon cancer patients, and 71%-93% of pancreatic cancer patients[19]. Some studies have revealed that in addition to the CEA level, the CA19-9 level is also related to the curative effect of chemotherapy[20,21]. In contrast to CEA, insufficient data are available to recommend the use of CA19-9 for evaluating treatment responses. The methodology of the published studies is heterogeneous, as several tumour marker cut-off levels and criteria for response assessment in mCRC patients have been used.

The inflammatory indices neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio, and systemic immune-inflammation index (SII) have been investigated as prognostic factors in many cancers, including mCRC[22-26]. The results of these studies suggest that the systemic inflammatory response is a more potent stimulator of cancer progression in patients with established disease.

With a focus on replacing the control CT examination with simplified and less toxic methods, we conducted a study to evaluate the efficacy of the tumour markers CEA and CA19-9 and the inflammatory indices NLR, PLR, LMR and SII for monitoring the chemotherapy response of patients with unresectable mCRC.

The primary endpoint of this study was to evaluate the concordance and correlation of dynamic changes in the tumour markers CEA and CA19-9, with response evaluation estimated by the RECIST 1.1, to find representative cut-off values for progressive disease (PD) and disease control (DC) and to evaluate the diagnostic characteristics of these markers.

The secondary endpoints were to evaluate the correlation of dynamic changes in inflammatory indices with the RECIST1.1 response and tumour marker kinetics and to test the diagnostic characteristics of these indices for monitoring the chemotherapy response in mCRC patients.

At the start of this study, tumour progression appeared to be the most relevant parameter for tumour response evaluation because typical clinical practice is to continue cytotoxic treatment until progression or unacceptable toxicity arises.
MATERIALS AND METHODS

The study was performed at the Department of Digestive Oncology of the Military Medical Academy, where we enrolled 102 patients with CRC and unresectable mCRC. Approval in concordance with the Declaration of Helsinki was obtained from the local ethics committee, and informed consent was obtained from the patients. The inclusion criteria were age > 18, histopathologically proven adenocarcinoma of the colon and rectum, confirmed unresectable metastatic disease with measurable metastases suitable for RECIST 1.1 evaluation with CT of the chest, abdomen and pelvis, Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2, positivity in at least one measurement of CEA or CA19-9 during evaluation, absence of contraindication for chemotherapy treatment, absence of concomitant infection, autoimmune disease, steroid treatment and any recognizable inflammatory condition, concomitant malignant tumour, no granulocyte colony-stimulating factor (GCSF) stimulation at least 2 wk before blood analysis, written informed consent, and the decision of a local multidisciplinary team to provide treatment with systemic chemotherapy. The exclusion criteria were age < 18, resectable metastatic disease, patients unsuitable for RECIST 1.1 evaluation, absence of both increased tumour markers during treatment, absence of regular CEA and CA19-9 monitoring, absence of complete blood count (CBC) monitoring, and absence of regular radiological monitoring according to the RECIST 1.1.

For all patients, we performed CT before beginning the treatment for the baseline CT scan, after 10-12 wk (three or four cycles of chemotherapy depending on the protocol) for the first control, and after another three or four cycles of chemotherapy or another 10-12 wk for the second control (third CT examination). Before the beginning of the first cycle of chemotherapy and at the time of each control radiological examination, we measured CEA and CA19-9 levels. In the second phase of the study, in the last 58 included patients, inflammatory indices were also measured, consisting of the NLR (Ne/Ly), LMR (Ly/Mo), PLR (Pt/Ly) ratio and SII [(Ne* Pt)/Ly].

Assessment of tumour response to chemotherapy and progression by radiology

Response rates were estimated according to the RECIST 1.1. PD was defined as an increase in the sum of the maximal longitudinal diameter > 20% in comparison with that at baseline or nadir, appearance of new non-target lesions, or unequivocal progression of non-target lesions. Complete response (CR) was defined as the absence of the tumour mass on CT imaging. Partial response (PR) was defined as a decrease in the sum of the maximal longitudinal diameter of at least 30%. Stable disease (SD) was defined as disease that met neither the PD or PR criteria. CT-evaluated response estimated by the RECIST 1.1 served as the gold standard of the response evaluation. All the CT images were examined by two radiologists with experience in abdominal image interpretation at the Institute for Radiology of the Military Medical Academy. The radiologists were blinded to each patient’s prognosis, tumour marker and inflammatory index data and chemotherapy schedule but were aware that the patients had been pathologically diagnosed with mCRC.

Determination of CEA and CA19-9 values and inflammatory indices and assessment of their change

All blood sampling procedures for CBC, and blood chemistry CEA and CA19-9 testing were performed up to 3 d before beginning the treatment, and each planned CT examination was performed after full recovery from the chemotherapy cycle. No GCSF was administered during the 14 d before blood sampling and response evaluation. We took at least 5 mL of blood from the peripheral vein and sent it to the Institute for Biochemistry of the Military Medical Academy. Serum CEA levels were measured using the Siemens Advia Centaur XP Direct Chemiluminescent Immunoassay DCL method (normal < 2.5 ng/L) and CA19-9 levels were measured with an Access GI Monitor assay using the Beckman Coulter UniCel DXi Indirect Chemiluminescent Immunoassay DCLIA method (normal < 31 U/mL).

Blood samples for CBC were collected in BD Vacutainer K2 EDTA tubes and analysed within 2 h of venepuncture. The CBC was determined by the Siemens Advia 120 haematology system, which is a flow cytometry-based system. Differentiation of white blood cells was performed by peroxidase and basophil channels. The peroxidase method is a primary differential method on Advia 120. Peroxidase in the granules of white blood cells reacts with hydrogen peroxide from reagent and forms dark precipitates within the cells. After measuring the light scatter, which represents the size of the cell and absorption showing the level of staining, the analyser separates
populations of neutrophils, monocytes, eosinophils, and large unstained cells, while lymphocytes and basophils appear as one cluster. These cells require a further method for differentiation. The basophil method uses the resistance of basophils to acid lysis and differentiates them from the rest of the white blood cell population. The Advia 120 analyser method of counting platelets is based on two-dimensional laser light scattering. The laser optics low-angle and high-angle scatter is used to determine the platelet count simultaneously with the red blood cells. The NLR, MLR, PLR and SII \([\text{Ne}^*\text{Pt}/\text{Ly}]\) were calculated as ratios of circulating neutrophil, monocyte, lymphocyte, and platelet counts, respectively. Normal ranges for these cell counts are as follows: Leukocytes 4-10.8 \(\times\) 10^9/L; neutrophils 1.9-8 \(\times\) 10^9/L; lymphocytes 0.9-5.2 \(\times\) 10^9/L; monocytes 0-1 \(\times\) 10^9/L; and platelets 130.0-400.0 \(\times\) 10^9/L (data from our laboratory).

The response indicated by tumour markers was estimated according to the change in the percent from the baseline value or at nadir calculated as \(\Delta\text{CEA}_1 = \left(\frac{\text{CEA}_2 - \text{CEA}_1}{\text{CEA}_1}\right) \times 100\), \(\Delta\text{CEA}_2 = \left(\frac{\text{CEA}_{\text{nadir or 2}} - \text{CEA}_{\text{nadir or 2}}}{\text{CEA}_{\text{nadir or 2}}}\right) \times 100\). The same formula was used for the CA19-9 and inflammatory indices.

Statistical analysis
Statistical analyses were conducted using IBM SPSS Statistics, version 26.0 (SPSS, Chicago, IL, United States), and statistical significance was defined as \(P < 0.05\) for all comparisons. Categorical variables are presented as frequencies and were analysed using the chi-squared test. All continuous variables are presented as the mean ± SD for normally distributed data or the median [interquartile range (IQR): 25-75 percentile] for nonnormally distributed data. The Kolmogorov-Smirnov test was used to test the normality of the data distribution. For intergroup comparisons, an independent \(t\)-test was used for parametric variables, and the Mann-Whitney \(U\) test was used for nonparametric variables. The relationship between variables was evaluated using Pearson’s coefficient correlation. The association between potential risk factors and disease progression was evaluated using binary logistic regression, expressing the strength of association by crude and adjusted odds ratios with 95% confidence intervals.

The Sensitivity (Se), specificity (Sp), negative predictive value (NPV), positive predictive value (PPV), efficiency and confidence intervals for each set of screening criteria for PD (CEA, CA19-9, NLR, PLR, LMR and SII) were obtained. Comparisons of receiver operating characteristic (ROC) curves were carried out to verify variations in the Se and false-positive fraction (1 - specificity) of different sets of markers using overall cut-off values. The accuracy and discriminative ability of tumour markers and inflammatory indices for the outcome of chemotherapy treatment were estimated with the Se, Sp, PPV, NPV, fraction correct (FC) and clinical utility index in the form of the case-finding utility or positive utility index (CUI Ve+) and screening utility or negative utility index \([\text{CUI- (Ve-)}]\). CUI- (Ve+) = Se \times PPV and CUI- (Ve-) = Sp \times NPV represents important indices for clinicians and estimates both the accuracy and discriminative ability of the test [27,28].

RESULTS
Patient characteristics
A total of 102 mCRC patients participated in this study from 2014 to 2019. All the patients were treated with chemotherapy as the first-line treatment. The baseline or at least one of three determined levels of CEA or CA19-9 for all patients included in the study were above normal (≥ 2.5 ng/mL, ≥ 31 ng/mL). CEA was present in all 102 patients, and 65 patients were positive for CA19-9 (63.7%). Inflammatory indices were recorded in 58 (55.8%) consecutive patients. The population of this study consisted of 71 men (69.6%) and 31 women (30.4%), and the average age was 63.37 years. In 42 patients (41.2%), the primary tumour was located in the rectum, in 44 (43.1%) the primary tumour was located in the left side of the colon, and in 16 (15.7%), the primary tumour was located in the right side of the colon. The localization of metastases was as follows: Liver 91 patients (89.2%), lung 38 (37.3%), peritoneum 13 (12.7%), and lymph nodes 38 (37.3%). The tumour histological grade was HG1- (low grade) in 51 patients (50.0%), HG2- (intermediate grade) in 45 patients (44.1%), and HG3- (high grade) in 6 patients (5.9%). Fluoropyrimidine-oxaliplatin-based chemotherapy was administered to 61 patients (59.8%), fluoropyrimidine-irinotecan to 21 patients (20.6%), bevacizumab to 20 patients (19.6%), and EGFR inhibitors to 4 (6.9%) patients before the first response evaluation, and in 53 (52.0%), 21 (20.6%), 15 (14.7%) and 13 (12.7%) patients,
before the second response evaluation (Table 1).

**Radiological response evaluation: RECIST**

We performed radiological response evaluation according to the previously described RECIST 1.1, but in the analysis, we mainly differentiated PD from DC (CR + PR + SD) based on the findings in the literature and personal experience indicating that the role of tumour markers could be useful for this purpose. We recorded 63 patients with PD (31%) and 141 patients with (69%) DC, including 0 patients who achieved CR, 31 patients who achieved PR (15%), and 110 patients who had SD (54%).

**CEA, CA19-9, and the inflammatory indices**

The values of the tumour markers CEA and CA19-9 were expressed as × upper normal limit (UNL) and absolute values of the inflammatory indices NLR, PLR, LMR and SII were expressed as the median (IQR) before beginning the treatment as a baseline measurement, and at the 1st and 2nd evaluation of chemotherapy response (Table 2).

An increase in CEA was recorded in 82% and 12% of patients with PD and DC, respectively, while a decrease was noticed in 18% of patients with PD and 72% of patients with DC. After applying the cut-off obtained with the ROC analysis, there was no significant difference in concordance between the kinetics of CEA and the RECIST-estimated response. There was a significant difference in the direction of CEA change between patients with PD and those with DC. CA19-9 showed similar results to CEA, with a significant difference in the direction of change between patients with PD and those with DC, and no significant change was observed when the cut-off obtained by ROC analysis was applied (Table 3).

The situation with the inflammatory indices was different. None of the inflammatory indices had a significant difference in kinetic direction between patients with PD and those with DC, when increases and decreases were analysed. In contrast to the previously mentioned cut-off based on the ROC analysis, the direction of change in the NLR was significantly altered in patients with DC (P < 0.05), leading to a significant difference in the CEA value direction of change between patients with PD and those with DC (P < 0.01). The PLR demonstrated no statistically significant change between patients with PD and those with DC after the application of the ROC analysis-based cut-off in separate analyses; however, this small change led to an ultimately significant difference in the PLR direction of change between patients with PD and those with DC (P < 0.05). The SII underwent a major change when we applied the cut-off value, leading to a dramatic turnover of the kinetics in patients with PD (P < 0.01) and an overall significant difference in the kinetics between patients with PD and those with DC (P < 0.01). The LMR was the only inflammatory index without any concordance with the RECIST-estimated response irrespective of the applied cut-off (Table 3).

**Correlation between the RECIST response, and CEA, CA19-9, and inflammatory index changes**

The relationship between variables was evaluated using Pearson’s coefficient correlation. CEA was significantly correlated (P < 0.001) with tumour response according to the RECIST 1.1 with a moderately strong correlation coefficient (r) (0.42 for the RECIST1.1, and 0.412 for the dichotomous RECIST 1.1 of PD vs DC). CA19-9 had a low r strength (r = 0.256 for the RECIST 1.1 and 0.27 for the dichotomous RECIST1.1 outcome) but a significant correlation with the RECIST 1.1 response (P < 0.05). The NLR had a moderately strong correlation with both the RECIST 1.1 and dichotomous RECIST 1.1 outcome (0.306 and 0.338, P < 0.01). The PLR had a low r strength (r = 0.205) but a significant correlation (P < 0.05) only with the dichotomous RECIST 1.1 outcome of PD vs DC. The SII had a low correlation with the RECIST 1.1 (r = 0.285, P < 0.05) and a moderate correlation with the dichotomous RECIST 1.1 outcome (r = 0.309, P = 0.001).

The change in CEA had a moderately strong correlation with CA19-9 (r = 0.406, P < 0.01) and a low r strength but a significant correlation with the NLR (r = 0.277, P < 0.05), PLR (r = 0.204, P < 0.05) and SII (r = 0.263, P < 0.05).

Unlike CEA and MSCT, CA19-9 had a moderately strong correlation with only the PLR (r = 0.417, P < 0.001).

The LMR did not have any significant correlation with the other variables.

**The best cut-off value for CEA, CA19-9 and inflammatory index changes for predicting tumour response**

We constructed ROC curves to determine the best cut-off value for changes in the patients’ CEA, CA19-9, NLR, PLR, LMR and SII values during the first and second
# Table 1 Patients' demographic and clinical features

<table>
<thead>
<tr>
<th>Patients' demographic and clinical features</th>
<th>n (%)</th>
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<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>71 (69.6)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (30.4)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>63.37 ± 10.21; (35-81)</td>
</tr>
<tr>
<td><strong>Primary localization</strong></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>42 (41.2)</td>
</tr>
<tr>
<td>Left colon</td>
<td>44 (43.1)</td>
</tr>
<tr>
<td>Right colon</td>
<td>16 (15.7)</td>
</tr>
<tr>
<td><strong>Localization of metastasis</strong></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>91 (89.2)</td>
</tr>
<tr>
<td>Lung</td>
<td>38 (37.3)</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>13 (12.7)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>38 (37.3)</td>
</tr>
<tr>
<td><strong>Tumour grade</strong></td>
<td></td>
</tr>
<tr>
<td>HG1</td>
<td>51 (50.0)</td>
</tr>
<tr>
<td>HG2</td>
<td>45 (44.1)</td>
</tr>
<tr>
<td>HG3</td>
<td>6 (5.9)</td>
</tr>
<tr>
<td><strong>Chemotherapy 1st-line</strong></td>
<td></td>
</tr>
<tr>
<td>Fluoropyrimidines + oxaliplatin</td>
<td>61 (59.8)</td>
</tr>
<tr>
<td>Fluoropyrimidines + irinotecan</td>
<td>14 (13.7)</td>
</tr>
<tr>
<td>Bevacizumab + (fluoropyrimidines + oxaliplatin); or (fluoropyrimidines + irinotecan)</td>
<td>20 (19.6)</td>
</tr>
<tr>
<td>EGFR inhibitors + (fluoropyrimidines + oxaliplatin); or (fluoropyrimidines + irinotecan)</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td><strong>Chemotherapy 1st-line (cont) or 2nd-line</strong></td>
<td></td>
</tr>
<tr>
<td>Fluoropyrimidines + oxaliplatin</td>
<td>53 (52.0)</td>
</tr>
<tr>
<td>Fluoropyrimidines + irinotecan</td>
<td>21 (20.6)</td>
</tr>
<tr>
<td>Bevacizumab + (fluoropyrimidines + oxaliplatin); or (fluoropyrimidines + irinotecan)</td>
<td>15 (14.7)</td>
</tr>
<tr>
<td>EGFR inhibitors + (fluoropyrimidines + oxaliplatin); or (fluoropyrimidines + irinotecan)</td>
<td>13 (12.7)</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD or n (%).

tumour response evaluations. The dependent variable of the ROC curve was categorized by the response as determined from a radiological scan and assessed using the RECIST 1.1 using PD and DC as variables. The best area under the curve (AUC), categorized as good, was obtained for CEA (0.842, $P < 0.01$), which suggests that a significant change in the CEA levels is a variable that can be used to predict the tumour response. CA19-9 (0.769), the NLR (0.713) and the SII (0.723) had AUC values categorized as acceptable ($P < 0.01$). The PLR had a poor but nevertheless statistically significant AUC value (0.62, $P < 0.05$), while the LMR AUC analysis was considered to have failed and was nonsignificant (Table 4, Figure 1).

The best PD cut-off value for CEA was 24.52%, with an Se of 80.3% and an Sp of 80.4%. The a CA19-9 best cut-off value was 21.49% with an Se of 67% and an Sp of 76%. For the NLR, the best cut-off value was 11.05% with an Se of 67% and an Sp of 66%; for the PLR, the best cut-off value was 5.9% with an Se of 53% and an Sp of 68%, and for the SII, the best cut-off value was -6.04% with an Se of 77% and an Sp of 63%. The cut-off with maximal Se (100%) for excluding PD without CT analysis with a maximal Sp of 35.7% was -60.85% for CEA, allowing for the safe avoidance of 25.49% of CT scans; for CA19-9 this optimal cut-off was -55.38% with an Sp of 39.6% and could be used to avoid 16.92% of CT control examinations (Table 4, Figure 1).
Table 2 Carcinoembryonic antigen and carbohydrate antigen and inflammatory index characteristics at baseline and at the 1st and 2nd evaluations

<table>
<thead>
<tr>
<th>Markers</th>
<th>Baseline</th>
<th>1st evaluation</th>
<th>2nd evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>6.99 (2.50-61.46)</td>
<td>5.86 (1.88-29.43)</td>
<td>6.53 (1.55-36.02)</td>
</tr>
<tr>
<td>CA19-9</td>
<td>2.68 (1.1-23.58)</td>
<td>2.76 (0.91-9.21)</td>
<td>3.13 (0.76-11.86)</td>
</tr>
<tr>
<td>NLR</td>
<td>2.22 (1.60-3.64)</td>
<td>1.77 (1.18-2.65)</td>
<td>2.19 (1.43-3.17)</td>
</tr>
<tr>
<td>PLR</td>
<td>144.87 (93.64-213.97)</td>
<td>115.43 (82.78-170.98)</td>
<td>141.19 (92.82-187.82)</td>
</tr>
<tr>
<td>LMR</td>
<td>3.92 (2.56-5.58)</td>
<td>4.21 (2.62-5.43)</td>
<td>3.56 (2.62-5.00)</td>
</tr>
<tr>
<td>SII</td>
<td>614.89 (298.31-1219.48)</td>
<td>339.34 (227.13-570.24)</td>
<td>475.02 (238.59-858.52)</td>
</tr>
</tbody>
</table>

1Values are expressed as × upper normal limit for carcinoembryonic antigen and carbohydrate antigen, not as the absolute value; the neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio and systemic immune-inflammation index calculated as the absolute value × 10⁹/L. Data are presented as median (interquartile range). CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; LMR: Lymphocyte-to-monocyte ratio; SII: Systemic immune-inflammation index.

Table 3 Any change in tumour markers and inflammatory indices and changes according to cut-off values

<table>
<thead>
<tr>
<th>204 RECIST</th>
<th>204 CEA</th>
<th>130 CA19-9</th>
<th>116 NLR, PLR, LMR and SII</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD 31%</td>
<td>PD/DC</td>
<td>CEA: 82%↑; 18%↓</td>
<td>CEA: 28%↑; 72%↓</td>
</tr>
<tr>
<td>∆CEA (24.52%): 80.3%↑; 19.7%↓</td>
<td>∆CEA (24.52%): 19.6%↑; 80.4%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA19-9: 75.6%↑; 24.4%↓</td>
<td>CA19-9: 33.7%↑; 66.3%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆CA19-9 (21.49%): 73.2%↑; 26.8%↓</td>
<td>∆CA19-9 (21.49%): 30.3%↑; 69.7%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLR: 45%↑; 55%↓</td>
<td>NLR: 54.2%↑; 45.8%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆NLR (11.05%): 66.7%↑; 33.3%↓</td>
<td>∆NLR (11.05%): 33.7%↑; 66.3%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLR: 55%↑; 45%↓</td>
<td>PLR: 44.8%↑; 55.2%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆PLR (5.90%): 52.8%↑; 47.2%↓</td>
<td>∆PLR (5.90%): 32.5%↑; 67.5%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMR: 45%↑; 55%↓</td>
<td>LMR: 41.7%↑; 58.3%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆LMR (30%↑): 70%↓</td>
<td>∆LMR (30%↑): 42.7%↑; 57.3%↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆SII (-6.04%): 72.2%↑; 27.8%↓</td>
<td>∆SII (-6.04%): 33.7%↑; 66.3%↓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aP > 0.01.
bP < 0.05.
cP > 0.05.

Carcinoembryonic antigen, carbohydrate antigen, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio (LMR) and systemic immune-inflammation index any increase or decrease of value; ∆- increase or decrease defined by cut-off obtained with receiver operating characteristic analysis; ↑ and ↔ statistical analysis with χ² test; ∆LMR cut-off was not determined. PD: Progressive disease; DC: Disease control; SD: Stable disease (CR + PR + SD); CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen; CA19-9: Carbohydrate antigen; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; LMR: Lymphocyte-to-monocyte ratio; SII: Systemic immune-inflammation index.

Binary logistic regression, and univariate and multivariate analyses. Dynamic change in markers and the dichotomous RECIST 1.1 outcome of PD vs DC

Univariate and multivariate binary logistic regression analyses were performed to explore the significance of the investigated markers as predictors of the outcome of
Table 4 Area under the curve and cut-off values

<table>
<thead>
<tr>
<th>Test result variable(s)</th>
<th>Area (s)</th>
<th>Asymptotic 95% CI</th>
<th>Lower bound</th>
<th>Upper bound</th>
<th>Cut-off value for PD, %</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cut-off value for sensitivity 1, %</th>
<th>Specificity for sensitivity 1</th>
<th>Avoidable CT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>△CEA</td>
<td>0.842 g</td>
<td>0.000</td>
<td>0.788</td>
<td>0.895</td>
<td>24.52</td>
<td>0.803</td>
<td>0.804</td>
<td>-60.85</td>
<td>0.357</td>
<td>52 (25.49)</td>
</tr>
<tr>
<td>△CA19-9</td>
<td>0.766 f</td>
<td>0.000</td>
<td>0.665</td>
<td>0.874</td>
<td>21.49</td>
<td>0.67</td>
<td>0.76</td>
<td>-55.38</td>
<td>0.396</td>
<td>22 (16.92)</td>
</tr>
<tr>
<td>△NelR</td>
<td>0.713 f</td>
<td>0.000</td>
<td>0.614</td>
<td>0.182</td>
<td>11.05</td>
<td>0.67</td>
<td>0.66</td>
<td>-77.85</td>
<td>0.013</td>
<td>2 (3.4%)</td>
</tr>
<tr>
<td>△LMR</td>
<td>0.451 g</td>
<td>P &gt; 0.05</td>
<td>0.309</td>
<td>0.562</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>△PLR</td>
<td>0.622 g</td>
<td>0.036</td>
<td>0.511</td>
<td>0.733</td>
<td>5.9</td>
<td>0.53</td>
<td>0.68</td>
<td>-59.33</td>
<td>0.075</td>
<td>6 (10.3)</td>
</tr>
<tr>
<td>△SII</td>
<td>0.723 f</td>
<td>P ≥ 0.9-1</td>
<td>0.625</td>
<td>0.82</td>
<td>-6.04</td>
<td>0.72</td>
<td>0.63</td>
<td>-88.62</td>
<td>0.013</td>
<td>2 (3.4%)</td>
</tr>
</tbody>
</table>

*P > 0.9-1 excellent.
*P ≥ 0.8-0.9 good.
*P ≥ 0.7-0.8 fair/acceptable.
*P ≥ 0.6-0.7 poor.
*P ≥ 0.5-0.6 failed, that’s a qualitative interpretation of the area under the curve (AUC). The area under the receiver operating characteristic curve (AUC) results were considered excellent for AUC values between 0.9 and 1, good for AUC values between 0.8 and 0.9, fair for AUC values between 0.7 and 0.8, poor for AUC values between 0.6 and 0.7 and failed for AUC values between 0.5 and 0.6. CEA: Carcinoembryonic antigen.

Figure 1 Receiver operating characteristic curve. A: Receiver operating characteristic (ROC) analysis of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA19-9); B: ROC analysis of CEA, CA19-9, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio and systemic immune-inflammatory index. CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen; NLR: Neutrophil-to-lymphocyte ratio; LMR: Lymphocyte-to-monocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammatory index; ROC: Receiver operating characteristic.

Chemotherapy response according to the dichotomous RECIST 1.1 outcome. In the univariate analysis, CEA was a significant predictor (P < 0.001), as was CA19-9 and all the inflammatory indices except the LMR (P < 0.05). In the multivariate analysis, only CEA was a significant predictor of outcome (P < 0.05), suggesting its robustness for monitoring response (Table 5).

Clinical utility index and fraction correct
CEA, CA19-9 and the inflammatory indices NLR, PLR and SII were analysed for diagnostic characteristics Se, Sp, PPV, NPV and CUI (Ve+) and CUI (Ve-) and FC for the differentiation of PD from DC.

CEA had the best Se (86.9%), NPV (93.4%), satisfactory case finding CUI (Ve+), good screening CUI (Ve-), and good overall utility FC. The Sp and PPV for CEA were the second best among the investigated markers and indices, and overall, CEA was found to be the best marker for monitoring tumour response.
Table 5 Binary logistic regression, and univariate and multivariate analyses

<table>
<thead>
<tr>
<th>Markers</th>
<th>Univariate logistic regression</th>
<th>Multivariate logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>1.005 (1.002-1.008), $P &lt; 0.001$</td>
<td>1.004 (1.000-1.007); $P = 0.044$</td>
</tr>
<tr>
<td>CA19-9</td>
<td>1.005 (1.001-1.008), $P = 0.015$</td>
<td>1.001 (0.998-1.004), $P &gt; 0.05$</td>
</tr>
<tr>
<td>NLR</td>
<td>1.011 (1.004-1.018), $P = 0.001$</td>
<td>1.015 (0.996-1.034), $P &gt; 0.05$</td>
</tr>
<tr>
<td>PLR</td>
<td>1.009 (1.001-1.016), $P = 0.034$</td>
<td>1.003 (0.996-1.002), $P &gt; 0.05$</td>
</tr>
<tr>
<td>LMR</td>
<td>1.000 (0.993-1.007), $P &gt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>SII</td>
<td>1.006 (1.002-1.010), $P = 0.004$</td>
<td>0.997 (0.985-1.009), $P &gt; 0.05$</td>
</tr>
</tbody>
</table>

Dynamic change in markers and dichotomous response evaluation criteria in solid tumour 1.1 outcomes progressive disease (PD) and non-PD (disease control). Data are presented as Exp(B) with 95%CI for EXP(B). CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; LMR: Lymphocyte-to-monocyte ratio; SII: Systemic immune-inflammation index.

CA19-9 had the second highest Se (80.6%) and NPV (89.2%), a good CUI (Ve-) value and a satisfactory overall utility FC.

The Inflammatory indices NLR, PLR and SII had poorer diagnostic characteristics than the tumour markers, with higher Se and NPV values for the NLR and SII than the PLR, but lower Sp and PPV values, leading to a satisfactory CUI (Ve-) value for all the indices, but poor overall utility (Table 6, Figure 2).

Discrepancies between the evolution of tumour markers and the radiologically assessed response to chemotherapy: Increase in tumour markers and anticipation of progression

Sequential follow-up of patients with three CT scans and two RECIST evaluations allowed us to record tumour marker flares, which are increases in tumour markers with subsequent decreases, followed by tumour regression or stabilization. Tumour marker prediction of PD manifests as an increase in tumour markers without supporting RECIST PD on the corresponding CT evaluation but with a further tumour marker increase and ultimately confirmed PD on the following CT evaluation.

CEA was expressed in flares in 11/102 (10.78%) patients and predicted PD in 8/102 (7.84%) patients. CA19-9 yielded similar results and was expressed in flares in 6/65 (9.23%) patients and predicted PD in 4/65 (6.15%) patients. The inflammatory indices NLR and PLR were expressed in flares in 3/58 (5.1%) and 4/58 (6.9%) patients, and predicted PD in 4/58 (6.9%) and 2/58 (3.4%) patients, respectively, while the SII was expressed in flares in 5/58 (8.6%) patients and predicted PD in 2/58 (3.4%) patients.

DISCUSSION

Response evaluation based on imaging is not always feasible because patients may have a disease that is difficult to measure by CT or MRI, such as diffuse peritoneal dissemination, or imaging results may be misleading early in the course of treatment, as is the case for immunotherapy. However, radiological imaging does not consider functional changes or tumour biology\[29,30\]. In addition, radiological imaging exposes patients to radiation and increases treatment costs. Therefore, the ideal follow-up strategy for mCRC patients undergoing systemic therapy uses a method that is accurate, reliable, simple, fast and inexpensive\[31\].

In our study, both the tumour markers CEA and CA19-9 expressed significant concordance in the direction of change along with the RECIST 1.1-estimated outcomes of PD and DC. One of the most important factors in the analysis of tumour marker utilization for monitoring response, the cut-off value, did not influence concordance with radiology-based response evaluation. The importance of any change in the CEA value, as reported by Hermunen et al\[32\], appears overly optimistic, as CEA values fluctuate for several reasons unrelated to the tumour response and many different cut-off values have been obtained using several methods\[30-33,39-55\], which can lead to significant differences in the statistical analysis. The question of how to interpret tumour marker changes in practice remains unresolved. Inflammatory indices have been investigated less often for this purpose; however, there are several different criteria and methods for differentiating between PD and DC. Any increase or decrease
Table 6 Diagnostic characteristics of carcinoembryonic antigen, carbohydrate antigen, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
<th>CUI-(Ve+)</th>
<th>CUI-(Ve-)</th>
<th>FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>86.9 (78.4-95.4)</td>
<td>79.7 (73.1-86.3)</td>
<td>64.6 (54.3-75.0)</td>
<td>93.4 (89.1-97.8)</td>
<td>0.56^ (0.44-0.68)</td>
<td>0.75^ (0.64-0.80)</td>
<td>81.9^*</td>
</tr>
<tr>
<td>CA19-9</td>
<td>80.6 (67.6-95.5)</td>
<td>72.5 (62.7-82.3)</td>
<td>56.9 (43.3-70.5)</td>
<td>89.2 (81.7-96.8)</td>
<td>0.46 (0.29-0.63)</td>
<td>0.65^ (0.57-0.73)</td>
<td>75^*</td>
</tr>
<tr>
<td>NLR</td>
<td>68.8 (53.2-84.0)</td>
<td>66.7 (56.4-76.9)</td>
<td>47.1 (33.4-60.8)</td>
<td>83.1 (74.0-92.2)</td>
<td>0.32 (0.14-0.50)</td>
<td>0.55^ (0.46-0.64)</td>
<td>67.2</td>
</tr>
<tr>
<td>PLR</td>
<td>53.8 (34.7-73.1)</td>
<td>81.3 (67.7-94.8)</td>
<td>70.0 (49.9-90.1)</td>
<td>68.4 (53.6-85.2)</td>
<td>0.38 (0.13-0.62)</td>
<td>0.55^ (0.43-0.68)</td>
<td>69</td>
</tr>
<tr>
<td>CII</td>
<td>70.3 (55.5-85.0)</td>
<td>65.9 (55.6-76.1)</td>
<td>48.1 (34.8-61.5)</td>
<td>83.1 (74.0-97.2)</td>
<td>0.34 (0.16-0.50)</td>
<td>0.55^ (0.46-0.64)</td>
<td>67.2</td>
</tr>
</tbody>
</table>

^1Satisfactory/adequate clinical utility index (CUI).
^2Good CUI.
^3Overall utility satisfactory/adequate.
^4Overall utility good. A qualitative interpretation of the clinical utility index: (E) ≥ 0.81 excellent; (G) ≥ 0.64 good; (SA) ≥ 0.49 satisfactory/adequate; (P) ≥ 0.36 poor; (VP) < 0.36 very poor. Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; CUI+: Clinical utility index positive; CUI-: Clinical utility index negative; FC: Fraction correct; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index.

Figure 2 Fraction correct of carcinoembryonic antigen, carbohydrate antigen, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index. ^1Fraction correct (FC) overall utility good; ^2FC overall utility acceptable; ^3FC overall utility poor. CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index.

in absolute value does not seem to be a prospective measure for evaluating tumour response in our study. However, while applying the cut-off value did not change the concordance of tumour markers and radiology-based evaluation of the response, it almost completely changed the possibility of using the inflammatory indices for this purpose, in our study (Table 3).

We performed a linear correlation method to evaluate the relationship between the RECIST response and changes in tumour markers and inflammatory indices. Several studies have published data about the correlation between tumour response and CEA kinetics[31,32,33] indicating a significant correlation, while Hermunen et al[32] separately analysed the correlation coefficient every 2 mo of treatment, showing variation from 0.37-0.47. In our study, there was a significant moderate correlation between CEA kinetics and both the RECIST 1.1 and the dichotomous RECIST 1.1 outcomes (PD, DC). In addition to correlating with the response according to the RECIST 1.1, CEA had a significant moderately strong correlation with CA19-9 and a low correlation with the inflammatory indices NLR, PLR and SII, while CA19-9 had a moderately strong correlation only with the PLR. The association between the CA19-9 change and platelet kinetics was previously described in pancreatic cancer[34].
Among the inflammatory indices, ΔNLR and ΔSII had moderate correlation strength with radiological evaluation, while ΔPLR had low correlation strength only with the dichotomous RECIST outcome. According to our results, the LMR had no correlation with the RECIST response or tumour markers, contrary to the published data about the significant prognostic importance of the LMR in mCRC [35,36,37].

The kinetics of changes in the CEA during chemotherapy treatment have been evaluated with three disease outcome measures: The objective response rate (RR), the progression-free survival (PFS) rate, and the overall survival (OS) rate in several studies. According to a published meta-analysis, the CEA response is highly correlated with the ORR (OR, 9.03), but the studies are extremely heterogeneous (I², 72%) and influenced by publication bias (Egger’s test of 2.67, P value, 0.004) [39].

We found 20 studies comparing CT and CEA for response evaluation in mCRC [30-33,39-55]. The setting differed slightly among these studies, as did their endpoints. CEA measurement and CT scans were repeated every 2 mo in all but three studies that used 1.5- and 3-mo CT intervals, respectively. There was no consensus on the cut-off values for CEA to define the response, PD or (SD). The definition of CEA progression varied between a 2.7 and 200% increase from baseline and between a 0 and 50% CEA decrease compared with that at baseline for the response. SD was defined as between these variable cut-offs.

The optimal cut-off value of CEA change was frequently determined arbitrarily on the basis of radiology-based criteria (WHO or RECIST), categorizing patients as “CEA responders” or “nonresponders”, or on the basis of ROC analysis [30-33,39-55].

In addition to the arbitrarily chosen cut-off, eight studies conducted from 2012 to 2020 determined the best cut-off for the response with ROC analysis [31,43,45,47,49,51,52,53]. All these studies used the RECIST 1.1. The cut-off for PD varied from 2.7% to 62%, among these studies, while in the same population, the cut-off depended on the line of chemotherapy, ranging from 7.5% to 51.3% (median 31%) and the type of treatment (for VEGFR treatment, the cut-off value is 62%) [52]. The AUC of the ROC analysis varied from 0.65 to 0.83 depending on the line of treatment and VEGF us. Therefore, the Se, Sp, PPV, NPV and accuracy among the studies also varied.

In our study, the kinetics of tumour marker and inflammatory index changes were evaluated with the ORR estimated by the RECIST 1.1 using the dichotomous outcomes PD and DC. The AUC of the ROC analysis for CEA was 0.842, which is categorized as good and is the highest AUC value for CEA published to date. The CEA cut-off value of 24.52% with the best Se of 80.2% and Sp of 80.4% is similar to that reported in published data. In our study, we considered all monitoring data together without stratification based on the 1st or 2nd line of chemotherapy or the use of biologics.

Information about CA19-9 and the best cut-off is sparse. To the best of our knowledge, only 3 papers have published the best CA19-9 cut-off value for PD using ROC analysis [43,45,49]. The published data were similar in the studies of Petrioli et al [46] (AUC 0.80, CA19-9 > 22%), Jia et al [50] (AUC 0.82, Ca 19-9 > 28%), and Trillet-Lenoir (AUC 0.69, CA19-9 > 20%); the first two analyses yielded good AUCs and the third analysis yielded poor AUCs [45]. In our ROC analysis with an acceptable AUC level, the best cut-off value of CA19-9 for PD was 21.49%, which is similar to the value reported in the published data, with a lower Se and Sp than those of CEA in the same analysis.

The inflammatory indices NLR, PLR, MLR and SII have been widely investigated and confirmed to be important prognostic factors in several cancers, including CRC and mCRC. The majority of studies are retrospective and devoted to the preoperative or perioperative values of the inflammatory indices, exploring the prognostic importance of these indices for the PFS, DFS or OS rate [35,36,37,56,57]. However, several articles have addressed the importance of changes in the inflammatory indices in patients with mCRC, gastric cancer, breast cancer, and lung cancer undergoing chemotherapy and their relationship with the PFS, OS and RR rates. The NLR has been suggested to be a prognostic marker in several solid tumours [57-61]. As with the tumour markers, the main question is how to find the optimal cut-off value for the differentiation of PD from DC. Nemoto et al [62] investigated the importance of increased vs decreased values of the inflammatory indices NLR, LMR, PLR, CEA and CA19-9 in patients with mCRC undergoing chemotherapy. All the inflammatory indices and both tumour markers, except for the LMR, significantly changed during chemotherapy, but the only NLR was a significant predictor of the OS and PFS rates [62]. Inflammation promotes tissue repair responses that induce the proliferation of premalignant cells, increase cell viability and stimulate angiogenesis, immunosuppression, inhibition of apoptosis, and DNA damage, ultimately contributing to metastatic spread [63,64]. Neutrophils are a factor related to systemic inflammation, which is associated with cancer growth, producing vascular endothelial growth factor
and various matrix proteases and contributing to metastatic spread\cite{65}. A high NLR indicates a relatively elevated neutrophil count and depressed lymphocyte count.

On the other hand, Shibutani et al\cite{66} confirmed the prognostic importance of the pretreatment value of the NLR for the OS rate in mCRC patients undergoing chemotherapy, but the posttreatment value was not predictive of response, making the NLR unsuitable for monitoring the chemotherapy response. Interestingly, contrary to the results obtained in a previous study, another study examined NLR changes in mCRC patients before and after two cycles of chemotherapy (FOLFIRI + bevacizumab) and revealed that an increased NLR led to significantly longer OS times than a decreased NLR in patients with SD\cite{67}. In discussing determination of the optimal cut-off value for the NLR, Nemoto was against the construction of ROC curves, instead favouring cut-off determination of good vs poor prognoses based on the median value. In our study, we used ROC analysis to determine the best cut-off value, as Guo analysed perioperative changes in NLR and ∆NLR and reported their association with the OS rate but not the PFS rate\cite{68}. ROC analysis used for cut-off determination has been reported in gastric cancer patients undergoing chemotherapy\cite{69} and in breast cancer patients\cite{70,71}.

Kim et al\cite{71} published a study with the largest number of patients (503) with mCRC undergoing chemotherapy and analysed the outcome of patients with different NLR dynamics, reporting that high prechemotherapy NLR, Glasgow prognostic score and CEA levels independently predicted poor survival and low chemotherapy response. In contrast, NLR reduction was an independent predictor of good prognosis and chemotherapy response. The cut-off for NLR was chosen on the basis of the median value. The authors concluded that the change patterns in NLR could be used to predict chemotherapy response and prognosis. Based on these results, they suggested that chemotherapy resistance is indicated by a continuously high NLR or a post-chemotherapy change to a high NLR, which indicates a persistent systemic inflammatory state. Moreover, NLR monitoring has been suggested to identify patients who will experience a low response to chemotherapy\cite{72}. In another study, the PLR along with the NLR were correlated with DC but not the ORR, and the PLR was a significant independent predictor of the PFS rate but not the OS rate in patients with mCRC and confined metastases to the liver in patients undergoing fluoropyrimidine-oxaliplatin chemotherapy\cite{73}.

The mechanism of the PLR in tumorigenesis might be derived from the role of platelets in promoting angiogenesis, adhesion, and invasion by increasing the production of vascular epidermal growth factor and transforming growth factors\cite{74}.

Our ROC curve analysis at the acceptable AUC level for the NLR and SII and the poor level for the PLR is one of the first to show the best cut-off of percent change in the NLR, PLR and SII values.

In patients receiving palliative therapy, DC is clinically meaningful and does not need to be characterized meticulously by radiology at short intervals. The main reason to develop good tumour markers for response is to at least partially replace expensive and toxic CT examinations.

CEA could replace CT evaluation if a reliable cut-off for DC can be identified. Trillet-Lenoir et al\cite{44} found that CT could be avoided in 13% of cases when progression was defined as a > 200% rise in CEA. Petrioli et al\cite{46} found that a CEA increase of more than 50% identified PD with an Sp of 96.4%. According to Hermunen et al\cite{32}’s study, increasing CEA levels could identify all patients with PD (Se = 1.0), and in 50%-74% of these patients, an increasing CEA level predicted PD earlier than CT. It was possible to replace CT with CEA monitoring in all patients with decreasing CEA levels, meaning that 23%-47% of CT scans could have been avoided at any given time point\cite{32}. Gulhati et al\cite{33} reported that with a 99% NPV, the clinical cut-off (for chemotherapy alone, -79.4; AUC 0.79, Se 97%, Sp 22.4%; for VEGFR, -88.7, AUC 0.72, Se 96.3%, Sp 16.7%) for the prediction of non-PD could avoid CT scans at the first response evaluation in 21.0% (chemotherapy alone) and 16.2% (chemotherapy with anti-VEGF antibody–treated) of patients. In all the studies, the cut-off value that could help to avoid at least some of the CT examinations was different from the best cut-off value. The value used to replace CT evaluation should be maximally sensitive and able to detect all PD. In our study, a CEA cut-off value of 60.85% with an Se of 100% Se and an Sp of 35.7% avoided 25% of CT control examinations in unresectable mCRC patients undergoing chemotherapy. We obtained different cut-off values but similar percentages of spared CT examinations as those reported by Hermunen et al\cite{32} and Gulhati et al\cite{33}.

The cut-off value of CA19-9, which could be a candidate to replace CT examinations, was investigated by Petrioli et al\cite{46}, who reported that a CA19.9 increase of more than 50% identified PD with an Sp of 92.6% and could be used to replace 25%-
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30% of CT evaluations; Trillet-Lenoir et al.\cite{44} reported that an increase of 200% could be used to substitute CT evaluation, avoiding 13% of CT scans. In our study, with an acceptable AUC value, a CA19-9 decrease of 55.38%, with and Se of 100% and an Sp of 39.6%, could be used to avoid 16% of CT examinations.

Our report is the first regarding the cut-off values of the NLR, PLR and SII with Se 1 and maximal Sp for PD; however, the number of CT scans that could be avoided with these values was negligible.

The association between potential risk factors and disease progression was evaluated using binary logistic regression and univariate analysis, which showed that both the markers and the all indices except for the LMR were significant predictors of PD. In the multivariate analysis, only CEA was a significant predictor of PD, confirming its independence from the other evaluated factors. On the other hand, the main problem in monitoring advanced disease treatment with chemotherapy is assuming that CEA value fluctuation during treatment is important and it is necessary to differentiate significant CEA value changes that represent disease progression, from so-called “physiological variation”, drug effects, liver damage, surges as pseudoprogression indicators and the influence of other nonmalignant conditions that can coexist with mCRC\cite{75,76}.

In our analysis, we were not satisfied with the significance obtained with classical statistical tests concerning increasing and decreasing values of tumour markers and inflammatory indices in patients with PD vs DC; instead, we tested for practicability and diagnostic features important for clinical use.

CUI, a practical multiattribute approach, appears to be useful for evaluating new diagnostic tests\cite{77}. To the best of our knowledge, no other studies have estimated the value of CEA, CA19-9 and the inflammatory indices in monitoring mCRC patients undergoing chemotherapy with the CUI. According to our data, CEA was good for screening PD, and acceptable for identifying patients, and its overall utility was good, confirming its robustness for monitoring unresectable mCRC and preventing a significant number of CT examinations. CA19-9 was good for screening PD, poor at identifying cases, and had satisfactory overall utility, making it acceptable in some circumstances, particularly when CEA is not expressed. The Inflammatory indices NLR, PLR and SII investigated under the conditions of our study yielded a positive signal with an acceptable level of screening for PD, justifying further investigation into their value for this purpose.

Tumour markers and inflammatory indices cannot completely substitute for CT monitoring of the response. Apart from true false elevation, there are two situations in which self-correction of tumour markers and correction of the CT-estimated response can occur during follow-up. The first is a surge in tumour markers; after temporary elevation, the value decreases, indicating patients who will benefit from chemotherapy with response or at least achieve DC. Our results indicating the surge in CEA and CA19-9 values are similar to those in other published studies, while we are the first to report surges in the inflammatory indices\cite{78-81}. From a practical point of view, surges are not a substantial problem and could be resolved with earlier unscheduled CT examination. The other problem with measuring tumour marker increases is that PD without confirmation on corresponding CT examination can be indicated and tumour markers may subsequently continue to rise with later confirmation of PD on subsequent CT, which represents true anticipation of PD. It is accepted that a continuous rise in tumour markers without corroborating CT could be considered PD\cite{7}. This situation demands frequent tumour marker measurement and it should be kept in mind that surges can sometimes persist for up to 4 mo and unscheduled CT examination may be required. It would be interesting to explore whether the synergistic action of tumour markers and inflammatory indices could help us solve this problem more easily. Fast increase and fast decrease in tumour markers also do not indicate the ultimate success or failure of chemotherapy, but rather dynamic change, which may be more informative regarding response and prognosis\cite{48}.

The limitation of our study is the small number of analysed patients, particularly those with inflammatory indices, and the lack of analysis of PFS and OS outcomes. Additionally, we used the same model for the evaluation of tumour markers and inflammatory indices. There are more options for analysing tumour marker kinetics, and the dynamics of changes\cite{47} and calculating the level of change\cite{82}, including construction of the slope\cite{83}, which could also influence the results of the study.
CONCLUSION

CEA is a useful marker for monitoring the chemotherapy response in unresectable mCRC patients and could replace a quarter of CT examinations. CA19-9 has poorer diagnostic characteristics than CEA but could be useful in some clinical circumstances, particularly when CEA values are not increased. Dynamic changes in the inflammatory indices NLR, PLR and SII could be promising for further investigation into their use for this purpose. A large, well-designed, multicentric, prospective study could help us define the role of tumour markers and inflammatory indices in monitoring patients with unresectable mCRC undergoing chemotherapy. scepticism regarding the possibility of conducting such a study has existed for a long time[49], but it is necessary to overcome this to rationalize and improve our approach to monitoring mCRC patients undergoing chemotherapy.

ARTICLE HIGHLIGHTS

Research background

The roles of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA19-9) in monitoring the patient response to chemotherapy for metastatic colorectal cancer (mCRC) are not well defined and accepted as standard practice. Inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII) are important predictors for disease course and outcome, but have not widely investigated in the monitoring of mCRC. There is unmet need for simple, safe, cheap and accurate method in monitoring of the patients response to chemotherapy for mCRC.

Research motivation

The main topic of the study was to evaluate the significance and usefulness of dynamic change of tumor markers CEA and CA19-9 in comparison with standard method for monitoring the chemotherapy response for mCRC. The key problem was to find representative cut-off values for PD and DC. The another topic was to evaluate correlation and concordance of the dynamic changes in the inflammatory indices with standard method for monitoring the chemotherapy response for MCRC and with the tumour markers. The significance of this study is to help to define role of CEA and CA19-9 in monitoring the chemotherapy response for mCRC, and to evaluate the potential role of the inflammatory indices in the same purpose.

Research objectives

The main objectives was to find correlation and concordance of the dynamic change of the tumor markers CEA and CA19-9 and the inflammatory indices with the standard method for monitoring the chemotherapy response, to find representative cut-off values for PD. The another main objective was to evaluate clinical significance of the tumor markers and the inflammatory indices using CUI. All main objectives were realized. Realization of our main objectives better defined the role of CEA, pointed out the role of CA19-9 and the potential role of inflammatory indices in the monitoring chemotherapy response in mCRC which should be further investigated.

Research methods

We performed baseline CT before beginning of the chemotherapy along with the tumour markers CEA and CA19-9 and CBC with the inflammatory indices NLR, PLR, LMR and SII. During monitoring of chemotherapy response we repeated all baseline values after 3 or 4 cycles of chemotherapy in the period of 10-12 wk, and after following 3 or 4 cycles of chemotherapy in the following 10-12 wk. CT- based evaluation of response was performed according to RECIST 1.1 criteria, and the tumors markers and inflammatory indices according to the change in the percent from the baseline value or at nadir calculated as ∆CEA1 = [(CEA2 - CEA1)/CEA 1] × 100, ∆CEA2 = [(CEA 3- CEA nadir or 2)/CEA nadir or 2] × 100. The same formula was used for the CA19-9 and inflammatory indices. Statistical analyses were conducted using IBM SPSS Statistics, version 26.0.

Research results

A total of 102 mCRC patients participated in this study. The tumour markers CEA and
CA19-9 and all inflammatory indices except LMR significantly correlated with the CT-based response to chemotherapy in patients with mCRC. The best PD cut-off value for CEA was 24.52%, for CA19-9 21.49%, for inflammatory indices NLR 11.05%, PLR 5.9%, SII -6.04%. The cut-off with maximal Se for excluding PD was -60.85% for CEA and -55.38% for CA19-9, allowing for the safe avoidance of 25.49% and 16.92% of CT control examinations. In the multivariate analysis, only CEA was a significant predictor of outcome. CEA had good overall utility FC, CA19-9 had a satisfactory overall utility FC, and the inflammatory indices poor overall utility.

Research conclusions
CEA is useful in monitoring of the chemotherapy response in patients with mCRC and can substitute a quarter of CT control examinations. CA19-9 could be useful in certain circumstances. The inflammatory indices NLR, PLR and SII should be further investigated into their use in chemotherapy monitoring for patients with mCRC.

Research perspectives
Future research should investigate potential of the combinations of the tumor markers and the inflammatory indices in monitoring chemotherapy response in mCRC.

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Observational Study

Prevalence of depression and anxiety and associated factors among geriatric orthopedic trauma inpatients: A cross-sectional study

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Author contributions: Chen JL performed study design, data collection and manuscript drafting and revision; Luo R performed data analysis and interpretation; Liu M performed language editing and data collection; all authors have read and approved the manuscript.

Institutional review board statement: This study was reviewed and approved by the Biomedical Research Ethical Committee of West China Hospital of Sichuan University (Approval No. 2020-29).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

Data sharing statement: The data used and/or analyzed during the current study are available from the corresponding author on request.

Abstract

BACKGROUND
Common mental disorders such as anxiety and depression in geriatric orthopedic trauma patients have received little attention in research.

AIM
To investigate the prevalence of emotional disorders among geriatric orthopedic trauma patients and identify demographic, social and clinical risk factors.

METHODS
This cross-sectional study was performed in geriatric patients (aged ≥ 60 years, both sexes) with orthopedic trauma admitted to a level I trauma center between May 2015 and December 2017. Demographic, social, and clinical characteristics were described. Huaxi Emotional-Distress Index (HEI) was used to evaluate the severity of anxiety and depression status. Differences in continuous variables were tested using the t-test, and differences in categorical variables were assessed using the Pearson χ² test. Binary logistic regression analyses were used to identify the factors associated with a HEI score > 8.

RESULTS
Among the 966 patients, 487 were male and 479 were female, with a mean age of 70.2 ± 7.1 years. The age ranged from 60 to 90 years. Seventy-five patients had an HEI score > 8, accounting for about 7.8% of all patients. A higher Injury Severity Score (4.17 ± 3.10 vs 7.96 ± 6.68, P < 0.001), higher Visual Analog Score (5.05 ± 1.09 vs 6.89 ± 1.23, P < 0.001), number of chronic diseases (P < 0.001), injury type (P = 0.038), and education level (P = 0.001) were significantly associated with HEI score > 8. On logistic regression, a higher education level was a protective factor for emotional disorders (P = 0.047), whereas Injury Severity Score (P = 0.024), Visual Analog Score (P < 0.001), two or more chronic diseases (P < 0.001) were the related independent risk factors.
Emotional disorders are common in geriatric patients with orthopedic trauma. Clinicians should remain vigilant of emotional disorders in geriatric patients and screen for anxiety and depression in higher risk groups.

**Key Words:** Anxiety; Depression; Geriatric; Trauma; Orthopedic

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**INTRODUCTION**

Throughout the lifespan, orthopedic trauma patients are often accompanied by anxiety and depression. In previous studies, the incidence of anxiety was 4.8%-39.8%[1-3] and the incidence of depression was 22.3%-87.6%[1,2,4,5]. From the perspective of age stratification, anxiety and depression are common in elderly people. The incidence of generalized anxiety disorder was reported at 0.7%-12%[6-9] and depression was 9%-11%[7,8]. In addition, anxiety and depression can occur separately or often together in elderly people[8]. However, until the last decade, common mental disorders such as anxiety and depression in geriatric orthopedic trauma patients received little attention in research, not to mention the huge burden of mental illness on families, society and the economy[6]. Therefore, it is urgent to understand, develop and evaluate evidence-based treatments for anxiety and depression among this specific group of patients. Before treatment, it is a top priority to establish the psychological characteristics and related factors of geriatric orthopedic trauma patients.

There are currently many scales assessing anxiety and depression among various target groups, such as the 15-item Geriatric Depression Scale[8], State-Trait Anxiety Inventory[10], Hospital Anxiety and Depression Scale[11], EuroQol (Quality of life)-5 Dimensions[12] and 7-item Generalized Anxiety Disorder Scale[8]. However, due to the time-consuming and professional evaluation, they have not been widely used in clinical practice. Therefore, based on the large size and unique cultural characteristics of Chinese people, Wang et al[13] designed a new screening scale [Huaxi Emotional-Distress Index, (HEI)] for identifying emotional disorders such as anxiety, depression and suicidal tendency.

HEI is extensively used in the West China Hospital of Sichuan University, Chengdu, China. HEI has shown good effect when used in non-psychiatric clinical settings. Therefore, the purpose of this study was to investigate the prevalence of emotional disorders among geriatric orthopedic trauma patients and identify demographic, social, and clinical risk factors for anxiety and depression.
MATERIALS AND METHODS

Study design
This cross-sectional study was performed in geriatric patients with orthopedic trauma admitted to West China Hospital between May 2015 and December 2017. Inclusion criteria were as follows: (1) Aged ≥ 60 years, both sexes; and (2) Musculoskeletal injury (including closed or open fracture, joint isolation, muscle/vessel/nerve soft tissue injury). Exclusion criteria were: (1) Cognitive impairment or consciousness disorder; (2) Refusal to participate; (3) Incomplete questionnaire; (4) Unable to communicate; (5) Central nervous system disorder due to acute trauma; and (6) Significant symptoms or a history of mental illness. The demographic, social and clinical data including age, sex, marital status, education level, Injury Severity Score (ISS), Visual Analog Score (VAS), injury type, surgery type and number of chronic diseases were collected from the Hospital Information System of West China Hospital.

HEI was used to evaluate the severity of anxiety and depression. The Cronbach’s α of HEI was 0.90, and sensitivity and specificity were 0.880 and 0.766, respectively[13]. There are nine self-reported items in total and all items are 5-point Likert-scaled with scale points 0-4. There are four grades based on the sum of the scores of nine items: normal (0-8 points), mild (9-12 points), moderate (13-16 points) and severe (17-36 points). The tenth and 11th item is not included in the total score (expanded to 11 items only in serious cases), but the results serve as a reference for medical staff. Details of HEI are presented in Supplementary material.

Assessment of variables
Age, sex, marital status, education level, and HEI were assessed using the standard version of questionnaires. Pain was measured with a VAS ranging from 0 (no pain) to 10 (worst pain). The VAS and HEI were calculated by trained nurses after patients filling in the results according to their actual situation. The ISS was used to measure the severity of the injury during the time of enrollment. Injury type, surgery type, and number of chronic diseases were determined by surgeons’ reports and patients’ reports of medical history–diagnosed hypertension, diabetes, cardiovascular disease, chronic lung disease, cerebrovascular disease, hepatic dysfunction, and renal dysfunction. For patients with an emotional disorder, psychological or psychiatric consultations were conducted for specialized treatment. The detailed process and response strategies are shown in Figure 1.

Statistical analysis
Continuous variables are expressed as mean ± SD, and categorical variables are expressed as absolute values and percentages. Differences in continuous variables were tested using the t-test, and differences in categorical variables were assessed using the Pearson χ² test. Binary logistic regression analyses were used to evaluate anxiety and depression, adjusted for age (continuous), sex (categorical), marital status (categorical), education level (categorical), ISS (continuous), VAS (continuous), injury type (categorical), surgery type (categorical), and number of chronic diseases (categorical). Odds ratios and 95% confidence intervals were calculated. All statistical analyses were carried out using SPSS version 21.0 (IBM, Chicago, IL, United States). A P value < 0.05 was regarded as statistically significant. The statistical methods of this study were reviewed by a member of the Clinical Study Design and Statistics Service from the West China Hospital, Sichuan University.

RESULTS

Patients’ characteristics
Among the 966 patients, 487 were male and 479 were female, with a mean age of 70.2 ± 7.1 years. The age ranged from 60 to 90 years. Of this sample, 89.2% of patients were married. Nearly two-thirds of the patients were admitted to the hospital with fractures. The average ISS was 4.47 ± 3.65. Illiteracy (12.9%) and semi-illiteracy (33.6%) accounted for almost half of the total number of patients. The vast majority (87.3%) of patients required elective surgery. Almost two-thirds of elderly patients suffered from chronic diseases. The basic demographic, clinical and social characteristics of the enrolled patients are shown in Table 1.
Table 1 Baseline data of the enrolled patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>477 (49.4)</td>
</tr>
<tr>
<td>70-79</td>
<td>397 (41.1)</td>
</tr>
<tr>
<td>≥ 80</td>
<td>92 (9.5)</td>
</tr>
<tr>
<td>ISS (points)</td>
<td>4.47 ± 3.65</td>
</tr>
<tr>
<td>VAS (points)</td>
<td>5.20 ± 1.20</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>487 (50.4)</td>
</tr>
<tr>
<td>Female</td>
<td>479 (49.6)</td>
</tr>
<tr>
<td>Injury types</td>
<td></td>
</tr>
<tr>
<td>Fracture</td>
<td>645 (66.8)</td>
</tr>
<tr>
<td>Joint dislocation⁠</td>
<td>65 (6.7)</td>
</tr>
<tr>
<td>Soft tissue injury</td>
<td>256 (26.5)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>862 (89.2)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>7 (0.7)</td>
</tr>
<tr>
<td>Divorced or widowed</td>
<td>97 (10.0)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>125 (12.9)</td>
</tr>
<tr>
<td>Primary school</td>
<td>325 (33.6)</td>
</tr>
<tr>
<td>High school</td>
<td>407 (42.1)</td>
</tr>
<tr>
<td>Junior college and above</td>
<td>109 (11.3)</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
</tr>
<tr>
<td>Emergency</td>
<td>94 (9.7)</td>
</tr>
<tr>
<td>Elective</td>
<td>843 (87.3)</td>
</tr>
<tr>
<td>None</td>
<td>29 (3.0)</td>
</tr>
<tr>
<td>Number of chronic diseases</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>326 (33.7)</td>
</tr>
<tr>
<td>1</td>
<td>438 (45.3)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>202 (20.9)</td>
</tr>
<tr>
<td>HEI score</td>
<td></td>
</tr>
<tr>
<td>≤ 8</td>
<td>891 (92.2)</td>
</tr>
<tr>
<td>&gt; 8</td>
<td>75 (7.8)</td>
</tr>
<tr>
<td>Total</td>
<td>966 (100)</td>
</tr>
</tbody>
</table>

¹Joint dislocation: If fracture and joint dislocation occurred at the same time, it was considered joint dislocation.
²Junior college: general college and technical secondary school.

**Prevalence of anxiety and depression and related factors**

Among the 966 elderly patients, 75 had an HEI score > 8, suggesting that about 7.8% of patients with orthopedic trauma had emotional disorders (Table 1). A higher ISS (4.17 ± 3.10 vs 7.96 ± 6.68, P < 0.001), higher VAS (5.05 ± 1.09 vs 6.89 ± 1.23, P < 0.001), number of chronic diseases (P < 0.001), injury type (P = 0.038), and education level (P = 0.001) were significantly associated with HEI score > 8 (Table 2). Binary logistic regression analysis indicated that a higher ISS (P = 0.024), higher VAS (P < 0.001), two or more chronic diseases (P < 0.001), and junior college education or above (P = 0.047) were independently associated with anxiety and depression (Table 3).

**DISCUSSION**

More than 70% of adults have experienced different traumatic events in their lifetime, and trauma such as traffic accidents, falling from height, and power tool injuries are common in China[14,15]. In addition, the global population is growing older.
Table 2 Association between Huaxi Emotional-distress Index score and related factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>HEI ≤ 8</th>
<th>HEI &gt; 8</th>
<th>t/χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 891)</td>
<td>(n = 75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>70.15 ± 7.08</td>
<td>70.32 ± 7.46</td>
<td>-0.2</td>
<td>0.842</td>
</tr>
<tr>
<td>ISS (points)</td>
<td>4.17 ± 3.10</td>
<td>7.96 ± 6.68</td>
<td>-4.862</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VAS (points)</td>
<td>5.05 ± 1.09</td>
<td>6.89 ± 1.23</td>
<td>-13.92</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>448 (50.3)</td>
<td>39 (52.0)</td>
<td>0.082</td>
<td>0.775</td>
</tr>
<tr>
<td>Female</td>
<td>443 (49.7)</td>
<td>36 (48.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury types, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fracture</td>
<td>602 (67.6)</td>
<td>43 (57.3)</td>
<td>6.526</td>
<td>0.038</td>
</tr>
<tr>
<td>Joint dislocation</td>
<td>62 (7.0)</td>
<td>3 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft tissue injury</td>
<td>227 (25.5)</td>
<td>29 (38.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>795 (89.2)</td>
<td>67 (89.3)</td>
<td>0.621</td>
<td>0.733</td>
</tr>
<tr>
<td>Unmarried</td>
<td>7 (0.8)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced or widowed</td>
<td>89 (10.0)</td>
<td>8 (10.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational level, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiteracy</td>
<td>105 (11.8)</td>
<td>20 (26.7)</td>
<td>17.652</td>
<td>0.001</td>
</tr>
<tr>
<td>Primary school</td>
<td>303 (34.0)</td>
<td>22 (29.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>376 (42.2)</td>
<td>31 (41.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junior college and above</td>
<td>107 (12.0)</td>
<td>2 (2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency</td>
<td>87 (9.8)</td>
<td>7 (9.3)</td>
<td>0.049</td>
<td>0.976</td>
</tr>
<tr>
<td>Elective</td>
<td>777 (87.2)</td>
<td>66 (88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>27 (3.0)</td>
<td>2 (2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of chronic diseases, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>325 (36.5)</td>
<td>1 (1.3)</td>
<td>213.385</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1</td>
<td>429 (48.1)</td>
<td>34 (12.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2</td>
<td>137 (15.4)</td>
<td>65 (86.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD or n (%). ISS: Injury Severity Score; VAS: Visual Analog Score; HEI: Huaxi Emotional-distress Index.

Table 3 Relationship of significant emotional distress predictors with Huaxi Emotional-distress Index score > 8

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>P value</th>
<th>Exp (B)/OR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>ISS</td>
<td>0.105</td>
<td>0.047</td>
<td>5.074</td>
<td>0.024</td>
<td>1.111</td>
<td>1.014</td>
</tr>
<tr>
<td>VAS</td>
<td>1.335</td>
<td>0.194</td>
<td>47.287</td>
<td>&lt; 0.001</td>
<td>3.8</td>
<td>2.597</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junior college and above</td>
<td>-1.778</td>
<td>1.032</td>
<td>3.959</td>
<td>0.047</td>
<td>0.169</td>
<td>0.029</td>
</tr>
<tr>
<td>Number of chronic diseases, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.547</td>
<td>0.894</td>
<td>19.397</td>
<td>&lt; 0.001</td>
<td>94.376</td>
<td>12.474</td>
</tr>
<tr>
<td>≥ 2</td>
<td>-13.545</td>
<td>1.59</td>
<td>72.583</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>714.031</td>
</tr>
</tbody>
</table>

ISS: Injury Severity Score; VAS: Visual Analog Score; SE: Standard error; OR: Odds ratio; CI: Confidence interval.

According to a UN report, as of 2020, the total population > 60 years old exceeded 1 million and by 2050, the number will peak at 1.6 million[16]. Therefore, the number of elderly orthopedic trauma patients has also increased annually as the population has shifted to older age.
Orthopedic trauma research in the past has been substantially focused on implant development and technique improvement involved in the treatment of these injuries [17]. The biopsychosocial model proposed by George Engel[18] in 1977 has not been fully applied in orthopedic trauma practice and research. Orthopedic trauma is often accompanied by various psychiatric symptoms, such as negative emotions, intrusion, and avoidance symptoms. According to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V), the psychiatric symptoms could manifest as acute stress disorder (ASD), post-traumatic stress disorder, depression, or anxiety [19]. Several studies have assessed the influence of the superimposed factors of old age and trauma on patients’ emotions. A study found that a few social and biological factors were related to the occurrence of ASD in elderly patients with osteoporotic fractures [20]. Unfortunately, orthopedic surgeons have paid insufficient attention to this.

The present study offers an introduction to the understanding of anxiety and depression and their associated factors affecting the recovery and healing among geriatric patients with orthopedic trauma in China. In the present study, the prevalence of emotional disorders was 7.8%. Our result was lower than 12.4% in Australia among hospitalized orthopedic trauma patients using Generalized Anxiety Disorder Scale Two item instrument[21], and 31.2% in the United States among orthopedic trauma patients using State-Trait Anxiety Inventory-S instrument[10], but higher than 6.25% in the UK among pelvic trauma patients using EuroQol (Quality of life)-5 Dimensions instrument[22]. This was most likely due to differences in sample size, timing and instruments used to measure these psychological parameters. In addition, most studies included adults of all ages and did not individually screen out elderly patients.

It needs to be emphasized that the ratio of male to female patients was almost equal in the present study, which was different from the high proportion of male patients in many previous studies[12,22,23]. Although a few studies indicated that the prevalence of depression and anxiety in women was higher than in men, no similar result was found in the present study[3,5]. This indicates that, in the Chinese elderly population, the prevalence of emotional disorders is not significantly different between men and women.

The present study revealed that independent variables like higher ISS, higher VAS, having two or more chronic diseases, and receiving a junior college education or above were statistically significant for HEI score > 8. The ISS score is often used to assess the severity of multiple traumas. A prospective cohort study found no association between depression and ISS[24]. However, the present study revealed that the severity of injury among geriatric orthopedic patients was significantly positively associated with HEI score > 8. The finding was in line with the study of Giannoudis et al[25] in the UK. The present study was conducted in a level I trauma center; therefore, this finding may be related to the various injury types, such as open fractures, polytrauma, and amputation, among the elderly patients. These severely injured patients often have to face multiple pressures of long hospitalizations, high costs, and even mutilation. Therefore, they are susceptible to negative emotions. Hawamdeh et al[26] found that factors associated with a high prevalence of anxiety and depression among amputees, included female sex, lack of social support, unemployment, and traumatic
amputation.

Pain plays an important role in the quality of life. Many studies have found that pain is closely related to depression and anxiety\cite{3,11,24,27}. Srahbu et al\cite{3} found that those who had pain within the last 24 h were 2.02 and 2.75 times more likely to develop depression and anxiety, respectively, than those without pain. In our study, those who had a higher VAS after orthopedic injury were 3.8 times more likely to develop anxiety and depression than those who had a lower VAS. In our experience, most elderly people have reduced tolerance to pain, so the severity and persistence of pain are more likely to lead to depression and anxiety. Most elderly people have sleep disorders. Pain can aggravate sleep disorders, which in turn exacerbate the pain. This vicious cycle is more likely to cause depression and anxiety.

Older adults with anxiety and depression frequently present with a variety of co-morbid chronic illnesses\cite{28,29}. In the present study, the number of chronic diseases was found to be associated with HEI > 8 on logistic regression. Those who had two or more chronic diseases had a higher risk of developing anxiety and depression when compared to those who did not have a chronic disease. A few studies indicated the presence of dysregulated homeostatic biological pathways in patients with depressed and anxiety, such as increased inflammation and disrupted energy-regulating neuroendocrine signaling (e.g., leptin, insulin)\cite{29-31}. However, the causal relationship between chronic diseases and emotional disorders seems to need clarification in the future. In addition, they are more like a pair of reciprocal relationships\cite{28}.

A few studies have found no association between education level and emotional disorders\cite{24,32}. However, the present study revealed that the education level among geriatric orthopedic patients was significantly positively associated with HEI score > 8. Those with a junior college education or above had a lower risk of developing anxiety and depression than those who were illiterate. In China, receiving better education and skill training increases job opportunities, and work brings better economic and social support so that people have more strength and resources to counteract frustrations and difficulties. This may explain why a higher educational level was a protective factor for emotional disorders. Lack of socioeconomic support and unemployment are risk factors for depression and anxiety\cite{3,5,26}.

Depression involves an entire clinical spectrum from mild to severe\cite{33}. Therefore, depression should be considered in the patients with HEI score < 8. Importance should be attached to the dynamic evaluation of emotions in elderly patients with orthopedic trauma as changes in disease progression or other serious stress events, such as loss of family members and appearance of malignant tumors, may occur during treatment.

This study had some limitations. First, it was a single-center study. Therefore, there must have been some selective bias. Second, this study did not investigate other possible risk factors, such as ethnicity, religion, insurance type, and substance abuse, that may have significantly affected the psychological condition of the patients. Third, this was a cross-sectional study, lacking longitudinal data, so it was difficult to confirm the causality. Hence, future studies need to be conducted to clarify these issues.

**CONCLUSION**

Emotional disorders, especially anxiety and depression, were common findings in geriatric patients who sustained orthopedic trauma. We would encourage clinicians to remain vigilant for emotional disorders and screen for emotional disorders in geriatric patients during the evaluation and treatment of other conditions. Psychological intervention or psychiatric treatment should be carried out.

**ARTICLE HIGHLIGHTS**

**Research background**

Common mental disorders such as anxiety and depression in geriatric orthopedic trauma patients have received little attention in research.

**Research motivation**

It is urgent to understand, develop and evaluate evidence-based treatments for anxiety and depression among geriatric orthopedic trauma patients. Before treatment, it is a top priority to establish the psychological characteristics and related factors.
Research objectives
This study aimed to analyze the data of geriatric orthopedic trauma patients from our hospital in order to investigate the prevalence of emotional disorders and identify demographic, social and clinical risk factors.

Research methods
This study was performed in elderly patients aged of 60 years or older with orthopedic trauma admitted to a level I trauma center between May 2015 and December 2017. Demographic, social, and clinical characteristics were described. Huaxi Emotional-Distress Index (HEI) was used to evaluate the severity of anxiety and depression status.

Research results
Among the 966 patients, 75 patients had an HEI score > 8, accounting for about 7.8% of all patients. A higher Injury Severity Score, higher Visual Analog Score, number of chronic diseases, injury type, and education level were significantly associated with HEI score > 8. On logistic regression, a higher education level was a protective factor for emotional disorders, whereas Injury Severity Score, Visual Analog Score, two or more chronic diseases were the related independent risk factors.

Research conclusions
Anxiety and depression are common in geriatric patients with orthopedic trauma. Clinicians should remain vigilant of emotional disorders in geriatric patients and screen for anxiety and depression in higher risk groups.

Research perspectives
Further investigations on larger samples are needed to confirm whether the results of our study are applicable on a broader scale.

ACKNOWLEDGEMENTS
The authors would like to thank the database manager for the technical support.

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Chen JL et al. Emotional disorders in geriatric trauma inpatients


Randomized Controlled Trial

Efficacy of acupuncture at ghost points combined with fluoxetine in treating depression: A randomized study

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Author contributions: Wang Y, Huang YW and Hu ZH designed the experiment; Wang Y and Huang YW used the work, Dilnur A, Lu Q and Zhang AJ, and Dong YQ collected data; Zeng FC, Xu JH and Wang W analyzed and interpreted data, and articles written by Wang Y, Huang YW and Hu ZH.

Institutional review board statement: The study was reviewed and approved by the Shanghai TCM-Integrated Hospital, Shanghai University of Traditional Chinese Medicine Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement:

Abstract

BACKGROUND
Depression affects more than 350 million people worldwide. In China, 4.2% (54 million people) of the total population suffers from depression. Psychotherapy has been shown to change cognition, improve personality, and enhance the ability to cope with difficulties and setbacks. While pharmacotherapy can reduce symptoms, it is also associated with adverse reactions and relapse after drug withdrawal. Therefore, there has been an increasing emphasis placed on the use of non-pharmacological therapies for depression. The hypothesis of this study was that acupuncture at ghost points combined with fluoxetine would be more effective than fluoxetine alone for the treatment of depression.

AIM
To investigate the efficacy of acupuncture at ghost points combined with fluoxetine for the treatment of patients with depression.
METHODS
This randomized controlled trial included patients with mild to moderate depression \((n = 160)\). Patients received either acupuncture at ghost points combined with fluoxetine \((n = 80)\) or fluoxetine alone \((\text{control group}, n = 80)\). Needles were retained in place for 30 min, 5 times a week; three treatment cycles were administered. The Mann–Whitney U test was used to compare functional magnetic resonance imaging parameters, Hamilton depression rating scale \((\text{HAMD})\) scores, and self-rating depression scale \((\text{SDS})\) scores between the acupuncture group and control group.

RESULTS
There were no significant differences in HAMD or SDS scores between the acupuncture group and control group, before or after 4 wk of treatment. The acupuncture group exhibited significantly lower HAMD and SDS scores than the control group after 8 wk of treatment \((P < 0.05)\). The acupuncture group had significantly lower fractional Amplitude of Low Frequency Fluctuations values for the left anterior wedge leaf, left posterior cingulate gyrus, left middle occipital gyrus, and left inferior occipital gyrus after 8 wk. The acupuncture group also had significantly higher values for the right inferior frontal gyrus, right insula, and right hippocampus \((P < 0.05)\). After 8 wk of treatment, the effective rates of the acupuncture and control groups were 51.25% and 36.25%, respectively \((P < 0.05)\).

CONCLUSION
The study results suggest that acupuncture at ghost points combined with fluoxetine is more effective than fluoxetine alone for the treatment of patients with mild to moderate depression.

Key Words: Traditional Chinese medicine; Acupuncture; Ghost point; Fluoxetine; Depression; Resting state magnetic resonance

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Core Tip: Acupuncture is an effective auxiliary method for the treatment of clinical depression. In this study, the authors found that the combined use of acupuncture at ghost points and fluoxetine may be more effective than fluoxetine alone for the treatment of patients with mild to moderate depression.


DOI: https://dx.doi.org/10.12998/wjcc.v10.i3.929

INTRODUCTION
Depression affects approximately 4.4% of the global population and is characterized by persistent mood depression, physical symptoms, intellectual disability, cognitive impairment, and decreased activity. It not only has adverse effects on physical and mental health, but also increases social and economic burden[1]. While selective serotonin reuptake inhibitors, tricyclic antidepressants, and other Western medicine treatments have satisfactory clinical effectiveness, they also have significant side effects, such as dependency and withdrawal reactions[2].

Acupuncture is an effective auxiliary method for the treatment of clinical depression. It mainly stimulates the nerve-related signaling pathway by adjusting neurotransmitter redistribution via the integration of the central nervous system, thus facilitating brain function through self-regulation of the human body. Nevertheless, its effect on prognosis remains to be further analyzed. While the results of a previous study have suggested that acupuncture can promote the onset of selective 5-hydroxytryptamine reuptake inhibitors and reduce adverse reactions[3], the related
mechanistic interactions are currently unclear. Therefore, this study explored the effect of acupuncture at ghost points combined with fluoxetine on clinical indicators of depression and resting-state functional magnetic resonance imaging (fMRI) parameters in patients with mild to moderate depression.

**MATERIALS AND METHODS**

**Patient recruitment and inclusion criteria**

A total of 160 patients diagnosed with mild to moderate depression between January 2019 and December 2021 were recruited. Patients were included if they (1) met the diagnostic criteria for depression, according to the fifth edition of the American Handbook on Diagnosis and Statistics of Mental Disorders[4,5]; (2) had a score between 17 and 24 points on the 17-item Hamilton depression rating scale (HAMD); and (3) were between 19 and 75 years of age. The exclusion criteria were as follows: history of brain tumor or cerebrovascular disease; cardiac or pulmonary dysfunction; infectious diseases; dementia or Alzheimer’s disease; other mental disorders; history of craniocebral trauma or surgery; and other serious diseases. The study protocol was approved by the appropriate medical ethics committee, and all patients provided written informed consent prior to examination and treatment.

**Treatment methods**

Patients were randomly allocated to receive either (1) acupuncture at ghost points combined with fluoxetine (acupuncture group, \( n = 80 \)); or (2) fluoxetine alone (control group, \( n = 80 \)). The following acupoints were selected: governor vessel, Dazhui, spine, and Mingmen, combined with 13 ghost points on the star (flat thorn), people, less business, Yinbai, Laogong, and Shenmai (Table 1).

Procedures were conducted in accordance with the national acupoint positioning standard for the selection of acupoints, acupoint positioning, and acupuncture depth, the “acupoint name and positioning” standard (GB/T12346-2006).

The Dazhui (GV14) acupoint is located in the spinal region (in the subspinous depression of the seventh cervical spine and on the posterior median line). The Jizhong (GV6) acupoint is located in the subspinal depression of the 11th thoracic spine and is on the posterior median line. The Mingmen (GV4) acupoint is located in the subspinous depression of the second lumbar spine and on the posterior midline. The Shaoshang (LU11) acupoint is located approximately 2.5 mm above the metacarpophalangeal joint of the finger, the radial side of the distal segment of the thumb and the medial side of the nail root. The Yinbai (SP1) acupoint is 2.5 mm posterior to the distal segment of the most medial toe, at the toenail corner.

The Laogong (PC8) acupoint is in the metacarpal region. It is oriented horizontally to the proximal third of the metacarpophalangeal joint, while the second and third metacarpal bones are inclined towards the third metacarpal bone. The Shenmai (BL62) acupoint is in the ankle region, below the tip of the lateral malleolus and in the depression between the lower edge of the lateral malleolus and the calcaneus.

Each patient assumed a sitting position and routine local skin disinfection was performed. A 40-mm disposable stainless-steel needle with a diameter of 0.25 mm (Huatuo brand) was first applied at the Du meridian points via the flat needling method. Patients then assumed a supine position and direct needling (with the retaining needle applied for 30 min) was performed at ghost acupoints via the Shangxingping acupuncture method. All acupuncture operators previously underwent uniform training and all operative procedures were standardized. Needles were retained in place for 30 min, 5 times a week (10 times as an observation course); a total of three treatment cycles were administered.

Fluoxetine capsules (20 mg/d for adult and elderly patients) were administered orally in accordance with the psychiatrist’s instructions. If necessary, the dose was re-evaluated and adjusted within the first 3–4 wk of treatment as higher doses increase the risk of adverse reactions. Some patients did not experience any obvious effects with a standard 20 mg dose; in such cases, doses were gradually increased to a maximum of 60 mg.

Resting-state fMRI scans were performed after routine scans to confirm the absence of organic brain lesions. A conventional structural image scan was performed using the T1 weighted image merge sequence. The scanning parameters were as follows: repetition time = 2300 ms; echo time = 2.2 ms; field-of-view = 256 mm × 256 mm; voxel = 1 mm × 1 mm × 1 mm; matrix = 256 × 256; number of layers = 192; number of excitations = 1; and a scanning time of approximately 5 min. The scanning parameters
Table 1 Comparison of general information of the two groups of patients, n (%)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Acupuncture group (n = 80)</th>
<th>Fluoxetine group (n = 80)</th>
<th>t/χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>47.96 ± 6.5</td>
<td>46.3 ± 7.2</td>
<td>1.475</td>
<td>0.142</td>
</tr>
<tr>
<td>Years of education (yr)</td>
<td>7.9 ± 2.2</td>
<td>8.1 ± 2.4</td>
<td>-0.549</td>
<td>0.583</td>
</tr>
<tr>
<td>HAMD score (points)</td>
<td>20.13 ± 2.20</td>
<td>19.75 ± 2.38</td>
<td>1.049</td>
<td>0.296</td>
</tr>
<tr>
<td>SDS score (points)</td>
<td>61.84 ± 4.55</td>
<td>60.63 ± 4.92</td>
<td>1.615</td>
<td>0.108</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (27.50)</td>
<td>28 (35.00)</td>
<td>1.477</td>
<td>0.224</td>
</tr>
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<td>Female</td>
<td>58 (72.50)</td>
<td>52 (65.00)</td>
<td>1.047</td>
<td>0.306</td>
</tr>
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<td>Yes</td>
<td>12 (15.00)</td>
<td>18 (22.50)</td>
<td>1.653</td>
<td>0.199</td>
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<td>No</td>
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<td>62 (77.50)</td>
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<tr>
<td>Drinking</td>
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<td>Yes</td>
<td>16 (20.00)</td>
<td>10 (12.50)</td>
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<td>0.257</td>
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<td>70 (87.50)</td>
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<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35 (43.75)</td>
<td>28 (35.00)</td>
<td>1.200</td>
<td>0.273</td>
</tr>
<tr>
<td>No</td>
<td>45 (56.25)</td>
<td>52 (65.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17 (21.25)</td>
<td>23 (28.75)</td>
<td>2.209</td>
<td>0.137</td>
</tr>
<tr>
<td>No</td>
<td>63 (78.75)</td>
<td>57 (71.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (18.75)</td>
<td>23 (28.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>65 (81.25)</td>
<td>57 (71.25)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HAMD: Hamilton depression rating scale.

for functional imaging using the echo planner imaging sequence were as follows: repetition time = 2000 ms; echo time = 30 ms; field-of-view = 220 mm; slice thickness = 3.5 mm; layer spacing = 0.6 mm; voxel = 3.44 mm × 3.44 mm × 3.44 mm; fractional anisotropy = 90; matrix = 64 × 64; number of layers = 33 (using layer-by-layer scanning); number of excitations = 1; time points = 240; and a scanning time of approximately 8 min. Pre- and post-treatment evaluations of fMRI parameters were performed and comparisons were made between the groups.

Clinical parameters and detection methods

Clinical parameters comprised the HAMD, self-rating depression scale (SDS), and traditional Chinese medicine (TCM) syndrome score. The HAMD consists of 17 test items[6]. Higher scores reflect more severe depression. The absence of depression is indicated by a score < 7. A total score in the range of 7–17 suggests that a patient may have depression, while a score between 17 and 24 confirms the diagnosis of depression. A total score > 24 indicates severe depression.

The SDS contains 20 items[7]. Norm data from the Chinese population defines mild, moderate, and severe depression by scores of 53, 53–62, and 63–72 points, respectively. The TCM syndrome score is based on the “Criteria for Diagnosis and Efficacy of TCM Symptoms”[8], which includes the three main syndromes of impatience and irritability, chest tightness, and rib distension. The secondary syndromes comprise headache, red eyes, bitter mouth, noise, constipation, and short yellow urine. Scores reflecting the severity of the main syndromes range from 0–6, while those for the secondary syndromes range from 0–3. Higher scores reflect greater syndrome severity.

The HAMD reduction rate ([before treatment - after treatment]/before treatment × 100%) was used to evaluate the treatment efficacy[9]. Reduction rates of ≥ 90,
70%–89%, and ≤ 69% indicated that the treatments were markedly effective, effective, and ineffective, respectively.

**Statistical analysis**

The distributions of the HAMD scores, SDS scores, and fMRI parameters were tested for normality. Following confirmation of approximate normal distributions (expressed as “mean ± SD”), the *t*-test was used for comparisons between the two groups. Enumeration data were expressed as *n* (%). The χ² test was used to compare nominal variables such as sex, combined diseases, and the presence of adverse reactions. The groups were compared in terms of ordinal variables using the Mann–Whitney *U* test to evaluate differences in clinical efficacy. The Statistical Package for Social Science version 21.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The level of statistical significance was set at α = 0.05.

**RESULTS**

There were no significant differences in HAMD or SDS scores between the acupuncture and control groups, before or after 4 wk of treatment. After 8 wk of treatment, the acupuncture group exhibited significantly lower HAMD and SDS scores than the control group (*P* < 0.05). Within-group comparisons showed that HAMD and SDS scores in both groups were significantly lower after 4 and 8 wk compared to those recorded before treatment (*P* < 0.05) (Table 2).

Comparisons between the groups before and after 4 wk of treatment did not yield significant differences in fractional amplitude of low frequency fluctuations (fALFF) values of the left posterior cingulate gyrus, left posterior cingulate gyrus, left anterior wedge leaf, left middle occipital gyrus, left inferior occipital gyrus, right inferior frontal gyrus, right cerebral island, or right hippocampus. After 8 wk of treatment, the fALFF values of the left posterior cingulate gyrus, left anterior wedge leaf, left middle occipital gyrus, and left inferior occipital gyrus were significantly lower in the acupuncture group than in the control group (*P* < 0.05). The fALFF values of the right inferior frontal gyrus, right insula, and right hippocampus in the acupuncture group were significantly higher than those in the control group (*P* < 0.05) (Table 3). TCM syndrome scores before treatment were not significantly different between the groups (Table 4). Plasma adrenocorticotropic hormone (ACTH), cortisol, and corticotropin-releasing hormone levels were not significantly different between the acupuncture and control groups. After 8 wk of treatment, the plasma ACTH and cortisol levels in the acupuncture group were significantly lower than those in the control group (*P* < 0.05) (Table 5). Within-group comparisons showed that the plasma ACTH, cortisol, and corticotropin-releasing hormone levels in the two groups were lower than those before treatment (*P* < 0.05). After 8 wk of treatment, 51.25% of the acupuncture group showed improvement, the effective rate was 41.25%, and the ineffective rate was 7.50%. The effective rate of the control group was 36.25%, the effective rate was 50.00%, and the ineffective rate was 13.75% (Table 6). The clinical effectiveness of the acupuncture group was significantly higher than that of the control group (*P* < 0.05) (Table 7).

**DISCUSSION**

The pathophysiology of depression is currently unclear, despite prior investigations of the intestinal flora hypothesis and the role of neurotransmitters, neurotrophic factors, and neuroendocrine-immune interactions[10]. Pharmacotherapy is the primary treatment modality for depression. Selective serotonin reuptake inhibitors and tricyclic antidepressants act on neurotransmitters, cytokines, and their receptors, thereby modifying complex inflammatory pathways. Although such drugs are highly effective in reducing symptoms, they are also associated with adverse reactions and relapse after drug withdrawal[11].

TCM theory postulates that the underlying basis of depression is a poor mood due to Qi stagnation, which leads to dysfunction of the viscera. Thus, acupuncture treatment for patients with depression often uses acupoints associated with soothing of the liver and depression relief, as well as tranquilization of the heart and mind. These acupoints include Baihui, Yintang, and other governor vessels. The selection of acupoints in patients with mild to moderate depression in the present study was based on the unique theory of Shen’s acupuncture treatment for depression syndrome, which
Table 2 Comparison of Hamilton depression rating scale scores and self-rating depression scale scores between the two groups (mean ± SD, scores)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before treatment HAMD score (points)</th>
<th>4 wk of treatment</th>
<th>8 wk of treatment</th>
<th>Before treatment SDS score (points)</th>
<th>4 wk of treatment</th>
<th>8 wk of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acupuncture</td>
<td>80</td>
<td>20.13 ± 2.20</td>
<td>16.60 ± 2.85</td>
<td>13.64 ± 2.75</td>
<td>61.84 ± 4.55</td>
<td>56.92 ± 5.10</td>
<td>51.14 ± 6.12</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>80</td>
<td>19.75 ± 2.38</td>
<td>17.21 ± 2.91</td>
<td>15.20 ± 2.48</td>
<td>60.63 ± 4.92</td>
<td>57.88 ± 5.53</td>
<td>54.63 ± 5.58</td>
</tr>
<tr>
<td>t value</td>
<td>1.049</td>
<td>-1.340</td>
<td>-3.768</td>
<td>1.615</td>
<td>-1.141</td>
<td>-3.769</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.296</td>
<td>0.182</td>
<td>0.000</td>
<td>0.108</td>
<td>0.255</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 vs this group before treatment.

HAMD: Hamilton depression rating scale; SDS: Self-rating depression scale.

Table 3 Comparison of fractional amplitude of low frequency fluctuations values between the two groups of patients (mean ± SD)

<table>
<thead>
<tr>
<th>Acupuncture group-fluoxetine group (fALFF difference)</th>
<th>MNI coordinates (mm)</th>
<th>Voxel</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cingulate back</td>
<td>-3</td>
<td>-11</td>
<td>13</td>
</tr>
<tr>
<td>Left precuneus</td>
<td>-4</td>
<td>-8</td>
<td>4</td>
</tr>
<tr>
<td>Middle occipital gyrus</td>
<td>-9</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Left suboccipital back</td>
<td>-14</td>
<td>-21</td>
<td>-9</td>
</tr>
<tr>
<td>Lower forehead of right frame</td>
<td>14</td>
<td>12</td>
<td>-2</td>
</tr>
<tr>
<td>Right insula</td>
<td>15</td>
<td>18</td>
<td>-5</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>11</td>
<td>-1</td>
<td>13</td>
</tr>
<tr>
<td>Post treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cingulate back</td>
<td>14</td>
<td>18</td>
<td>-11</td>
</tr>
<tr>
<td>Left precuneus</td>
<td>5</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Middle occipital gyrus</td>
<td>12</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>Left suboccipital back</td>
<td>7</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Lower forehead of right frame</td>
<td>-18</td>
<td>-16</td>
<td>9</td>
</tr>
<tr>
<td>Right insula</td>
<td>-17</td>
<td>-13</td>
<td>14</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>-13</td>
<td>12</td>
<td>-11</td>
</tr>
</tbody>
</table>

fALFF: Fractional amplitude of low frequency fluctuations.

Involves “regulating yang and eliminating pathogenic factors”[12]. After 4 and 8 wk of treatment, the HAMD and SDS scores in both the acupuncture and control groups were significantly lower than those before treatment.

Patients in the acupuncture group had significantly lower HAMD and SDS scores than patients in the control group. This suggests that the long-term clinical effect of acupuncture at ghost points combined with fluoxetine is better than fluoxetine alone for the treatment of mild to moderate depression. The decrease in scores between weeks 4 and 8 was not as notable as that observed from baseline to week 4. This could be attributed to the fact that the scores for each scale have a lower limit; indeed, both HAMD and SDS scores had entered a relatively stable range after 8 wk of treatment, even approaching values observed in patients without depression.

The assessment of the neurophysiology and neuroanatomy of affective disorders with fMRI can be used to facilitate the diagnosis of depression. Some studies have suggested that fALFF and regional homogeneity values can be used as indicators for
Table 4 Comparison of traditional Chinese medicine syndrome scores between the two groups of patients (mean ± SD, scores)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TCM syndrome points</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>Post treatment</td>
<td></td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>80</td>
<td>21.73 ± 4.20</td>
<td>7.96 ± 1.55</td>
<td>27.511</td>
</tr>
<tr>
<td>Fluoxetine group</td>
<td>80</td>
<td>20.68 ± 4.47</td>
<td>10.20 ± 2.39</td>
<td>18.493</td>
</tr>
</tbody>
</table>

TCM: Traditional Chinese medicine.

Table 5 Comparison of plasma adrenocorticotropic hormone, Cor and corticotropin-releasing hormone levels before and after treatment in the two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ACTH (ng/L)</th>
<th>Cor (ng/L)</th>
<th>CRH (ng/L)</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>8 wk of treatment</td>
<td>Before treatment</td>
<td>8 wk of treatment</td>
<td>Before-treatment</td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>80</td>
<td>38.74 ± 7.20</td>
<td>28.64 ± 5.51</td>
<td>122.64 ± 14.81</td>
<td>98.13 ± 11.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>132.85 ± 17.20</td>
</tr>
<tr>
<td>Fluoxetine group</td>
<td>80</td>
<td>40.01 ± 8.14</td>
<td>31.47 ± 7.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120.28 ± 16.57</td>
<td>105.25 ± 13.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.51 ± 15.83</td>
</tr>
<tr>
<td>t value</td>
<td></td>
<td>-1.045</td>
<td>-2.821</td>
<td>1.950</td>
<td>-3.541</td>
<td>0.767</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.297</td>
<td>0.005</td>
<td>0.001</td>
<td>0.000</td>
<td>0.192</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < 0.05 vs this group before treatment.

ACTH: Adrenocorticotropic hormone; CRH: Corticotropin-releasing hormone.

Table 6 Comparison of clinical efficacy between the two groups of patients, n (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Markedly effective</th>
<th>Efficient</th>
<th>Invalid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acupuncture group</td>
<td>80</td>
<td>41 (51.25)</td>
<td>33 (41.25)</td>
<td>6 (7.50)</td>
</tr>
<tr>
<td>Fluoxetine group</td>
<td>80</td>
<td>29 (36.25)</td>
<td>40 (50.00)</td>
<td>11 (13.75)</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>-2.041</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7 Comparison of the incidence of adverse reactions between the two groups of patients, n (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Insomnia</th>
<th>Nausea</th>
<th>Irritable</th>
<th>Anxiety</th>
<th>Tremor</th>
<th>Complication rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acupuncture group</td>
<td>80</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4 (5.00)</td>
</tr>
<tr>
<td>Fluoxetine group</td>
<td>80</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>13 (16.25)</td>
</tr>
<tr>
<td>χ²</td>
<td></td>
<td>5.331</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

the early diagnosis and monitoring of depression[13]. After 8 wk of treatment in the present study, the fALFF values of the left posterior cingulate gyrus, left precuneus, left middle occipital gyrus, and left inferior occipital gyrus were significantly lower in the acupuncture group than in the control group. In contrast, the fALFF values of the right inferior frontal gyrus, right insula, and right hippocampus in the acupuncture group were higher than those in the control group[14,15]. These results suggest that the combined use of acupuncture at ghost points and fluoxetine has a greater effect on the regulation of neurological function compared to fluoxetine alone when used in patients with mild to moderate depression[16]. Acupuncture combined with fluoxetine
may play an antidepressant role by reducing the fALFF values of patients in the frontal lobe, middle frontal gyrus, parietal lobe, anterior central gyrus, precuneus, and parietal lobule, which are higher than those of healthy people. The combined use of acupuncture and fluoxetine affects a wider range of brain regions than fluoxetine alone, thus indicating that each treatment may regulate brain function via different mechanisms. Acupuncture may have a more significant regulatory role in a greater number of brain regions compared to pharmacotherapy[17,18].

Previous studies have reported significantly lower TCM syndrome scores in an acupuncture group compared to a fluoxetine group after 8 wk of treatment; a greater clinical efficacy was achieved with acupuncture treatment[19,20]. In addition, the incidence of adverse reactions in the acupuncture group (5.71%) was significantly lower than that in the fluoxetine group (25.71%). This suggested that acupuncture at ghost points combined with fluoxetine had a greater clinical efficacy than fluoxetine alone in patients with mild to moderate depression. The results of the present study provide additional evidence for the efficacy of acupuncture in the treatment of depression. Acupuncture exerts its effects via multiple targets and mechanisms; these comprise the regulation of neurotransmitters and receptors, nerve plasticity, the neuroendocrine-immune network, and the brain-gut axis. This study demonstrated that the combined use of acupuncture and pharmacotherapy may provide synergistic benefits in patients with mild to moderate depression. Additional studies are warranted to further investigate the efficacy of different acupuncture and drug combinations.

CONCLUSION

The combined use of acupuncture at ghost points and fluoxetine may be more effective than fluoxetine alone for the treatment of patients with mild to moderate depression. At the same time, it has a better effect on improving the TCM syndrome of patients and regulating the neurological function of brain functional areas.

ARTICLE HIGHLIGHTS

Research background
Depression affects more than 350 million people worldwide. In China, 4.2% (54 million people) of the total population suffers from depression.

Research motivation
This study provided data reference for the treatment of depression.

Research objectives
This study explored the effect of acupuncture at ghost points combined with fluoxetine on clinical indicators of depression and resting-state functional magnetic resonance imaging (fMRI) parameters in patients with mild to moderate depression.

Research methods
They were divided into acupuncture group (acupuncture at ghost points combined with fluoxetine) and fluoxetine group (fluoxetine alone) with 80 cases in each group.

Research results
The acupuncture group was better than the fluoxetine group.

Research conclusions
Acupuncture at ghost points combined with fluoxetine is more effective than fluoxetine.

Research perspectives
The combined use of acupuncture at ghost points and fluoxetine may be more effective than fluoxetine alone for the treatment of patients with mild to moderate depression.
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Wang Y et al. Acupuncture combined with fluoxetine in depression treatment

DOI: 10.1111/bdi.12871
Atrial fibrillation burden and the risk of stroke: A systematic review and dose-response meta-analysis

Sheng-Yi Yang, Min Huang, Ai-Lian Wang, Ge Ge, Mi Ma, Hong Zhi, Li-Na Wang

Abstract

BACKGROUND
The increased stroke risk associated with atrial fibrillation (AF) burden exceeding 5 min is a matter of debate. In addition, the potential linear or nonlinear relationship between AF burden and stroke risk has been largely unexplored.

AIM
To determine the association between AF burden > 5 min and the increased risk of stroke and explore the potential dose-response relationship between these two factors.

METHODS
Sixteen studies from six databases with 53141 subjects (mean age 65 years) were included. Fifteen studies were observational studies, and one was a randomized controlled trial study. The potential nonlinear dose-response association was characterized using a restricted cubic splines regression model. AF burden for each 1 h and 2 h was associated with an increased risk of stroke. Trial sequential analysis with a random-effect model was used to evaluate the robustness of the evidence from the included 16 studies.

RESULTS
AF burden > 5 min was associated with an increased risk of clinical AF [adjusted risk ratio (RR) = 4.18, 95% confidence interval (CI): 2.26-7.74]. However, no
Atrial fibrillation (AF) is one of the most frequent cardiac arrhythmias. Reports suggest that an estimated 12.1 million people will suffer from this condition in the United States by 2030 and 17.9 million people in Europe by 2060[1,2]. It has been established that patients with AF have a 3 to 5-fold increased risk of stroke, and subjects with AF-related embolic stroke have a worse progression than those who experience stroke not related to AF[3-5]. With the widespread use of cardiac implantable electronic devices (CIEDs) and wearable devices, it is now possible to monitor the time and frequency of AF episodes. The American Heart Association recommends that the AF burden should be defined as the duration of the longest AF episode during a defined monitoring period[6]. Some studies demonstrated an association between AF burden and stroke risk, but few mentioned the existence of a dose-response effect. The Italian AT 500 registry study showed that patients with device-detected AF episodes of > 24 h had a 3.1-fold increased risk of stroke. In contrast, patients with AF episodes of > 5 min and < 24 h experience no significant increase in stroke risk[7]. Moreover, the ASSERT Clinical Trial reported episodes lasting > 6 min were associated with an increased risk of ischemic stroke or systemic embolism[8]. A recent systematic review demonstrated the AF burden exceeding different thresholds was associated with an increased risk of stroke; however, they did not provide a definite threshold for AF burden at stroke risk[9]. It is a matter of controversy whether an AF burden of > 5 min can increase the risk of stroke, and no studies have reported the potential dose-response effect on stroke. Accordingly, we performed a systematic review and meta-analysis to determine the association between AF burden > 5 min and the increased risk of stroke and explored the dose-response effect between these two factors.

**CONCLUSION**

AF burden was a significant risk factor for clinical AF and future stroke. A significant linear association was documented between increased AF burden and risk of future stroke.

**Key Words:** Atrial fibrillation; Stroke; Dose-response; Meta-analysis; Risk

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**Core Tip:** We performed a systematic review and meta-analysis to determine whether atrial fibrillation (AF) burden > 5 min was associated with increased risk of stroke and to explore the dose response effect of AF burden on the future stroke. A significant linear association was documented between increased AF burden and risk of future stroke.

---

**INTRODUCTION**

INTRODUCTION

Atrial fibrillation (AF) is one of the most frequent cardiac arrhythmias. Reports suggest that an estimated 12.1 million people will suffer from this condition in the United States by 2030 and 17.9 million people in Europe by 2060[1,2]. It has been established that patients with AF have a 3 to 5-fold increased risk of stroke, and subjects with AF-related embolic stroke have a worse progression than those who experience stroke not related to AF[3-5]. With the widespread use of cardiac implantable electronic devices (CIEDs) and wearable devices, it is now possible to monitor the time and frequency of AF episodes. The American Heart Association recommends that the AF burden should be defined as the duration of the longest AF episode during a defined monitoring period[6]. Some studies demonstrated an association between AF burden and stroke risk, but few mentioned the existence of a dose-response effect. The Italian AT 500 registry study showed that patients with device-detected AF episodes of > 24 h had a 3.1-fold increased risk of stroke. In contrast, patients with AF episodes of > 5 min and < 24 h experience no significant increase in stroke risk[7]. Moreover, the ASSERT Clinical Trial reported episodes lasting > 6 min were associated with an increased risk of ischemic stroke or systemic embolism[8]. A recent systematic review demonstrated the AF burden exceeding different thresholds was associated with an increased risk of stroke; however, they did not provide a definite threshold for AF burden at stroke risk[9]. It is a matter of controversy whether an AF burden of > 5 min can increase the risk of stroke, and no studies have reported the potential dose-response effect on stroke. Accordingly, we performed a systematic review and meta-analysis to determine the association between AF burden > 5 min and the increased risk of stroke and explored the dose-response effect between these two factors.
MATERIALS AND METHODS

This systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines[10].

Search strategy

The literature search was performed by two researchers (YSY and HM) with the help of an experienced medical reference librarian. Studies were retrieved by searching electronic databases (PubMed, EMBASE, Medline, Cochrane, Web of Science) from inception until February 28, 2020. The following search terms were used: AF, physiological monitoring, implantable cardiac monitor, artificial pacemaker, electrocardiograph, burden, stroke, cerebrovascular disorders, brain infarction and thromboembolic event. The language of publication was restricted to English. We also retrieved the reference lists of included articles and previous reviews to identify potential studies as comprehensively as possible. All retrieved references were exported to EndNote X9, and duplicate citations were removed.

Inclusion criteria and exclusion criteria

Two investigators (YSY, HM) independently assessed the eligibility of the studies identified. The inclusion criteria included: (1) Studies that described AF burden within 1 d or more; (2) Studies that described the method used to quantify AF burden such as a pacemaker, implantable cardioverter-defibrillator and cardiac-resynchronization device; and (3) Studies where clinical outcomes included stroke, ischemic stroke, systemic embolism, transient ischemic attack or other thromboembolic events. The combined endpoint of these outcomes was also included: (1) Studies that directly and/or indirectly provided the relative risk of the outcome, including hazard ratio (HR), risk ratio (RR) and odds ratio (OR) values; (2) Observational studies or randomized controlled trials (RCTs); and (3) Studies where the study design and methods were described in detail.

However, reviews, conference abstracts, editorials, case reports, duplicate publications and cross-sectional studies were excluded.

Data extraction

Two researchers (YSY and HM) independently extracted the following information from the included studies: Study type, significant AF burden definition, adverse outcomes, sample size, follow-up period, the method for AF monitoring and others. The number of cases and HR, RR, OR for the risk of the adverse outcomes for different AF burdens were also recorded. HRs provided by original studies were considered as adjusted RRs. We also contacted the authors for additional data or any clarification if necessary. Disagreements were resolved by a consensus-based discussion.

Quality assessment and the level of evidence

The quantitative assessment tool ‘QualSyst’[11] and the Oxford Centre for Evidence-Based Medicine 2009 Level of Evidence Tool[12] were used to assess the methodological quality and the evidence levels of the included studies by two researchers (YSY and HM). The ‘QualSyst’ scoring system included 14 criteria with three possible answers: Yes, No, and Partial. “Yes” = 2 points, “No” = 0 points and “Partial” = 1 points. Items not applicable to a particular study design were marked ‘NA’ and were excluded from calculating the summary score. A summary score was calculated for each article based on the evaluation criteria. A score greater than 75% of the summary score indicated strong quality, a score ranging from 55% to 75% indicated moderate quality, and a score lower than 55% indicated poor quality. The level of evidence was assessed according to the type of study, and each subgroup level included five levels.

Data synthesis and statistical analysis

Sufficient data were obtained to calculate the incidence of AF burden and stroke. Adjusted RRs and 95% confidence interval (CI) were extracted from each study. A meta-analysis was used to pool the relative risks of each study. Chi-squared-based Q test and the I^2 value were used to evaluate the heterogeneity within the studies. The random-effects meta-analysis model was used when the heterogeneity was statistically significant (I^2 > 50%, P < 0.05)[13]. Publication bias was assessed by Egger’s test. A P value < 0.05 was statistically significant.

The potential linear or nonlinear dose-response effect was evaluated using a restricted cubic splines regression model, where the AF burden was associated with an increased risk of stroke every 1 min[14]. We further explored the increased risk of
stroke per hour. Four knots at the 5th, 35th, 65th and 95th percentiles of AF burden were used in the regression model. The nonlinear $P$ value was calculated by testing the null hypothesis that the second spline coefficient was equal to zero$^{[15]}$. If $P_{\text{nonlinear}}$ was greater than 0.05, the linear dose-response effect was statistically significant.

Moreover, when the AF burden was not a definite value, the midpoint between the upper and lower boundaries was considered as the average AF burden; when the lowest level was an open interval, the lowest dose was assumed to be 0; when the highest category was open-ended, a value with 1.5 times the boundary of the highest dose was considered the dose$^{[16]}$. Trial sequential analysis (TSA) was used to evaluate the statistical power of the current sample size and provide robust evidence of the effect of AF burden on the stroke risk$^{[17]}$. Heterogeneity-adjusted required information size was calculated with $\alpha = 0.05$, $\beta = 0.2$ and a relative risk reduction of 30%.

The meta-analysis was conducted using Review Manager (v5.3). The potential dose-response association was conducted by STATA software (v15.0, College Station, TX, United States). TSA was conducted with TSA 0.9.5.10 Beta software (http://www.ctu.dk.tsa)$^{[18]}$.

RESULTS

Identification of studies

The search strategy yielded a total of 10479 abstracts from five English databases, while a manual search of the references cited in other available included articles and previous reviews yielded an additional 372 abstracts. After removing duplicates, 7827 studies remained. After abstract screening, 7004 studies were excluded. The remaining 823 full-texts were assessed for eligibility based on the inclusion and exclusion criteria, and 807 studies were excluded for the following reasons: 412 were not original articles, 218 lacked detailed data on AF burden and 126 did not provide information on the clinical outcomes, 44 had a history of AF or stroke, and seven were cross-sectional studies. Finally, 16 studies were included in the quantitative synthesis (Figure 1).

Characteristics of the involved studies

Table 1 shows the characteristics of the included 16 studies, all except one were RCT studies$^{[7,8,19-32]}$. The detected devices for AF burden included one or more of the three following devices: Pacemaker, implantable cardioverter-defibrillator and cardiac-resynchronization device. The 16 studies included 53141 subjects with mean or median ages > 65 years. Except for case-crossover study, subjects in all studies were followed up for at least 1 year to ascertain the clinical outcomes$^{[25]}$. Four studies were multinational consortium studies; six were conducted in European countries, four in North American countries and two in Asian countries.

Table 2 shows the quality evaluation and the evidence level for each study. Twelve studies were associated with scores higher than 21. The levels of evidence ranged from 1b to 3a, and most were considered level 2b evidence.

The incidence of AF burden > 5 min and stroke

Eleven studies provided data on the incidence of AF burden > 5 min. The detectable rate of AF burden > 5 min ranged from 10.12% to 70.77% among CIED patients, and AF burden > 24 h ranged from 6.70% to 39.26%. Overall, AF burdens > 5 min and > 24 h were detected in 26% (95%CI: 1%-52%) and 15% (95%CI: 6%-35%) of patients within the follow-up period, respectively, and the pooled incidence of stroke was 2.80% (95%CI: 1.56%-4.03%).

Association between AF burden > 5 min and future stroke risks

Sufficient data were obtained to calculate the crude RR for stroke associated with AF burden > 5 min in each study. The average follow-up for the 11 studies ranged from 12 to 67 mo (mean = 36.18 mo). The random-effects pooled analysis revealed that patients with AF burden > 5 min had a 67% increased risk of stroke (RR = 1.67, 95%CI: 1.25-2.25) compared with patients with AF burden < 5min (Figure 2A). Significant heterogeneity was found within the included studies ($I^2 = 52\%$, $P = 0.020$). The funnel plot was symmetrical, and Egger’s test showed no significant publication bias ($t = 1.56$, $P = 0.150$).

Six of the included studies provided adjusted RRs on the strength of association between AF burden > 5 min and the stroke risk. In these six studies, the average
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Study type</th>
<th>Significant AF burden definition</th>
<th>Adverse outcomes</th>
<th>Sample size</th>
<th>Follow-up period</th>
<th>AF monitoring</th>
<th>Age (male/female)</th>
<th>Nation</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glotzer et al. [19], 2003, Ancillary MOST</td>
<td>Secondary analysis of multicenter RCT</td>
<td>AF rate &gt; 220 bpm, AF burden ≥ 5 min</td>
<td>Stroke/systemic embolism</td>
<td>312</td>
<td>Median: 27 mo</td>
<td>PM</td>
<td>74 yr (141/171)</td>
<td>United States</td>
<td>Patients with sinus node disease who required PM for bradycardia and a history of AF</td>
</tr>
<tr>
<td>Capucci et al. [7], 2005, Italian AT 500 Registry</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 174 bpm, AF burden ≥ 5 min or ≥ 1 d</td>
<td>Thromboembolic event</td>
<td>725</td>
<td>Median: 22 mo</td>
<td>PM</td>
<td>72 yr (360/365)</td>
<td>Italy</td>
<td>Patients with symptomatic atrial tachyarrhythmias and a history of AF. Permanent AF were excluded</td>
</tr>
<tr>
<td>Botti et al. [20], 2009, NA</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 174 bpm, AF burden ≥ 5 min or ≥ 1 d</td>
<td>Stroke/systemic embolism</td>
<td>568</td>
<td>Mean: 1 yr</td>
<td>PM</td>
<td>70 yr (NA)</td>
<td>Italy</td>
<td>Patients with a class I or II American College of Cardiology/American Heart Association indication for dual-chamber PM, symptomatic atrial tachyarrhythmias and a history of AF. Permanent AF were excluded</td>
</tr>
<tr>
<td>Glotzer et al. [21], 2009, TRENDS</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 175 bpm, AF burden ≥ 20 s</td>
<td>Ischemic stroke, TIA, and systemic embolism</td>
<td>2486</td>
<td>Mean: 1.4 yr</td>
<td>PM, ICD or CRT</td>
<td>70 yr (1650/836)</td>
<td>International</td>
<td>Patients with an established class I/II indication for an ICD or stroke risk factor and a history of AF. Permanent AF were excluded</td>
</tr>
<tr>
<td>Healey et al. [8], 2012, ASSERT ClinicalTrials</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 190 bpm, AF burden: ≥ 6 min</td>
<td>Ischemic stroke or systemic embolism</td>
<td>2580</td>
<td>Mean: 2.5 yr</td>
<td>PM or ICD</td>
<td>77 yr (1506/1074)</td>
<td>International</td>
<td>Patients who had a history of hypertension, but no AF</td>
</tr>
<tr>
<td>Shanmugam et al. [22], 2012, Home Monitor CRT</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 180 bpm, AF burden ≥ 14 min</td>
<td>Thromboembolic event</td>
<td>560</td>
<td>Median: 370 d</td>
<td>PM or ICD</td>
<td>66 yr (434/136)</td>
<td>Europe</td>
<td>Patients with a heart failure, CRT and a history of AF. Permanent AF were excluded</td>
</tr>
<tr>
<td>Gonzalez et al. [23], 2014, NA</td>
<td>Retrospective, observational study</td>
<td>AF rate &gt; 178 bpm, AF burden ≥ 5 min</td>
<td>Stroke and all-cause mortality</td>
<td>224</td>
<td>Median: 6.6 yr</td>
<td>PM</td>
<td>74 yr (118/106)</td>
<td>United States</td>
<td>Consecutive patients with no history of AF who underwent dual-chamber PM implantation</td>
</tr>
<tr>
<td>Boriani et al. [24], 2014, SOS AF project (PANORAMA, TRENDS, ClinicalService)</td>
<td>Prospective studies</td>
<td>AF rate &gt; 175 bpm, AF burden ≥ 5 min</td>
<td>Ischemic stroke or TIA events</td>
<td>10016</td>
<td>Median: 2 yr</td>
<td>PM or ICD</td>
<td>70 yr (6889/3157)</td>
<td>International</td>
<td>Patients who had at least months of follow-up and with a history of AF. Permanent AF were excluded</td>
</tr>
<tr>
<td>Turakhia et al. [25], 2015, NA</td>
<td>Case-Crossover</td>
<td>AF burden &gt; 5.5 h in a day during a defined 30-d period</td>
<td>Ischemic Stroke</td>
<td>9850</td>
<td>Case period: 1-30 d Control period: 91-120 d</td>
<td>PM or ICD</td>
<td>NA</td>
<td>United States</td>
<td>Patients with CIEDs remotely monitored in the Veterans Administration Health Care System and a history of AF</td>
</tr>
<tr>
<td>Witt et al. [26], 2015, NA</td>
<td>Retrospective, observational study</td>
<td>AF burden &gt; 6 min</td>
<td>Thromboembolic events</td>
<td>394</td>
<td>Median: 4.2 yr</td>
<td>CRT</td>
<td>67 yr (290/104)</td>
<td>Denmark</td>
<td>Patients with a CRT device, and no history of AF</td>
</tr>
<tr>
<td>Benezet-Mazuecos et al. [27], 2015, NA</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 225 bpm, AF burden ≥ 5 min</td>
<td>Silent ischemic brain lesions</td>
<td>109</td>
<td>Median: 2 yr</td>
<td>PM, ICD or CRT</td>
<td>74 yr (61/48)</td>
<td>Europe</td>
<td>Patients with PMs, ICDs, and CRT capable of atrial activity monitoring, and with no history of AF</td>
</tr>
<tr>
<td>Van Gelder et al. [28], 2017, ASSERT ClinicalTrials</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 190 bpm, AF burden &gt; 6 min</td>
<td>Ischemic stroke or systemic embolism</td>
<td>2455</td>
<td>Mean: 2.5 yr</td>
<td>PM or ICD</td>
<td>NA</td>
<td>International</td>
<td>Patients with hypertension but no prior AF requiring medical therapy</td>
</tr>
</tbody>
</table>
Yang SY et al. AF burden and the stroke risk

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Type of Study</th>
<th>AF Rate</th>
<th>AF Burden</th>
<th>Outcome</th>
<th>Median Follow-up Time</th>
<th>Country</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Chu et al [29], 2020</td>
<td>NA</td>
<td>Retrospective, observational study</td>
<td>AF rate &gt; 250 bpm, AF burden &gt; 6 min</td>
<td>Ischemic stroke, transient ischemic attack, or systemic embolism</td>
<td>152</td>
<td>Median: 67 mo</td>
<td>China</td>
<td>Patients who were with a dual-chamber PM and a history of AF</td>
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<tr>
<td>Kaplan et al [30], 2019</td>
<td>NA</td>
<td>Retrospective, observational study</td>
<td>AF burden &gt; 6 min</td>
<td>Ischemic Stroke and systemic embolism</td>
<td>21768</td>
<td>NA</td>
<td>United States</td>
<td>Patients who had a cardiovascular diagnosis code or had a cardiovascular related procedure performed during the data collection period and with a history of AF</td>
</tr>
<tr>
<td>Li et al [31], 2019</td>
<td>The West Birmingham Atrial Fibrillation Project</td>
<td>Prospective, observational study</td>
<td>AF rate &gt; 175 bpm, AF burden &gt; 5 min</td>
<td>Thromboembolic event</td>
<td>594</td>
<td>Median: 4.2 yr</td>
<td>United Kingdom</td>
<td>Patients receiving a PM, ICD, or CRT between January 1999 and January 2017</td>
</tr>
<tr>
<td>Nakano et al [32], 2019</td>
<td>NA</td>
<td>Retrospective, observational study</td>
<td>AF rate &gt; 200 bpm</td>
<td>Embolic stroke</td>
<td>348</td>
<td>Median: 65 yr</td>
<td>Japan</td>
<td>Patients receiving PMs and ICDs between May 1980 and May 2016</td>
</tr>
</tbody>
</table>

1Healey et al [8], 2012 and Van Gelder et al [28], 2017 were both from ASSERT clinical Trials and were used for analysis the association between atrial fibrillation burden > 5 min and future stroke, the dose-response association, respectively. PM: Pacemaker; ICD: Implantable cardioverter-defibrillator; CRT: Cardiac-resynchronization device; NA: Not applicable; AF: Atrial fibrillation.

Follow-up time ranged from 24 to 67 mo (mean = 36.90 mo). Notwithstanding that Li et al [31] found a higher annual incidence of stroke in patients with AF burden > 5 min (1.85% vs 1.14%), the difference was not statistically significant (adjusted RR = 1.31, 95% CI: 0.51-3.38) [31]. The other five studies indicated that the annual incidence of stroke for AF burdens > 5 min and < 5 min ranged from 1.69 to 3.1 and 0.58 to 1.4 per 100 patient-years, respectively. The fixed-effect pooled analysis revealed that patients with AF burden > 5 min had a 2.49-fold increase in the risk of stroke (adjusted RR = 2.49, 95% CI: 1.79-3.47) compared with patients with AF burden < 5 min (Figure 2B). There was no significant heterogeneity (I² = 0%, P = 0.620) and publication bias (t = 1.08, P = 0.340) among these studies.

TSA of ten studies showed that 71.5% (37144 out of 51978 patients) of the heterogeneity-adjusted information size required was accrued. We also found that the cumulative Z curve crossed the trial sequential monitoring boundary, providing robust evidence of the association between the AF burden > 5 min and increased risk of stroke based on the sample size (Figure 3).

Subgroup analyses of association between AF burden > 5 min and the future stroke risk

The fixed-effect pooled analysis performed with adjusted RRs revealed that patients with AF burden > 5 min had a 1.23-fold increase in risk of stroke (RR = 2.23, 95% CI: 1.48-3.53) compared to AF burden < 5 min among patients with no history of AF. Moreover, patients with AF burden > 5 min had a 2.14-fold increase in the risk of stroke (adjusted RR = 2.14, 95% CI: 1.23-3.72) compared to AF burden < 5 min among patients not on anticoagulation therapy. The detailed results of subgroup analyses...
### Table 2 Quality evaluation and the evidence level for each study

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Question described</th>
<th>Appropriate study design</th>
<th>Appropriate subject selection</th>
<th>Characteristics described</th>
<th>Random allocation</th>
<th>Investigators blinded</th>
<th>Subjects blinded</th>
<th>Outcome and measures well defined and robust to bias</th>
<th>Sample size appropriate</th>
<th>Analytic methods appropriate</th>
<th>Estimate of variance reported</th>
<th>Controlled for confounding</th>
<th>Results reported in detail</th>
<th>Conclusion supported by results?</th>
<th>Rating</th>
<th>Levels of evidence</th>
</tr>
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<tbody>
<tr>
<td>Glotzer et al [19], 2003</td>
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<tr>
<td>Healey et al [8], 2012</td>
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<td>Chu et al</td>
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</tbody>
</table>
with different populations are shown in Supplementary Table 1.

**Does-response relationship between AF burden and the future stroke risk**

Seven studies were included in the dose-response meta-analysis on the association between AF burden and stroke. The potential linear or nonlinear dose-response association was evaluated using a restricted cubic splines regression model. A linear dose-response relationship \( (P_{\text{nonlinear}} = 0.656) \) was found (Figure 4), and AF burden was associated with 2.0% and 3.0% increased risks of stroke for every 1 h (\( \text{RR} = 1.02, 95\% \text{CI: 1.01-1.03} \)) and 2 h (\( \text{RR} = 1.03, 95\% \text{CI: 1.02-1.05} \)), respectively.

**AF burden and risk of clinical AF**

Three of the included studies, including 3286 patients, provided adjusted RRs values of the AF burden > 5 min on the risk of clinical AF. The random-effect pooled analysis reveal that patients with AF burden > 5 min had a 3.18 fold increased risk of clinical AF (adjusted \( \text{RR} = 4.18, 95\% \text{CI: 2.26-7.74} \)) compared with the patient suffering AF burden < 5 min (Figure 5). The heterogeneity was significant among the different study designs \( (I^2 = 77\%, P = 0.010) \), RCT [19] and two retrospective observational studies [8,26]. The funnel plot was symmetrical and no significant publication bias was found in the Egger’s test \( (t = 0.80, P = 0.570) \).

**AF burden and the risk of all-cause mortality**

The reported adjusted RRs for the strength of association between AF burden > 5 min and risk of all-cause mortality in three studies differed. An ancillary study of the Mode Selection Trial trial [19] included patients with sinus node disease who were in sinus rhythm at the time of pacemaker implantation and aged > 21 years. Two studies [23,26] included patients with no history of AF. The random-effects pooled analysis found that patients with AF burden > 5 min had a 55% increased risk of all-cause mortality (adjusted \( \text{RR} = 1.55, 95\% \text{CI: 0.87-2.75} \)) (Figure 6); however, significant heterogeneity \( (I^2 = 68\%, P = 0.040) \) and publication bias \( (t = -21.13, P = 0.030) \) were present in this
DISCUSSION

In this systematic review and dose-response meta-analysis on the association between AF burden and the risk of stroke, 16 original studies were included, including 53141 CIED patients. First of all, we found that patients with an AF burden > 5 min had an increased risk of stroke. Moreover, a linear dose-response relationship was found; the risk of stroke was increased by 2.0% per hour among subjects with AF burden > 5 min. Last but not least, we found AF burden > 5 min was associated with a significantly increased risk of clinical AF but not associated with an increase in all-cause mortality.

AF burden: A significant risk factor for stroke

Data from each study were extracted to calculate the crude RRs without considering the time-to-event endpoints. The pooled results indicated that patients with AF burden > 5 min had a higher stroke risk. That significant heterogeneity was detected for the pooled analysis of the relationship between AF burden and stroke risk ($I^2 = 52\%, P = 0.02$). The heterogeneity might be associated with the variations in patient populations, hypertension, prior AF and antithrombotic therapy, etc. [33]. The population included in our study had different comorbidities, including patients with symptomatic atrial tachyarrhythmias [7,20], sinus node disease [19] and heart failure [22]. Moreover, some studies provided no information on patient history of AF [8,23,26-28]. Besides, in the study by Chu et al. [29], patients with oral anticoagulants for any reason were excluded. However, even though anticoagulants were used in different proportions of patients at baseline, we found that the heterogeneity was not significant. With the pooled data of HRs adjusted for one or more known embolism predictors [including age, sex, heart failure, prior stroke diabetes, congestive heart failure, hypertension, age 75 years, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 years, sex category (CHA2DS2-VASc) score], we found that an AF burden > 5 min was associated with an increased risk of stroke ($F = 0, P = 0.62$).

We found that subjects with AF burden of > 5 min had a 67% increased risk of stroke. Recently, a meta-analysis also found that subclinical AF (pooled with highest AF duration cut-off values from the original studies) was associated with a 2.4-fold increased risk of stroke [9]. These results indicated that the risk of stroke was higher among the subjects with the serious AF burden. This finding provides novel insights...
Yang SY et al. AF burden and the stroke risk

**Figure 2** Meta-analysis forest plot: Atrial fibrillation burden and the risk of future stroke. A: Crude risk ratio (RR) model; B: Adjusted RR model; SE: Standard error; CI: Confidence interval.

**Figure 3** Trial sequential analysis of atrial fibrillation burden > 5 min. Heterogeneity adjusted required information size of 51978 participants calculated on basis of incidence of 2.37% in control group, relative risk reduction of 30%, $\alpha = 5\%$, $\beta = 20\%$, and $I^2 = 30\%$. Actually, accrued number of participants was 37144, 71.5% of required information size. AF: Atrial fibrillation.

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>log [Risk ratio]</th>
<th>SE</th>
<th>Weight</th>
<th>Risk ratio IV, random, 95%CI</th>
<th>Risk ratio IV, fixed, 95%CI</th>
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</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakano et al., 2019</td>
<td>-0.5798</td>
<td>0.5515</td>
<td>5.7%</td>
<td>0.56 [0.19, 1.65]</td>
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<tr>
<td>Li et al., 2019</td>
<td>-0.2107</td>
<td>0.3998</td>
<td>8.8%</td>
<td>0.81 [0.37, 1.77]</td>
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<tr>
<td>Kaplan et al., 2019</td>
<td>0.2311</td>
<td>0.0882</td>
<td>21.6%</td>
<td>1.26 [1.06, 1.50]</td>
<td></td>
</tr>
<tr>
<td>Capucci et al., 2005</td>
<td>0.2546</td>
<td>0.6372</td>
<td>4.5%</td>
<td>1.29 [0.37, 4.50]</td>
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</tr>
<tr>
<td>Chu et al., 2019</td>
<td>0.5128</td>
<td>0.7043</td>
<td>3.8%</td>
<td>1.67 [0.42, 6.64]</td>
<td></td>
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<tr>
<td>Borlani et al., 2014</td>
<td>0.6313</td>
<td>0.2123</td>
<td>16.0%</td>
<td>1.88 [1.24, 2.85]</td>
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<tr>
<td>Witt et al., 2015</td>
<td>0.8372</td>
<td>0.2376</td>
<td>14.8%</td>
<td>2.31 [1.45, 3.68]</td>
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</tr>
<tr>
<td>Botto et al., 2009</td>
<td>0.9083</td>
<td>0.7592</td>
<td>3.4%</td>
<td>2.48 [0.56, 10.98]</td>
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</tr>
<tr>
<td>Healey et al., 2012</td>
<td>0.9746</td>
<td>0.3329</td>
<td>10.9%</td>
<td>2.65 [1.38, 5.09]</td>
<td></td>
</tr>
<tr>
<td>Benezet-Mazuecos et al., 2015</td>
<td>1.1119</td>
<td>0.4658</td>
<td>7.2%</td>
<td>3.04 [1.22, 7.57]</td>
<td></td>
</tr>
<tr>
<td>Glotzer et al., 2003</td>
<td>1.335</td>
<td>0.7824</td>
<td>3.2%</td>
<td>3.80 [0.82, 17.61]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95%CI)</strong></td>
<td></td>
<td></td>
<td></td>
<td>1.67 [1.25, 2.25]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0.10; Chi^2 = 20.87; df = 10 (P = 0.02); I^2 = 52\%$

Test for overall effect: $Z = 3.41 (P = 0.0006)$

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>log [Risk ratio]</th>
<th>SE</th>
<th>Weight</th>
<th>Risk ratio IV, fixed, 95%CI</th>
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</thead>
<tbody>
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<td>Li et al., 2019</td>
<td>0.27</td>
<td>0.4813</td>
<td>12.4%</td>
<td>1.31 [0.51, 3.36]</td>
</tr>
<tr>
<td>Witt et al., 2015</td>
<td>0.8329</td>
<td>0.381</td>
<td>19.8%</td>
<td>2.30 [1.09, 4.65]</td>
</tr>
<tr>
<td>Healey et al., 2012</td>
<td>0.9636</td>
<td>0.3416</td>
<td>24.6%</td>
<td>2.50 [1.28, 4.88]</td>
</tr>
<tr>
<td>Glotzer et al., 2003</td>
<td>1.026</td>
<td>0.3132</td>
<td>29.3%</td>
<td>2.79 [1.51, 5.15]</td>
</tr>
<tr>
<td>Benezet-Mazuecos et al., 2015</td>
<td>1.1151</td>
<td>0.5392</td>
<td>9.9%</td>
<td>3.05 [1.06, 8.78]</td>
</tr>
<tr>
<td>Chu et al., 2019</td>
<td>1.9095</td>
<td>0.8404</td>
<td>4.1%</td>
<td>6.75 [1.30, 35.05]</td>
</tr>
<tr>
<td><strong>Total (95%CI)</strong></td>
<td></td>
<td></td>
<td></td>
<td>2.49 [1.79, 3.47]</td>
</tr>
</tbody>
</table>

Heterogeneity: $Chi^2 = 3.51; df = 5 (P = 0.62); I^2 = 0\%$

Test for overall effect: $Z = 5.38 (P < 0.00001)$

Consistently, Shanmugam et al[22] found that a higher AF burden (AF burden > 3.8 h) was associated with a 9.4-fold risk of stroke among CIED patients. Two studies[28, 30] also reported that patients with AF burden > 24 h had an increased risk of stroke. However, these results were inconsistent with a study by Healey et al[8], which could be accounted for by the fact that patients who experienced long periods of sinus rhythm and the better treatment of stroke had no history of AF[8].

The European and American[34] guidelines recommend estimating stroke risk in AF patients based on the CHA2DS2-VASc score. Moreover, an oral anticoagulant is recommended to reduce thromboembolic stroke risk in patients with AF, especially male patients with a CHA2DS2-VASc score of 1 and female patients with a CHA2DS2-
VASc score of 2. Interestingly, some studies explored the association between AF burden and CHA2DS2-VASc scores. Botto et al[20] indicated that patients with a CHADS2 score of 1 or 2 had either a high or low stroke risk consistent with a high or low detected AF duration, respectively. Kaplan et al[30] also found an interaction between AF duration and CHA2DS2-VASc score. The risk of systemic embolism in patients with intermediate CHA2DS2-VASc scores was variable and correlated with the maximum AF burden. Accordingly, the stroke risk among AF patients should be evaluated based on the CHA2DS2-VASc score and AF burden to provide better personalized anticoagulation decisions.

**Association between AF burden and risk of clinical AF or all-cause mortality**

Clinical AF is a chaotic heart rhythm characterized by an irregular and often rapid heart rate documented with a 12-lead electrocardiogram. Electrocardiogram-documented AF was confirmed in 38.9% of patients with AF burden and 2.1% without
Our study found that AF burden > 5 min was associated with an increased risk of clinical AF. Furthermore, progression from paroxysmal to persistent or permanent AF might be faster in patients with subclinical AF who did not receive treatment. Consequently, more emphasis should be placed on screening patients with AF burden > 5 min and providing timely therapy.

Our study demonstrated that AF burden was not associated with all-cause mortality. However, there was significant heterogeneity in this meta-analysis. Indeed, further research is required to explore the role of AF burden on all-cause mortality.

**Limitations**
Even though this meta-analysis was performed utilizing crude RRs and adjusted RRs, there are still some limitations. Owing to the lack of adjusted RRs corresponding to three or more groups of AF burden, this meta-analysis was conducted without considering the time-to-event points and adjusting for confounding factors. Furthermore, patients with CIEDs might have diabetes, hypertension and other stroke risk factors, which might lead to an overestimation of the effect of AF on stroke. Underreporting of stroke and prescribing an oral anticoagulant to patients with higher AF burden might also lead to underestimating the impact of AF burden on the stroke risk. However, anticoagulation was used in the different subgroups of patients who had comorbidities at the baseline. Finally, publication bias was present in this study. Our results might have been influenced by non-published studies or language bias as we only included studies published in English.

**CONCLUSION**
This meta-analysis demonstrated that AF burden is a significant risk factor for clinical AF and stroke. There is a linear dose-response between AF burden and risk of stroke. Further studies are needed to validate this effect and evaluate the cut-off value for AF burden among patients requiring anticoagulation treatment.

**ARTICLE HIGHLIGHTS**

**Research background**
With the widespread use of cardiac implantable electronic devices and wearable devices, it is nowadays possible to monitor the atrial fibrillation (AF) burden. However, whether an AF burden of > 5 min can increase the risk of stroke is still highly controversial, and the potential linear or nonlinear relationship between them remains largely unexplored.

**Research motivation**
A comprehensive systemic review and meta-analysis can summarize the results of available studies and help doctors in the clinical decision-making process.

**Research objectives**
This meta-analysis aimed to determine the association between AF burden > 5 min and the increased risk of stroke and explore a dose-response effect of AF burden on the risk of stroke.

**Research methods**
Studies were identified by searching electronic databases (PubMed, EMBASE, Medline, Cochrane and Web of Science) from inception until February 28, 2020. The potential nonlinear dose-response association was evaluated using a restricted cubic splines regression model. AF burden was associated with an increased risk of stroke for every 1 h and 2 h. Trial sequential analysis with a random-effect model was used to evaluate the robustness of the evidence from the included 16 studies. Data from these studies were pooled using RevMan software and Stata.

**Research results**
The meta-analysis indicated that an AF burden > 5 min was associated with an increased risk of clinical AF [adjusted risk ratio (RR) = 4.18, 95% confidence interval (CI): 2.26-7.74] but was not associated with an increased risk of all-cause mortality.
(adjusted RR = 1.55, 95%CI: 0.87-2.75). Patients with an AF burden > 5 min had an increased risk of stroke (adjusted RR = 2.49, 95%CI: 1.79-3.47). The linear dose-response analysis showed that the risk of stroke was increased by 2.0% per hour as the AF burden was increased (P_nonlinear = 0.656, RR = 1.02, 95%CI: 1.01-1.03). Trial sequential analysis provided robust evidence of the association between AF burden > 5 min and increased risk of stroke.

**Research conclusions**

AF burden is a significant risk factor for clinical AF and stroke. A significant linear association is present between increased AF burden and the risk of stroke.

**Research perspectives**

More emphasis should be laid on patients with AF burden to minimize the stroke risks.

**ACKNOWLEDGEMENTS**

We are grateful to Meng-Jiao He and Fei-Hong Chen for their helpful advice of methodology and statistics and to Mr. Satyajit Kundu for language polishing.

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AF burden and the stroke risk


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Li YG, Miyazawa K, Pastori D, Szekely O, Shahid F, Lip GYH. Atrial high-rate episodes and...


Effectiveness of Maitland and Mulligan mobilization methods for adults with knee osteoarthritis: A systematic review and meta-analysis

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ORCID number: Ling-Ling Li 0000-0002-4812-960X; Xin-Jie Hu 0000-0003-4395-9433; Yong-Hui Di 0000-0002-5351-9573; Wei Jiao 0000-0003-0233-2271.

Author contributions: Li LL and Hu XJ designed the research; Li LL, Hu XJ and Di YH performed the research; Li LL and Hu XJ contributed new reagents/analytic tools; Li LL and Di YH analyzed the data; Li LL and Hu XJ wrote the paper.

Conflicts-of-interest statement: Dr. Jiao reports grants from National Key Research and Development Program of China, during the course of the study.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Supported by the National Key Research and Development Program of China, No. 2018YFF0301104.

Country/Territory of origin: China

Specialty type: Medicine, research and experimental

Abstract

BACKGROUND
As a serious global problem, knee osteoarthritis (KOA) often leads to pain and disability. Manual therapy is widely used as a kind of physical treatment for KOA.

AIM
To explore further the efficacy of Maitland and Mulligan mobilization methods for adults with KOA.

METHODS
We searched PubMed, the Cochrane Library, EMBase, Web of Science and Google Scholar from inception to September 20, 2020 to collect studies comparing Maitland and Mulligan mobilization methods in adults with KOA. The quality of the studies was assessed using the Physiotherapy Evidence Database Scale for randomized controlled trials. Data analyses were performed using Review Manager 5.0 software.

RESULTS
A total of 341 articles were screened from five electronic databases (PubMed, the Cochrane Library, EMBase, Web of Science and Google Scholar) after excluding duplicates. Ultimately, eight trials involving 471 subjects were included in present systematic review and meta-analysis. The mean PEDro scale score was 6.6. Mulligan mobilization was more effective in alleviating pain (standardized mean difference (SMD) = 0.60; 95% confidence interval (CI): 0.17 to 1.03, P = 0.007; I² = 60%, P = 0.020) and improving Western Ontario and McMaster Universities function score (SMD = 7.41; 95% CI: 2.36 to 12.47, P = 0.004; I² = 92%, P = 0.000). There was no difference in the effect of the two kinds of mobilization on improving the range of motion (SMD = 9.63; 95% CI: -1.23 to 20.48, P = 0.080; I² = 97%, P = 0.000).
Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

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Published online: January 21, 2022
P-Reviewer: Labusca L, Sun C
S-Editor: Wang LL
L-Editor: Filipodia
P-Editor: Wang LL

CONCLUSION
Mulligan mobilization technique is a promising intervention in alleviating pain and improving function score in KOA patients.

Key Words: Mulligan mobilization; Maitland mobilization; Manipulation; Manual therapy; Knee osteoarthritis; Meta-analysis

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Core Tip: Manual therapy is widely used as a kind of physical treatment for knee osteoarthritis. Maitland and Mulligan mobilization are two types of manual therapy used in osteoarthritis treatment. There was no systematic reviews and meta-analyses to compare the efficacy of different mobilization techniques, such as Maitland vs Mulligan mobilization. This study aims to explore further the efficacy of Maitland and Mulligan mobilization and fill the research gaps. Mulligan mobilization was found to be a promising alternative option for knee osteoarthritis treatment. Particularly, the Mulligan mobilization has been recommended to be applied in alleviating pain and improving Western Ontario and McMaster Universities function scores.

Citation: Li LL, Hu XJ, Di YH, Jiao W. Effectiveness of Maitland and Mulligan mobilization methods for adults with knee osteoarthritis: A systematic review and meta-analysis. World J Clin Cases 2022; 10(3): 954-965
DOI: https://dx.doi.org/10.12998/wjcc.v10.i3.954

INTRODUCTION
Osteoarthritis (OA) is the most common type of arthritis, with 1 in 3 people over age 65 affected and a higher prevalence in women[1,2]. The knees are among the most commonly affected joints in OA[3,4]. Knee osteoarthritis (KOA) is characterized as pain, joint stiffness, functional impairment and even disability, contributing to a heavy burden on healthcare service[5,6]. Considering the severe socioeconomic burden, non-pharmacological, pharmacological and surgical approaches were applied[7]. Physical therapy has been known to play a vital role in pain relief and restoration of mobility and function in KOA patients[8]. Manual therapy is a widely used physical treatment for KOA[9]. Several studies have reported positive effects of manual physical therapy on KOA[9-11]. The American College of Rheumatology recommends the combination of manual therapy with exercise for KOA patients under the supervision of a physiotherapist[12]. Besides, for the patients with deficits in range of motion (ROM), manual therapy plays a role to restore or maximize ROM improvement before surgeries[13].

Maitland and Mulligan mobilization are two types of manual therapy used in OA treatment[14]. Mulligan mobilization allows the patients to perform the offending movements in a functional position, hence, leading to a rewarding outcome[15]. Maitland mobilization aims to reestablish the spinning, gliding and rolling motions of the two joints[14]. In clinical practice, movement quality can be increased via improving joint stability of weak muscles by applying Maitland mobilization combined with psychological effects (self-confidence and motivating factors) and corrected mechanical loading. Maitland and Mulligan mobilization therapies have been used to treat multiple diseases, such as primary adhesive capsulitis of the shoulder[16], hip osteoarthritis[17] and knee osteoarthritis[18]. As reported by previous studies, Maitland or Mulligan mobilizations were used by 99.8% of physical therapists to treat cervicogenic dizziness[19].

Recently, some reviews have found that the manual therapies might be effective and safe in ameliorating osteoarthritis symptoms[16,18,20]. A meta-analysis by Qinguang Xu et al[18] demonstrated that manual therapy effectively and safely alleviated pain, reduced stiffness and restored physical function in KOA patients, and thus it could be considered as a complementary and alternative option. In the studies on primary adhesive capsulitis of the shoulder, Noten et al[16] identified the efficacy of mobilization techniques. Although Maitland mobilization was recommended in these
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studies[16,20], there still was no systematic review and meta-analysis to compare the efficacy of different mobilization techniques, such as Maitland vs Mulligan mobilization. Therefore, this study used an evidence-based method to determine the efficacy of Maitland and Mulligan mobilization methods in adults with KOA.

MATERIALS AND METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA)[21] and the Cochrane Collaboration Handbook[22]. The protocol of this systematic review and meta-analysis was registered on the International Prospective Register of Systematic Reviews (PROSPERO: CRD42020182532) on April 28, 2020.

Information sources

Two reviewers performed literature search individually in the following electronic databases: PubMed, The Cochrane Library, Web of Science, Embase and Google Scholar, from the time of inception to September 20, 2020. We also reviewed the reference lists of relevant reviews and meta-analyses[23,24].

Search strategy

The search terms included related text words and medical subject headings regarding ‘Mulligan’ or ‘Maitland’ or ‘Mobilization with Movement’ or ‘MWM’ or ‘Passive joint mobilization’ or ‘PJM’ or ‘musculoskeletal manipulations’ or ‘mobilization’ or ‘manual therapy’ and “Knee Osteoarthritis” or “knee osteo-arthritis” or “Knee Osteoartritides” or “Osteoarthritis of Knee” or “Osteoarthritis of the Knee”. We had tailored search strategy for each database, and details of the predefined search criteria are provided in Supplementary Table 1.

Eligibility criteria

Trials were considered eligible if the following items were met: (1) Adult patients with KOA at any stage according to Kellgren and Lawrence grading system; (2) Containing data about Maitland joint mobilization or mobilization with movement technique with or without other interventions; (3) Reporting pain, range of motion, functional performance/ability or other relevant outcomes; and (4) Controlled clinical trials.

Since our aim was to explore the different efficacy of these two techniques in ROM, pain and functional performance in KOA, some experiments containing the combination of joint mobilization (Maitland or Mulligan) with other common treatments were also included, as long as they mainly focused on assessing the effect of these two types of joint mobilization methods.

Study selection

Two independent reviewers (Li LL, Hu XJ) removed duplication, screened titles, abstracts and full texts and agreed on the final eligibility. Negotiation was required when there was disagreement[25]. We recorded the reasons for exclusion of full texts.

Data collection process and data items

Two independent reviewers (Li LL, Di YH) extracted the data from included articles using a pre-designed form, including the following parameters: Author’s name, publication year, sample size, study design, type/frequency/duration of the intervention and outcome assessment. Any disagreements were discussed and resolved by the two authors.

Risk of bias in individual studies

The quality of the included articles was assessed by two reviewers individually using PEDro scale. The results given by the two reviewers were compared and any disagreements were resolved by all three authors. The PEDro scale is based on the Delphi list and reported to be reliable for randomized controlled trials (RCTs) of physical therapy in systematic reviews. The PEDro scale consists of 11 items, including: (1) Specified eligibility criteria of studies; (2) Random allocation of studies; (3) Concealed allocation; (4) Similarity between groups at baseline; (5) Blinding of all subjects; (6) Blinding of all therapists; (7) Blinding of all assessors; (8) Less than 15% dropouts; (9) Intention-to-treat analysis; (10) statistical comparisons between groups; and (11) Point measures and variability data. PEDro score was calculated by assessing
the items 2-11. Each item was scored as either 1 or 0 according to whether the item was met or not, respectively. The total score of the scale is 10. Articles were classified into three distinct categories, including high (7-10), moderate (4-6) and low (0-3) quality.

**Statistical analysis**
All data were analyzed by using Cochrane Collaboration software (Review Manager Version 5.2 for Windows). Only continuous variables (range of motion, pain, function scale) were identified, therefore, the difference in means between the intervention groups with 95% confidence intervals (CI) was used as the main summary measures to determine the effect size of the results[26]. The final value and the standard deviation of the results were recorded as well as the number of patients in each treatment group at the last time of the follow-up. To evaluate the heterogeneity of the included studies, the chi² statistical test and P statistic were performed. The extent of heterogeneity was measured by the P statistical test and presented as the total percentage of variation between the studies. The P value was considered low if P was 0%-25%, moderate if P was 25%-50% and high if P was 50%-90%. A random effect model was employed if the heterogeneity was relatively high. Conversely, in case of low heterogeneity, a fixed effect model was used to analyze the data with inverse variance weighting[27]. Sensitivity analysis was conducted to identify the potential sources of high heterogeneity[28]. The statistical significance was assessed by using the Z index of overall effects[27]. Funnel plots was used to assess potential publication biases. If the included trials were < 10, we did not test for publication bias[29].

**RESULTS**
A total of 341 articles were screened from five electronic databases. After removing 333 articles, of which 125 were duplicates, 206 articles were screened out through title and abstract review, 10 articles were still for further consideration. After excluding two studies, eight trials involving 471 subjects were included in the present systematic review and meta-analysis (the reasons for their exclusion were given in Figure 1).

**Characteristics of included studies**
The characteristics regarding the study population, intervention, follow-up period and main results of the studies are presented in Table 1.

**Risk of bias**
All the articles included were assessed with the PEDro Scale (Table 2). The total score of methodological quality varied from 5 to 10 out of 11. The score of most studies exceeded the cut-off point 6, but only two studies scored 9. Many studies missed points on blinding of patients[14,15,30-32], therapists[14,15,30-34] and assessors[14,15,30,32]. In addition, there was often a lack of the concealment of allocation. These are shortcomings for RCTs.

**Pain**
Seven studies[14,15,30-34] with continuous data on pain degree were included in the meta-analysis, with a total of 354 participants. Five studies[14,15,30,31,33] reported the severity of pain using visual analogue scale, while the other two studies[32,34] adopted another Numeric Pain Rating Scale. The Numeric Pain Rating Scale is a segmented numeric version of the visual analogue scale, and both scales use a horizontal bar or line to rate the degree of pain. Thus, these two scales could be considered as the same. According to the forest plot (Figure 2), the pooled standardized mean difference (SMD) was 0.60 (SMD = 0.60; 95%CI: 0.17 to 1.03; \(P = 0.007\)).

**ROM**
Data were collected from five studies[14,30,31,33,35] with continuous data containing a total population of 204 participants. According to the forest plot (Figure 3), random effect model showed that there was no difference in the effect of the two mobilization methods on improving ROM (SMD = 9.63; 95%CI: -1.23 to 20.48; \(P = 0.08\)).

**Western Ontario and McMaster Universities (WOMAC) function score**
Six studies, with 297 participants, reported WOMAC function score[14,15,31-33,35], and one study[14] reported WOMAC function and pain score. According to the forest
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Table 1 Characteristics of the included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Comparators</th>
<th>Sample size</th>
<th>Age (yr)</th>
<th>Interventional type</th>
<th>Interval</th>
<th>Treatment period</th>
<th>Outcomes</th>
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</thead>
<tbody>
<tr>
<td>Sambandam et al [31], 2011</td>
<td>Mulligan</td>
<td>20</td>
<td>61.0 ± 5.8</td>
<td>Unspecific; CT: 10 min hot pack and quad isometrics, 10 rep; HE: 10 rep isometric quadriceps</td>
<td>Once per day</td>
<td>2 wk</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Maitland</td>
<td></td>
<td>20</td>
<td>61.0 ± 5.8</td>
<td></td>
<td></td>
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<tr>
<td>Kiran et al [14], 2018</td>
<td>Mulligan</td>
<td>31</td>
<td>47.5 ± 0.6</td>
<td>Depending on pain; CT: 10 min hot pack and quad isometrics</td>
<td>3 times per week</td>
<td>2 wk</td>
<td>1, 2, 3</td>
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<tr>
<td>Maitland</td>
<td></td>
<td>31</td>
<td>47.5 ± 0.6</td>
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<tr>
<td>Jeyakumar et al [30], 2017</td>
<td>Mulligan</td>
<td>20</td>
<td>51.0 ± 1.5</td>
<td>Medial, lateral, and rotational Mulligan without a belt; one glide per sec for 30 sec; 3 sets per treatment session; CT: hot pack, quad isometrics and SLR</td>
<td>Once per day</td>
<td>3 wk</td>
<td>1, 2</td>
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<tr>
<td>Maitland</td>
<td></td>
<td>20</td>
<td>51.0 ± 1.5</td>
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<tr>
<td>Multu et al [33], 2018</td>
<td>Mulligan</td>
<td>21</td>
<td>54.2 ± 7.3</td>
<td>A sustained manual glide of the tibia (medial, lateral, or rotation) during active; knee flexion and extension, 3 sets of 10 rep; EP: aerobic, active ROM, strength, stretching exercises</td>
<td>3 times per week</td>
<td>4 wk</td>
<td>1, 2, 3</td>
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<tr>
<td>Maitland</td>
<td></td>
<td>21</td>
<td>56.3 ± 6.6</td>
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<tr>
<td>Lahnumpuii et al [35], 2016</td>
<td>Mulligan</td>
<td>15</td>
<td>49.5 ± 5.5</td>
<td>Medial, lateral, rotation and dorsal mobilization with active knee flexion; 3 sets of 10 rep; EP: isometric strengthening (quad, hamstring, VMO), Stretch (quad, hamstring), ROM</td>
<td>3 sessions per week</td>
<td>4 wk</td>
<td>2, 3</td>
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<tr>
<td>Maitland</td>
<td></td>
<td>15</td>
<td>48.5 ± 6.9</td>
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<tr>
<td>Giri [32], 2019</td>
<td>Mulligan</td>
<td>30</td>
<td>61.7 ± 70</td>
<td>A sustained manual glide of the tibia (either medial, lateral, anterior, posterior or rotation) during active knee flexion and extension; 3 sets of 10 rep. Mulligan taping; tape with brown rigid tape</td>
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<td>2 wk</td>
<td>1, 3</td>
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<tr>
<td>Maitland</td>
<td></td>
<td>30</td>
<td>51.2 ± 9.2</td>
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<tr>
<td>Rao et al [34], 2017</td>
<td>Mulligan</td>
<td>30</td>
<td>51.8 ± 9.2</td>
<td>According to individual condition</td>
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<tr>
<td>Maitland</td>
<td></td>
<td>30</td>
<td>51.8 ± 9.2</td>
<td>AP, PA, medial, lateral, compression and distraction glides; rotation</td>
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<tr>
<td>Lalit et al [15], 2012</td>
<td>Mulligan</td>
<td>30</td>
<td>51.7 ± 7</td>
<td>Internal rotation the tibia, 3 reps for 3 sets; EP: multiple angle isometrics, terminal arc knee extension, mini-squats, partial lunges one-leg balances, cross-body leg swings, 10 rep per session, 3 sessions per day</td>
<td>3 sessions per day</td>
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<td>1, 3</td>
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<tr>
<td>Maitland</td>
<td></td>
<td>30</td>
<td>54.7 ± 7</td>
<td>Anterior and posterior glide for tibial-femoral joint, superior-inferior and medio-lateral glides for patellar femoral joint, 10 rep per session, 3 sessions per day; EP: multiple angle isometrics, terminal arc knee extension, mini squats, partial lunges one-leg balances, cross-body leg swings (10 rep per session, 3 sessions per day)</td>
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1 mean ± SD.
2 Range.

plot (Figure 4), Mulligan dynamic joint mobilization was more effective in improving the WOMAC function score of patients with knee arthritis. (SMD = 7.41; 95%CI: 2.36 to 12.47; P = 0.004).
Table 2 Quality of included studies by PEDro Scale

<table>
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<tr>
<th>Author</th>
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C1: Eligibility criteria were specified; C2: Subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received); C3: Allocation was concealed; C4: The groups were similar at baseline regarding the most important prognostic indicators; C5: There was blinding of all subjects; C6: There was blinding of all therapists who administered the therapy; C7: There was blinding of all assessors who measured at least one key outcome; C8: Measurements of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups; C9: All subjects for whom outcome measurements were available received the treatment or control condition as allocated, or where this was not the case, data for at least one key outcome were analyzed by “Intention to treat”; C10: The result of between-group statistical comparisons were reported for at least one key outcome; C11: The study provided both point measurements and measurements of variability for at least one key outcome; 0: Does not meet the included criteria; 1: Meets the included criteria. TS: Total score.

Figure 1 Preferred reporting items for systematic review and meta-analyses flow diagram.

Publication bias
The analysis of the funnel plot for publication bias suggested the absence of bias because of plot symmetry (Figure 5).
**DISCUSSION**

**Summary of evidence**

In this systematic review and meta-analysis of eight randomized controlled trials including 471 KOA patients, Mulligan mobilization was found to be a promising alternative option for KOA treatment. Particularly, the Mulligan mobilization has been recommended to be applied in alleviating pain and improving WOMAC function score. Because of the poor methodological quality of included studies, more studies are needed to assess the effect of manual therapies on pain, WOMAC function score and ROM.

Limited preliminary evidence showed that Mulligan mobilization could reduce pain and improve WOMAC function compared with Maitland mobilization. This conclusion was consistent with that in previous studies. Gomes et al.[36] found that pain was significantly improved in KOA patients who received a course of Mulligan mobilization. Besides, Bhagat et al.[37] showed that Mulligan technique was effective in alleviating pain and improving functional mobility in KOA patients. However, in these studies[36, 37], the intervention periods were not analyzed and the sample size was small. In the previous meta-analysis, Stathopoulos et al.[17] found that the efficacy of Mulligan mobilization method on KOA was unclear due to the high heterogeneity. We speculated that shorter intervention periods or the small sample size might contribute to this heterogeneity. Therefore, further studies will be required to identify the biomechanical rationale behind the effect of mobilization in a longer treatment period.
Figure 4 Forest plot of Western Ontario and McMaster Universities function score. SD: Standard deviation; CI: Confidence interval.

Figure 5 Publication bias.

For ROM, Mulligan mobilization might have the same efficacy as Maitland mobilization. Mulligan and Maitland mobilization, as two kinds of manual therapies, have been found to improve the mechanical loading, joint stability and strength of weak muscles through mechanical, self-confidence and motivating factors. In a cohort study, KOA patients received a manual physical therapy program focusing on passive extension mobilization of the knee, and the restoration effects in Mulligan mobilization group was not better than that in the exercise group[38]. In another study, ROM in Mulligan mobilization was improved in the long term[33]. According to the studies by Stathopoulos et al[17], Mulligan mobilization could only ameliorate joint dysfunctions of the upper and lower extremities and facilitated the immediate recovery of full and pain-free ROM. However, no studies have focused on the treatment period and the site of arthritis. In our study, we focused on the ROM of knees and included studies with various treatment periods. Besides, the high heterogeneity might decrease the reliabilities of the results. Further study and follow-ups will be needed to validate the conclusion.

Overall, KOA is regarded as a complex disorder with multiple risk factors, such as generalized constitutional factors (age, female sex, etc.)[39] and local adverse mechanical factors (trauma, malalignment, etc.)[40]. Confined to the current evidence, we did not limit sex, age, body weight or even history, which may influence the representativeness and application of conclusions. In addition, it was found that the heterogeneity of most included RCTs was high. Thus, the positive effects of the Mulligan mobilization should be interpreted with caution. Finally, because manual therapies require hands-on treatments, it is not possible to perform the study in a blinded way, resulting in the poor score on the PEDro Scale. In the future clinical trials, attention should be paid to all the points above in study design.
Strengths and limitations

Our research has several strengths. First, as far as we are aware, this is the first systematic review and meta-analysis aiming to determine the efficacy of Maitland vs Mulligan mobilization with movement in KOA patients. Secondly, this meta-analysis included as many relevant outcomes as possible and was completed according to the accepted guideline[41]. Thus, the results were relatively comprehensive.

However, similar to other meta-analyses, there were also limitations[42]. Firstly, since not all the grey literature could be searched, some studies might have been missed[43]. This may be negligible with comprehensive and reliable research strategy. Secondly, the sample size in this review might not be enough, which could affect the quality of evidence. Thirdly, due to less than 10 included studies, interpretation of publication bias assessment should be done with caution[29]. Finally, we did not report the cost due to the lack of data. Thus, more RCTs should be conducted, including novel interventions, and more data on adverse effects (AEs) safety will be of necessity.

CONCLUSION

Mulligan joint mobilization is a promising intervention with the potential to improve the pain and joint function for patients with KOA. Based on real-world and other epidemiological settings, more data and surveillance will be necessary to identify the efficacy. Also, further studies are necessary to explore the cost of KOA in other ethnicities.

ARTICLE HIGHLIGHTS

Research background
Knee osteoarthritis (KOA) is the most common type of arthritis, with heavy burden on healthcare service. Manual therapy is an effective method for the treatment of KOA, but the efficacy of Maitland vs Mulligan mobilization techniques is still controversial.

Research motivation
Some reviews have found that the manual therapies might be effective and safe in ameliorating osteoarthritis symptoms, and Maitland mobilization was recommended in these studies. However, there still was no systematic review and meta-analysis to compare the efficacy of different mobilization techniques, such as Maitland vs Mulligan mobilization. Therefore, it is necessary to conduct a meta-analysis to fill this gap in our understanding.

Research objectives
To determine the efficacy of Maitland and Mulligan mobilization methods in adults with KOA.

Research methods
We searched PubMed, Embase, Web of Science, the Cochrane Library and Google Scholar from inception to September 20, 2020 to collect studies comparing Maitland and Mulligan mobilization methods in adults with KOA. Data processing and statistical analyses were performed using Cochrane Collaboration software (Review Manager Version 5.2 for Windows). The odds ratio and 95% confidence interval (CI) were employed to analyze the dichotomous variables. Meanwhile, the standardized mean difference (SMD) with a 95% CI was used to analyze the continuous variables.

Research results
A total of 341 articles were screened from five electronic databases (PubMed, the Cochrane Library, EMBASE, Web of Science and Google Scholar) after excluding duplicates. Ultimately, eight trials involving 471 subjects were included in present systematic review and meta-analysis. Mulligan mobilization is more effective in alleviating pain (SMD = 0.60; 95%CI: 0.17 to 1.03, \(P = 0.007; I^2 = 60\% \), \(P = 0.020\)) and improving Western Ontario and McMaster Universities function score (standardized mean difference = 7.41; 95%CI: 2.36 to 12.47, \(P = 0.004; I^2 = 92\%, P = 0.000\)). There was no difference in the effect of the two kinds of mobilization on improving the range of
motion (standardized mean difference = 9.63; 95% CI: -1.23 to 20.48, \( P = 0.080; F = 97\%\), \( P = 0.000\)).

**Research conclusions**
The Mulligan mobilization has been recommended to be applied in alleviating pain and improving Western Ontario and McMaster Universities function score.

**Research perspectives**
Our meta-analysis revealed that Mulligan mobilization will be a promising alternative option for KOA treatment. Unfortunately, because of the poor methodological quality of included studies, more data and surveillance will be necessary to identify the efficacy. Also, further studies are needed to explore the cost of KOA in other ethnicities.

**ACKNOWLEDGEMENTS**
We would like to thank all authors of the included primary studies.

**REFERENCES**


Patients with inflammatory bowel disease and post-inflammatory polyps have an increased risk of colorectal neoplasia: A meta-analysis

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Author contributions: Shi JL and Huang X designed the research; Shi JL, Lv YH and Huang J performed the research; Huang J and Shi JL contributed analytic tools; Lv YH, Huang J, Huang X and Liu Y analyzed the data; Shi JL wrote the paper.

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Abstract

BACKGROUND
Longstanding intestinal inflammation increases the risk of colorectal neoplasia in patients with inflammatory bowel disease (IBD). Accurately predicting the risk of colorectal neoplasia in the early stage is still challenging. Therefore, identifying visible warning markers of colorectal neoplasia in IBD patients is the focus of the current research. Post-inflammatory polyps (PIPs) are visible markers of severe inflammation under endoscopy. To date, there is controversy regarding the necessity of strengthened surveillance strategies for IBD patients with PIPs.

AIM
To determine whether IBD patients with PIPs carry an increased risk of colorectal neoplasia.

METHODS
Researchers searched the following databases up to July 31, 2021: MEDLINE (PubMed), MEDLINE (Ovid), EMBASE, Cochrane Library, China National Knowledge Infrastructure, Wan-Fang Data, China Science and Technology Journal Database and Chinese BioMedical Literature Database. Cohort and case-control studies that compared the risk of colorectal neoplasia between IBD patients with or without PIPs and published in English or Chinese were included. Methodological quality was assessed using the Risk of Bias in Nonrandomized Studies-of Interventions assessment tool. The outcomes of interest were the rates of various grades of colorectal neoplasia. The pooled risk ratio (RR) and 95% confidence interval (95%CI) were calculated using the random-effects model. Begg’s test and Egger’s test were used to calculate the publication bias. Sensitivity and subgroup analyses were performed to verify the robustness of the results. The Grading of Recommendations, Assessment, Development and Evaluation
approach was used to assess the overall quality of evidence supporting the outcomes of interest.

RESULTS
Nine studies involving 5424 IBD patients (1944 with PIPs vs 3480 without PIPs) were included. The overall bias in each included study ranged from moderate to serious. Compared with nonconcurrent PIPs, patients with PIPs had a higher risk of colorectal neoplasia (RR = 1.74, 95%CI: 1.35-2.24, P < 0.001, I² = 81.4%; aHR = 1.31, 95%CI: 1.01-1.70, P = 0.04, F = 26.2%; aOR = 2.62, 95%CI: 1.77-3.88, P < 0.001, F = 0%), advanced colorectal neoplasia (RR = 2.07, 95%CI: 1.49-2.87, P < 0.001, F = 77.4%; aHR = 1.63, 95%CI: 1.05-2.53, P = 0.03, P = 10.1%) and colorectal cancer (RR = 1.93, 95%CI: 1.32-2.82, P = 0.001, F = 83.0%). Publication bias was not observed in Begg’s test or Egger’s test. Sensitivity and subgroup analyses showed that the results are robust. The overall quality of evidence was assessed as moderate to low.

CONCLUSION
IBD patients with PIPs may have an increased incidence of colorectal neoplasia.

Key Words: Inflammatory bowel disease; Ulcerative colitis; Pseudopolyps; Inflammatory polyps; Colorectal cancer; Colorectal neoplasia

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Core Tip: This study is the first systematic review and meta-analysis to separately evaluate the potential risk between post-inflammatory polyps (PIPs) and colorectal neoplasia, advanced colorectal neoplasia, and colorectal cancer. Interestingly, we found that although malignant transformation from PIPs is rare, inflammatory bowel disease (IBD) patients with PIPs still bear an increased incidence of various grades of colorectal neoplasia. As an early warning of the increasing risk of colorectal neoplasia, IBD patients with PIPs should undergo strengthened surveillance to detect early dysplastic changes to allow for appropriate management so that there are improvements in both quality of life and survival rates.

INTRODUCTION
Longstanding intestinal inflammation increases the risk of colorectal neoplasia in patients with inflammatory bowel disease (IBD)[1,2]. Unlike sporadic colorectal neoplasms, IBD-related colorectal neoplasms are usually characterized by a younger onset age, more malignant behavior and a poorer prognosis[3-5]. Therefore, clinical guidelines recommend regular endoscopic surveillance for IBD patients to enable the early detection of colorectal neoplasms. Furthermore, patients with certain risk factors need to undergo an intensified surveillance strategy; these risk factors include extensive colitis, family history of colorectal cancer, concurrent primary sclerosing cholangitis or post-inflammatory polyps (PIPs)[6-9].

Post-inflammatory polyps (PIPs) are usually formed from the alternating cycling of intestinal inflammation and mucous epithelial cell regeneration. According to published data, PIPs are not rare in IBD patients, with their prevalence ranging from 4% to 74%[10,11]. To date, there is controversy in the literature regarding the necessity of a strengthened surveillance strategy for IBD patients with PIPs. Some earlier case-control studies showed an increased risk of colorectal neoplasia in patients with PIPs [12,13]. For this reason, clinical guidelines suggest a strengthened surveillance strategy for IBD patients with previous or present PIPs in endoscopy. However, the
recommended endoscopic surveillance intervals for IBD patients with PIPs vary considerably from country to country. In addition, some recent multicenter cohort studies showed no significant correlation between PIPs and colorectal neoplasia in IBD patients, in contrast to prior views and clinical guidelines[14,15]. Unnecessary and frequent endoscopic surveillance not only decreases the quality of life of IBD patients but also increases the burdens of health care and resource stewardship. Therefore, it is crucial to explore the potential risk association between PIPs and colorectal neoplasia and to clarify the safe and reasonable endoscopic surveillance intervals for IBD patients with PIPs.

Because of the lack of large, randomized trials and meta-analyses specifically focused on the risk of PIPs and colorectal neoplasia, most of the current data are from small-scale, observational, nonrandomized studies. Therefore, researchers systematically identified and analyzed data from observed trials and evaluated the association between PIPs and colorectal neoplasia, advanced colorectal neoplasia, and colorectal cancer in IBD patients separately. This study aimed to determine whether IBD patients with PIPs bear an increased risk of various grades of colorectal neoplasia.

MATERIALS AND METHODS

This meta-analysis was conducted and presented according to the PRISMA and MOOSE guidelines. The methods were established prior to the conduct of the review. The protocol of this study was registered in PROSPERO (CRD42020172539).

Search strategy

The following databases were searched systematically from inception up to July 31, 2021: MEDLINE (PubMed), MEDLINE (Ovid), EMBASE, Cochrane Library, China National Knowledge Infrastructure (CNKI), Wan-Fang Data, China Science and Technology Journal Database (VIP) and Chinese BioMedical Literature Database (CBM). The search items included “post-inflammatory polyps”, “colorectal neoplasms”, “inflammatory bowel diseases” and their associated words. The search strategy is detailed in the Supplementary data. Additional records were identified through hand searches of reference lists in clinical guidelines and relevant articles.

Study eligibility criteria

PIPs were defined as nonneoplastic lesions originating from the mucosa after the alternating cycling of intestinal inflammation and mucous epithelial cell regeneration and were proposed to be related to excessive healing processes. PIPs are usually diagnosed by endoscopists and pathologists and have been described as inflammatory polyps, pseudopolyps or post-inflammatory polyps in the literature[10].

The inclusion criteria were as follows: (1) Participants with confirmed IBD (including ulcerative colitis, Crohn’s disease and unclassified IBD); (2) Comparison of the colorectal neoplasia burden and prognosis between patients with PIPs and patients without PIPs; (3) Reported outcomes of interest (such as colorectal neoplasia, advanced colorectal neoplasia, colorectal cancer); and (4) Cohort study or case-control study published in English or Chinese. The exclusion criteria were as follows: (1) Participants with a known history of colorectal neoplasm before IBD diagnosis; (2) Participants with synchronous diagnoses of IBD and colorectal neoplasm; (3) Full-text versions were not available for assessing risk of bias; and (4) Reviews, case reports, or poster abstracts. Two researchers (Lv YH and Huang J) applied eligibility criteria and selected studies for inclusion in the systematic review independently. Disagreements between individual judgments were resolved by discussion and consultation with a third researcher (Jialing Shi) until a consensus was reached.

Risk of bias assessment

The methodological quality of each included study was assessed using the Risk of Bias in Nonrandomized Studies-of Interventions (ROBINS-I) assessment tool[16]. Two researchers (Yehong Lv, Jun Huang) assessed the methodological quality of each included study independently. Researchers were blinded to each other’s decisions. Disagreements between individual judgments were resolved by discussion and consultation with a third researcher (Jialing Shi) until a consensus was reached. The final score was listed in a homemade Excel form.
Outcomes of interest
The outcomes of interest were the related variables of IBD-associated colorectal neoplasia, including dysplastic number, pathologic grading, cytologic type, and time from diagnosis to dysplastic change. However, many published studies reported only 1-2 relevant indices, and most of them focused on tumor incidence. This aspect made it difficult to synthesize and analyze many other useful outcome variables for colorectal neoplasia. Because the incidence of colorectal neoplasia (including the number of cases and its effect size) well reflected the potential associations between risk factors and tumorigenesis, the researchers ultimately chose the incidence of various grades of colorectal neoplasia (including colorectal neoplasia, advanced colorectal neoplasia and colorectal cancer) as the outcome of interest in this review. Neoplasia in this review was defined as not only the malignant transformation of PIPs but also the malignant transformation of colorectal mucosa. All cases of neoplasia were diagnosed by pathological examination. Colorectal neoplasia was defined as low-grade dysplasia, high-grade dysplasia and colorectal cancer. Advanced colorectal neoplasia was defined as high-grade dysplasia and colorectal cancer. All relevant dysplasia data were extracted from final pathology reports or electronic medical records. Relevant clinical data for cases were extracted from electronic medical records.

Data extraction
The following data were collected: study characteristics (first author, publication year, study design, follow-up time, study conclusions), participant characteristics (numbers of PIPs and control group, IBD phenotypes, country of origin, primary sclerosing cholangitis (PSC), family history of colon cancer, extensive colitis), and outcome assessment (occurrence of various grades of colorectal neoplasia, including the numbers of colorectal neoplasia and its specific effective size). If the data were not reported in texts or tables, researchers contacted the corresponding author of the eligible study for additional information when necessary. Two researchers (Yehong Lv, Jun Huang) performed data extraction independently. Disagreements between individual judgments were resolved by discussion and consultation with a third researcher (Xue Huang until consensus was reached). The extracted data were listed in a homemade Excel form.

Data synthesis and analysis
Data synthesis was performed using STATA 15.0. The random-effects model was used for all data synthesis and statistical analysis. The pooled risk ratio (RR) and 95% confidence interval (95%CI) were calculated to evaluate the potential risk between PIPs and colorectal neoplasia. When adjusted ratios were available, pooled adjusted ratios, such as the pooled adjusted hazard ratio (aHR), the pooled adjusted relative risk (aRR), or the pooled adjusted odds ratio (aOR), and their 95%CI:s were also calculated.

Researchers used the $I^2$ statistic to quantify statistical heterogeneity. An $I^2$ less than 25% was considered low-level heterogeneity, 25% to 50% was considered moderate-level heterogeneity, and more than 50% was considered high-level heterogeneity. Because the number of included studies was less than ten, funnel plots for evaluating the potential publication bias were not constructed. Instead, Begg’s test and Egger’s test were used to calculate the publication bias.

In the sensitivity analysis, the following two methods were performed to verify the robustness of the results: (1) The use of the fixed-effects model; and (2) The exclusion of outliers or studies with significant clinical heterogeneity.

For further analysis, subgroup analysis was performed according to study design (cohort vs case-control study) and methodological quality (serious/critical vs low/moderate/unclear risk of bias) for screening the heterogeneous origin. Because geography plays a role in IBD-associated colorectal cancer, the recommended endoscopic surveillance intervals vary considerably in different countries and societies. The geographic heterogeneity between PIPs and colorectal neoplasia was investigated in further analysis. The potential risk between PIPs and colorectal neoplasia in different IBD phenotypes (ulcerative colitis, Crohn’s disease, unclassified IBD) was also investigated in further analysis. A $P$ value less than 0.05 was considered significant.

Statistical analysis
The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach was used to assess the overall quality of evidence supporting the outcomes of interest[17]. The final quality of evidence was classified as high, moderate,
low or very low. The quality of evidence was assessed using GRADE profiler 3.6.

**RESULTS**

**Study selection**
A literature search was conducted up to July 31, 2021, with 779 records identified through database searching and 13 additional records identified through other sources. After removing duplicates, 207 articles were eligible for screening. Researchers excluded 160 articles after screening the titles and abstracts, and 47 articles remained. In the full-text articles assessed, 38 articles were excluded for the following reasons: review (n = 10), case report (n = 14), conference abstracts (n = 7), and paper written in Korean (n = 1). All participants were IBD patients with colorectal cancer (n = 1). All participants were ulcerative colitis patients with low-grade dysplasia (n = 1), interventions focused on endoscopy techniques (n = 3), and there were no reports of the outcomes of interest (n = 1). Ultimately, 9 studies met the inclusion criteria and were all included in the qualitative and quantitative synthesis[12-15,18-22] (Figure 1).

**Included study characteristics**
Four cohort studies and five case-control studies were included in this study. The sample sizes of participants ranged from 204 to 1582. PIPs were present in 1944/5424 (35.8%) IBD patients (median prevalence, 29.7%). The median follow-up durations ranged from 3.0 to 22.9 years (median follow-up, 13.0 years). In different IBD phenotypes, five studies exclusively focused on ulcerative colitis (UC), and four remaining studies focused on mixed IBD phenotypes. In different cohort geographies, the included studies were conducted in the Netherlands (n = 4), the United States of America (n = 3), the United Kingdom (n = 2), Belgium (n = 1) and China (n = 1). The summarized characteristics from the included studies are presented in Table 1.

**Risk of bias assessment**
Methodological quality was assessed using the ROBINS-I. The overall bias in each included study ranged from moderate to serious. Overall, five studies had a moderate risk of bias, three studies had a serious risk of bias, and one study had an unknown risk of bias. Because of the lack of information on missing data, the study by M D Rutter had unknown risks of missing data and overall bias. The outcomes of interest in our research were not the main outcomes in some studies, which may have led to the lack of detailed data and processing methods. For this reason, studies commonly have a moderate or serious risk in the sections of “bias due to confounding”, “bias in the selection of participants for the study”, and “bias in classification of interventions”. The risk of bias assessment from each included study is presented in Table 2.

**Association of PIPs with colorectal neoplasia**
All nine included studies evaluated the association between PIPs and colorectal neoplasia and involved 5424 IBD patients (1944 with PIPs vs 3480 without PIPs). A total of 553 (28.4%) IBD patients with PIPs were diagnosed with colorectal neoplasia, compared with 546 (15.7%) IBD patients without PIPs. Using a random-effects model, IBD patients with PIPs were significantly associated with a higher risk of colorectal neoplasia than IBD patients without PIPs (RR = 1.74, 95% CI: 1.35-2.24, P < 0.001, F = 81.4%) (Figure 2A). Four studies reported the adjusted aHR ratio, three studies reported the adjusted aOR ratio, and one study reported the adjusted aRR ratio. When pooling the aHR and aOR, significant differences between these two groups were still observed (pooled aHR = 1.31, 95% CI: 1.01-1.70, P = 0.04, F = 26.2%; pooled aOR = 2.62, 95% CI: 1.77-3.88, P < 0.001, F = 0%) (Figure 2B, 2C). Publication bias was not observed in Begg’s test or Egger’s test.

In the sensitivity analysis, IBD patients with PIPs were still significantly associated with a higher risk of colorectal neoplasia than IBD patients without PIPs when researchers used the fixed-effects model (RR = 1.67, 95% CI: 1.50-1.85, P < 0.001, F = 81.4%). The results did not change after excluding outliers or studies with significant clinical heterogeneity.

In the subgroup analysis, different study designs and methodological qualities did not change the results or heterogeneity of each group. In different IBD phenotypes, five studies exclusively focused on UC and involved 2280 patients (921 with PIPs vs 1359 without PIPs). PIPs were also significantly associated with a higher risk of colorectal neoplasia in UC patients (RR = 1.76, 95% CI: 1.18-2.63, P = 0.006, F = 81.6%).
Table 1 The summarized characteristics of the included studies

<table>
<thead>
<tr>
<th>Included studies</th>
<th>Study design</th>
<th>IBD phenotypes</th>
<th>Country</th>
<th>Median disease duration (yr)</th>
<th>PSC (n, %)</th>
<th>Family history of CRC (n, %)</th>
<th>Extensive colitis (n, %)</th>
<th>Median follow-up time (yr)</th>
<th>The risk of various grades of colorectal neoplasia (PIPs vs nonPIPs)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jong MEd 2019[21]</td>
<td>Cohort Study</td>
<td>UC, CD, UNCLASSIFIED IBD</td>
<td>Netherlands</td>
<td>≥ 8.0</td>
<td>27 (5.2%)</td>
<td>74 (14.3%)</td>
<td>345 (66.5%)</td>
<td>21.6 years in PIPs, 22.9 yr in nonPIPs</td>
<td>CRN 36/154 vs 65/365 (aHR = 1.08, 95%CI: 0.66-1.75); ACRN 9/154 vs 10/365 (aHR = 1.38, 95%CI: 0.52-3.68); CRC 6/154 vs 7/365</td>
<td>PIPs did not increase the risk of CRN, ACRN or CRC</td>
</tr>
<tr>
<td>Mahmoud R 2019[15]</td>
<td>Cohort Study</td>
<td>UC, CD, UNCLASSIFIED IBD</td>
<td>Netherlands, America</td>
<td>≥ 8.0</td>
<td>234 (14.8%)</td>
<td>93 (5.9%)</td>
<td>1275 (80.6%)</td>
<td>5.4 years in PIPs, 4.5 years in nonPIPs</td>
<td>CRN 64/462 vs 124/1120 (aHR = 1.25, 95%CI: 0.88-1.77); ACRN 17/462 vs 24/1120 (aHR = 1.17, 95%CI: 0.59-2.31)</td>
<td>PIPs did not increase the risk of CRN or ACRN</td>
</tr>
<tr>
<td>Xu W 2020[22]</td>
<td>Cohort Study</td>
<td>UC</td>
<td>China</td>
<td>6.0</td>
<td>10 (4.1%)</td>
<td>NR</td>
<td>116 (47.2%)</td>
<td>13.0</td>
<td>CRN 11/57 vs 8/189 (aOR = 5.46, 95%CI: 1.69-17.638)</td>
<td>PIPs increased the risk of ACRN</td>
</tr>
<tr>
<td>Choi C-HR 2017[14]</td>
<td>Cohort Study</td>
<td>UC</td>
<td>United Kingdom</td>
<td>≥ 8.0</td>
<td>42 (4.3%)</td>
<td>48 (4.9%)</td>
<td>987 (100%)</td>
<td>13.0</td>
<td>CRN 66/447 vs 31/540 (aHR = 1.20, 95%CI: 0.80-1.80)</td>
<td>PIPs did not increase the risk of CRC</td>
</tr>
<tr>
<td>Jegadeesan R 2016[20]</td>
<td>Case-Control Study</td>
<td>UC</td>
<td>American</td>
<td>12.5</td>
<td>47 (10.1%)</td>
<td>65 (13.1%)</td>
<td>457 (97.9%)</td>
<td>3.0</td>
<td>CRN 32/138 vs 79/329</td>
<td>PIPs did not increase the risk of CRN</td>
</tr>
<tr>
<td>Lutgens M 2015[19]</td>
<td>Case-Control Study</td>
<td>UC, CD, UNCLASSIFIED IBD</td>
<td>Netherlands, Belgium</td>
<td>NR</td>
<td>30 (5.7%)</td>
<td>33 (6.2%)</td>
<td>349 (65.7%)</td>
<td>NR</td>
<td>CRC 126/260 vs 62/270 (aHR = 2.30, 95%CI: 1.20-4.10)</td>
<td>PIPs increased the risk of CRC</td>
</tr>
<tr>
<td>Baars JE 2011[13]</td>
<td>Case-Control Study</td>
<td>UC, CD, UNCLASSIFIED IBD</td>
<td>Netherlands</td>
<td>9.0</td>
<td>22 (4.3%)</td>
<td>34 (6.6%)</td>
<td>156 (30.4%)</td>
<td>15.5</td>
<td>CRC 71/147 vs 68/366 (aRR = 1.92, 95%CI: 1.28-2.88)</td>
<td>PIPs increased the risk of CRC</td>
</tr>
<tr>
<td>Velayos FS 2006[12]</td>
<td>Case-Control Study</td>
<td>UC</td>
<td>American</td>
<td>17.0</td>
<td>50 (13.3%)</td>
<td>24 (6.4%)</td>
<td>318 (84.6%)</td>
<td>NR</td>
<td>CRC 105/184 vs 83/192 (aOR = 2.50, 95%CI: 1.40-4.60)</td>
<td>PIPs increased the risk of CRC</td>
</tr>
<tr>
<td>Rutter MD 2004[18]</td>
<td>Case-Control Study</td>
<td>UC</td>
<td>United Kingdom</td>
<td>22.0</td>
<td>NR</td>
<td>NR</td>
<td>204 (100%)</td>
<td>NR</td>
<td>CRN 42/95 vs 26/109 (aOR = 2.29, 95%CI: 1.28-4.11)</td>
<td>PIPs increased the risk of CRN</td>
</tr>
</tbody>
</table>

*Adjusted factors: IBD type, sex, concomitant PSC, age at IBD diagnosis, maximum disease extent, medication use, family history of CRC, and the mean inflammation score.

*Adjusted factors: concomitant PSC, and the mean inflammation score.
Study did not report.

Thirty-eight patients (including 1 ACRN) were excluded due to missing values.

Adjusted factors: colorectal stricture, the presence of PIPs, age at IBD diagnosis, disease duration, and concomitant PSC.

Adjusted factors: patient's age, average number of biopsies, surveillance interval, and colonoscopy type (i.e., white-light or chromoendoscopy).

Adjusted factors: IBD type, concomitant PSC, microscopic disease extent, and the presence of PIPs.

Adjusted factors: age at IBD diagnosis, sex, duration of PSC, disease extent at onset, and the presence of PIPs.

Adjusted factors: backwash ileitis, shortened colon, tubular colon, scarring, segment of severe inflammation, normal colonic appearance, the presence of PIPs, and colonic stricture. IBD: Inflammatory bowel disease; PSC: Primary sclerosing cholangitis; CRC: Colorectal cancer; PIPs: Post-inflammatory polyps; UC: Ulcerative colitis; CD: Crohn's disease; UNCLASSIFIED IBD: Unclassified inflammatory bowel disease; CRN: Colorectal neoplasia; ACRN: Advanced colorectal neoplasia; NR: Not reported.

Because of the lack of CD and UNCLASSIFIED IBD data, the effects of PIPs on colorectal neoplasia in CD and UNCLASSIFIED IBD patients are not available. In different cohort geographies, patients with PIPs had an increased risk of colorectal neoplasia in Europe (RR = 2.05, 95%CI: 1.62-2.59, \( P < 0.001 \), \( I^2 = 60.7\% \)) and Asia (RR = 4.56, 95%CI: 1.93-10.79, \( P < 0.001 \), \( I^2 \) not available). No association was observed in the US (RR = 1.17, 95%CI: 0.86-1.59, \( P = 0.314 \), \( I^2 = 56.1\% \)) (Table 3).

### Association of PIPs with advanced colorectal neoplasia

Three cohort studies and three case-control studies evaluated the association between PIPs and advanced colorectal neoplasia and involved 3766 IBD patients (1264 with PIPs vs 2502 without PIPs). A total of 339 (26.8\%) IBD patients with PIPs were diagnosed with advanced colorectal neoplasia, compared with 255 (10.2\%) IBD patients without PIPs. Using a random-effects model, IBD patients with PIPs were significantly associated with a higher risk of advanced colorectal neoplasia than IBD patients without PIPs (RR = 2.07, 95%CI: 1.49-2.87, \( P < 0.001 \), \( I^2 = 77.4\% \)) (Figure 3A).

Three studies reported the adjusted aHR ratio, two studies reported the adjusted aOR ratio, and one study reported the adjusted aRR ratio. When pooling the aHR, significant differences between these two groups were still observed (pooled aHR = 1.63, 95%CI: 1.05-2.53, \( P = 0.03 \), \( I^2 = 10.1\% \)) (Figure 3B). Publication bias was not
Table 3 The results of subgroup analysis in colorectal neoplasia and advanced colorectal neoplasia

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Study</th>
<th>Pooled RR (95%CI)</th>
<th>P value</th>
<th>I² value%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colorectal neoplasia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study design</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort study</td>
<td>4</td>
<td>1.88 (1.18-3.00)</td>
<td>0.008</td>
<td>80.0</td>
</tr>
<tr>
<td>Case-control study</td>
<td>5</td>
<td>1.68 (1.20-2.35)</td>
<td>0.002</td>
<td>85.6</td>
</tr>
<tr>
<td>Methodological quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serious/Critical risk of bias</td>
<td>3</td>
<td>1.74 (1.00-3.01)</td>
<td>0.049</td>
<td>87.5</td>
</tr>
<tr>
<td>Low/Moderate/Unclear risk of bias</td>
<td>6</td>
<td>1.74 (1.28-2.36)</td>
<td>0.000</td>
<td>80.7</td>
</tr>
<tr>
<td><strong>IBD phenotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>5</td>
<td>1.76 (1.18-2.63)</td>
<td>0.006</td>
<td>81.6</td>
</tr>
<tr>
<td>CD</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>UNCLASSIFIED IBD</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Geographic regions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>5</td>
<td>2.05 (1.62-2.59)</td>
<td>0.000</td>
<td>60.7</td>
</tr>
<tr>
<td>America</td>
<td>2</td>
<td>1.17 (0.86,1.59)</td>
<td>0.314</td>
<td>56.1</td>
</tr>
<tr>
<td>Asia</td>
<td>1</td>
<td>4.56 (1.93,10.79)</td>
<td>0.000</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Advanced colorectal neoplasia (ACRN)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study design</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort study</td>
<td>3</td>
<td>2.42 (1.36-4.32)</td>
<td>0.003</td>
<td>40.1</td>
</tr>
<tr>
<td>Case-control study</td>
<td>3</td>
<td>1.92 (1.27-2.90)</td>
<td>0.002</td>
<td>88.6</td>
</tr>
<tr>
<td>Methodological quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serious/Critical risk of bias</td>
<td>1</td>
<td>2.11 (1.64-2.71)</td>
<td>0.000</td>
<td>NA</td>
</tr>
<tr>
<td>Low/Moderate/Unclear risk of bias</td>
<td>5</td>
<td>2.1 (1.35-3.27)</td>
<td>0.001</td>
<td>80.6</td>
</tr>
</tbody>
</table>

RR: Risk ratio; CRN: Colorectal neoplasia; IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn’s disease; UNCLASSIFIED IBD: Unclassified inflammatory bowel disease; ACRN: Advanced colorectal neoplasia; NA: Not available.

In the sensitivity analysis, IBD patients with PIPs were still significantly associated with a higher risk of advanced colorectal neoplasia than IBD patients without PIPs when researchers used the fixed-effects model (RR = 1.91, 95%CI: 1.67-2.18, P < 0.001, I² = 77.4%). The results did not change when researchers excluded outliers or studies with significant clinical heterogeneity. In the subgroup analysis, different study designs and methodological qualities did not change the results or heterogeneity of each group (Table 3).

**Association of PIPs with colorectal cancer**

One cohort study and three case-control studies evaluated the association between PIPs and colorectal cancer and involved 1938 IBD patients (745 with PIPs vs 1193 without PIPs). A total of 308 (41.3%) IBD patients with PIPs were diagnosed with colorectal cancer, compared with 220 (18.4%) IBD patients without PIPs. Using a random-effects model, IBD patients with PIPs were significantly associated with a higher risk of developing colorectal cancer than IBD patients without PIPs (RR = 1.93, 95%CI: 1.32-2.82, P = 0.001, I² = 83.0%) (Figure 4). Publication bias was not observed in Begg’s test or Egger’s test. Because the adjusted ratios were not available, the pooled adjusted ratio was not calculated. Because few studies were included in this section, sensitivity analysis and subgroup analysis were not performed.

**Quality of evidence**

The GRADE approach was used to assess the overall quality of evidence. There is low-quality evidence to support that IBD patients with PIPs bear an increased risk of observed in Begg’s test or Egger’s test.
colorectal neoplasia and colorectal cancer. There is moderate-quality evidence to support that IBD patients with PIPs bear an increased risk of advanced colorectal neoplasia. A summary of the assessment is presented in Table 4.

**DISCUSSION**

This study aimed to explore the potential association between PIPs and colorectal neoplasia in IBD patients. The results indicated that IBD patients with PIPs bear an increased risk of colorectal neoplasia, advanced colorectal neoplasia, and colorectal cancer.

In contrast to sporadic colorectal cancer, IBD-related colorectal cancer follows a sequence of “inflammation-dysplasia-carcinoma”. In IBD patients, recurrent mucosal inflammation is the primary risk factor for intestinal neoplasia. The alternating cycling of intestinal inflammation and mucous epithelial cell regeneration provides more opportunities for transcription errors and the subsequent development of neoplasia by activating procarcinogenic genes and inhibiting tumor suppressor genes. The development of colorectal neoplasia is frequently associated with mutations, methylation and dysregulation of genes. It induces microsatellite instability, telomere shortening, and chromosomal instability and further induces tumor progression[23-26]. The related genes and molecules involve the adenomatous polyposis coli (APC) gene, k-ras, deleted in colorectal cancer (DCC) genes, deleted in pancreatic cancer-4 (DPC4) genes, and tumor protein 53 (p53), among others[27-31]. Meanwhile, the inflammatory microenvironment of IBD, which consists of a variety of immune cells, epithelial cells, stromal cells, cytokines and chemokines, has many similarities to the microenvironment of cancer[32]. The innate and adaptive immune systems are involved in tumor development by the release of reactive oxygen species, nitrogen species and cytokines [25]. The use of immunosuppression may also allow neoplasia to progress at a faster rate. Moreover, intestinal dysbacteriosis also appears to play a role in IBD-related colorectal neoplasia, such as Escherichia coli, Bacteroides fragilis, Enterococcus faecalis and Fusobacterium nucleatum[24,33,34].

These changes were detectable not only in dysplastic mucosa but also in morphologically normal intestinal mucosa. Their accumulation will lead to extensive genomic and epigenomic alterations and then create a favorable microenvironment for tumor progression. This phenomenon is called field cancerization[35-37]. In theory, the earlier the field cancerization can be detected, the earlier the interventions will be to slow or stop tumor progression. Unfortunately, the above changes are invisible under...
Table 4 Assessing the overall quality of evidence supporting each outcome using Grading of Recommendations, Assessment, Development and Evaluation

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Illustrative comparative risksa (95%CI)</th>
<th>Relative effect (95%CI)</th>
<th>No of Participants (studies)</th>
<th>Quality of the evidence(GRADE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assumed risk</td>
<td>Corresponding risk</td>
<td>NonPIPs</td>
<td>PIPS</td>
<td></td>
</tr>
<tr>
<td>Association of PIPs with colorectal neoplasia; Follow-up: 3.0-22.9 yr</td>
<td>Study populationb</td>
<td>157 per 1000</td>
<td>273 per 1000 (212 to 351)</td>
<td>RR 1.74 (1.35 to 2.24)</td>
</tr>
<tr>
<td>Association of PIPs with advanced colorectal neoplasia; Follow-up: 3.0-22.9 yr</td>
<td>Study populationb</td>
<td>102 per 1000</td>
<td>211 per 1000 (151 to 293)</td>
<td>RR 2.07 (1.48 to 2.87)</td>
</tr>
<tr>
<td>Association of PIPs with colorectal cancer; Follow-up: 3.0-22.9 yr</td>
<td>Study populationb</td>
<td>184 per 1000</td>
<td>356 per 1000 (243 to 520)</td>
<td>RR 1.93 (1.32 to 2.82)</td>
</tr>
</tbody>
</table>

aThe basis for the assumed risk (e.g., the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95%CI).
bLabel: Moderate. GRADE Working Group grades of evidence: High quality: Further research is very unlikely to change our confidence in the estimate of effect; Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; Very low quality: We are very uncertain about the estimate. CI: Confidence interval; RR: Risk ratio.

endoscopy. Accurately predicting the risk of colorectal neoplasia in IBD patients in the early stage is still challenging. Therefore, looking for visible warning markers of colorectal neoplasia in IBD patients is the focus of current research.

PIPs are formed as a consequence of repeated cycles of active inflammation and regeneration of the intestinal epithelium. Under endoscopy, PIPs look like polyps or loose mucosal tags[10,38]. Although malignant transformation from PIPs is rare, IBD patients with PIPs are at an increased risk of various grades of colorectal neoplasia. Previous studies have shown that PIPs positively correlate with the severity of inflammation and are considered surrogate markers of significant cumulative inflammatory burden[26,39,40]. Given this finding, researchers have proposed that PIPs are visible markers of severe inflammation under endoscopy and an early warning of an increased risk of colorectal neoplasia in IBD patients.

In different IBD phenotypes, the colorectal neoplasia burden of UC patients with PIPs is also increased, which is consistent with the burden of IBD patients. Thus, compared with UC patients without PIPs, a strengthened surveillance strategy is preferable for UC patients with PIPs. Meanwhile, because of the lack of data on Crohn’s colitis patients, there is still doubt whether surveillance intervals should be independent of IBD phenotypes. Additional well-designed trials are needed for further research.

Geographic heterogeneity exists in the incidence of IBD and IBD-associated colorectal cancer[41-43]. Currently, there is controversy regarding reasonable endoscopic surveillance intervals for patients with PIPs. The recommended intervals vary considerably from country to country. Therefore, what actual role does geography play in PIPs and colorectal neoplasia? In this study, compared with patients without PIPs, patients with PIPs had an increased risk of colorectal neoplasia in Europe and Asia. Conversely, no association between PIPs and colorectal neoplasia has been observed in the United States. The reason for this geographic heterogeneity is multifactorial and includes genetics, diet, IBD phenotype, inflammation burden, treatment options, and differences in endoscopic surveillance. However, it is important to note that this result should be interpreted and applied cautiously because
Figure 2 Forest plot showing the association of post-inflammatory polyps with colorectal neoplasia in inflammatory bowel disease patients. A: Forest plot of pooling unadjusted risk ratio; B: Forest plot of pooling adjusted hazard ratio; C: Forest plot of pooling adjusted odds ratio.
More well-designed trials are needed to verify this variation in future research. In contrast to these results, the American Society of Gastrointestinal Endoscopy (ASGE) recommends annual endoscopic surveillance for IBD patients with PIPs, which is more frequent than the every 2-3 years that is recommended by the European Crohn’s and Colitis Organization (ECCO), the British Society of Gastroenterology (BSG), the Association of Coloproctology for Great Britain and Ireland (ACPGBI) and the National Institute for Clinical Excellence (NICE)\[6,8,44,45]\.

When an endoscopist identifies an IBD patient with concurrent PIPs, what should they do? Because IBD patients with PIPs bear an increased risk of colorectal neoplasia, it is necessary for them to enroll in a rigorous treatment program that includes strengthened endoscopic surveillance strategies to achieve complete histological mucosal healing and identify colorectal neoplasia in an early stage. The purpose of endoscopic surveillance is to detect early dysplastic changes to allow for appropriate management so that there are improvements in quality of life and survival rates. To reduce the rate of missing dysplasia, surveillance should be performed by an experienced gastroenterologist in IBD when the disease is in remission. Adequate bowel preparation, meticulous inspection with slow withdrawal, and the application of advanced endoscopic equipment are key for high-quality surveillance. Detailed recommendations of various societies for IBD patients with PIPs are summarized in

**Figure 3 Forest plot showing the association of post-inflammatory polyps with advanced colorectal neoplasia in inflammatory bowel disease patients.** A: Forest plot of pooling unadjusted risk ratio; B: Forest plot of pooling adjusted hazard ratio.
When considering endoscopic surveillance intervals, societies recommend different intervals that range from one to three years. European societies suggest that PIPs are an intermediate risk factor for developing colorectal cancer in IBD patients and that IBD patients with PIPs should undergo endoscopic surveillance every 2-3 years\[6,44,45\]. Nevertheless, US and Australian societies suggest shortening the surveillance interval to every year because they believe that IBD patients with PIPs are at high risk of colorectal cancer\[8,46\]. In China and Japan, current guidelines and specifications do not mention a definite interval for patients with PIPs. Correspondingly, these Asian societies advocate initiating endoscopic surveillance from 8-10 years after disease onset and recommend annual or biennial endoscopic surveillance for patients with left-sided colitis or extensive colitis\[47-49\]. To summarize, the optimal interval of endoscopic surveillance for IBD patients with PIPs has not been established, and additional well-designed trials are needed for further research.

How can colonoscopy screening be performed for IBD-associated colorectal cancer? During recent decades, new technology has improved in terms of endoscopic devices, including white light endoscopy (WLE), chromoendoscopy, magnifying endoscopy, endomicroscopy, narrow band imaging (NBI), and endoscopic molecular imaging. Among them, the majority of clinical guidelines recommend methylene blue or indigo carmine chromoendoscopy with targeted biopsies for surveillance colonoscopy. Under chromoendoscopy, the visualization of the colonic epithelium is improved by highlighting the areas of mucosal irregularities and delineating the borders of suspected lesions. Studies have shown that 61%-84% of neoplastic lesions could be visualized by recent endoscopy\[50-53\]. In this context, targeted biopsies have the advantage of fewer samples. Therefore, although chromoendoscopy takes a longer time and may be more cumbersome, chromoendoscopy with targeted biopsies has a higher dysplasia detection rate and is more cost-effective than conventional colonoscopy\[54-58\]. However, random biopsies are beneficial for monitoring disease progression, evaluating histologic stage and assessing treatment efficacy. In special circumstances, such as a known history of dysplasia, concomitant PSC or a foreshortened colon, random biopsies are still recommended regardless of the screening method. With advances in optical imaging techniques, it is unclear whether chromoendoscopy should still be used when surveillance is performed with high-definition colonoscopy or new endoscopic imaging. Additional well-designed trials are needed for further research.

The increased risk of colorectal neoplasia in IBD patients with PIPs probably reflects the increased risk of previous severe inflammation rather than the PIPs themselves having malignant potential. In a multicenter cohort study, researchers found that most patients with PIPs undergo colectomy due to uncontrolled inflammation but not colorectal neoplasia\[15\]. Therefore, it is not necessary to remove PIPs conventionally.
unless there is diagnostic uncertainty or concerning malignant features or clinical symptoms, such as bleeding or intussusception. Features of underlying malignancy include uneven redness, nodularity, villous texture, slight elevation or depression, friability, obscured vascular pattern, ulcerated or velvety surface, disruption of innominate lines, and inability to lift with submucosal injection[57,59,60]. In patients with multiple PIPs or uncontrolled inflammation, a terrible intestinal mucosal environment makes it difficult for endoscopists to identify abnormal lesions, and prophylactic colectomy should be considered[18]. To summarize, the management of IBD patients with PIPs, including prophylactic colectomy and enhanced endoscopic surveillance, requires careful consideration of the individual patient, their disease, and endoscopic and histologic factors and involves a multidisciplinary team discussion that should include gastroenterologists, surgeons and pathologists.

In this study, the overall quality of evidence was assessed as moderate to low. There are several obstacles to designing and performing randomized controlled trials for endoscopic surveillance of IBD patients, such as ethical issues and the relatively low incidence of colorectal neoplasia. Thus, robust and available evidence usually comes from well-designed multicenter observational trials. Having recognized these limitations, we systematically searched several databases, undertook a meta-analysis of the latest and most favorable evidence, and used multiple methods to verify the robustness of the potential risk between PIPs and colorectal neoplasia. In the three outcomes of interest, the results did not change when researchers excluded outliers or studies with significant clinical heterogeneity. This result indicated that based on the current studies, the results of this meta-analysis are robust and that individual studies have less influence.

A meta-analysis that focused on the prognostic factors for ACRN in IBD patients was published in 2021[61]. Similar to our study, the researchers found that patients with PIPs were at higher risk for ACRN based on three cohort studies and two case-control studies (OR = 3.29, 95%CI: 2.41-4.48, \( P < 0.001, \ I^2 = 0 \)). However, this association was not confirmed in the pooled HR analysis (univariable HR = 1.67, 95%CI: 0.99-2.82, \( P = 0.11, \ I^2 = 56 \%). A probable reason for this result was that the number of available studies and patients included was too small for an accurate performance assessment. In contrast, we extended the search cutoff time to July 31, 2021 to include additional literature and participants. Finally, three cohort studies and three case-control studies involving 3766 IBD patients (1264 with PIPs vs 2502 without PIPs) were included. The results showed

### Table 5 Societal recommendations for colorectal cancer surveillance in inflammatory bowel disease patients with post-inflammatory polyps

<table>
<thead>
<tr>
<th>Society</th>
<th>Surveillance intervals</th>
<th>Surveillance techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA 2010</td>
<td>More frequent surveillance (No specific interval recommended)</td>
<td>Chromoendoscopy with targeted biopsies OR Standard or high-definition colonoscopy along with random biopsies</td>
</tr>
<tr>
<td>ASGE 2015</td>
<td>Every year</td>
<td>Chromoendoscopy with targeted biopsies OR Random biopsies (2-4 biopsies every from 10 cm) and targeted biopsies if chromoendoscopy is not available</td>
</tr>
<tr>
<td>Cancer Council Australian 2019</td>
<td>Every year</td>
<td>Chromoendoscopy with targeted biopsies</td>
</tr>
<tr>
<td>BSG/ACPGBI 2010</td>
<td>Every 3 yr</td>
<td>Chromoendoscopy with targeted biopsies OR Random biopsies (2-4 biopsies every from 10 cm) and targeted biopsies if chromoendoscopy is not available</td>
</tr>
<tr>
<td>NICE 2011</td>
<td>Every 3 yr</td>
<td>Chromoendoscopy with targeted biopsies</td>
</tr>
<tr>
<td>ECCO 2013/2017</td>
<td>Every 2-3 yr</td>
<td>Chromoendoscopy with targeted biopsies OR White light endoscopy with random biopsies (4 biopsies every from 10 cm) and targeted biopsies</td>
</tr>
<tr>
<td>JSGE 2018/2020</td>
<td>Not mention the definite interval (Every 1-2 yr for patients with left-sided colitis or extensive colitis)</td>
<td>Chromoendoscopy with targeted biopsies OR Available endoscopic technology with targeted biopsies to increase the neoplasia detection rate</td>
</tr>
<tr>
<td>Chinese Society of Gastroenterology 2018/2020</td>
<td>Not mention the definite interval (Every 1-2 yr for patients with left-sided colitis or extensive colitis)</td>
<td>Chromoendoscopy/magnifying endoscopy with targeted biopsies</td>
</tr>
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</table>

that patients with PIPs were at higher risk for ACRN, which was confirmed in both pooled RR analysis and pooled HR analysis.

This study is the first meta-analysis to separately assess the relationship between PIP and CRN, ACRN and CRC. This study has several strengths. First, this study evaluated the association between PIPs and colorectal neoplasia, advanced colorectal neoplasia, and colorectal cancer separately. Disparity in the risk stratification of different grades of colorectal neoplasia can provide bases for surveillance strategy, treatment options and prognosis judgment. Second, this study used a new tool (ROBINS-I) to assess the methodological quality of each included study. Third, this study used multiple methods to identify the robustness of the results.

This study also has some limitations. First, the heterogeneity of outcomes is high. Therefore, researchers used multiple methods to identify the robustness of the results and conducted subgroup analyses to search for the source of heterogeneity. Second, a family history of colon cancer and concurrent primary sclerosing cholangitis have been reported as risk factors for colorectal neoplasia in several studies. However, because of missing data in the target population, no high-quality evidence could be obtained.

**CONCLUSION**

IBD patients with PIPs may have an increased incidence of various grades of colorectal neoplasia. Due to the lower rate of malignant transformation, PIPs do not need to be removed conventionally. However, due to the increased risk of colorectal neoplasia, IBD patients with PIPs should undergo strengthened surveillance to detect early dysplastic changes to allow for appropriate management to improve quality of life and survival rates. Meanwhile, there are still many gaps in this field of research, such as information on safe and reasonable endoscopic surveillance intervals for patients with PIPs and the pathogenic process of PIPs in colorectal neoplasia. Therefore, additional well-designed multicenter trials are needed.

**ARTICLE HIGHLIGHTS**

**Research background**

Longstanding intestinal inflammation increases the risk of colorectal neoplasia in patients with inflammatory bowel disease (IBD). Accurately predicting the risk of colorectal neoplasia in IBD patients in the early stage is still challenging. Post-inflammatory polyps (PIPs) are visible markers of severe inflammation under endoscopy. To date, there is controversy in the literature regarding the necessity of a strengthened surveillance strategy for IBD patients with PIPs.

**Research motivation**

Unnecessary and frequent endoscopic surveillance not only decreases the quality of life of IBD patients but also increases the burdens of health care and resource stewardship. Therefore, it is crucial to explore the potential risk association between PIPs and colorectal neoplasia. A better insight into this topic would help physicians to clarify the safe and reasonable endoscopic surveillance intervals for IBD patients with PIPs.

**Research objectives**

To determine whether IBD patients with PIPs bear an increased risk of various grades of colorectal neoplasia.

**Research methods**

Researchers systematically searched eight databases up to July 31, 2021. Cohort and case-control studies that compared the risk of colorectal neoplasia between IBD patients with or without PIPs and published in English or Chinese were included. Methodological quality was assessed using the Risk of Bias in Nonrandomized Studies-of Interventions (ROBINS-I) assessment tool. The outcomes of interest were the rates of various grades of colorectal neoplasia. The pooled risk ratio (RR) and 95% confidence interval (95%CI) were calculated using the random-effects model. Begg’s test and Egger’s test were used to calculate the publication bias. Sensitivity and subgroup analyses were performed to verify the robustness of the results. The Grading
of Recommendations, Assessment, Development and Evaluation (GRADE) approach was used to assess the overall quality of evidence supporting the outcomes of interest.

**Research results**

Of 792 records, four cohort studies and five case-control studies involving 5424 IBD patients (1944 with PIPs vs 3480 without PIPs) were included in this study. The overall bias in each included study ranged from moderate to serious. After meta-analyses, IBD patients with PIPs were significantly associated with a higher risk of colorectal neoplasia than IBD patients without PIPs (RR = 1.74, 95%CI: 1.35-2.24, \( P < 0.001, I^2 = 81.4\%\)). Meanwhile, patients with PIPs also had a higher risk of advanced colorectal neoplasia (RR = 2.07, 95%CI: 1.49-2.87, \( P < 0.001, I^2 = 77.4\%\)) and colorectal cancer (RR = 1.93, 95%CI: 1.32-2.82, \( P = 0.001, I^2 = 83.0\%\)). Publication bias was not observed. And Sensitivity and subgroup analyses showed that the results are robust. The overall quality of evidence was assessed as moderate to low.

**Research conclusions**

IBD patients with PIPs may have an increased incidence of various grades of colorectal neoplasia. Due to the lower rate of malignant transformation, PIPs do not need to be removed conventionally. However, due to the increased risk of colorectal neoplasia, IBD patients with PIPs should undergo strengthened surveillance to detect early dysplastic changes to allow for appropriate management to improve quality of life and survival rates.

**Research perspectives**

There are still many gaps in this field of research, such as information on safe and reasonable endoscopic surveillance intervals for patients with PIPs and the pathogenic process of PIPs in colorectal neoplasia. Therefore, additional well-designed multicenter trials are needed.

**ACKNOWLEDGEMENTS**

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Intravascular fasciitis involving the external jugular vein and subclavian vein: A case report

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Author contributions: Meng XH contributed to the study conception and writing; Meng XH and Fang X contributed to the data collection and investigation; Meng XH and Xie LS contributed to the analysis; all authors contributed to the critical review and revision, final approval of the article and accountability for all aspects of the work.

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Country/Territory of origin: China

Abstract

BACKGROUND
Intravascular fasciitis (IVF) is a rare nodular fasciitis that often involves the layers and lumens of blood vessels; therefore, it is easily misdiagnosed as a malignant tumor with invasion into blood vessels.

CASE SUMMARY
A 13-year-old boy was admitted due to a mass on the left side of his neck. Duplex ultrasonography revealed a circular solid hypoechoic mass in the external jugular vein, and magnetic resonance imaging revealed an enhanced longitudinal mass-like lesion in the left supraclavicular fossa. Surgical treatment was arranged and completed, histopathological analysis showed a large amount of spindle cell proliferation, and immunohistochemistry showed that the spindle cells were positive for the expression of vimentin, caldesmon, and smooth muscle actin and negative for the expression of S-100 protein, desmin, CD34, and c-kit; Ki-67 staining revealed a low proliferative index (5%-10%), which confirmed the differentiation characteristics of myofibroblasts. Fluorescence in situ hybridization detected the rearrangement of USP6. IVF was subsequently diagnosed.

CONCLUSION
IVF is characterized by intraluminal, intramural and extramural involvement of small to large arteries or veins. Unless the doctor has a deep understanding of the disease or suspects that there is an initial indicator, IVF may be confused with other intravascular malignancies, leading to unnecessary radical surgery. Imaging examination combined with histopathological examination can improve the
INTRODUCTION

Intravascular fasciitis (IVF) was first proposed by Patchefsky et al[1] in 1981 as a special type of nodular fasciitis involving the small and medium veins or arteries. The disease is rare and rarely reported domestically or internationally. Intravascular fasciitis is a variant of nodular fasciitis, which is caused by the reactive intravascular proliferation of myofibroblasts. The histology of IVF is very similar to that of typical nodular fasciitis, but it often involves the layers and lumens of blood vessels, frequently leading to an overdiagnosis of vascularized malignancies[2,3]. IVF most commonly occurs in the upper extremities, head and neck, followed by the lower extremities and trunk, but there have been reports of rare sites, such as the mouth and maxillofacial region[4-7]. This study reports intravascular nodular fasciitis of the external jugular vein and subclavian vein and explores its clinical and pathological features, diagnosis and differential diagnosis combined with a literature review to raise awareness of rare lesions and avoid misdiagnosis.

CASE PRESENTATION

Chief complaints
A 13-year-old boy came to our clinic with his parents due to a mass on the left side of his neck for 1 mo.

History of present illness
One month prior to admission, the patient accidentally felt a mass on his left neck, approximately 2 cm × 2 cm in size, and there was no local discomfort such as redness, swelling and tenderness. After learning this, his parents brought him to the hospital for treatment.

History of past illness
The patient had been in good health until he showed evidence of the disease.

Personal and family history
The patient was not from a consanguineous marriage and there was no family history of similar disease.

Physical examination
During the physical examination, a clear soft tissue mass was palpated on the left supraclavicular area and was determined to be an ovoid-shaped mass of approximately 2 cm × 2 cm in size.
**Laboratory examinations**
The results of laboratory examinations on admission were normal, including routine blood and coagulation function tests, liver and kidney function tests, tumor markers, urine tests and stool tests.

**Imaging examinations**
Duplex ultrasonography showed that a circular solid hypoechoic mass could be seen on the left clavicle along the external jugular vein, with a general length of approximately 4.2 cm (Figure 1A). There was no blood flow signal passing through the lumen, and the mass invaded into the subclavian vein along the external jugular vein. Hypoechoic masses were observed in some areas of the subclavian vein, involving a length of approximately 2.1 cm, and no obvious involvement was observed in the left internal jugular vein (Figure 1A). Similarly, the left supraclavicular fossa showed irregular patchy patterns on cervical magnetic resonance imaging. The lesion was mostly located between the left common jugular vein and the sternocleidomastoid muscle, and the boundary was not clear. The contrast-enhanced scan showed slight enhancement, and the wall of the adjacent left subclavian vein was thickened. Lesions with abnormal signals in the left supraclavicular fossa were tested by biopsy, considering the possibility of lymphatic or granulomatous lesions (Figure 1B). The abnormal signal in the left supraclavicular fossa was considered to indicate lymphatic or granulomatous lesions, and biopsy was recommended.

The pathologist reported that the mass was a spindle cell mesenchymal tumor without definite malignant features, but the specific tumor type and nature needed to be determined by routine histology and immunohistochemistry. Surgery was then performed; because only the mass was removed, no vein reconstruction was required.

A microscopic investigation was based on a microscopic examination that reported the mass from the left jugular vein was a fibroblast/myofibroblast tumor, consistent with intravascular nodular fasciitis. Hematoxylin-eosin staining showed that the mass was composed of spindle cells, which were mainly fibroblasts and myofibroblasts. The cell morphology was relatively consistent, with an irregular fascicular-shaped arrangement. The atypia was mild, with a small amount of nuclear division, a small amount of mucous degeneration and collagen deposition in the stroma, as well as scattered inflammatory cell infiltration and erythrocyte extravasation (Figure 2). Immunohistochemistry studies showed that the spindle cells were positive for vimentin, caldesmon and smooth muscle actin and negative for S-100 protein, desmin, CD34, and c-kit; Ki-67 staining revealed a low proliferative index (5%-10%), confirming their myofibroblastic differentiation (Figure 3). The USP6 rearrangement test was also positive (Figure 3). Overall, the results were consistent with IVF.

**FINAL DIAGNOSIS**
Left external jugular vein and subclavian vein IVF.

**TREATMENT**
Conservative treatment of this disease has a certain cure rate, so both clinical observation with conservative treatment and surgical treatment options are available for this disease. Surgical indications were present in this patient, and the surgical treatment option was selected by the patient and his family. Therefore, the patient underwent left neck mass resection after the relevant examinations. After surgical treatment, he had no neurological symptoms or swelling of his arm.

The surgery began with an 8 cm skin incision that was made along the left supraclavicular vein, and the solid mass in the external jugular vein was visible on the deep surface of the left supraclavicular platysma muscle. The solid mass, approximately 4 cm long, grew along the blood vessel and involved the left subclavian vein and its surrounding branches. As requested by the patient's family members, no claviclectomy was performed during the operation. Only the external segment of the jugular vein and the mass in the accessible superficial branch segment were completely removed. Considering that the mass could be malignant and that there was a possibility of extensive excision, a piece of tissue was sent for rapid frozen pathology.
Figure 1 Imaging examination. A: Duplex ultrasonography: a circular solid hypoechoic mass could be seen along the external jugular vein, with a general length of approximately 4.2 cm; there was no blood flow signal passing through the lumen, and the mass invaded into the subclavian vein along the external jugular vein. Hypoechoic masses were observed in some areas of the subclavian vein, involving a length of approximately 2.1 cm; B: Magnetic resonance imaging revealed an enhanced longitudinal mass-like lesion in the left supraclavicular fossa. EJV: External jugular vein; SV: Subclavian veins.

OUTCOME AND FOLLOW-UP
The patient had no thoracic duct injury or pneumothorax after the operation and was discharged four days after the operation. The outpatient review was good one month after discharge. Through telephone follow-up one year after discharge, the patient noted that the left neck did not have a lump or discomfort. The next follow-up will be two years after discharge. The disease will be mainly monitored for potential malignant behavior.

DISCUSSION
Nodular fasciitis is a nonneoplastic myofibroblastic hyperplasia that grows quickly, is sometimes rich in cells and has certain atypicality. This condition can be seen with a high number of mitotic figures and is easily misdiagnosed as various types of soft tissue sarcoma; therefore, it is also known as pseudosarcoma fasciitis. IVF is a rare and special type of nodular fasciitis; thus far, only seven cases have been reported in the domestic literature, and thirty-six cases have been reported internationally. IVF generally occurs in adolescent and young adult patients, with an average age of onset of 26.5 years worldwide. Of these thirty-six patients, 80% were under the age of thirty. There is no significant difference in the incidence rate between males and females, and the ratio is close to 1:1\[8-10\]. However, among the cases reported domestically, the
epidemiological characteristics are inconsistent with those of foreign cases, which may be caused by an insufficient number of reported cases. Usually, before the onset of the disease, the patients were healthy, and even after disease development, the patients generally had no feelings of discomfort, such as pain, tenderness or decreased mobility. IVF is most commonly found in the upper extremities, head and neck, followed by the lower extremities and trunk, and there have been reports of rare sites. Most of the clinical manifestations have been painless masses that grow slowly under the skin. A few may have pain or tenderness. Most of the lesions were solitary nodules, and a few patients presented with multiple nodules, the size of the lesion ranged from a minimum of 0.6 cm to 5 cm, and the course of the disease ranged from 2 wk to 8 years. IVF is a benign disease that is usually cured by simple local excision. Thus far, the pathogenesis of endovascular fasciitis is still unclear. It was confirmed that spindle cells are derived from myofibroblasts based on their immunohistochemical characteristics. A few cases have suggested that this condition may be related to previous trauma, thrombosis, and high levels of estrogen.

IVF mainly involves small veins or arteries, where lesions extend longitudinally along the vessels and pass through the vessel wall into surrounding connective tissue and adjacent vessels. Due to the small size of the vessels, the lesions may have a polynodular appearance, and intracavitary lesions are separated from the main tumor. The histological features are myofibroblast proliferation with a fractured structure, erythrocyte exosmosis, and a mucous background, but interstitial mucous degeneration is not evident, and there is no pleomorphism and rarely mitosis. The intravascular component of IVF can be identified by immunohistochemical staining for elastin and smooth muscle actin. Immunohistochemical staining for smooth muscle actin, caldesmon and vimentin indicates positivity for spindle cells, Ki-67 staining reveals a low proliferative index (< 10%), and staining for c-kit, S100 protein, desmin, CD31 and CD34 is negative, suggesting that IVF involves myofibroblastic differentiation. USP6 gene rearrangement has been recently demonstrated in nodular fasciitis, and the presence of fusions involving the USP6 gene in most cases provides a useful tool for diagnostic confirmation. Because of its vessel involvement, IVF may be mistaken for a malignancy. When IVF cells undergo atypical changes, more mitotic signs may occur, often involving various layers and lumens of blood vessels, which may lead to the overdiagnosis of malignant tumors, such as fibrosarcoma, leiomyosarcoma, and mucinous liposarcoma. Despite the invasive growth of these lesions, there is no evidence of invasive clinical behavior, recurrence or metastasis. The absence of large atypical hyperchromatic nuclei, and the presence of abnormal mitotic figures and immunohistochemical characteristics of the spindle cells can identify IVF.

CONCLUSION

Intravascular fasciitis is a rare vascular-associated nodular fasciitis that is easily misdiagnosed as other tumors. We report a case of this rare disease and provide insights on IVF, with the hope of improving the recognition of non-neoplastic lesions.
Figure 3 Immunohistochemistry and USP6 gene rearrangement. A: The spindle cells were positive for smooth muscle actin; B: The spindle cells were positive for vimentin; C: The spindle cells were positive for caldesmon; D: Ki-67 staining of spindle cells revealed a low proliferative index (5%-10%); E: The spindle cells were negative for CD34; F: The spindle cells were negative for S-100; G: The spindle cells were negative for desmin; H: The spindle cells were negative for c-kit; I: Fluorescence in situ hybridization with a separate probe for USP6 indicated that there might be one yellow or red-green adjacent fusion signal and two red-green separation signals in most cells.

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Occurrence of human leukocyte antigen B51-related ankylosing spondylitis in a family: Two case reports

Mie Jin Lim, Eul Noh, Ro-Woon Lee, Kyong-Hee Jung, Won Park

BACKGROUND
Ankylosing spondylitis (AS) is strongly associated with the human leukocyte antigen (HLA) B27 haplotype. In regions where conventional polymerase chain reaction for HLA typing is available for antigens such as HLA B27 or HLA B51, it is common to perform the HLA B27 test for evaluation of AS. While HLA B27-associated clustered occurrences of AS have been reported in families, we report the first case series of HLA B51-related occurrences of AS in a family.

CASE SUMMARY
A father and his daughters were diagnosed with AS and did not have the HLA B27 haplotype. Although they were positive for HLA B51, they exhibited no signs of Behçet’s disease (BD). Of the five daughters, one had AS, and three, including the daughter with AS, were positive for HLA B51. The two daughters with the HLA B51 haplotype (excluding the daughter with AS) exhibited bilateral grade 1 sacroiliitis, whereas the daughters without the HLA B51 haplotype did not have sacroiliitis. Thus, this Korean family exhibited a strong association with the HLA B51 haplotype and clinical sacroiliitis, irrespective of the symptoms of BD.

CONCLUSION
It is advisable to check for HLA B51 positivity in patients with AS/spondyloarthropathy who test negative for HLA B27.

Key Words: Ankylosing spondylitis; Spondyloarthropathy; Human leukocyte antigen B51; Human leukocyte antigen B27; Sacroiliitis; Case report
INTRODUCTION

Ankylosing spondylitis (AS) is a chronic, immune-mediated arthritis that primarily affects the spine and sacroiliac joints. Inflammation of the sacroiliac joints is a hallmark feature of the disease, and grade ≥ 2 radiological sacroiliitis on both sides or unilateral grade ≥ 3 radiological sacroiliitis are the criteria (per the modified New York criteria) for the diagnosis of AS[1] and comprise some of the criteria required for a diagnosis of spondyloarthropathy (SpA)[2]. A strong association between the human leukocyte antigen (HLA) B27 allele and AS was discovered in the early 1970s[3]. AS is also known to run strongly within families, and HLA B27 positivity was observed to be higher in familial AS patients than in their sporadic AS counterparts[4]. According to Jung et al.[5], the proportion of HLA B27 positivity among Korean AS patients was 80%, and in a Korean population study, the HLA B27 positivity rate in Korean AS patients was 83.3% compared to a rate of 4.0% in healthy controls[6].

The HLA B51 antigen is a well-known genetic factor associated with Behçet’s disease (BD)[7]. In countries such as Korea and Japan where conventional polymerase chain reaction (PCR) for HLA genotyping is available for antigens such as HLA B27 or HLA B51, it is common to perform the HLA B27 test for the evaluation of AS and HLA B51 testing for BD. Interestingly, two cases of Reiter’s syndrome associated with HLA B51 have been reported[8,9] and the possibility of HLA B51-related arthropathy in individuals with HLA B27-negative reactive arthritis or seronegative SpA has also been proposed[10,11]. To date, there have been no reports of familial AS occurrence related to HLA B51. Here, we report the first cases of HLA B51-related AS in a family and the impact of HLA B51 positivity on sacroiliitis.

CASE PRESENTATION

Chief complaints

Case 1: In 2018, an 82-year-old man visited our clinic with the chief complaint of inflammatory low back pain.

Case 2: In 2020, the eldest daughter of the patient described in case 1 visited the clinic. She was 56 years old and complained of back pain, which had started 3 years previously and worsened as she woke up in the morning.

History of present illness

Case 1: He had previously been diagnosed with AS at another hospital. He did not complain of any additional pain in the Achilles tendon or the peripheral joints. He did not have any symptoms of BD, such as oral or genital ulcers.

Case 2: She did not complain of any other pain in the Achilles tendon or peripheral joints. She did not have any symptoms of BD, such as oral or genital ulcers.
not have any symptoms related to the eyes.

History of past illness
Case 1: He was suffering from interstitial lung disease.
Case 2: She had no previous medical history.

Personal and family history
Case 1: There is no personal and family history.
Case 2: The patient was the first of five daughters of the patient in case 1.

Physical examination
Case 1: The patient’s blood pressure was 126/27 mmHg, pulse rate was 79 beats/min, and respiratory rate was 24 breaths/min at the time of presentation. The body temperature was within the normal range. No abnormal skin lesions were observed on the body. The Schober’s test showed a positive result of 1 cm, and the distance between the occiput and wall was 10 cm. Chest wall expansion test could not be performed because of dyspnea related to interstitial lung disease. Ophthalmologic examination revealed no evidence of iridocyclitis.

Case 2: Her blood pressure was 98/52 mmHg, pulse rate was 70 beats/minute, and respiratory rate was 20 breaths/min at the time of presentation. Her body temperature was within the normal range. No abnormal skin lesions were found, and no heart murmur was heard. Schober’s test showed a positive result of 2.5 cm. The distance between the occiput and wall and the chest wall expansion test were within normal limits.

Laboratory examinations
Case 1: Laboratory tests showed a white blood cell count of 10190/μL, C-reactive protein (CRP) of 14.3 mg/L (0-5), erythrocyte sedimentation rate (ESR) of 52 mm/h (1-15) and positive antinuclear antibodies with a titer of 1:640. The tests for extractable nuclear antigen antibodies were negative. Rheumatoid factors were not observed. The patient tested negative for HLA B27 and positive for HLA B51, using conventional PCR. With help from the laboratory department, simple HLA genotyping was performed, which further confirmed the presence of HLA B51.

Case 2: Laboratory tests showed a white blood cell count of 5950/μL, CRP of 0.4 mg/L (0-5), and ESR of 14 mm/h (1-15). Neither rheumatoid factor nor antinuclear antibodies were present. She tested negative for HLA B27 and was positive for HLA B51, using conventional PCR.

Imaging examinations
Case 1: Radiographic imaging of the sacroiliac joints revealed complete ankyloses, and his spine exhibited a “bamboo” appearance (Figures 1A and 1B). Transthoracic echocardiography revealed a sclerotic mitral and aortic valve.

Case 2: Radiographic imaging of the sacroiliac joints revealed multiple definite erosions with sclerotic changes compatible with grade III bilateral sacroiliitis (Figure 1C). Magnetic resonance imaging of her spine revealed fat deposition at the corners of the vertebral bodies, suggesting changes caused by AS (Figure 1D).

FINAL DIAGNOSIS

Case 1
The final diagnosis in this case was AS. Laboratory findings of leukocytosis and high levels of inflammatory markers were thought to be caused by interstitial lung disease, as the patient did not complain much about back pain and no other symptoms of AS were reported.

Case 2
The final diagnosis of the presented case was AS.
Figure 1 Radiographic images of the father (case 1) and the daughter (case 2) with ankylosing spondylitis. A: Sacroiliac joint of the father showing complete ankyloses; B: Lumbar spine imaging of the father in the shape of “bamboo spine”; C: Computed tomography image of the sacroiliac joint of the daughter showing multiple definite erosions with sclerotic changes on both sacroiliac joints; D: Magnetic resonance image of the lumbar spine of the daughter revealing fat deposition at the corners of the lumbar spine, probably due to ankylosing spondylitis.

TREATMENT

Case 1
The patient was prescribed non-steroidal anti-inflammatory drugs (NSAIDs).

Case 2
The patient was prescribed NSAIDs.

OUTCOME AND FOLLOW-UP

Case 1
The patient reported that his back pain was under control at the 2 mo follow up. In addition, he complained of dyspnea, and 2 years after the diagnosis of AS in our hospital, the patient passed away due to worsening of interstitial lung disease.

Case 2
She reported that her back pain was under control at 2 mo follow up.

Family of cases 1 and 2
The father (case 1) had five daughters, including the first daughter (case 2) who was previously diagnosed with AS. The family was concerned about the possibility of familial inheritance of AS, and all five daughters agreed to undergo full HLA-B genotyping and computed tomography (CT) of the sacroiliac joint(s) to assess the possibility of AS. HLA-B genotyping was performed using a commercially available polymerase chain reaction sequencing-based kit (AlleleSEQR HLA-B Sequencing Kit, Genome Diagnostics B. V., Utrecht, The Netherlands) for experimental purposes. This study was approved by the Institutional Review Board of Inha University Hospital (Incheon, Korea; IRB 2020-03-003), and written informed consent was obtained from all participants.
Symptoms
None of the daughters reported signs or symptoms of oral ulcers or genital ulcers, and only the youngest daughter complained of inflammatory back pain. No abnormal skin lesions were observed.

Laboratory examinations
Three daughters, including the patient in case 2 and the youngest daughter, tested positive for HLA B51, and two other daughters were negative for HLA B51. None of the patients tested positive for HLA B27.

Imaging examinations
A radiologist who was blinded to patient information interpreted the images. Three daughters had the HLA B51:01 allele, among whom only the eldest daughter (case 2) was diagnosed with AS. However, the other two daughters, including the youngest daughter, were found to exhibit grade 1 sacroiliitis, upon performing pelvic bone CT [(Figures 2A and 2B for 4th daughter) and (Figures 2C and 2D for the youngest daughter)]. Two daughters without the HLA B51:01 allele did not exhibit sacroiliitis. The family pedigree is shown in Figure 3.

DISCUSSION
To the best of our knowledge, this is the first report to describe the occurrence of HLA B51-related AS in a family. Three of the five daughters had the HLA B51:01 allele and developed either AS or clinical sacroiliitis; however, the daughters without the HLA B51:01 allele did not exhibit any clinical signs or symptoms of SpA. Low-grade sacroiliitis is indicative of early AS in patients with undifferentiated SpA [12]. Thus, the high prevalence of HLA B51:01 in the daughters with sacroiliitis suggests a strong association of HLA B51 with AS/SpA in the family. It was also interesting to observe that no one in the family manifested clinical symptoms of BD, although the association between HLA B51 and BD is known to be strong.

The clinical significance of HLA B51-related familial AS familial is that this family is from Korea, where HLA B51 is highly prevalent. A previous case control study performed in Korea showed that the prevalence of HLA B51 positivity in patients with BD was reported to be 55.7%, compared to 15.7% in healthy controls [13]. The prevalence of HLA B51 in BD has been reported to be higher in countries adjacent to the ancient Silk Road, which include Turkey, Iraq, China, Japan, and Korea. In accordance with Korean data, the positivity of HLA B51 in Han Chinese was 55.83% in BD patients and 12% in controls [14], and in Japan it was 59.4% in BD patients and 13.6% in controls [15]. The HLA B51 in the family seemed to be inherited from the father (case 1), and three out of five daughters were positive for HLA B51. The wife of the patient in case 1 passed away years before this study, and HLA genotyping could not be performed. We assumed that she would be positive for HLA B40 and HLA B58 because of occurrence of homogeneous HLA B40 and HLA B58 among daughters. All HLA B51 positive daughters had sacroiliitis. Thus, in regions where HLA B51 is prevalent, it could play a role in the development of HLA B27-negative reactive arthritis or seronegative SpA [9,10].

Second, unlike in previous studies, sacroiliitis was observed in most of the daughters (three of five) in this family. There are conflicting reports regarding the prevalence of sacroiliitis in patients with BD. Chang et al [7] reported that sacroiliitis was diagnosed in 58.9% of SpA patients, 10.3% of those with BD, and 3.6% of healthy controls. Olivieri et al [16] conducted a similar study using CT scans and reported sacroiliitis in 30% of BD patients and 5% of controls. The difference in the prevalence of sacroiliitis between patients with BD and controls was clinically significant in both the aforementioned studies [7,16]. However, another study showed contrasting results with the prevalence of sacroiliitis seen in 7.4% of individuals with BD and 8% of the control group [17]. Kotevoglu et al [18] conducted a study using CT and found sacroiliitis in 5% of patients with BD and in 7% of healthy controls. In this family, sacroiliitis was found in 60% of all daughters who exhibited no clinical features of BD. Thus, the high prevalence of sacroiliitis in this family should be interpreted in the context of SpA and not a clinical feature of BD.

Lastly, all family members with sacroiliitis, tested positive for HLA B51 and negative for HLA B27. There are studies about HLA B51 and HLA B27 in SpA, and HLA B27 remains the major factor in AS. In two studies, Chang et al [7] reported that the majority of SpA patients (67.9%) were HLA B27 positive, whereas the prevalence
Figure 2 Computed tomography image of the sacroiliac joint(s) of the two daughters who tested positive for human leukocyte antigen B51. A, B: Multiple small undulating lesions are apparent on both sacroiliac joints, suggesting grade 1 bilateral sacroiliitis were seen in one daughter; C, D: Small undulating lesions in both sacroiliac joints are apparent, suggesting grade 1 bilateral sacroiliitis in the other daughter. The lesions are indicated by the red circle.

Figure 3 Pedigree of the family. Human leukocyte antigen B51:01 carriers had either ankylosing spondylitis (black arrow) or grade 1 sacroiliitis. HLA: Human leukocyte antigen.

of HLA B51 positivity was only 21.4% in those with SpA. Similarly, a study by Jung et al[5] reported that 106 of 153 patients with AS were HLA B27 positive/HLA B51 negative, whereas eight were HLA B51 positive/HLA B2-negative, and 16 patients were both HLA B27 and HLA B51 positive. In clinical practice, HLA typing is usually carried out for determining the presence of HLA B27 for the evaluation of AS, and HLA B51 for the evaluation of BD. If a patient suspected of having SpA, is negative for HLA B27, additional testing of HLA B51 could help facilitate the diagnosis of SpA even if the patient does not have the clinical features of BD.

A genome-wide association study of AS, which used “immunochip” technology, reported that the presence of the HLA B51:01 allele was associated with an increased risk of AS[3]. In addition, AS is strongly associated with the presence of specific amino acids at position 97 in HLA-B, and position 97 is associated with the cell surface expression of HLA B51[19]. Therefore, in accordance with the present case report,
HLA B51 could also potentially contribute to the development of AS.

**CONCLUSION**

This is the first report of familial inheritance of HLA B27-negative AS and HLA B51 positivity associated with either mild or definite radiological sacroiliitis. No patient in the family exhibited any signs or symptoms of BD. Therefore, it is advisable to check for HLA B51 positivity in patients with HLA B27-negative AS or SpA, even in the absence of clinical signs of BD.

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Multicentric recurrence of intraductal papillary neoplasm of bile duct after spontaneous detachment of primary tumor: A case report

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Abstract

BACKGROUND

Intraductal papillary neoplasm of the bile duct (IPNB) rarely recurs in a multicentric manner. We encountered a patient with multiple recurrences of the gastric subtype of IPNB one year after spontaneous detachment of the primary tumor during peroral cholangioscopy (POCS).

CASE SUMMARY

A 68-year-old woman on maintenance hemodialysis because of lupus nephritis had several cardiovascular diseases and a pancreatic intraductal papillary mucinous neoplasm (IPMN). She was referred to our department for dilation of the common bile duct (CBD) and a tumor in the lumen, detected using ultrasonography. She had no complaints, and blood tests of hepatobiliary enzymes were normal. Magnetic resonance cholangiopancreatography (MRCP) showed a papillary tumor in the CBD with a filling defect detected using endoscopic retrograde cholangiography (ERC). Intraductal ultrasonography revealed a papillary tumor and stalk at the CBD. During POCS, the tumor spontaneously detached with its stalk into the CBD. Pathology showed low-intermediate nuclear atypia of the gastric subtype of IPNB. After 1 year, follow-up MRCP showed multiple tumors distributed from the left hepatic duct to the CBD. ERC and POCS showed multicentric tumors. She was alive without hepatobiliary symptoms at least two years after initial diagnosis of IPNB.

CONCLUSION

The patient experienced gastric subtype of IPNB without curative resection. Observation may be reasonable for patients with this subtype.

Key Words: Bile duct neoplasm; Neoplasm Recurrence; Pancreatic intraductal neoplasms; Magnetic resonance cholangiopancreatography; Endoscopic retrograde cholangiography;
and hepatology

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Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): D
Grade E (Poor): 0

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Core Tip: Multiple occurrences of intraductal papillary neoplasm of bile duct (IPNB) are rare. Here we present the case of a patient with multicentric recurrence of IPNB after spontaneous detachment of the primary tumor. She harbored an asynchronous intraductal papillary mucinous neoplasm and experienced gastric subtype of IPNB without complete resection.

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INTRODUCTION

Intraductal papillary neoplasm of the bile duct (IPNB) is a subtype of biliary epithelial tumors and a counterpart of pancreatic intraductal papillary mucinous neoplasm (IPMN)[1]. Despite numerous case reports of IPNB, just 12 cases describe multicentric recurrence[2-14]. Furthermore, we possess insufficient knowledge of the variations in patterns of recurrence and prognosis of IPNB[15]. Here we describe the case of a patient with the gastric subtype of IPNB that developed multicentric recurrence after spontaneous detachment of the primary tumor during peroral cholangioscopy (POCS). We obtained sufficient samples for pathological examination. In contrast, other case reports analyzed pathology after surgery or biopsy. Present case got enough pathogens by POCS. The patient experienced an unusual course without undergoing curative resection.

CASE PRESENTATION

Chief complaints

A 68-year-old woman was diagnosed with intraductal papillary mucinous neoplasm (IPMN) using ultrasonography (US) that was performed to address her abdominal aortic aneurysm (AAA). Five months later, follow-up US revealed a dilated common bile duct (CBD) and a hyperechoic tumor in the lumen. She was referred to our department for further evaluation. She had no particular complaints.

History of present illness

The patient underwent maintenance hemodialysis for 30 years because of end-stage renal disease associated with lupus nephritis. She also regularly visited the Department of Cardiology after percutaneous coronary intervention for myocardial ischemia, severe aortic stenosis.

History of past illness

She underwent artificial graft replacement for her AAA and a cholecystectomy for acute cholecystitis.

Personal and family history

There is no specific family history of illness.

Physical examination

Upon examination, the patient had no icteric sclera, and the abdominal region and her vital signs were normal.
Laboratory examinations

Laboratory tests did not detect elevated levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, or γ-glutamyl transpeptidase. The levels of tumor markers such as carcinoembryonic antigen and carbohydrate antigen 19-9 were normal as well.

Imaging examinations

Magnetic resonance cholangiopancreatography (MRCP) showed a filling defect in the CBD (Figure 1A). Endoscopic ultrasound (EUS) showed a papillary tumor in the CBD (Figure 1B) and branch-duct type multiple IPMN without worrisome features, high-risk stigmata, or both (Figure 1C). Endoscopic retrograde cholangiography (ERC) showed a filling defect of contrast agent in the CBD (Figure 1D). Intraductal ultrasonography (IDUS) revealed a papillary tumor with a stalk at the CBD, which spontaneously detached with its stalk during peroral cholangioscopy (POCS) (Figure 1E and F).

MULTIDISCIPLINARY EXPERT CONSULTATION

We recovered a sufficient amount of the detached tumor for histopathological analysis. Hematoxylin and eosin staining showed low to intermediate nuclear atypia, although interstitial invasion was unclear (Figure 2B). Immunohistochemical analyses of tumor markers were as follows (-, undetectable; +, positive): CEA (-); p53 (-); MIB-1 index 5%-15%; mucins (MUC) 2 (-), MUC5AC (+), and MUC6 (+) (Figure 2C-E).

FINAL DIAGNOSIS

The IPNB showed multicentric recurrence after detachment from the primary tumor.

TREATMENT

Additional resection of the bile duct was considered. However, her age and numerous complications indicated that she was at high risk for surgery itself. Among possible treatment options, she selected observation.

OUTCOME AND FOLLOW-UP

We performed MRCP 6 mo after the diagnosis of the primary tumor. The primary tumor showed no recurrence. However, a new papillary tumor appeared in the left intrahepatic duct (Figure 3A). After 1 year, MRCP showed further multiple papillary tumors in the extrahepatic duct (Figure 3B). There was no finding of obstruction of the bile duct, cholangitis, or both. We subsequently repeated the ERC and POCS. ERC showed multiple filling defects of contrast agent in the extrahepatic and intrahepatic ducts (Figure 3C), and POCS showed multiple papillary tumors in the extrahepatic and intrahepatic ducts (Figure 3C). Histopathological analysis of tumor specimens of the left intrahepatic duct and in the CBD showed similarities to the previous specimens. The IPNB showed multicentric recurrence after detachment from the primary tumor. There were no symptoms or evidence of cholangitis. Further observation was selected, and she remained asymptomatic with normal levels of hepatobiliary enzymes. During this time, MRCP showed slight growth of the tumors. She continued her typical daily activities for at least 1 year after the diagnosis of multicentric recurrence of IPNB.

DISCUSSION

IPNB, which is a rare variant of bile duct tumors, is characterized by papillary growth within the bile duct lumen and is considered a biliary counterpart of intraductal papillary mucinous neoplasm of the pancreas. Untreated IPNB, although benign, causes recurrent cholangitis and jaundice[3]. In most cases, surgical resection is
selected because of the malignant potential of IPNB[16]. A retrospective cohort study of 39 cases of IPNB conducted by Rocha et al[17] found that R0 resection is significantly superior to R1 resection (median survival, 82 mo vs 36 mo), leading to the recommendation of complete resection of the IPNB.
Kim et al.[16] found that R1 resection reduced survival outcomes of patients with IPNB and suggested that concurrent bile duct resection should be performed if the resection margin of the bile duct is not reliably free of neoplastic involvement. According to these reports, additional bile duct resection may have been required for the remnant stalk of IPNB of our patient. However, her other severe pathologies contraindicated surgery, which she declined.

A case report of multicentric IPNB, including a literature review, convincingly demonstrates that recurrent tumors typically develop in the lower bile duct compared with the primary IPNBs[2]. Furthermore, 84% of IPNBs develop in the intrahepatic or hilar bile duct, or both[18]. In contrast, 80% of recurrent IPNBs occur in the CBD. These findings suggest that multicentric recurrence is likely caused by dissemination in the bile duct, rather than through a multicentric origin[2]. Our present case is atypical, because the primary tumor was located in the CBD, and multicentric recurrence was distributed through the intrahepatic and extrahepatic ducts, which is unlikely explained by dissemination. Our present case therefore may represent a true multicentric or disseminated recurrence of IPNB. Future studies are therefore required to identify the molecular mechanism underlying multicentric development of IPNB.

POCS directly observes tumors, their features, and the extent of dissemination[3]. Here we obtained an amount of tumor specimens sufficient for analysis, because the primary tumor spontaneously detached during POCS. The grade of unclear atypia was low-to-intermediate grade with no evidence of invasive cancer. The results of immunohistochemical analysis were consistent with the gastric subtype of IPNB. IPNB is histologically classified into subtypes as follows: pancreatobiliary, intestinal, gastric, and oncocystic[15]. The gastric subtype is characterized by gastric foveae. Immunohistochemical analysis detects the expression of MUC5AC and MUC6, but often not that of MUC1[19].
Conflicting data make it difficult to determine the subtype of IPNB associated with poor prognosis. For example, Schlitter et al.[20] found no difference between survival rates of subtypes. In contrast, Kim et al.[15] found that the prognosis of the gastric subtype was better than that of the others subtypes after curative resection (5-year overall survival, 83.9%). Zen et al.[21] found that adenoma is the most frequent gastric subtype, whereas the pancreatobiliary subtype occasionally comprises cells with high-grade nuclear atypia and an invasive component. Gordon-Weeks et al.[22] found that the pancreatobiliary subtype contains an invasive tumor with worse prognosis compared with those of other subtypes. Furukawa et al.[23] found that the prognosis of the gastric subtype of IPMN of the pancreas was better compared with those of other subtypes. However, the diagnostic methods and populations vary among these reports, and further studies are required to determine the associations between subtypes and prognosis.

Our literature search uncovered 10 related cases of synchronous occurrence of IPMN and IPNB[24-33]. Among them, only one case involves IPNB in the CBD, similar to our present case, and the others involve the intrahepatic duct[23]. Date et al.[34] detected GNAS and KRAS mutations in IPMN and IPNB, which appeared metachronously in the same patient. Although IPMN and IPNB share similarities of imaging findings, the relationship between the mutational status of each is unknown. Further studies are therefore required to accumulate more cases with co-occurrence of IPNB and IPMN.

Our present patient has survived for at least two years after the diagnosis of multicentric recurrence. Further studies are required, although our present experience indicates that observation may suffice for certain subtypes of IPNB because of better prognosis as well as to monitor multicentric recurrence or dissemination.

**CONCLUSION**

IPNB varies widely in appearance and clinical features. Multicentric recurrence of IPNB is rare, and the present case is atypical. Early surgery is required if IPNB is diagnosed. However, there is conflicting evidence regarding the subtype of IPNB associated with poor prognosis and its potential for recurrence. The gastric subtype of IPNB may have a good prognosis.

Here we encountered a patient with multicentric recurrence who survived after diagnosis for at least two years without curative resection. Thus, if there is no histopathological evidence of malignancy, observation may serve as a reasonable alternative for patients with the gastric subtype of IPNB. Further investigation is required to unambiguously identify the subtype of IPNB that may be selected for observation.

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Case of primary extracranial meningioma of the maxillary sinus presenting as buccal swelling associated with headache: A case report

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Peer-review model: Single blind

Abstract

BACKGROUND
Meningiomas are benign tumors that originate from the meningothelial arachnoid cells, but they rarely develop extracranially. There is no specific surgical guideline for resecting them in the maxillary sinus, and little is known about their biological behavior and operative management.

CASE SUMMARY
We present a 54-year-old female patient referred to our department with a primary extracranial meningioma that presented as buccal swelling associated with headache. On clinical examination the mass was non-tender, fixed, sessile and non-pulsatile situating in the right maxillary sinus. Computed tomography scan showed a well-defined mass of 7 cm × 6 cm × 6 cm compressing the surrounding structures. Magnetic resonance imaging revealed a well circumscribed heterogenous lesion with necrotic center and relatively hypointense on T2-weighted imaging. Imaging studies revealed no evidence of intracranial extension and metastatic nests. Biopsy showed grade I primary extracranial with low mitotic activity. Total maxillectomy with excision of tumor and adjacent paranasal structures following reconstruction of the orbit and maxilla with tissue patch was done by the maxillofacial surgeon. The biopsy reported fibrous meningioma based on the hematoxylin and eosin section. On immunohistochemistry the tumor cells were positive for vimentin, focally positive for epithelial membrane antigen and CD99 and negative for signal transducer and activator of transcription 6. The mass was removed surgically with reconstruction, and the pathological studies confirmed the diagnosis to be an extracranial meningioma. The present study briefly reviews the current knowledge concerning the diagnosis and treatment of extracranial meningiomas in the head and neck area and offers suggestions for managing extracranial meningiomas in the paranasal sinuses.
CONCLUSION
To conclude, extracranial meningiomas in the paranasal sinuses may be successfully managed by surgical treatment without evident post-surgery complications.

Key Words: Primary extracranial meningioma; Maxillary sinus; Diagnosis; Surgical treatment; Buccal swelling; Case report

Core Tip: Meningiomas are rare benign tumors originating from the meningotheial arachnoid cells that rarely occur in an extracranial location. So herein we present a rare case of extracranial meningioma in the maxillary sinus of a 54-year-old female presenting with right buccal swelling. Headache was the only symptom. This case mainly focuses on the diagnosis and surgical management with reduced post-surgical complication and provides an insight and surgical guideline in treatment planning.

INTRODUCTION
Meningiomas are one of the largest groups of brain tumors. They come in two forms: intracranial and extracranial. The extracranial location is very rare. Approximately 6%-17% of all meningiomas can be found in extracranial regions[1]. Male patients are more likely to have extracranial meningiomas[2]. We describe a rare case of primary extracranial meningioma of the maxillary sinus in a 54-year-old female patient presenting as buccal swelling and headache. Regardless of the grade, the recommended treatment is complete surgical excision if possible; we used a combined surgical approach to achieve complete excision of the lesion. The clinical, histological and immunohistochemical features are described. The possible histogenesis and the differential diagnosis are also discussed. Subsequently, we reviewed the literature on this respect.

CASE PRESENTATION
Chief complaints
A 54-year-old female patient presented with right buccal swelling for 2 years and headache for 2 mo to the Department of Head and Neck Oncology Surgery, West China College of Stomatology, Sichuan University.

History of present illness
The patient visited a local hospital and started anti-inflammatory and analgesic drugs as they considered the symptoms to be caused by cold and toothache, but the pain did not improve significantly. The patient again visited Guanyuan People’s Hospital seeking further treatment. The biopsy taken showed the spindle cell tumor in the right maxillary sinus, which was further examined by immunohistochemistry. The patient denied any shortness of breath, nausea, dysphagia, hoarseness, loss of consciousness and any neurological or constitutional symptoms at any time.

History of past illness
The patient had no previous medical history.
Personal and family history
She was a non-smoker with no specific family history.

Physical examination
On extra-oral examination the mass located in the right face was non-tender, fixed and non-pulsatile and sessile (Figure 1A). The patient did not have any palpable lymph nodes or associated neck masses. Upon intra-oral examination, an obvious buccal swelling covered with slightly red oral mucosa was present. The majority of the mass was located in the right maxillary sinus and involved the base of the maxilla.

Laboratory examinations
The patient underwent biopsy of the mass using gingival incision extending as far as the upper first molar teeth under local anesthesia. Biopsy reported a grade I primary extracranial meningioma with low mitotic activity. Hematological examinations were within normal limits.

Imaging examinations
Magnetic resonance imaging revealed a well-circumscribed heterogenous lesion with a necrotic center and was relatively hypointense on T2-weighted imaging (Figure 1B and C).

Computed tomography (CT) demonstrated the presence of a large, well-defined soft tissue mass measuring about 7 cm × 6 cm × 6 cm occupying the entirety of the right maxillary sinus, affecting nearby sphenoid and ethmoid sinuses, without affecting the dura mater or endocrinal structures. The surrounding structures were compressed by the mass, and the mass extended from the roof of the oral cavity into the skull base. CT on bone window setting showed an expansive mass with a high density area in the right maxillary sinus. The anterior and lateral walls of the maxillary sinus were thinned and destructed by the expanding mass, with erosion of the wall of the right maxillary sinus as well as orbital floor. (Figure 1D and E). Imaging study based on comprehensive detection of the lesion revealed that there was no evidence of intracranial extension and metastatic nests.

FINAL DIAGNOSIS
Right maxillary meningioma.

TREATMENT
As intracranial invasive meningioma was excluded, the surgery was decided to be performed by the Oral and Maxillofacial Surgeons. Total maxillectomy together with the excision of the tumor and the adjacent paranasal structures, following reconstruction of the orbit and maxilla with tissue patch was performed.

On June 6, 2016, under general anesthesia “extended resection of right maxillary meningioma; right maxillary extended resection; inferior turbinate partial resection; middle turbinate partial resection; right-sided canal neurotomy; right trigeminal peripheral branch transection; A1 extraction; A1-A7 gingival flap; and free skin patch repair” was performed.

Frozen pathology showed spindle cell tumor with extensive necrosis in the right maxilla, which was confirmed by extensive biopsy and immunohistochemical staining. The operation lasted for 2 h and 5 min. The blood loss was 650 mL, and the fluid infused was 2600 mL during the operation. After the operation, the patient returned to the intensive care unit. The vital signs of the patient were observed. Ceftriaxone 2.0g IV BD for 4 d was used to treat infection and prevent intracranial infection. Postoperative nutritional support and antitumor therapy were used.

The entire tumor specimen was submitted for histology, and fresh tissue was fixed in formaldehyde solution for ultrastructural analysis. Histologically, the specimen consisted of epithelioid lobulated tissue, separated by abundant collagen fibers (Figure 2A and B). Image analysis at high magnification showed a thick fibrous capsule and was composed of interwoven fascicles of spindle-shaped meningiocytes and collagen fibers that were arranged into lobules. The tumor cells had abundant cytoplasm and indistinct cytoplasmic borders, arranged in whorled and lobulated patterns. There was osteoid formation in the tumor. No cytologic atypia or necrosis
Figure 1 Characterization of imaging studies and gross finding. A: Facial swelling measured about 4 cm in diameter on right side; B: Magnetic resonance imaging (sagittal section) demonstrated a soft tissue mass with a necrotic center compressing adjacent structures; C: Magnetic resonance imaging (coronal view) demonstrated a soft tissue mass with a necrotic center compressing the right maxilla; D and E: Computed tomography imaging demonstrated a soft tissue mass with a necrotic center compressing adjacent structures, red arrow showing the mass compressing the anterior wall of the right maxilla; F: The mass appeared to be lobulated and yellow-white measuring about 8 cm in diameter; G: On hemisection, the mass showed a well-circumscribed heterogenous lesion with a necrotic center.

were discovered, but some mitoses were present. The specimen showed abundant cytoplasm and indistinct cytoplasmic borders, arranged in whorled and lobulated patterns (Figure 2C and D). Based on the hematoxylin and eosin sections, the lesion was diagnosed as a fibrous meningioma.

Immunohistochemically, the tumor cells were strongly positive for vimentin (Figure 3A), focally positive for epithelial membrane antigen (Figure 3B) and CD99 (Figure 3C). The cells showed negative staining for signal transducer and activator of transcription 6 (Figure 3D) and CD34 (Figure 3E). The MIB-1 (Ki-67) labeling index was 15% (Figure 3F), i.e. focally positive.

OUTCOME AND FOLLOW-UP

The patient was discharged with the following advice: perform mouth opening exercises; fabricate lumbar appendage in Prosthodontic Department a month later; radiotherapy should be done; proper nutritional support, proper oral hygiene and proper wound care; avoid spicy, acidic and irritating foods; and review after discharge for 1 mo and follow-up for discomfort.

DISCUSSION

Meningiomas can exist as intracranial or extracranial brain tumors and are benign, slow-growing tumors. The extracranial location accounts for 2% of all these tumors[3] and found most often in male patients and in young individuals[4]. Due to their unusual symptoms and lack of prevalence, primary extracranial meningiomas are often misdiagnosed[5]. Fortunately, 80% of extracranial tumors are benign[6]. Cases of extracranial meningioma of the sinonasal tract[7], retromolar area[8], eyebrows[9], pelvis[10], etc. have also been reported. Some of the published reports of extracranial meningiomas are listed in Table 1. Histologically, primary extracranial meningiomas do not differ from intracranial, and most of these tumors are sporadic with unclear
Table 1 Published case reports of primary extracranial meningioma

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Year of publication</th>
<th>Site of primary extracranial meningioma</th>
<th>Diagnostic tests</th>
<th>Histology</th>
<th>Treatment performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maharjan et al[15]</td>
<td>2018</td>
<td>Nasal cavity</td>
<td>Contrast-enhanced CT of the nose and paranasal sinuses</td>
<td>WHO grade II atypical transitional meningioma</td>
<td>Endoscopic excision of the mass</td>
</tr>
<tr>
<td>Kim et al [18]</td>
<td>2018</td>
<td>Forehead</td>
<td>CT scan</td>
<td>Lobular architecture composed of tumor cells with eosinophilic cytoplasm and indistinct cell border</td>
<td>Excisional biopsy under local anesthesia</td>
</tr>
<tr>
<td>El-Daly et al [1]</td>
<td>1997</td>
<td>Maxillary antrum</td>
<td>CT scan</td>
<td>Interlacing bundles of bland-appearing spindle cells associated with calcific deposit</td>
<td>Medial maxillectomy with complete removal of the tumor</td>
</tr>
<tr>
<td>Ho et al[3]</td>
<td>1980</td>
<td>Right nasal cavity</td>
<td>Sinus x-ray and CT</td>
<td>Clearly demarcated meningioma with fibrous capsule and well-preserved pseudostratified respiratory epithelium</td>
<td>Ablation of the right frontal sinus, external ethmoidectomy and excision of the right middle turbinate</td>
</tr>
<tr>
<td>Nur et al[12]</td>
<td>2006</td>
<td>Right pelvic cavity</td>
<td>Pelvic sonogram</td>
<td>Lobulated pattern composed of solid sheets of tumor cells separated by connective tissue septae</td>
<td>Exploratory laparotomy with optimal debulking of the pelvic tumor</td>
</tr>
<tr>
<td>Allsoul et al [11]</td>
<td>2015</td>
<td>Right side neck mass</td>
<td>CT and MRI</td>
<td>Meningothelial cells with intranuclear inclusion and multiple psammoma bodies</td>
<td>Partial excision of the mass</td>
</tr>
<tr>
<td>Takeshima et al[9]</td>
<td>2004</td>
<td>Right ovary</td>
<td>Abdominal CT</td>
<td>Mature cerebral tissue was also noted. Melanocytes with black pigment were scattered in the peripheral region of the brain tissue</td>
<td>Right salpingo-oophorectomy</td>
</tr>
<tr>
<td>Lingen et al [9]</td>
<td>1995</td>
<td>Right maxillary sinus</td>
<td>CT</td>
<td>Bundles of ovoid and spindle-shaped cells arranged in broad bands</td>
<td>Total maxillectomy</td>
</tr>
<tr>
<td>Rege et al [16]</td>
<td>2017</td>
<td>Right retromolar area</td>
<td>CBCT</td>
<td>Spindle cell neoplasm, without evidence of atypia, whorls suggesting meningothelial origin</td>
<td>Partial resection of the mandible and reconstruction with autogenous iliac tricortical bone</td>
</tr>
<tr>
<td>Lee et al[17]</td>
<td>2017</td>
<td>Left eyebrow</td>
<td>CT</td>
<td>Tumor cells arranged in sheets or whorls, with occasional psammomabodies</td>
<td>Surgical excision</td>
</tr>
<tr>
<td>This Study (Present case)</td>
<td></td>
<td>Maxillary sinus</td>
<td>CT and MRI</td>
<td>Epithelioid lobulated tissue, separated by abundant collagen fibers</td>
<td>Total maxillectomy with excision of tumor</td>
</tr>
</tbody>
</table>

MRI: Magnetic resonance imaging; CT: Computed tomography; WHO: World Health Organization; CBCT: Cone-beam computed tomography.

etiology[11]. Primary extracranial meningiomas have been considered as arising independently from cranial nerve sheaths or from extracranial embryonic rests of arachnoid cells and as extracranial metastases of a primary intracranial meningioma, but their origin has not been completely established[12].

The present case shows the clinical and imaging aspects of extracranial meningioma of the maxillary sinus in an elderly lady. Primary extracranial meningioma of the paranasal sinuses is rare[13]. In general, the most common signs and symptoms of paranasal sinus meningiomas may mimic cases of sinusitis with nasal obstruction, anosmia, facial pressure or pain, epistaxis and rhinorrhea[14,15]. Meningiomas in the extracranial space often present with nonspecific symptoms until the tumor has reached a significant size. This was the case with our patient who had buccal swelling for 2 years, which has been neglected by the patient until the headache started. Clinical examination should be comprehensive because more than 10% of cases may remain asymptomatic even in advanced stages[15]. Imaging studies, especially CT and magnetic resonance imaging scans, have proved to be useful in the diagnosis and management of meningiomas. The differential diagnosis should include a variety of benign and malignant neoplasms such as melanoma, olfactory neuroblastoma, carcinoma, hemangioma, sarcoma and aggressive psammomatoid ossifying fibroma [10,14]. Histology is therefore essential, and the general histologic features and immunohistochemically findings can usually differentiate between these tumors, as extracranial meningioma presents with solid nests of meningothelial cells arranged in sheets or whorls with a fibroadipose background[5,13]. Immunohistochemistry is helpful in confirming the diagnosis; extracranial meningiomas tend to show strong positivity towards vimentin and epithelial membrane antigen, as indeed occurred in
our patient, and are focally positive for CD99 and Ki-67.

Both CT and magnetic resonance imaging are essential in preoperative surgical planning. Surgery is the only curative treatment, and surgical excision of the mass should be performed if possible. External beam radiation therapy has been shown to be effective and therefore reserved as a palliative approach[16,17]. In the present study, surgical therapy was determined to be the optimal treatment approach for
several reasons. The various treatment previously performed on the current patient did not result in an evident recession of the mass. Without surgical intervention, a firm mass and unbearable headache would remain.

CONCLUSION

The present study reports successful surgical treatment of a patient with a rare primary extracranial meningioma in the maxillary sinus. The present study demonstrated that imaging studies can aid in the diagnosis and biopsy and is useful to specify diagnosis. Surgical treatment is a viable option for the successful management of extracranial meningiomas in the maxillary sinus, and complete postoperative care often requires a multidisciplinary approach.

ACKNOWLEDGEMENTS

The authors thank Dr. Aladimi MT from the West China school of Stomatology, Sichuan University (Chengdu, China) for his kind help in the manuscript preparation and for certain important suggestions for the present manuscript.

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Pulmonary amyloidosis and multiple myeloma mimicking lymphoma in a patient with Sjogren’s syndrome: A case report

Joa Kim, Yun Sung Kim, Hee Jeong Lee, Sang Gon Park

Abstract

BACKGROUND
Sjogren’s syndrome (SS), which affect salivary gland function, is an autoimmune disease. SS may involve extraglandular organs. Approximately 10 to 20 percent of SS patients have clinically significant lung disease, but presentation of pulmonary amyloidosis is extremely rare. The incidence of benign monoclonal gammopathy in SS patients is high, but multiple myeloma is rare. No case involving the simultaneous occurrence of two rare diseases, pulmonary amyloidosis and multiple myeloma, in the same patient with SS has been reported so far.

CASE SUMMARY
A 41-year-old male patient was referred to our hematology department due to incidentally detected gastric plasmacytoma. He had been diagnosed with SS four years earlier. Multiple miliary nodules, ground glass opacity in both lung fields, and enlargement of both inguinal lymph nodes was observed on chest and abdomen computer tomography. Based on the pathological findings of lung and lymph node biopsied specimens, the patient was diagnosed with pulmonary amyloidosis and multiple myeloma. Pulmonary amyloidosis and multiple myeloma associated with SS has rarely been reported.

CONCLUSION
This is an extremely rare case of simultaneous pulmonary amyloidosis and multiple myeloma in the same patient with SS.

Key Words: Case report; Sjogren’s syndrome; Amyloidosis; Multiple myeloma; Plasma-
DOI: https://dx.doi.org/10.12998/wjcc.v10.i3.1016

INTRODUCTION

Primary Sjogren’s syndrome (SS) is a chronic systemic autoimmune disease characterized by decreased organ function by lymphocyte infiltration, not only exocrine gland but also extraglandular organ[1]. Major B cell activation is the main pathogenesis of SS and continuous activation of B cells can lead to clonal proliferation and malignant lymphoma[2-4]. Clonal proliferation of B cells may lead to both monoclonal gammopathy of undetermined significance, a benign monoclonal gammopathy, and multiple myeloma (MM), a hematologic malignancy[5-9]. However, in practice, multiple myeloma is very rare[6,7,10]. Amyloidosis is a heterogeneous group of disorders characterized by deposits in the extracellular matrix of abnormal protein material. The incidence of amyloidosis is low and that of amyloidosis with involvement of only the respiratory system is extremely low[11,12]. In particular, amyloidosis is a rare cause of pulmonary infiltration in SS[13]. Simultaneous occurrence of pulmonary amyloidosis and multiple myeloma associated with SS is extremely rare.

CASE PRESENTATION

Chief complaints

A 46-year-old male patient was admitted for treatment of an incidental gastric plasmacytoma.

History of present illness

The patient was checked by a positron emission tomography (PET) scan due to enlargement of both inguinal lymph nodes more than two months ago. The PET scan revealed hypermetabolic activity in the gastric body, both inguinal lymph nodes (Figure 1A). A gastroscopic biopsy of the stomach lesion was performed and diagnosed with plasmacytoma (Figure 2).

History of past illness

The patient had not been diagnosed with any diseases.

Personal and family history

The patient had been diagnosed with SS four years ago due to dry eyes and dry mouth, and his symptoms improved after treatment with methotrexate, hydroxychloroquine. However, prednisolone was added two months ago due to swelling of the parotid glands and enlargement of lymph nodes.
Figure 1 The positron emission tomography scan, chest computed tomography, and abdominal computed tomography. A and B: Diffuse hypermetabolic activity in the gastric body and mild increased metabolic activity in both inguinal areas found on an axial view of the positron emission tomography scan; C: In the axial view of the chest computed tomography (CT), ground glass opacities and centrilobular nodules were found in both lung fields; D: Abdominal CT showing multiple enlarged lymph nodes in the bilateral inguinal area.

Figure 2 A gastroscopic biopsy of the stomach lesion. A: In mid power field, dense, monotonous infiltrate of plasma cells in lamina propria are noted; B: In high power field, plasma cells vary from mature to binucleated (black arrow); C-E: Plasma cells show immunoreactivity to CD138 and a Kappa restricted monoclonal pattern (kappa: lambda ≥ 20:1) which is consistent with plasmacytoma.

**Physical examination**
A physical examination revealed painless lymph nodes of variable size in both inguinal areas and both neck areas.

**Laboratory examinations**
The complete blood count results, with normal ranges in parentheses, were as follows: white blood cells, $5160 \times 10^3/\mu\text{L}$ ($4.0-10.0 \times 10^3/\mu\text{L}$); hemoglobin, $13.3 \text{ g/dL}$ (12-16
Kim J et al. Pulmonary amyloidosis and MM in SS

Blood biochemistry results were as follows: total protein 9.03 g/dL (5.3-7.4 g/dL); albumin 3.74 g/dL (3.5-5.2 g/dL); A/G ratio 0.71 (1.0-2.0); C-reactive protein, 0.49 mg/dL (0-0.3 mg/dL). The results of serum protein electrophoresis showed an increase in the total protein amount, a decrease of albumin, and a slightly sharp increase of gamma-globulin. The results of serum immunofixation electrophoresis revealed oligoclonal gammopathy; multiple dense bands were observed in IgG, IgA, kappa, and lambda antisera. The results of Ig quantification showed IgG 2540 mg/dL (700-1600); IgM 18.3 mg/dL (40-230 mg/dL); IgA 1220 mg/dL (70-400 mg/dL). B2-microglobulin level was increased to 2.8 mg/L (0.0-2.4 mg/L). The results of a serum free light chain assay revealed increased kappa light chain (420.60 mg/L, normal 3.3-19.40 mg/L) and an increased kappa/lambda light chain ratio (34.9, normal 0.26-1.65). Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, calcium, and creatinine were all within normal limits.

Imaging examinations

The PET-computed tomography (CT) revealed hypermetabolic activity in the gastric body, a mild increase in metabolic activity in both inguinal areas, and ground glass opacities (GGO) and centrilobular nodules in both lung fields (Figure 1A). A chest CT showed patch GGO and multiple nodules in both lung fields (Figure 1C) and an abdominal CT showed multiple enlarged lymph nodes in the bilateral inguinal area (Figure 1D).

FURTHER DIAGNOSTIC WORK-UP

An excisional lymph node biopsy was performed for differential diagnosis of pulmonary tuberculosis with tuberculous lymphadenitis from lymphoma. Biopsy of the right inguinal lymph node revealed diffuse infiltration of plasma cells, which were positive for CD 138, Kappa, and lambda chain immunohistochemical staining (Figure 3). The results of a bone marrow biopsy showed that plasma cells were increased to 34% and immunostain of IgG, IgA, kappa, and lambda was positive.

A lung biopsy was also performed to rule out pulmonary tuberculosis, interstitial lung disease, or lung involvement of multiple myeloma. Lung biopsy revealed chronic inflammation showing multinucleated giant cells and deposition of amorphous proteinaceous material (Figure 4A). Infiltration of lymphoplasmacytes was observed in the interstitium and perivascular area (Figure 4B). In addition, Congo-red stain for amorphous proteinaceous material revealed apple green birefringence under a polarized microscope. Mycobacterium tuberculosis nested polymerase chain reaction to exclude pulmonary tuberculosis was negative.

FINAL DIAGNOSIS

We diagnosed the patient with pulmonary amyloidosis and multiple myeloma involving extramedullary organs (stomach, lymph nodes) associated with SS.

TREATMENT

Systemic chemotherapy was planned for the multiple myeloma, however the patient was transferred to a larger hospital.

OUTCOME AND FOLLOW-UP

The patient was transferred to another hospital and underwent chemotherapy. He is on regular follow up for SS to our rheumatology department and is on medication.

DISCUSSION

Although SS is typically known as an autoimmune disorder, it can also present as...
Amyloidosis is a disease group; certain proteins such as amyloid fibrils are deposited in extracellular tissue. Amyloidosis can be classified as primary and secondary. Primary amyloidosis is caused by clonal plasma cell proliferation which appears in monoclonal gammopathy or myeloma and the associated abnormal lymphoproliferative disease. The form of lymphoproliferative disease can present as polyclonal lymphocytic infiltration of the salivary glands, oligo- or monoclonal B cell proliferation resulting in clonally derived lymphoproliferative disorders such as monoclonal gammopathy, light-chain amyloidosis, and malignant lymphoma[3,4,6-9]. SS often involves interstitial lung disease, sometimes primary pulmonary lymphoma, pleuritis, but occurrence of pulmonary amyloidosis is very rare[14].
proteins consist of fragments of immunoglobulin light chain such as kappa and lambda (light chain amyloidosis)\[15\]. Secondary amyloidosis is the reactive systemic amyloidosis usually associated with chronic inflammatory diseases or neoplasms and the associated fibrils are composed of fragments of the acute phase reactant serum amyloid A (reactive amyloidosis)\[15\]. These two types are the most common form of amyloidosis. Amyloid deposition is rare in SS patients, but when it does occur, multiple organs are affected, such as skin, kidney, breast, tongue, and lymph nodes, as well as the lung\[16-21\]. However, only about 50 cases related to primary SS and pulmonary amyloidosis have been reported so far\[22\]. Amyloidosis of the lungs has three different clinicopathologic forms: diffuse alveolar-septal amyloidosis (diffuse parenchymal amyloidosis), nodular pulmonary amyloidosis (nodular parenchymal amyloidosis), and tracheobronchial amyloidosis\[23\]. Diffuse alveolar-septal amyloidosis is characterized by amyloid deposition in the alveolar septa and vessel walls, and transbrachial amyloidosis is characterized by amyloid deposition in various segments of the tracheobronchial tree\[23\]. Nodular pulmonary amyloidosis can be defined as one or more tumor-like amyloid deposits involving the lungs\[23\]. Pathologically, well circumscribed nodules with homogeneous and dense eosinophilic material, lymphocytes and plasma cells are generally found within or nearby nodules \[23\]. Other pathologic findings that may appear include foreign body giant cells, calcifications, and bony or cartilaginous areas\[23\]. Lung biopsy of our patient revealed chronic inflammatory nodules showing multinucleated giant cells and deposition of amorphous proteinaceous material (Figure 4A). Infiltration of lymphoplasmacytomas was observed in the interstitium and perivascular area (Figure 4B). Congo-red stain for amorphous proteinaceous material revealed apple green birefringence under a polarized microscope.

In MM, monoclonal immunoglobulin is produced by plasma cell neoplastic proliferation of plasma cells. There are two categories of MM, according to diagnostic criteria for plasma cell proliferative disorders. The first is asymptomatic multiple myeloma (smoldering multiple myeloma), which is serum monoclonal protein more than 3 g/dL and/or clonal bone marrow plasma cells more than 10% but absence of end-organ damage\[5\]. Symptomatic multiple myeloma satisfies all of the following: more than 10% clonal bone marrow plasma cells and presence of serum and/or urinary monoclonal protein and evidence of end-organ damage\[5\]. This patient had increased serum protein IgG, IgA, and Kappa light chain, and plasma cells in the bone marrow were increased to 34%. Although there was no end-organ damage such as anemia, renal insufficiency, hypercalcemia, and bone lesions, plasmacytomas were observed in multiple lymph nodes and stomach as end-organ involvement. This patient was expressed as the type of extramedullary plasmacytoma in multiple myeloma.

Plasmacytomas, tumors composed of plasma cells, are histologically identical to those observed in MM\[24\]. Those that occur solely in the bone are designated solitary plasmacytoma of bone\[25\]. Those that arise outside bone in soft tissues are called solitary extramedullary plasmacytoma (EMP)\[25\]. EMP can arise anywhere in the body. The incidence of extramedullary disease with newly diagnosed MM is variable, ranging from 7%-13%\[26\]. EMP arise most commonly from direct extension of primary bone tumors, but rarely they may also result from hematogenous spread involving distant organs\[26\].

The incidence of benign monoclonal gammopathy in SS patients is relatively high and a prevalence of monoclonal gammopathy in primary SS patients of 7% to 22% was recently reported\[6-8\], however, the prevalence of MM is very rare\[6,7,10\]. IgG is the most common class associated with MM\[7\]. This report describes a patient with SS associated with IgG and IgA-kappa-type MM.

Amyloidosis and MM associated primary SS is rare but can occur respectively. However, no cases of simultaneous occurrence of MM and amyloidosis in patients with SS have been reported. Of course, there is a limitation which cannot rule out the occurrence of pulmonary amyloidosis caused by MM. However, abnormal proteins of pulmonary amyloidosis caused by MM consist of fragments of immunoglobulin light chains such as kappa and lambda. Pulmonary amyloidosis was diagnosed in our patient’s lung biopsy, but immunohistochemistry of kappa and lambda was negative. Therefore, we report a rare case diagnosed simultaneously with pulmonary amyloidosis and multiple myeloma associated with SS.
CONCLUSION

SS is a chronic inflammatory disease characterized by decreased organ function by lymphocyte infiltration, not only exocrine gland but also extraglandular organ. Interstitial lung disease is the most common pulmonary abnormality in primary SS, but pulmonary amyloidosis is rare. Monoclonal gammopathy can also occur, but progression to MM is rare in primary SS. To the best of our knowledge, simultaneous development of MM and pulmonary amyloidosis in primary SS patients has not been reported so far. Herein, we report the extremely rare case of pulmonary amyloidosis and multiple myeloma associated with SS.

REFERENCES


Concomitant Othello syndrome and impulse control disorders in a patient with Parkinson’s disease: A case report

Tian Xu, Zhao-Sheng Li, Wei Fang, Lan-Xiao Cao, Guo-Hua Zhao

BACKGROUND

Othello syndrome (OS) is characterized by delusional beliefs concerning the infidelity of a spouse or sexual partner, which may lead to extreme behaviors. Impulse control disorders refer to behaviors involving repetitive, excessive, and compulsive activities driven by an intense desire. Both OS and impulse control disorders in Parkinson’s disease (PD) may be side effects of dopamine agonists. At present, there are only a few case reports and studies related to PD with concomitant OS and impulse control disorders.

CASE SUMMARY

We describe a 70-year-old male patient with PD, OS, and impulse control disorders, who presented with a six-month history of the delusional belief that his wife was having an affair with someone. He began to show an obvious increase in libido presenting as frequent masturbation. He had been diagnosed with PD ten years earlier and had no past psychiatric history. In his fourth year of PD, he engaged in binge eating, which lasted approximately one year. Both OS and hypersexuality were alleviated substantially after a reduction of his pramipexole dosage and a prescription of quetiapine.

CONCLUSION

Given its potential for severe consequences, OS should be identified early, especially in patients undergoing treatment with dopamine agonists.

Key Words: Othello syndrome; Delusional jealousy; Impulse control disorders; Hypersexuality; Parkinson’s disease; Case report

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A 70-year-old right-handed man, who showed the first signs of PD at 60 years of age, was admitted to our hospital for behavioral alterations. He presented with a six-month history of a delusional belief that his wife was having an affair with someone. At the same time, he began to show an obvious increase in libido presenting as frequent masturbation.

History of present illness
The patient noticed he had a right-hand tremor ten years ago and then developed akinesia and rigidity. He was diagnosed with PD by a neurologist approximately one year later. Following treatment with levodopa-benserazide (200-50 mg/d), he initially showed significant improvement. His symptoms then progressed to difficulty turning during a walk, constipation, olfactory dysfunction, and vivid anxiety-provoking dreams. Seven years ago, the patient engaged in binge eating with a significant increase in food consumption, eating 3-4 times at night, and the symptoms lasted approximately one year. Six years ago, pramipexole (0.75 mg/d) and selegiline (1 mg/d) were prescribed. His pramipexole dosage was titrated up to 1.5 mg/d. In the past year, his memory has declined, especially his recent memory. About six months ago, he began to accuse his wife of having an affair with someone, although he could not provide any evidence of infidelity. The delusional belief was confirmed by his wife.
and two children. At the same time, he began to show an obvious increase in libido presenting as frequent masturbation. Three months before his current admission to the hospital, he developed visual hallucinations of seeing ghosts in the window. This visual hallucination was so vivid that he often asked family members to exorcise the ghosts.

**History of past illness**
The patient was otherwise healthy. He denied a history of hypertension, diabetes mellitus, prior cerebrovascular disease, or other neurological complications. He had no past psychiatric history.

**Personal and family history**
The patient had a college diploma and was retired from the Municipal People’s Procuratorate. He denied a past history of drug or alcohol abuse, smoking, and sexual promiscuity. One of his five siblings had PD, but there was no family history of psychiatric illness. The patient is married and has two children who are living independently.

**Physical examination**
The patient’s general examination was unremarkable. The neurologic examination revealed a masked-like facial expression. The motor examination revealed moderate bradykinesia and rigidity of all four limbs. Mild resting tremor was present in the patient’s right upper extremity, and he exhibited difficulty in the initiation of walking and turning. A reduced arm swing was observed when walking, and his performance on the pull-back test was negative. No other positive neurological signs were found. The patient’s scores on the rating scales were as follows: 29 on Part III of the Unified PD Rating Scale, stage II on the Hoehn and Yahr scale, 9/30 on the Mini-Mental State Examination, 13/30 on the Montreal Cognitive Assessment Scale, 11 on the Hamilton Depression Rating Scale, and 11 on the Hamilton Anxiety Rating Scale.

**Laboratory examinations**
The following laboratory tests were within normal limits: blood cell count, liver and renal function, thyroid function, electrolytes, vitamin B12, folate, syphilis, and tumor markers.

**Imaging examinations**
Magnetic resonance imaging (MRI) showed mild bilateral frontotemporal atrophy (Figure 1). The T1, T2 and FLAIR sequence showed temporal atrophy, with broadening of the posterior horn of the lateral ventricle (Figure 1A-C); and the magnetic resonance sagittal view showed mild frontal lobe atrophy (Figure 1D).

**FINAL DIAGNOSIS**
PD with OS, ICDs, and dementia were diagnosed based on the patient’s symptoms and findings from the neurologic examination.

**TREATMENT**
The patient’s dose of pramipexole was reduced to 50% of the current dosage, and quetiapine 25 mg/d was prescribed. Entacapone was added to alleviate the worsening of his motor symptoms.

**OUTCOME AND FOLLOW-UP**
The patient’s symptoms showed marked improvement at the follow-up visit two months later, the delusion concerning his wife’s infidelity subsided and his motor syndrome remained stable.
Xu T et al. PD with OS and ICDs

Figure 1 Magnetic resonance imaging shows mild bilateral frontotemporal atrophy. A-C: The T1, T2 and FLAIR sequence showed temporal atrophy, with broadening of the posterior horn of the lateral ventricle; D: Magnetic resonance sagittal view showed mild frontal lobe atrophy.

DISCUSSION

Although PD is a common degenerative neurological disorder with typical motor symptoms, its non-motor symptoms have received increasing attention. The most recent update on treatments for non-motor symptoms of PD authored by the Evidence-Based Medicine Committee of the International Parkinson and Movement Disorder Society includes ICDs[7], whereas OS, which has a relatively lower prevalence, is reported less often. In a cross-sectional study of ICDs and OS[6] in 1063 PD patients, 81 of them presented with ICDs (7.61%) and 23 presented with OS (2.16%), while 9 patients presented with both OS and ICDs. A diagnosis of OS is infrequent in PD patients, but its occurrence may have severe consequences. Here, we report the case of a 70-year-old male PD patient with concomitant OS and ICDs, who had a good response to a reduction of his pramipexole dosage and the addition of quetiapine to his medication regimen.

OS in PD is reported infrequently; thus, we conducted a search of the English-language research literature from 2000-2021 in the MEDLINE database (https://www.ncbi.nlm.nih.gov), using the following keywords: Othello syndrome, delusional jealousy, delusions, jealousy and PD. The search yielded one case report[8], two case series[9,10], and four studies[6,11-13], which we reviewed in addition to our case report. The characteristics of a total of 28 patients who had PD with concomitant OS and ICDs are presented in Table 1.

Concomitant OS and ICDs were more common in males (24 patients) and in middle-aged patients. In a retrospective case series study in the Mayo Clinic, 61.9% (65/105) of the patients were male[3]. Similar results were found in studies on PD with ICDs. A prospective multi-center study found that PD patients with ICDs were more likely to develop in males, younger patients, and patients with an earlier onset of PD[14]. The average age of PD onset was 47.00 ± 8.63 years, and only two patients who developed OS were older than 70 years. The mean duration of PD at OS onset was 7.04 ± 3.99 years, which was similar to the previous study at the Mayo Clinic[11].
## Table 1 Clinical characteristics of 28 Parkinson’s disease patients with concomitant Othello syndrome and impulse control disorders

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Patient</th>
<th>Sex</th>
<th>Age at PD onset</th>
<th>Age at OS onset</th>
<th>PD duration at OS onset</th>
<th>ICDs</th>
<th>Dopamine agonist</th>
<th>Visual hallucinations</th>
<th>Psychiatry history</th>
<th>Neuroimaging</th>
<th>Dementia</th>
<th>Measures undertaken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poletti et al[6]</td>
<td>Patient 1</td>
<td>M</td>
<td>45</td>
<td>60</td>
<td>15</td>
<td>PG + HS</td>
<td>Pramipexole</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
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<td>Patient 2</td>
<td>M</td>
<td>50</td>
<td>62</td>
<td>12</td>
<td>HS + PG</td>
<td>Ropinirole</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Poletti et al[6]</td>
<td>Patient 3</td>
<td>M</td>
<td>67</td>
<td>76</td>
<td>9</td>
<td>HS</td>
<td>Pramipexole</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Poletti et al[6]</td>
<td>Patient 4</td>
<td>M</td>
<td>42</td>
<td>52</td>
<td>10</td>
<td>HS</td>
<td>Ropinirole</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Poletti et al[6]</td>
<td>Patient 5</td>
<td>M</td>
<td>34</td>
<td>40</td>
<td>6</td>
<td>HS + PG</td>
<td>Pramipexole</td>
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<td>57</td>
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<td>PG + punding + HS</td>
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<td>44</td>
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<td>Anxiety, depression</td>
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<tr>
<td>Adam et al[10]</td>
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<td>38</td>
<td>39</td>
<td>1</td>
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### Table

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<td>60</td>
<td>69</td>
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PD: Parkinson’s disease; OS: Othello syndrome; ICDs: Impulse control disorders; M: Male; F: Female; N/A: Not applicable; PG: Pathological gambling; HS: Hypersexuality; BE: Binge eating; DDS: Dopamine dysregulation syndrome; CS: Compulsive shopping.

Among the ICDs in our review, hypersexuality (HS) was most prevalent (23/28 patients). Pathological gambling was observed in 9 patients, compulsive shopping in 6 patients, binge eating in 2 patients, punding in 2 patients, and dopamine dysregulation syndrome in 2 patients. HS is characterized by excessive sexual thoughts or behaviors or an atypical change from baseline behavior, such as an inappropriate or excessive sexual desire for the partner, compulsive masturbation, or the development of paraphilias[5]. A functional MRI study compared a group of 12 PD patients with HS with a control group with PD without HS or other ICDs[13]. The results showed an increase in sexual desire in PD patients with HS after exposure to sexual cues; and the increased sexual desire correlated with enhanced activation in the ventral striatum, cingulate cortex, and orbitofrontal cortex. The pathophysiology of OS remains unclear.

A previous study showed that OS is associated with the dopaminergic frontostriatal circuits, ventromedial prefrontal cortex, and insula[16]. Overall, both HS and OS were found to be associated with hyperdopaminergic behaviors[13].

Previous studies have shown a relationship between dopamine agonists and OS[9, 17-19]. One of these studies, a cross-sectional prevalence study of 805 consecutive PD patients, revealed a significant association between dopamine agonists and OS (odds ratio, 18.1)[17]. Other dopaminergic medications have been reported to have an association with OS, such as amantadine[20], levodopa[9], and selegiline[21]. In our review, all 28 patients were using dopamine agonists at the onset of OS, consistent with the results of previous studies. Pramipexole and ropinirole were used most frequently by 11 patients, pergolide was used by 3 patients, and cabergoline, piribedil, and rotigotine were each used by 1 patient. The duration of treatment with dopamine agonists at the onset of OS varied from a few months to several years under a stable dose. For example, one of the patients[12] developed OS one month after receiving ropinirole treatment, whereas our patient exhibited characteristics of OS more than five years after receiving pramipexole. In early PD, the dopamine depletion is greatest in the ventrolateral tier of the substantia nigra pars compacta, which projects primarily into the dorsal striatum. Thus, the functioning of the dorsolateral frontostriatal circuit (linking the dorsolateral prefrontal cortex and the dorsal striatum), which mediates executive functions, can be restored by dopaminergic medication[22]. However, dopaminergic medication may cause oversensing of the relatively intact orbital frontostriatal circuit (linking the orbitofrontal cortex and the ventral striatum), which mediates reward processing. Dopamine agonists may induce non-physiological tonic dopaminergic stimulation of the orbital frontostriatal circuit, which can lead to an evaluation of the stimulus as a positive reward, thereby inducing an aberrant salient relationship with a loved one[16], and consequently, a greater fear of losing the relationship, resulting in OS. Furthermore, excessive motivation to achieve sexual
goals may lead to HS.

The concurrent development of OS and ICDs in our review was more common among patients without dementia and with moderate motor deterioration. Two age peaks in the incidence of PD with OS have been reported: The first peak is in young patients with mild motor impairment and a negligible decline in cognition and the second peak occurs in advanced PD patients with severe motor and cognitive decline \[9\]. We believe that OS in our patient was associated with both cognitive impairment and the use of dopamine agonists. In addition to having OS and ICDs, 11 of 16 patients in our review had visual hallucinations, and 6 of 11 patients had a psychiatric history; however, the true prevalence could be much higher. The MRI of most of the patients showed normal findings; only one patient’s MRI showed an old infarct of the right basal ganglia, and another patient’s MRI showed mild left frontotemporal atrophy. In our case, the patient showed mild bilateral frontotemporal atrophy, consistent with dementia.

OS may lead to marital discord and breakdown or have other negative effects. The treatment of OS in patients with PD includes the withdrawal or dosage reduction of dopamine agonists, plus a prescription for atypical antipsychotics at low doses. In 10 of 17 patients in our review, the syndrome was relieved or eliminated with a dosage reduction or withdrawal of the dopamine agonists. Atypical neuroleptics had to be added to 5 patients’ prescriptions: Clozapine for 1 patient and quetiapine for 4 patients. In our case report, it was necessary to use an antipsychotic (quetiapine), which was tolerated quite well. Improvement in our patient’s symptoms was progressive, although slow and gradual. In a case series of 3 young PD patients with OS receiving dopamine agonists, the OS resolved with the withdrawal of the drug and subsequent treatment with bilateral subthalamic nucleus deep brain stimulation (STN DBS)\[11\]. In another case report, psychotic symptoms in the form of OS appeared after undergoing bilateral STN DBS, and a gradual resolution was achieved by adding a low dosage of quetiapine\[23\].

CONCLUSION

Both OS and ICDs in PD may be side effects of dopamine agonist therapy. There is a frequent association between OS and ICDs; thus, when the features of either syndrome appear, the features of the other syndrome should be investigated. Clinicians should be aware of OS in patients with PD so they can identify it early, especially in patients treated with dopamine agonists, to help them avoid the devastating psychosocial consequences of this syndrome. PD patients may consider them unrelated to dopamine replacement therapies and even conceal the syndrome to their physician, resulting in challenging and late diagnoses. Patients and their partners should be warned about this uncommon but consequential syndrome. Withdrawal or reduction of dopamine agonists, plus prescriptions of atypical antipsychotics, can usually alleviate symptoms of the syndrome.

ACKNOWLEDGEMENTS

We are grateful to the patient for giving us his permission to submit this paper for publication.

REFERENCES


Multiple endocrine neoplasia type 1 combined with thyroid neoplasm: A case report and review of literatures

Jia-Lu Xu, Su Dong, Le-Le Sun, Jin-Xin Zhu, Jia Liu

Abstract

BACKGROUND
Multiple endocrine neoplasia type 1 (MEN1) is a rare hereditary tumor syndrome inherited in an autosomal dominant manner and presents mostly as parathyroid, endocrine pancreas (such as gastrinoma) and anterior pituitary tumors. At present, papillary thyroid carcinoma (PTC) and nodular goiter are not regarded as components of MEN1.

CASE SUMMARY
A 35-year-old woman presented with MEN1 accompanied by cointaneous PTC and nodular goiter. The pathological diagnosis was PTC with cervical lymph node metastasis, nodular goiter, parathyroid cyst and adenomatoid hyperplasia. Genetic testing was performed and a MEN1 gene mutation was detected. The patient underwent unilateral lobectomy of the thyroid gland and surgical removal of the parathyroid tumors. At 18 mo of follow-up, ultrasonic examination of the neck showed no abnormality. Serum calcium and parathyroid hormone levels were normal. No new MEN1-associated tumors were detected.

CONCLUSION
The role of inactivating mutations of MEN1 gene in tumorigenesis of PTC and/or nodular goiter remains to be determined by more case reports and further research.

Key Words: Multiple endocrine neoplasia type 1; Thyroid cancer; Papillary thyroid carcinoma; Nodular goiter; Case report
Multiple endocrine neoplasia type 1 (MEN1) is a rare hereditary tumor syndrome inherited in an autosomal dominant manner and characterized by a predisposition to a multitude of endocrine neoplasms, mostly of the parathyroid, endocrine pancreas and anterior pituitary tumors. Other endocrine tumors in MEN1 include gastroenteropancreatic neuroendocrine tumors, adrenocortical tumors, and rarely pheochromocytoma and medullary thyroid carcinoma (MTC). MEN1 is caused by inactivating mutations of \textit{MEN1} gene. The incidence is 1/10000–1/100000. \textit{MEN1} is a tumor suppressor gene and is located on human chromosome 11q13. The \textit{MEN1} gene is 9 kb and contains 10 exons. Exons 2–10 are coding regions. \textit{MEN1} gene encodes the protein menin, which contains 610 amino acids. Menin plays an important role in cell division and proliferation, cell cycle regulation and genomic stability. Mutations of \textit{MEN1} gene can lead to functional loss of menin, and occurrence of multiple tumors.\textit{MEN1} gene mutation analysis is an important diagnostic method for MEN1.

It has been reported that expression of menin is preserved in human normal thyroid tissue and thyroid tumors, but it can be decreased or absent in certain types of thyroid tumors. Currently, little is known about the prevalence of papillary thyroid carcinoma (PTC) and nodular goiter in MEN1 patients, and it is unclear whether tumorigenesis of these thyroid tumors is \textit{MEN1} related. The role of menin protein deficiency in tumorigenesis of PTC and/or nodular goiter is still controversial. There may be a potential correlation between \textit{MEN1} syndrome and papillary thyroid carcinoma/ nodular goiter.

**Core Tip:** Multiple endocrine neoplasia type 1 (MEN1) is a rare hereditary tumor syndrome inherited in an autosomal dominant manner and presents mostly as parathyroid, endocrine pancreas and anterior pituitary tumors. We here report a case of MEN1 combined with papillary thyroid carcinoma (PTC) and nodular goiter, and review the literature. The role of inactivating mutations of \textit{MEN1} gene in tumorigenesis of PTC and/or nodular goiter is still controversial. There may be a potential correlation between \textit{MEN1} syndrome and papillary thyroid carcinoma/ nodular goiter.

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**INTRODUCTION**

Multiple endocrine neoplasia type 1 (MEN1) is a rare hereditary tumor syndrome inherited in an autosomal dominant manner and characterized by a predisposition to a multitude of endocrine neoplasms, mostly of the parathyroid, endocrine pancreas and anterior pituitary tumors. Other endocrine tumors in MEN1 include gastroenteropancreatic neuroendocrine tumors, adrenocortical tumors, and rarely pheochromocytoma and medullary thyroid carcinoma (MTC). MEN1 is caused by inactivating mutations of \textit{MEN1} gene. The incidence is 1/10000–1/100000. \textit{MEN1} is a tumor suppressor gene and is located on human chromosome 11q13. The \textit{MEN1} gene is 9 kb and contains 10 exons. Exons 2–10 are coding regions. \textit{MEN1} gene encodes the protein menin, which contains 610 amino acids. Menin plays an important role in cell division and proliferation, cell cycle regulation and genomic stability. Mutations of \textit{MEN1} gene can lead to functional loss of menin, and occurrence of multiple tumors.\textit{MEN1} gene mutation analysis is an important diagnostic method for MEN1.

It has been reported that expression of menin is preserved in human normal thyroid tissue and thyroid tumors, but it can be decreased or absent in certain types of thyroid tumors. Currently, little is known about the prevalence of papillary thyroid carcinoma (PTC) and nodular goiter in MEN1 patients, and it is unclear whether tumorigenesis of these thyroid tumors is \textit{MEN1} related. The role of menin protein deficiency in tumorigenesis of PTC and/or nodular goiter is still controversial. Here, we present a patient with \textit{MEN1} accompanied by coincidental PTC and nodular goiter and review the related literature.

**CASE PRESENTATION**

**Chief complaints**

A 35-year-old woman presented with a neck mass on physical examination, but without abnormal feelings.

**History of present illness**

The patient immediately came to our hospital after discovery of the neck mass.

**History of past illness**

The patient underwent partial resection of the pancreas and stomach for pancreatic and gastroduodenal neuroendocrine tumor 4 years ago. The patient had a history of pituitary microadenoma for 2 years, which was not treated but under observation.

**Personal and family history**

Her father had a history of stomach surgery, but the details were unknown since he died 20 years ago. Other relatives of the patient had no symptoms of \textit{MEN1} syndrome.
Physical examination
Her father had a history of stomach surgery, but the details were unknown since he died 20 years ago. Other relatives of the patient had no symptoms of MEN1 syndrome.
There was an anterior neck mass which was movable due to breathing.

Laboratory examinations
The results of biochemical tests were as follows: serum calcium 2.82 mmol/L (reference range: 2.11–2.52 mmol/L); albumin 47.7 g/L (reference range: 40–55 g/L); serum intact parathyroid hormone (iPTH) elevated to 676.3 pg/mL (reference range: 12–88 pg/mL); gastrin 17: 0.8 pmol/L (reference range: 1–15 pmol/L); thyroid function was normal; thyroid peroxidase antibody was 23.98 IU/mL (reference range: < 35 IU/mL).

Imaging examinations
Neck ultrasound revealed a 64 mm × 28 mm × 45 mm cystic mass located below the right lobe of the thyroid gland with a well-defined smooth border, and several solid nodules were detected in the right thyroid lobe, with the largest (18 mm × 10 mm × 8 mm) in the right lower thyroid lobe. The largest nodule in the thyroid had an unclear boundary, dotted calcification and abundant internal blood flow (Figure 1A and B). Computed tomography (CT) or magnetic resonance imaging examination showed changes in the pituitary region; lesions in the right thyroid lobe and superior mediastinum; and changes after partial gastrectomy and in the tail of the pancreas (Figure 2A–D). In 99mTc-methoxyisobutyl isonitrile scintigraphy, tracer uptake was increased in the right lower region of the thyroid gland and mediastinum, and no abnormal retention of the tracer in the late phase was observed. No uptake was detected in other regions. Preoperative sestamibi single-photon emission computed tomography (SPECT)/CT found a lesion in the right lower thyroid lobe and part of which extended to the superior mediastinum (Figure 3A). Bone scanning showed T-scores -2.6 and Z-scores -2.0.

FINAL DIAGNOSIS
The final diagnosis of the presented case was thyroid neoplasm (right lobe).

TREATMENT
Because the patient had a large functional parathyroid cyst, her serum calcium and iPTH levels were significantly abnormal and several solid nodules were detected in the right thyroid lobe (the largest nodule was suspected to be malignant by ultrasound). Fine needle aspiration (FNA) could not be performed for parathyroid cysts. And the patient refused FNA of the thyroid nodule before operation for fear of additional injury and requested to perform rapid intraoperative pathological diagnosis. Parathyroidectomy and unilateral thyroid lobectomy were recommended and performed with the patient’s consent.
During the operation, gross examination of the largest cyst showed that it was partially surrounded and contiguous with the right lower thyroid lobe and extended to the superior mediastinum, and it was peeled off easily from the right lower thyroid lobe and mediastinum. Furthermore, it was filled with clear watery fluid, suggesting that it was a parathyroid cyst (Figure 3B). The largest cyst and right lobe of the thyroid were removed. The central lymph nodes were cleared. Intraoperative frozen section pathology showed that the largest cyst was the source of parathyroid, and parathyroid carcinoma was excluded. A PTC (maximum diameter 1 cm, invading the capsule) was found in the thyroid right lobe. In the central lymph nodes, 4/5 had cancer metastasis. iPTH at 20 min after resection was decreased to 253.4 pg/mL. Other parathyroid glands were explored. Two upper parathyroid glands were normal. The left lower parathyroid was enlarged and removed. Rapid intraoperative pathological examination revealed that the left lower parathyroid had adenomatoid hyperplasia. After another 20 min, serum iPTH decreased to 63.9 pg/mL. The postoperative pathological results were PTC (maximum diameter 1cm, invading the capsule) in the right thyroid lobe and nodular goiter. In the central lymph nodes, 4/5 had PTC metastasis. In the right cervical cysts, parathyroid cysts (monolocular) showed adenomatous hyperplasia. In the left lower parathyroid, adenomatous hyperplasia
Figure 1 Thyroid ultrasonography. A: Solid nodule with multiple punctate microcalcifications and relatively regular shape within the right lobe of the thyroid (white arrow); B: A huge cystic mass with a clear boundary located in the lower right lobe of the thyroid (white arrow).

Figure 2 Computed tomography/magnetic resonance imaging examination. A: A lesion located in the right side of the trachea (white arrow); B: Enlarged pituitary structure (white arrow); C: Remnant stomach anastomosed to the jejunum (white arrow); D: Remnant pancreas body and tail (white arrow).

was observed (Figure 4). The patient had indications for a total thyroidectomy because of central compartment lymph node metastasis. But the patient had a strong desire to preserve the thyroid gland and refused to remove the left lobe.

After surgery, the patient was closely monitored on serum calcium and was pumped calcium gluconate 2.0 g/day through a central venous catheter for 5 d. After taking 1.5 g calcium carbonate daily for 3 mo, her serum calcium levels returned to normal. Meanwhile the patient received endocrine suppression therapy after the operation.

OUTCOME AND FOLLOW-UP

After the operation, genetic analysis was performed, and a germline MEN1 gene mutation was detected. There was a heterozygous mutation in the second exon of MEN1 gene which was 357-360delCTGT. During follow-up, there was no hypoparathyroidism or other complications. The laboratory data on postoperative day 2 showed that serum calcium was 1.98 mmol/L and iPTH was 24.1 pg/mL. After taking 1.5 g
calcium carbonate daily for 3 mo, the patient’s laboratory data improved: calcium 1.98 mmol/L and iPTH 43.7 pg/mL. Calcium was 2.47 mmol/L and iPTH was 61 pg/mL after 18 mo. One month after the operation, the dose of levothyroxine was reduced from 75 mg to 50 mg.

**DISCUSSION**

Currently, the role of inactivating mutations of *MEN1* gene in tumorigenesis of PTC and/or nodular goiter is still controversial. It remains to be determined by more case reports and further research.

*MEN1* is a rare hereditary tumor syndrome inherited in an autosomal dominant manner and presents mostly as the parathyroid[15], endocrine pancreas (such as
gastrinoma\textsuperscript{[16]} and anterior pituitary tumors\textsuperscript{[17]}. Other endocrine and nonendocrine lesions of MEN1, such as adrenal cortical tumors, carcinoids of the bronchi, gastrointestinal tract and thymus lipomas, angiofibromas, and collagenomas, have also been described\textsuperscript{[1,18]}. MEN1 with a large functioning parathyroid cyst is rare. Cavalli \textit{et al}\textsuperscript{[18]} found that approximately 300 cases of sporadic parathyroid cysts had been reported up to 2017, and only two cases have been described in MEN1. Parathyroid cysts can be divided into functioning and nonfunctioning, and most parathyroid cysts are nonfunctioning. The functioning parathyroid cysts are more likely to be caused by degenerative changes in parathyroid adenoma than hyperplasia in our case\textsuperscript{[19]}. In many cases, it is difficult to diagnose the nature of the cyst merely by ultrasound before surgery. Parathyroid cysts need to be differentiated from lymphatic cysts, cystic thyroid nodules and hemangioma\textsuperscript{[20,21]}. The diagnostic rate can be improved by laboratory examination and other imaging examinations. Preoperative SPECT/CT is useful in localizing parathyroid cysts in most patients, with an accuracy rate up to 79% if it is interpreted in combination with cervical ultrasound images\textsuperscript{[22]}. Postoperative pathological diagnosis is the gold standard. The clinical features of hyperparathyroidism (HPT) with MEN1 are similar to those with sporadic HPT, but the former is often more aggressive. For patients who have HPT with MEN1, early surgical treatment is preferred. Surgical treatment should be considered in asymptomatic patients when (1) serum calcium is higher than the reference range 2.52 mmol/L; (2) glomerular filtration < 60 mL/min; (3) bone mineral density at any point is 2.5 or lower, or patient has fragility fractures; and (4) age < 50 years. Whether early surgery can reduce the incidence rate and mortality is not clear. For patients who have HPT with MEN1, especially in asymptomatic or mildly young patients, early parathyroidectomy can reduce the long-term effects of HPT on the patients, especially reducing the bone loss. Although our case was a young woman with normal upper parathyroid glands on both sides, and bilateral lower parathyroid glands showed adenoma-like changes and hyperplasia, parathyroid hormone and serum calcium levels had increased significantly. To avoid a permanent hyperparathyroidism, we did not perform subtotal parathyroidectomy or a total parathyroidectomy with parathyroid tissue autotransplantation. And based on our past experience, unless it is a parathyroid cancer, surgery can achieve a better treatment effect by removing the problematic parathyroid glands. In the present case, the parathyroid hormone had decreased from 676.3 pg/mL to 63.9 pg/mL after the removal of the bilateral inferior parathyroid glands, and the patient’s serum parathyroid hormone was 47.6 pg/mL at 27 mo postoperatively. FNA is the most useful means for diagnosis of thyroid nodules, however, it is not widely accepted for diagnosis of parathyroid tumors due to the risk of dissemination of tumor cells.

Two different forms of MEN1, sporadic and familial, have been described\textsuperscript{[23]}. The sporadic form presents with two of the three principal MEN1-related endocrine tumors (parathyroid adenoma, enteropancreatic tumor and pituitary tumor) within a single patient, while the familial form consists of MEN1 with at least one first-degree relative showing one of the endocrine characteristic tumors\textsuperscript{[24-27]}. In our case, the patient did not provide a clear family history. It is still unclear what form of MEN1 our patient had. There is no evidence to exclude the accidental occurrence of MEN1 with PTC and nodular goiter in this patient.

So far, few cases of thyroid carcinoma and/or nodular goiter combined with MEN1 have been reported. Whether there is a correlation among them is still controversial\textsuperscript{[12,28,29]}. Hill \textit{et al}\textsuperscript{[30]} investigated the probability of concomitant thyroid cancer in patients with MEN1. They found that in patients with MEN1, a 28% substantial incidence of thyroid cancer was observed and all cancers in MEN1 patients were common PTCs histologically (100%). We noticed that only PTCs that measured >1 cm in diameter were considered in the report by Hill \textit{et al}\textsuperscript{[30]} But at present, papillary thyroid microcarcinoma (PTMC) accounts for more than half of all PTCs in clinical practice. If we take these PTMC cases into account, the actual incidence of papillary thyroid carcinoma would be higher in MEN1 patients. Our case was PTMC. MEN1 is caused by inactivating mutations of the \textit{MEN1} gene that encodes the protein menin\textsuperscript{[2]}. Menin is a nuclear protein whose interaction with several other nuclear proteins indicates a role in transcriptional regulation. Previous studies have supported a role for MEN1 in controlling cell growth and differentiation, and in sensing or repairing DNA damage as well. The loss of menin function in tumor precursor cells is involved in the mechanism underlying tumor formation in MEN1\textsuperscript{[8-10,31-33]}. Research showed that the inactivation of menin in the thyroid gland of young mice affected the proliferation of follicular cells\textsuperscript{[13]}. Capraru \textit{et al}\textsuperscript{[11]} showed that the expression of menin was positive, identical to normal thyroid tissue, but it could be decreased or absent in some thyroid tumors including PTC. As is well known, mutations of the \textit{MEN1} gene
cause deficiency in the menin protein in MEN1 patients. There was a heterozygous 357-360delCTGT mutation in the second exon of MEN1 gene in our case. Further molecular studies are needed to evaluate the role of menin protein deficiency in tumorigenesis of PTC and nodular goiter. Kazub skaia et al.[34] investigated follicular cell (papillary and follicular) thyroid carcinoma, genetic inheritance and molecular diagnostic markers. They believed that familial PTC and PTC may be a component of multitu m syndrome, such as MEN1, Cowden syndrome, and familial adenomatous polyposis. We speculate upon possible reasons for the association between MEN1 and PTC/nodular goiter. Loss of heterozygosity (LOH) studies have been used to identify sites harboring tumor suppressor genes involved in tumor initiation or progression. MEN1 is a tumor suppressor gene located on human chromosome 11q13. Specific genomic areas, such as 3p22, 7q31, 11q13 and 11q23, have been reported to be involved in some epithelial or endocrine tumor types[35]. PTC is also one of the endocrine tumors. Whether inactivating mutations of 11q13 can induce PTC remains to be further studied. Chu et al.[36,37] found MEN1 deletion in neurotrophic tyrosine kinase receptor (NTRK)-rearranged PTC patients. They also proved that nucleotide variants and indels in pTERT, MEN1 and CDH1 were observed in several kinase fusion-related PTCs. The relationship among MEN1, PTC and NTRK needs to be further studied. In the analysis of MEN1 gene, about 20% may have false negative results due to the diversity of the causative mutation and scattered position in the entire open reading frame. Moreover, approximately 10% new germline mutations are being detected in the overall MEN1 patients, which may be the reason why the genotype-phenotype correlations could not be identical in 10%-30% of patients[38]. In 2008, Kim et al.[29] reported the first case of PTC combined with MEN1 in Korea. Their patient’s genetic analysis of DNA had revealed no germline mutation in MEN1 gene locus. But there was a genetic mutation in our case. Menin, the protein encoded by MEN1 gene is ubiquitously expressed in endocrine tissues, is less in many endocrine tumors including PTC. Deletion of the MEN1 tumor suppression still might be etiologically related to the oncogenesis of PTC. DNA analysis of more samples with PTC combined with MEN1 may be helpful. Now it is very difficult to confirm the LOH of MEN1 gene completely and accurately, which has dozens of polymorphic markers[29]. The clinical aspects and molecular genetics of MEN1 were reviewed together with the reported 1336 mutations[39]. It has been proved that many of the diseases that have been widely believed to be associated with MEN1 mutations, such as pituitary tumors, lung carcinoids, etc., sometimes failed to exhibit meaningful LOH at 11q13[40]. For PTC, some people have used three of polymorphic markers to test one patient’s sample, with limited results[12]. The exact significance remains to be determined by more case reports and further research. It is another possibility that MEN1 patients who develop PTC may have specific MEN1 mutations of the affected allele that act like dominant oncogenes with regard to thyroid cancer oncogenesis. If any of these scenarios was the case, the MEN1 gene could play a role in the development of the papillary cancer without obvious LOH of the gene locus.

CONCLUSION

In summary, we presented a rare case of MEN1 combined with PTC and nodular goiter, in which a germline mutation of the MEN1 gene was detected. It is possible there is a potential correlation between MEN1 syndrome and PTC/nodular goiter. However, further studies and additional case reports are required to clarify it.

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Full recovery from chronic headache and hypopituitarism caused by lymphocytic hypophysitis: A case report

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Abstract

BACKGROUND
Lymphocytic hypophysitis (LYH) is an important condition to consider in the differential diagnosis of patients with a pituitary mass. The main clinical manifestations of LYH include headache, symptoms related to sellar compression, hypopituitarism, diabetes insipidus and hyperprolactinemia. Headache, which is a frequent complaint of patients with LYH, is thought to be related to the occupying effect of the pituitary mass and is rapidly resolved with a good outcome after timely and adequate glucocorticoid treatment or surgery.

CASE SUMMARY
Here, we report a patient with LYH whose initial symptom was headache and whose pituitary function assessment showed the presence of secondary hypoadrenalism, central hypothyroidism and hypogonadotropic hypogonadism. Pituitary magnetic resonance imaging showed symmetrical enlargement of the pituitary gland with suprasellar extension in a dumbbell shape with significant homogeneous enhancement after gadolinium enhancement. The size of the gland was approximately 17.7 mm × 14.3 mm × 13.8 mm. The pituitary stalk was thickened without deviation, and there was an elevation of the optimal crossing. The lesion grew bilaterally toward the cavernous sinuses, and the parasternal dural caudal sign was visible. The patient presented with repeatedly worsening and prolonged headaches three times even though the hypopituitarism had fully resolved after glucocorticoid treatment during this course.

CONCLUSION
This rare headache regression suggests that patients with chronic headaches should also be alerted to the possibility of LYH.
Lymphocytic Hypophysitis with Headache

A 56-year-old female patient presented with an intermittent throbbing headache located in the left temporal region. She was admitted to our hospital for neurosurgery.

History of present illness

Two months before hospitalization, the patient did not complain about headache. One month before hospitalization, the headache worsened and became more pronounced at night, and she experienced vision loss with bilateral temporal visual field defects.
History of past illness
The patient had a history of hypertension for more than 20 years. There was no family history of autoimmune disease.

Personal and family history
There was no personal or family history.

Physical examination
The patient presented with a height of 160 cm, a weight of 65 kg, a temperature of 36.5 °C, and a blood pressure of 140/108 mmHg. The clinical neurological examination showed no abnormalities. Our initial clinical diagnosis was cellar area occupancy.

Laboratory examinations
The laboratory data showed a potassium level of 3.2 mmol/L, uroprotein+, and a urinary specific gravity of 1.025. The patient was negative for antinuclear antibodies, immunoglobulin G (IgG), IgM, IgA, and IgG4, which did not support IgG4-related hypophysitis, and no antithyroid antibodies were detected. The remaining biochemical and coagulation test results were unremarkable. The cortisol, adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), growth hormone (GH), thyroid-stimulating hormone (TSH), free triiodothyronine 3 (FT3), and free triiodothyronine (FT4) levels were measured (Table 1). A water deprivation test was not performed because the patient did not have polyhydramnios or polyuria.

Imaging examinations
The chest computerized tomography was normal. The electrocardiography was characterized by a flat t wave. The MRI of the pituitary gland showed symmetrical enlargement with suprasellar extension in a dumbbell shape with significant homogeneous enhancement after gadolinium enhancement (Figure 1), high signal in the posterior pituitary lobe in the T1 sequence, low signal in the anterior pituitary gland in the T1 sequence and high signal in the T2 sequence. The pituitary stalk was thickened, but not deviated, approximately 3.1 mm at the optic cross and approximately 3.1 mm at the pituitary insertion with an elevation of the optimal crossing (Figure 1). The lesion grew bilaterally toward the cavernous sinuses and encircled the bilateral internal carotid arteries, and the parasternal dural caudal sign was visible (Figure 2), all of which are specific MRI manifestations consistent with LYH. According to the scoring system by Gutenberg et al[7], our patient was -8, strongly suggesting the diagnosis of LH.

MULTIDISCIPLINARY EXPERT CONSULTATION
Ophthalmologic assistance was requested for the examination, and the assessment suggested temporal visual field defects in both eyes, a corrected visual acuity of 0.5 in the left eye and a visual acuity of 0.8 in the right eye.

FINAL DIAGNOSIS
Considering the rapid progression of hypopituitarism and the combination of the MRI features, laboratory tests, clinical manifestations and epidemiological features, LYH was strongly considered.

TREATMENT
Glucocorticoid therapy was administered in conjunction with an endocrinology consultation, starting with a daily intravenous infusion of 50 mg hydrocortisone. This treatment was changed to 30 mg/d combined with 25 μg/d levothyroxine tablets after 3 d and 20 mg/d after 4 d. The patient’s symptoms significantly improved, but the pituitary function did not significantly improve upon reassessment at 1 wk of the steroid treatment. The pituitary-adrenal axis and pituitary-thyroid axis were improved, but the pituitary-gonadal axis did not recover (Table 1). After an evaluation of the
Table 1 Pituitary, cortical, and thyroid hormones

<table>
<thead>
<tr>
<th>Hormones</th>
<th>ACTH</th>
<th>Cortisol nmol/L</th>
<th>TSH μIU/mL</th>
<th>FT3 pmol/L</th>
<th>FT4 pmol/L</th>
<th>LH mIU/mL</th>
<th>FSH mIU/mL</th>
<th>PRL mIU/L</th>
<th>GH ng/mL</th>
</tr>
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<tbody>
<tr>
<td>Normal value</td>
<td>1.6-13.9</td>
<td>240-619</td>
<td>0.27-4.2</td>
<td>3.1-6.8</td>
<td>12.0-22.0</td>
<td>10.87-58.64</td>
<td>16.74-113.6</td>
<td>58-416.4</td>
<td>0.010-3.607</td>
</tr>
<tr>
<td>Onset</td>
<td>-</td>
<td>124.59</td>
<td>0.242</td>
<td>3.56</td>
<td>6.05</td>
<td>&lt; 0.2</td>
<td>4.31</td>
<td>75.8</td>
<td>0.142</td>
</tr>
<tr>
<td>1 mo</td>
<td>0.89</td>
<td>176.99</td>
<td>0.073</td>
<td>3.09</td>
<td>13.18</td>
<td>0.590</td>
<td>5.12</td>
<td>82.83</td>
<td>-</td>
</tr>
<tr>
<td>3 mo</td>
<td>-</td>
<td>-</td>
<td>2.29</td>
<td>4.08</td>
<td>16.05</td>
<td>18.38</td>
<td>48.89</td>
<td>183.19</td>
<td>-</td>
</tr>
<tr>
<td>6 mo</td>
<td>-</td>
<td>-</td>
<td>0.115</td>
<td>3.42</td>
<td>21.05</td>
<td>26.85</td>
<td>56.11</td>
<td>397.19</td>
<td>-</td>
</tr>
<tr>
<td>8 mo</td>
<td>-</td>
<td>-</td>
<td>2.11</td>
<td>3.89</td>
<td>20.84</td>
<td>32.64</td>
<td>65.75</td>
<td>400.78</td>
<td>-</td>
</tr>
<tr>
<td>11 mo</td>
<td>-</td>
<td>-</td>
<td>0.634</td>
<td>3.77</td>
<td>13.82</td>
<td>21.49</td>
<td>57.79</td>
<td>280.57</td>
<td>-</td>
</tr>
<tr>
<td>12 mo</td>
<td>6.97</td>
<td>293.63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15.73</td>
<td>46.18</td>
<td>415.4</td>
<td>-</td>
</tr>
</tbody>
</table>

ACTH: Adrenocorticotropic hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; PRL: Prolactin; GH: Growth hormone; TSH: Thyroid-stimulating hormone; FT3: Free triiodothyronine 3; FT4: Free triiodothyronine.

Figure 1 Pretreatment coronal magnetic resonance imaging showing pituitary enlargement (arrowhead) and optic chiasm elevation (arrow). A: In the T1 sequence; B: In the T2 sequence; C: Postgadolinium-enhanced coronal magnetic resonance imaging showing an enlarged pituitary gland with significant homogeneous enhancement (arrowhead) and an elevation of the optic chiasm (arrow); D: Posttreatment coronal magnetic resonance imaging showing an almost normal pituitary gland in the T1 sequence; E: Posttreatment coronal magnetic resonance imaging showing an almost normal pituitary gland in the T2 sequence; F: With gadolinium enhancement in the coronal position.

pituitary function, the treatment was changed to continuous 120 mg/d- methylprednisolone pulse therapy due to the poor treatment effect and then 80 mg/d after 5 d. Subsequently, pulse therapy was continued for 1 mo and finally discontinued; the headache was significantly relieved, and the visual field returned to normal, suggesting LYH rather than pituitary adenoma.

OUTCOME AND FOLLOW-UP

Repeat pituitary MRI showed a decrease in the size of the suprasellar mass with homogeneous enhancement, thinning and no deviation of the pituitary stalk, no
elevation of the chiasm, and no abnormal signal in the cavernous sinus. However, the patient was found to have concomitant glucocorticoid-related diabetes mellitus, was treated with insulin and tested negative for diabetic autoimmune antibodies. Methylprednisolone was continued at 60 mg/d. The dose was reduced to 40 mg/d after 1 wk, and a regimen of 4 mg reduction every 2 wk and discontinuation of levothyroxine tablets was employed. Unfortunately, 6 mo after the diagnosis of LYH, the patient again presented with headaches of the same nature that were worse than before. Ophthalmologic assistance was requested for the examination, and the assessment suggested bilateral refractive error, no abnormalities in the bilateral visual fields and symptomatic treatment. Repeat MRI showed no significant change from the previous MRI. The pituitary function assessment suggested complete recovery of the thyroid and gonadal axes (Table 1), the patient was considered to have no recurrence of LYH, and methylprednisolone was continued with a regimen of 4 mg reduction every 3 wk. The patient's headache worsened for the third time two months later, and she presented with features similar to those previously noted. On examination, she had a full-moon face, centripetal obesity, weight gain and hirsutism. However, repeated pituitary MRI and endocrine function assessment did not show any deterioration. The patient was discharged from the hospital on 6 mg/d methylprednisolone and was instructed to adjust her glucocorticoid dose to 4 mg/d after 1 mo. However, 3 mo later, the patient's headache worsened for the fourth time, with the same features. Repeated pituitary MRI and assessment of the pituitary function showed no significant changes; therefore, LYH was considered stable. Thus, glucocorticoid therapy was stopped, but other treatments, such as glucose-lowering treatments, were continued. Finally, 1 year after the diagnosis, the patient's pituitary function was evaluated to have no abnormalities, and the patient was considered to have completely recovered pituitary function. The patient's headache did not recur (Figure 3).
LYH is the most common subtype of PAH and is characterized by diffuse lymphocytic and plasma cell infiltration with fibrosis in the pituitary gland[13], and the pathological process involves inflammatory changes, edema and enlargement of the pituitary gland, followed by destruction of pituitary cells, parenchymal fibrosis and, ultimately, pituitary atrophy with hypopituitarism[5]. However, the natural course of LYH is poorly understood, and the condition may spontaneously resolve or deteriorate rapidly[14]. In addition, the clinical presentation can widely vary depending on the course of the disease[5].

However, the most common complaint in 60% of patients with LYH is headache[15], followed by amenorrhea/erectile dysfunction (59%) and diplopia (27%)[9]. Headache is also the most common complaint in the first neurosurgical consultation, with an incidence of 89%[15]. LYH has been reported to present as frontal, temporal, or occipital headache[16-18], severe dull or progressive headache[18], or even trigemino-autonomic cephalalgia[17]. Our patient initially presented with intermittent headaches and later with persistent frontal pain that fluctuated in nature that was characterized by a long duration. The diagnosis of chronic postintracranial disorder headache (CPIDH) is reasonable when the etiologic disease is effectively treated or resolves on its own, but the headache does not resolve or significantly improve after 3 mo[19]. Our patient’s headache persisted and repeatedly worsened for more than 8 mo and could be considered CPIDH. It has been suggested that headache is associated with cerebrospinal fluid lymphocytosis, but Honegger et al[20] did not identify a clear correlation between the degree of headache and the cerebrospinal fluid leukocyte count. It has been suggested that pituitary masses causing cavernous sinus involvement and mechanical pulling of the dura are potential pathological mechanisms underlying secondary headaches associated with LYH[11]. Meningeal and dural compression[21] and cavernous sinus involvement that can cause headache[22] are frequently reported. It has also been reported that headache is combined with hyperprolactinemia in 50% of LYH patients[19], but this was not a problem in our case. It has also been reported that the severity of symptoms and the speed of onset of LYH manifestations are typically not related to the degree of pituitary enlargement and compression of peripheral structures but are related to endocrine cell destruction mediated by autoimmune factors[5,22], and the long-term chronic headache manifestations in our patient may be related to this pathological feature.

ACTH deficiency is the most common endocrine disorder in LYH (60%), followed by TSH deficiency, gonadotropin deficiency and hyperprolactinemia[23]; thus, the pattern of ACTH > TSH > LH/FSH > GH axis deficiency and the specific vulnerability of ACTH secretion to LYH have been suggested in several reports[5,24]. Our patient exhibited ACTH, TSH, and LH/FSH deficiency, which is consistent with the above reports. Another feature of LYH is that the degree of hypopituitarism is disproportionate to the size of the mass, which is also supported by findings in some cases[25]. One case report describes pituitary inflammation with pituitary enlargement exhibiting hypopituitarism with a long delay in onset[26]. However, this phenomenon was not significantly represented in the present report.

In recent years, the application of MRI in the sellar region has contributed to the feasibility of clinical diagnosis[8] and has become the preferred modality for the study of pituitary lesions. Typical MRI of LYH shows symmetrical enlargement of the pituitary gland with suprasellar extension with marked homogeneous enhancement, thickening of the pituitary stalk without deviation, disappearance of the bright spot of the pituitary gland in the T1 sequence, and the dural tail sign[20,27-30]. A lingual suprasellar and retrosellar extension of the saddle mass in contact with the basal
hypothalamus and even infiltration of the basal hypothalamus is a relatively typical finding in granulomatous pituitary inflammation[20], but this feature was not present in our patient. These features have been confirmed in a larger number of cases, rendering the clinical diagnosis of LYH increasingly simple[31]. However, as an increasing number of cases have been reported, pituitary MRI features, including heterogeneity and ring enhancement, which may also be found on pituitary MRI of LYH, are being updated[25]. Relatively low signals on T1-weighted images and relatively high signals on T2-weighted images may also be MRI manifestations of LYH[14]. The MRI findings of different types of PAH are similar, and it is difficult to differentiate it from pituitary adenoma based on only on MRI[32]. The scoring system by Gutenberg et al[7] can distinguish between nonesecretory pituitary tumors and LYH with a sensitivity of 92% and specificity of 99%, which aids in the differential diagnosis of LYH. Our patient's score was -8, favoring a diagnosis of LYH, although granulomatous hypophysitis could also present with similar sellar infiltration; however, granulomatous hypophysitis is very rare and often found by autopsy[33]. The patient did not agree to biopsy of the pituitary gland and pancreas, and case reports of IgG4-related diseases suggest that the pituitary gland is the least involved[34]. Therefore, although it cannot be excluded, it cannot be diagnosed as IgG4-related hypophysitis temporarily according to the corresponding diagnostic criteria[34]. Necrotizing hypophysitis present with a lack of contrast enhancement and sudden-onset hypopituitarism[35], diabetes insipidus and radiologic findings of the ischemic pituitary are three characteristics of necrotizing hypophysitis[35]. Our patient presented with marked homogeneous anterior pituitary enhancement without ischemic manifestations. Xanthomatous hypophysitis commonly exhibits cystic enlargement and peripheral ring enhancement after contrast[36,37] and is rarely known to improve in response to glucocorticoid therapies[38]. This situation is inconsistent with our patient's findings; thus, necrotizing hypophysitis and xanthomatous hypophysitis were not considered.

The objective of LYH treatment is to rectify the hormone deficiency and relieve the symptoms associated with the effects of the mass. Although glucocorticoids are the preferred pharmacological treatment for LYH, surgery may be considered in the presence of severe neurological or ophthalmic manifestations or the absence of a response to pharmacological treatment[39]. In 2015, Khare et al[24] described 15 patients from western India with pituitary masses that regressed with conservative treatment. Therefore, unless the symptoms are severe or progressively worsen, conservative treatment may be considered[40]. In addition, postoperative hypopituitarism may occur, and deterioration caused by surgery or biopsy may be avoided[9]. Surgical treatment may contribute to the permanent relief of headache, and headache and visual field defects usually improve shortly after surgery[12]. However, considering the risk of hypopituitarism associated with surgery[41], we did not perform surgery but adhered to long-term glucocorticoid treatment and follow-up, and the outcome was good[42]. Although some patients with LYH may show spontaneous recovery, it is also too late to initiate glucocorticoid therapy 3 mo after symptom onset[7,24]. Our time window for initiating glucocorticoid therapy was 2 mo. Thus, the pituitary function completely recovered, and the headaches, despite the longer duration, were eventually relieved. One study indicates that the first pulse of methylprednisolone was the most effective at less than 6 mo of onset[42], and Wang et al[23] reported a lower relapse rate associated with longer steroid administration because these authors found a significant difference in relapse rates with steroid drug dose administration times of 6 and < 6 mo. Fortunately, in the absence of significant efficacy with short-term hydrocortisone pulse therapy combined with continuous oral hydrocortisone treatment, the endocrinologist administered long-term methylprednisolone pulse therapy shortly after the onset of the disease to the patient in this case study, and the pituitary function significantly improved until it completely recovered. However, the headache recurred before eventually disappearing completely due to early detection and timely management, and the patient was satisfied with the outcome[5].

CONCLUSION

This report presents a rare case of LYH in combination with chronic headache despite complete resolution of hypopituitarism. Although the patient experienced long-term recurrent exacerbation of chronic headache, all symptoms eventually resolved in the patient after adequate evaluations of the clinical and MRI features due to early diagnosis and long-term high-dose glucocorticoid therapy.
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Novel method of primary endoscopic realignment for high-grade posterior urethral injuries: A case report

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Author contributions: Ho CJ and Yang MH designed the report, collected the patient’s clinical data, analyzed the data, and wrote the paper.

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Abstract

BACKGROUND
A male urethral disruption injury is a urological emergency. Primary endoscopic realignment (PER) refers to reestablishment of urethral alignment via indwelling urethral catheter by cystoscope, which is recommended as the optimal emergent treatment approach for reducing the likelihood of complications following injury. However, the prior literature suggests the success rate of PER to be relatively low due to complicated urethral disruption. We report a modified PER approach that serves to improve both the success rate and safety of the treatment.

CASE SUMMARY
A 19-year-old male patient presented with multiple pelvic fractures and complete urethral disruption following a high-velocity traffic accident. The patient’s abdominal computed tomography and retrograde urethrography results revealed complete urethral disruption at the bulbar urethra, with hematoma and contrast medium extravasation that extended into the extraperitoneal space. The conventional retrograde PER by cystoscope failed due to severe disruption and considerable hematoma. Modified simultaneous antegrade and retrograde PER was performed by means of semi-rigid ureteroscopy via a suprapubic Foley catheter and cystoscopy via the external urethra. An antegrade guidewire was passed through the bladder neck and then pulled out through the external urethral meatus with a cystoscope. Urethral continuity was achieved after a 16-Fr silicone Foley catheter was indwelled into the bladder along the guidewire. The patient recovered well, achieving voiding continence and avoiding further operation for urethral stricture.

CONCLUSION
Modified PER via suprapubic Foley catheter represents a promising and safe treatment approach in patients with posterior urethral injuries.
Key Words: Posterior urethral injury; Emergent treatment; Primary endoscopic realignment; Novel method; Case report

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Core Tip: We report a modified primary endoscopic realignment to improve both the success rate and intraoperative safety of a patient with high-grade urethral disruption injury. The surgery was performed with simultaneous antegrade and retrograde endoscopes. We used a suprapubic Foley catheter to serve as an access route of the antegrade cystoscope. The antegrade guidewire was passed through the bladder neck and pulled out through the external urethra, with assistance of the retrograde cystoscope. For the 19-year-old male who presented with high-grade complete urethral disruption after traffic accident, modified endoscope realignment was achieved, and the patient recovered well.

INTRODUCTION

Although traumatic posterior urethral injuries (PUIs) remain uncommon, they have been reported to co-occur with pelvic fractures[1]. Indeed, prior studies have estimated the incidence of PUIs following pelvic fractures to be as high as 25%[2]. While a PUI does not represent a life-threatening injury, it may profoundly compromise a patient’s quality of life due to being associated with morbidities such as urethral stricture, erectile dysfunction, and urinary incontinence[1,3]. The European Association of Urology guidelines on urological trauma recommend primary endoscopic realignment (PER) as the preferred technique for the treatment of PUIs[4]. Several studies have shown that when treated with PER, patients exhibit a lower rate of urethral stricture as well as a reduced need for subsequent urethroplasty[3]. Retrograde cystoscopy has traditionally been the most commonly used method for performing PER. However, a previous study reported that the success rate when using retrograde cystoscopy was only 21%[5]. The high failure rate is likely due to several reasons, including the fact that high-grade PUIs are usually associated with a significant gap between disruption-ends of the urethra. In addition, the peripheral hematoma typically seen following a PUI can limit the surgeon’s vision when using a scope, which can make performing the realignment more difficult.

In an effort to increase the success rate of PER, one retrospective study suggested performing antegrade realignment via the suprapubic tract using semi-rigid ureteroscopy[6]. However, this technique requires the use of a unique peel-away sheath to establish a suprapubic tract, which is not currently widely accessible.

In the present study, we report a modified approach involving simultaneous antegrade and retrograde PER performed by means of semi-rigid ureteroscopy in a case of total disruption PUI. More specifically, the antegrade PER was performed via a suprapubic tract established with a Foley catheter, which could serve as a safer and more efficient method.

CASE PRESENTATION

Chief complaints

A 19-year-old male patient was brought to our emergency room after suffering multiple pelvic fractures complicated by urethral injury following a high-velocity motorcycle accident.
**History of present illness**
The patient developed acute urinary retention after the initial injury, followed by lower abdomen distention without urethral meatus bleeding.

**History of past illness**
The patient had no relevant prior medical history.

**Personal and family history**
The patient had no personal and family history.

**Physical examination**
The patient’s physical examination revealed no obvious ecchymosis on the penis, scrotum, or perineum.

**Laboratory examinations**
The patient had no specific laboratory abnormalities.

**Imaging examinations**
Due to the suspicion of urethral trauma, abdominal computed tomography with retrograde urethrography was performed in the emergency room. Contrast medium extravasation at the bulb urethra was noted, with a massive hematoma surrounding and extending into the extraperitoneal space. A so-called “pie in the sky” sign was also observed, indicating that the urethra was totally disrupted, causing the prostate to be displaced upward (Figure 1).

**FINAL DIAGNOSIS**
The patient was diagnosed with American Association for the Surgery of Trauma grade IV-V complete urethral disruption. Further, Tile type-A2 stable pelvic ring fractures were also identified over the bilateral pubic ramus.

**TREATMENT**
The patient underwent an emergency operation involving PER. First, a 22.5-Fr cystoscope was inserted into the patient’s urethra; although, this attempt at retrograde realignment failed. Next, simultaneous antegrade and retrograde endoscopic examinations were performed. The procedure is illustrated in Figure 2. A suprapubic cystostomy puncture under ultrasound guidance was performed using a cystostomy trocar and a side-slit cannula. Once the trocar was engaged in the intravesical space, a 22-Fr three-way Foley catheter was placed through the cystostomy tunnel and then fixed by means of balloon inflation. The tip of the Foley catheter was incised before it was inserted to perform the subsequent procedure (Figure 2A). Following the removal of the cystostomy side-slit cannula, a semi-rigid 4.5/6.5-Fr ureteroscope was inserted into the bladder via the cystostomy Foley catheter in order to examine the bladder and bladder neck (Figure 2B). A hydrophilic guidewire (ZIPwire™; Boston Scientific, Boston, MA, United States) was passed through the bladder neck and then pulled out through the external urethral meatus using grasping forceps designed for use with a cystoscope (Figure 2C). The retrograde cystoscope was indwelled along the guidewire, which was then replaced with a rigid guidewire (Fixed Core Wire Guide; Cook Medical, Bloomington, IN, United States). Urethral continuity was achieved after a 16-Fr silicone Foley catheter was indwelled into the bladder along the guidewire. Finally, both the suprapubic cystostomy and the urethral Foley catheter were left in place.

**OUTCOME AND FOLLOW-UP**
The patient recovered well and did not experience any complications. He was discharged on the seventh postoperative day. The urethral Foley catheter was removed on the 28th postoperative day, and the patient exhibited voiding continence and complete voiding. The cystourethroscopy results revealed that the patient’s injuries had healed well without bulbomembranous urethra stricture (Figure 3).
Figure 1 Contrast medium extravasation at bulbar urethra (arrow) with massive hematoma (arrow heads), and “pie in the sky” sign of prostate was noted.

Figure 2 Procedure of primary endoscopic realignment. A: The tip of the Foley catheter was incised and a semi-rigid 4.5/6.5-Fr ureteroscope was inserted; B: Simultaneous antegrade and retrograde endoscopy; C: The guidewire was antegrade and then pulled out through the external urethral meatus using grasping forceps with a cystoscope.

Moreover, the patient reported nocturnal penile tumescence and erection following sexual stimulation.

DISCUSSION

The classic presentation of high-grade urethral injury involves bloody discharge over the urethral meatus and difficulty in relation to urination. Clinicians should perform retrograde urethrography for diagnostic purposes following pelvic trauma. Immediate suprapubic tube (SPT) placement used to be the standard treatment approach in the case of PUI. However, patients who were treated with only SPTs were found to always develop complete stenosis, which necessitated further treatment by means of posterior urethroplasty[7]. Thus, PER was suggested as a more promising treatment approach.
for a number of reasons, including the fact that PER of urethral distraction defects may allow for any subsequent strictures to be managed endoscopically or via easier posterior urethroplasty due to realigning the distracted urethra and shortening the stricture length[8]. Hadjizacharia et al[9] reviewed the outcomes of 21 patients, including 14 treated with PER and 7 with SPT. The patients treated with PER were found to have a shorter time to self-voiding as well as a lower stricture rate when compared with the SPT group. Johnsen et al[3] found that PER could serve as the definitive therapy in more than a third of all treated patients. It could also prevent the need for formal urethroplasty, even in cases in which the PER failed.

As endoscopic equipment and techniques have developed, PER with simultaneous antegrade and retrograde flexible cystoscopy has become increasingly common. However, the high intraoperative irrigation flow and crude scope diameter could exacerbate any soft tissue injury and increase the risk of the pelvic hematoma becoming infected. Tausch et al[10] reviewed a sample of patients with PUI. They concluded that while PER may result in the restoration of urethral continuity without the need for further intervention, PER patients may require multiple interventions and experience more adverse events than SPT patients. Zhang et al[6] reported a modified procedure involving the use of standard semi-rigid ureteroscopy to reduce manipulation-related trauma, which resulted in a good success rate and a low subsequent comorbidity rate. However, the suprapubic route was established by means of serial sheath dilatations and then completed using a 16-Fr peel-away sheath. Unfortunately, this equipment is not readily available, especially in rural regions.

In our case, we used 4.5/6.5-Fr semi-rigid ureteroscopy to perform the antegrade cystoscopy in order to achieve realignment. In contrast to the above-mentioned studies, the access route was already established using a 22-Fr Foley catheter, which is a common piece of medical equipment found in every hospital. Furthermore, endoscopic realignment using a Foley catheter offers several advantages. First, the Foley balloon can serve as a seal between the Foley catheter and the bladder wall, which should prevent further fluid extravasation into the extraperitoneal space during a prolonged procedure. Thus, the postoperative infection rate, ileus rate, and abdominal discomfort level should be minimized. Second, the urinary bladder is a flexible organ, meaning that the established access route when using a non-fixable sheath could be dislocated when the bladder deflates. The use of a Foley balloon can ensure more secure access and a lower risk of dislocation during the procedure. Third, most urologists are familiar with the ultrasound-guided percutaneous cystostomy procedure, which could help to reduce the operative time without the requirement for a steep learning curve when compared with other innovative procedures. Fourth, due to the likelihood of multi-traumatic and complex comorbidities, a patient with a PUI usually carries a high risk when undergoing anesthesia. With the use of an established suprapubic Foley catheter, the operation could be terminated at any time with a sufficient outflow tract available for urinary diversion.

CONCLUSION

Pelvic fractures accompanied by posterior urethral disruption can be encountered during emergency medical practice. To avoid further morbidity and reduce the need for urethroplasty, PER could serve as a promising initial management approach. This case report presented a modified PER procedure with a high success rate and an easy-to-learn method that involves the insertion of a ureteroscope into a suprapubic Foley

Figure 3 Healed urethra at 28 d after realignment.
catheter. Because the Foley catheter is available in most hospitals and the cystostomy procedure is familiar to most urologists and physicians, the described procedure could be widely adopted by general emergency facilities.

REFERENCES


Congenital muscular dystrophy caused by beta1,3-N-acetylgalactosaminyltransferase 2 gene mutation: Two case reports

Wen-Juan Wu, Su-Zhen Sun, Bao-Guang Li

Abstract

BACKGROUND
Mutations in the beta1,3-N-acetylgalactosaminyltransferase 2 (B3GALNT2) gene can lead to impaired glycosylation of α-dystroglycan, which, in turn, causes congenital muscular dystrophy (CMD). The clinical phenotypes of CMD are broad, and there are only a few reports of CMD worldwide.

CASE SUMMARY
This report describes the cases of two children with CMD caused by B3GALNT2 gene mutation. The main manifestations of the two cases were abnormal walking posture, language development delay, and abnormal development of the white matter. Case 2 also had unreported symptoms of meningocele and giant arachnoid cyst. Both cases had compound heterozygous mutations of the B3GALNT2 gene, each containing a truncated mutation and a missense mutation, and three of the four loci had not been reported. Nineteen patients with CMD caused by B3GALNT2 gene mutation were found in the literature. Summary and analysis of the characteristics of CMD caused by B3GALNT2 gene mutation showed that 100% of the cases had nervous system involvement. Head magnetic resonance imaging often showed abnormal manifestations, and more than half of the children had eye and muscle involvement; some of the gene-related symptoms were self-healing.

CONCLUSION
B3GALNT2 gene can be used as one of the candidate genes for screening CMD, cognitive development retardation, epilepsy, and multiple brain developmental malformations in infants.

Key Words: Beta1,3-N-acetylgalactosaminyltransferase 2 gene; Congenital muscular dystrophy; Epilepsy; Language development retardation; Autism; Case report
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Core Tip: Mutations in the beta1,3-N-acetylgalactosaminyltransferase 2 (B3GALNT2) gene can lead to impaired glycosylation of alpha-dystroglycan, which, in turn, causes congenital muscular dystrophy (CMD). The clinical phenotypes of CMD are broad, and there are only a few reports of CMD worldwide. This paper introduces two cases of congenital dystrophy caused by mutation of the B3GALNT2 gene. Briefly, 19 children with B3GALNT2 gene mutation published in the world were reviewed. Clinical characteristics and mutation genotypes of 21 children were analyzed, and the pathogenesis is discussed.

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DOI: https://dx.doi.org/10.12998/wjcc.v10.i3.1056

INTRODUCTION

Congenital muscular dystrophy (CMD) refers to the primary and progressive myopathy that occurs at birth or within a few months after birth, usually causing considerable burden to the child and their families and society. The main clinical manifestations of CMD include premature myasthenia, hypodystonia, motor development retardation, and joint contracture with or without central nervous system involvement. The creatinase level tends to increase to varying degrees, myogenic damage can be seen on electromyography, and muscular dystrophy is the most common muscle pathology. Ge et al.[1] reported that the prevalence rate of CMD in China was 0.017-0.083/100000. CMD is a group of hereditary diseases with strong heterogeneity caused by anti-dystroglycan (DG) deficiency. DG is a protein that is widely expressed in a variety of tissues, including the brain, Schwann cells of peripheral nerves, neuromuscular junction, muscle, liver, lung, and skin[2]. DG is cleaved into alpha-DG and beta-DG after translation. The core structure of alpha-DG is dumbbell-shaped, with a spherical C-terminal and an N-terminal at the two ends, respectively, while the middle is connected by a mucoprotein-like structure. Alpha-DG undergoes O-linked glycosylation, which rarely occurs in vivo[3], and then forms an anti-dystrophin-glycoprotein complex with beta-DG. Then, it binds with ligands containing a laminin globular domain in the extracellular matrix, such as laminin, perlecan, agglutinin, axon protein, and pikacurin, to maintain muscle cell integrity[4], thus playing an important role in brain development[5]. Alpha-DG has many glycosylation sites, short glycoprotein fragments, complex functions, and diverse pathogenic genes[6]. Once the glycosylation of alpha-DG is impaired, it can trigger many pathophysiological processes, such as cell migration disorder[7] or alpha-dystroglycanopathy (alpha-DGP)[8]. Recent studies[9] have shown that the gene located in 1q42.3 containing 64292 base pairs is one of its pathogenic genes, which encode beta1,3-N-acetylgalactosaminyltransferase2 (B3GALNT2). The deficiency of this enzyme can lead to the damage of alpha-DG glycosylation, consequently impairing the function of alpha-DG and affecting cell proliferation, migration, differentiation, and maintenance of tissue integrity, resulting in alpha-DGP[10]. Its pathogenic type was autosomal recessive inheritance.

At present, there are few reports about the B3GALNT2 gene causing CMD, and the relationship between genotype and phenotype is still not fully understood. In this paper, the pathogenic characteristics of this gene in two cases of alpha-DGP caused by B3GALNT2 gene mutation found in our hospital are summarized and discussed in view of previous reports.

CASE PRESENTATION

Chief complaints

Case 1: A 49-mo-old Chinese Han boy presented with abnormal walking posture in...
January 2019.

**Case 2:** A 45-mo-old Chinese Han boy presented with delayed growth and development from childhood and an abnormal scalp in June 2019.

**History of present illness**

**Case 1:** The child showed signs of developmental delay since childhood. He could raise his head at 5 mo, sit at 8 mo, walk alone at 1 year and 8 mo, and say the Chinese words for “mother” and “father” at 28 mo.

**Case 2:** The child could raise his head at 6 mo, sit alone at 12 mo, and walk at 24 mo. At the time of this visit, he still walked with a spastic gait. At present, he could pronounce a small number of disyllabic words such as the Chinese words for “father” and “mother”.

**History of past illness**

**Case 1:** The patient had no special past history.

**Case 2:** Meningocele was seen in the parietal and occipital region after birth, which then gradually healed.

**Personal and family history**

**Case 1:** The child was the fifth fetus and the second delivered by cesarean section at 38 wk of pregnancy. There was no significant family history.

**Case 2:** The child was the second fetus and delivered by full-term natural delivery. There was no significant family history.

**Physical examination**

**Case 1:** Physical examination revealed that the child could understand and respond to instructions, walked with “duck step”, and had no skin abnormalities or abnormal palpebral fissures. The bilateral pupils were round, equal in size, and sensitive to light reflex. The patient had no stiff neck, and no obvious heart, lung, and abdomen abnormalities. He had normal muscle strength of the extremities, bilateral biceps reflex, knee-tendon reflex, and active Achilles tendon reflex. He had low muscle tone and positive bilateral Babinski signs.

**Case 2:** Physical examination revealed that the child could understand and respond to instructions well, and walked with a spastic gait. An abnormal area of approximately 3 cm × 2 cm was visible in the parietal occipital area of the scalp; it was blue and purple in color, lacked hair growth, and showed no rupture, no tenderness, and no fluctuation. The bilateral eye fissure had no deformity, and bilateral pupils were round, equal in size, and sensitive to light reflex. There were no limitations of eye movement; no stiff neck; and no obvious abnormalities in the heart, lung, and abdomen. The muscle strength of the extremities was normal, and muscle tension was high. Bilateral biceps reflex, knee-tendon reflex, and Achilles tendon reflex were active, and bilateral Babinski signs were negative.

**Laboratory examinations**

**Case 1:** The leukoencephalopathy gene package (McKinnon) was used for sequencing, and the results demonstrated that the **B3GALNT2** gene had a compound heterozygous mutation (**Figure 1**), and the allele mutations included c.1068dupT (p.D357_D358 delinsX) from the father (**Figures 1A and 1B**) and c.40G>C (p.G14R) from the mother (**Figures 1C and 1D**). The former was a nonsense mutation, which led to protein truncation, and the latter was a missense mutation.

**Case 2:** Genetic examination showed that the **B3GALNT2** carried a compound heterozygous mutation (**Figure 2**). The mutation sites on the allele included c.261-2A>G (splicing) from the mother (**Figures 2A and 2B**) and c.979G>A (p.D327N) from the father (**Figures 2C and 2D**). The former was a missense mutation, and the latter was a nonsense mutation, which led to protein truncation.

**Imaging examinations**

**Case 1:** At the age of 2 years and 4 mo, electromyography of the extremities was generally normal. Visual evoked potentials (**Figure 3A**) showed that both eyes were stimulated, each wave was well-differentiated, and P1 latency was significantly...
Wu WJ et al. Muscular dystrophy caused by B3GALNT2 mutation

Figure 1: Gene detection results for case 1. A: c.1068dup T (p.D357_D358delinsX) mutant type; B: c.1068dup T (p.D357_D358delinsX) wild type; C: c.40G>C (p.G14R) mutant type; D: c.40G>C (p.G14R) wild type.

Figure 2: Gene detection results for case 2. A: c.261-2A>G mutant type; B: c.261-2A>G wild type; C: c.979G>A (p.D327N) mutant type; D: c.979G>A (p.D327N) wild type.

Prolonged somatosensory evoked potentials (Figure 3B) showed that both upper limbs were stimulated, the cortical and surrounding waves were well-differentiated, and the latencies were roughly normal. Auditory evoked potentials (Figure 3C) suggested that bilateral ears were stimulated, the waveform of the left side was relatively poor, the latencies of the right III, IV, and V waves were prolonged. Head magnetic resonance imaging (MRI) showed cerebellar cortical cysts (Figure 4A), multiple flaky and linear equal/slightly long T1, long T2, and high fluid-attenuated inversion recovery signals around the bilateral ventricles of the brain (Figures 4B and 4C).

Case 2: The first head MRI (Figure 5) at 10 mo of age showed an abnormal signal of the flat pons (Figure 5A), an arachnoid cyst in the right temporal pole (Figure 5B), and periventricular white matter neuronal migration disorder in bilateral frontal and parietal lobes-polymicrogyria malformation (Figures 5C and 5D). The second head MRI at 2 years and 11 mo revealed an abnormal signal of periventricular white matter (Figures 5E and 5F), which was absorbed and considerably improved compared to that seen in the previous MRI; neuronal migration disorder in bilateral frontotemporal parietal lobes-polymicrogyria malformation, an arachnoid cyst in the right temporal pole, and a flat pons.
Figure 3 Visual and auditory evoked potentials and somatosensory evoked potentials of the child at 2 years and 3 mo of age. A: Visual evoked potentials of the child; B: Somatosensory evoked potentials of the child; C: Auditory evoked potentials of the child.

Figure 4 Head magnetic resonance imaging of case 1 at 2 years and 3 mo of age. A: Cerebellar cyst; B and C: Multiple flaky high FLAIR signals around the bilateral ventricles.

**FINAL DIAGNOSIS**

The final diagnosis for both cases was B3GALNT2 gene mutation-related α-DGP. Case 2 was also diagnosed with a giant arachnoid cyst.
TREATMENT

Both cases received coenzyme Q10 and levocarnitine. The giant arachnoid cyst in case 2 was resected.

OUTCOME AND FOLLOW-UP

Case 1

The patient could pronounce 2- to 3-syllable words, and his walking posture was improved over the course of the follow-up.

Case 2

At the latest follow-up visit, the child’s walking posture was improved compared to before, although language development did not significantly improve. He recovered well after resection of the giant arachnoid cyst, and the meningocele gradually self-healed.

A total of 21 articles were found using the search key word “B3GALNT2” on the PubMed website, of which five articles were related to human CMD. The first article was published in 2013. The five articles included four pedigrees and seven sporadic patients. A total of 21 patients were reported, including the two patients in the present case report. Tables 1 and 2 present a summary of all these cases: (1) The nervous system was involved in all cases, and cognitive and motor developmental disorders were the most commonly encountered clinical symptoms (100%), followed by seizures (52.6%) and low muscle tension (64.3%); (2) Abnormal head MRI may be manifested as periventricular white matter lesion (81.3%), neuronal migration disorder (68.8%), brainstem and/or cerebellar dysplasia (56.3%), cerebellar cortical cyst (50%), and severe hydrocephalus (31.25%); (3) Muscle involvement was seen in 63.2% of patients, and the increased creatinase level ranged from 300 to 1740 U/L, which was even higher in patients with a more severe condition; and (4) Eye involvement occurred in 28.6% of the cases. The common symptoms were optic nerve atrophy and eye fissure deformities. In severe cases, glaucoma, cataract, and even blindness could be observed.
Table 1 Summary of clinical manifestations of 21 children reported in the literature and in this study since 2013

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<th>Source in the order of report time</th>
<th>Serial number</th>
<th>Gender</th>
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<th>Epilepsy (9, 52.6%)</th>
<th>Low muscle tension (11, 64.3%)</th>
<th>Optic nerve atrophy</th>
<th>Palpebral fissure deformity</th>
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<tr>
<td>2018 AI et al [14]</td>
<td>Sporadic 21</td>
<td>Male</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (952)</td>
</tr>
</tbody>
</table>

*+* indicates presence of the manifestation; *–* indicates absence of the manifestation; and *“NA”* indicates that results were not obtained; Blo: Bilateral lens opacities; Reg: Right-sided congenital glaucoma; Lc: Left-sided microphthalmia and cataract.

**DISCUSSION**

This study reports two cases of CMD caused by B3GALNT2 gene mutation. Both cases had an abnormal gait and significant delays in language development. Head MRI was performed for both children and revealed the presence of white matter lesions in the brain. The present report suggested that the cognitive language retardation and white matter lesions in CMD were caused by B3GALNT2 gene mutation. New symptoms of meningocele and giant arachnoid cyst were also encountered in one of our cases. To our knowledge, this is the first study to use electrophysiological examination of the
Table 2 Summary of the examinations of 21 children reported in the literature and in this study since 2013

<table>
<thead>
<tr>
<th>Source in the order of report time</th>
<th>Serial number</th>
<th>Head MRI</th>
<th>White matter lesions (13, 81.3%)</th>
<th>Cerebellar cortical cyst (8, 50%)</th>
<th>Neuronal migration disorder (polymicrogyria and cobblestone-like no gyrus) (11, 68.8%)</th>
<th>Brain stem and/or cerebellum dysplasia (9, 56.3%)</th>
<th>Mutant genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sporadic 2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>c.51_73dup; p.525Cfs*38 (homozygous)</td>
</tr>
<tr>
<td></td>
<td>Sporadic 3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>c.726_727del (p.V243Efs<em>2); c.822_823dup (p.I276fs</em>26)</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>c.308_309del (p.V103Gfs*10); c.755T&gt;G (p.V252E)</td>
</tr>
<tr>
<td>Pedigree 1</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Sporadic 6</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>c.802G&gt;A (homozygous) (p.V268M)</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>c.1423C&gt;T (homozygous) (p.Gln475*)</td>
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<tr>
<td>2017 Ho et al[12]</td>
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<tr>
<td></td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>11</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Pedigree 4</td>
<td>14</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>c.979C&gt;A (homozygous) (p.D327N)</td>
</tr>
<tr>
<td></td>
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<td>NA</td>
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<td>NA</td>
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<td>NA</td>
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<tr>
<td>This report</td>
<td>Sporadic 20</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>c.1068dup T (p.D335T_D338delinsX); c.40G&gt;C (p.G14R)</td>
</tr>
<tr>
<td>Sporadic 21</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>c.261-2A&gt;G (splicing); c.979C&gt;A (p.D327N)</td>
</tr>
</tbody>
</table>

“+” indicates presence of the manifestation; “-” indicates absence of the manifestation; and “NA” indicates that results were not obtained; MRI: Magnetic resonance imaging.
audiovisual conduction pathway in children with this type of gene mutation. The findings suggest that even if audiovisual function was normal, the evoked audiovisual potential should be examined to investigate for potential abnormalities. The white matter lesions in case 2 were gradually absorbed and improved with age. He recovered well after surgical resection of the giant arachnoid cyst, and the meningocele gradually self-healed. These results suggested that some of the symptoms associated with the gene mutation might have a tendency to self-heal, which was consistent with the report of Hedberg et al.[11]. Further studies are required to determine whether this is related to the peak age of gene expression. Both of the present cases had compound heterozygous mutations, the gene mutation sites were from their parents, and the mutations were compatible with autosomal recessive inheritance.

Case 1 had two compound heterozygous mutations in the B3GALNT2 gene; one of them was c.1068dupT (p.D357_D358delinsX), which was a nonsense (zero-effect) mutation that may lead to loss of gene function. The frequency in the normal population database was 0.00010, indicating that this was a low-frequency variation (PM2). The bioinformatics protein function prediction software packages SIFT, PolyPhen_2, and REVEL all predicted it as unknown. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, the variation was preliminarily determined as a suspected pathogenic variation. The other was c.40G>C (p.G14R), which was a missense mutation. It was (-) in the normal population database, which indicated a low-frequency variation (PM2). It existed in trans with another pathogenic variation (PM3). SIFT, PolyPhen_2, and REVEL predicted it as harmful, harmful, and benign, respectively. According to the ACMG guidelines, the variation was preliminarily determined as being of unknown clinical significance. In case 2, there were two compound heterozygous mutations in the B3GALNT2 gene: One was c.261-2A>G, which was located in the classical splicing site (± 1, 2). It was likely to affect the normal splicing of mRNA and lead to protein truncation. The frequency in the normal population database was 0.00004, which showed that this was a low-frequency variation (PM2). According to the ACMG guidelines, this variation was preliminarily determined to be a suspected pathogenic variation (PS1). The other site was c.979G>A (p.D327N), which was a known PS1 in the Human Gene Mutation Database, and the frequency of variation in the normal population was 0.00003 (PM2). In the transposition, the suspected pathogenicity variation c.261-2A>G (PM3) was detected. SIFT, PolyPhen2, and Mutation Taster all predicted that the mutation was harmful (PP3). According to the ACMG guidelines, the variation was preliminarily determined to be a suspected PS1. According to the clinical symptoms and gene results, both children were diagnosed as having CMD caused by the B3GALNT2 gene mutation. Each child had one missense mutation, and each had a mutation leading to protein truncation. Case 1 had a frameshift mutation leading to protein truncation, whereas case 2 had a splicing mutation leading to protein truncation, suggesting that truncation mutation may be related to a severe clinical phenotype.

In this study, data from two hospitalized patients and data of 19 patients obtained from a search of the previous literature were collected, yielding data from a total of 21 patients with α-DGP caused by B3GALNT2 gene mutation. Clinical manifestations included common symptoms such as nervous system, muscular dystrophy, eye development malformation, and rare symptoms such as sensorineural deafness. Moreover, 100% of the 21 patients had nervous system involvement, and delayed cognitive and motor development. In severe cases, patients had no cognitive and motor development, and most cognitive disorders included language retardation. Patients with a mild lesion on MRI only had periventricular white matter lesions, while patients with severe lesions seen on MRI had brainstem and/or cerebellar dysplasia and neuronal migration disorders. Among the 21 patients, 63.2% had elevated creatinase that was mostly between 300 and 1740 U/L, and 26.6% of the patients had eye involvement. The common symptoms were optic nerve atrophy and eye fissure deformities (excessive small or large). Patients with extensive nervous system and muscle involvement had severe ocular symptoms such as glaucoma, cataracts, and even blindness.

There were 12 pairs of alleles in 21 patients involving 15 Loci. Among them, c.979G>A (p.D327N) appeared three times, and c.822_823dup (p.L276fs*26) appeared twice, suggesting that the above two sites may be focal mutations. It was further speculated that the severity of the B3GALNT2 gene mutation was still related to its mutation type and site. All four patients with biallelic truncated mutations in sporadic cases 2, 3, 7, and 19 had neuronal migration disorders (polymicrogyria or cobblestone-like surface with no gyri). Pedigree 1 and sporadic cases 8 and 21 had severe truncated mutations of a single allele (the sites were all before the 103th amino acid), and they also had neuronal migration disorders or developmental brain malformations.
Pedigree 3 and sporadic case 20 had mild truncated mutations of a single allele (the sites were all after the 276th amino acid), and they had only white matter involvement. Other forms of mutation had relatively mild symptoms. The above results suggested that patients with a truncated mutation of the gene located more at the front of the mutation site had more severe symptoms. The missense mutation was often associated with relatively mild symptoms. Sporadic type 1 is special, in that it is a compound heterozygous missense mutation that is associated with severe symptoms such as polymicrogyria malformation and eye and muscle involvement. Further studies are required to study the mechanism underlying sporadic type 1 mutation.

CONCLUSION
This article reports two cases of CMD caused by B3GALNT2 gene mutation. Each case carried a truncated mutation and a missense mutation, while three of the four variants have not been previously reported; thus, our findings in this case report expand the gene database. Case 2 had two new phenotypes: Giant arachnoid cyst, which recovered well after surgical resection, and meningocoele after birth, which gradually self-healed. These data broadened the phenotypic spectrum of the gene. Clinical characteristics of the gene mutation are summarized and analyzed through a literature search, providing clues for clinicians to diagnose and treat this gene mutation. Due to the limitation of a small number of patients, absence of functional verification, and lack of evidence at the cellular level, the clinical characteristics and pathogenic mechanisms of this mutation were not completely clarified. Further studies at a clinical level are required to support our findings.

REFERENCES
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Novel α-galactosidase A gene mutation in a Chinese Fabry disease family: A case report

An-Yi Fu, Qi-Zhi Jin, Ya-Xun Sun

Abstract

BACKGROUND
Fabry disease (FD) is a rare X-linked lysosomal storage disease caused by a deficiency of the enzyme α-galactosidase A.

CASE SUMMARY
Herein, we analyzed a four-generation Chinese family. The proband is a 57-year-old woman who was diagnosed with left ventricular hypertrophy and atrial fibrillation 7 years ago. Echocardiography showed an end-diastolic diameter of the interventricular septum of 19.9 mm, left ventricular end-diastolic diameter of 63.1 mm, and moderate-to-severe mitral regurgitation. Cardiac magnetic resonance indicated an enlarged left heart and right atrium, decreased left ventricular systolic and diastolic function, a left ventricular ejection fraction of 20%, and thickening of the left ventricular septum. In March 2019, gene and enzyme activity tests confirmed the diagnosis of FD. Her son was diagnosed with FD after gene and enzyme activity assay, and was prescribed agalsidase-β for enzyme replacement therapy in July 2020. Two sisters of the proband were also diagnosed with FD by genetic testing. All authors read and approved the final manuscript.

CONCLUSION
A novel mutation was identified in a Chinese family with FD, in which the male patient had a low level of enzyme activity, early-onset, and severe organ involvement. Comprehensive analysis of clinical phenotype genetic testing and enzyme activity testing helped in the diagnosis and treatment of this FD family.
INTRODUCTION

Fabry disease (FD, OMIM 301500) is a progressive, X-linked inherited disorder of glycosphingolipid metabolism caused by a deficiency of α-galactosidase A (α-GAL) activity[1]. The prevalence of FD was once believed to be very rare, occurring approximately in 1:50000 patients[2]. Substrates of this lysosomal enzyme accumulate, resulting in cellular dysfunction in multiple organs. FD is commonly known as a silent disease that appears later in life and could be easily misdiagnosed. Patients lacking α-GAL activity exhibit a 10-20 year shortened life span: Male patients with FD have a median survival of 57 years, and the median female survival is 72 years[3].

Classically affected FD males with no residual α-GAL activity, may display neurological (pain and acroparesthesia), cutaneous (angiokeratoma), renal (proteinuria and kidney failure), cardiovascular (cardiomyopathy, arrhythmia, and valvulopathy), cochleovestibular, and cerebrovascular (transient ischemic attacks and strokes) signs while heterozygous females have symptoms ranging from mild to severe[1]. Male patients are usually severely affected, while the clinical presentation in female patients may be more variable[4].

There are currently 967 known GLA mutations, including 671 missense/nonsense mutations, listed in the Human Gene Mutation Database[5]. The type of amino acid exchange domain in the α-GAL 3D-structure determines the disease severity and temporal course of clinical presentation. Patients with active site or buried mutations showed a severe phenotype with multi-organ involvement and early disease manifestation. Patients with certain mutations showed a milder phenotype with less organ impairment and later disease onset[6]. In male patients, the α-GAL enzyme activity is often significantly decreased, while about a third of female patients have enzymes within the normal range.

Enzyme replacement therapy (ERT) and chaperone therapy are currently considered the main targeted treatments for FD. As two representative drugs of enzyme replacement therapy, agalsidase-a and agalsidase-β have been shown to be clinically effective for patients with FD; yet, these are very expensive (approximately $200000 per patient annually in China). Some patients with amenable GLA mutations have residual activity in α-GAL. In these patients, small molecular chaperones could promote enzyme stability and are clinically effective.

In the present study, we describe a novel frameshift mutation in GLA and different α-GAL enzymatic activity in a Chinese family in which both male and female members presented with left ventricular hypertrophy and atrial fibrillation.
CASE PRESENTATION

Chief complaints
The proband was a 57-year-old woman who has experienced paroxysmal chest tightness and shortness of breath for 7 years.

History of present illness
A 57-year-old woman was diagnosed with left ventricular hypertrophy and atrial fibrillation 7 years ago. Echocardiography showed an end-diastolic diameter of the interventricular septum of 19.9 mm, left ventricular end-diastolic diameter of 63.1 mm, moderate-to-severe mitral regurgitation, and a left ventricular ejection fraction (LVEF) of 45%. Cardiac magnetic resonance (CMR) indicated an enlarged left heart and right atrium, decreased left ventricular systolic and diastolic function, an LVEF of about 20%, and thickening of the left ventricular septum. In March 2019, gene and enzyme activity tests confirmed the diagnosis of FD. Her son was diagnosed with FD after gene and enzyme activity assay, and was prescribed agalsidase-β for enzyme replacement therapy in July 2020. Two sisters of the proband were also diagnosed with FD by genetic testing. Both of them had a history of atrial fibrillation.

History of past illness
The proband had a history of hypertension for more than 20 years.

Personal and family history
The proband did not have any significant personal history. Her father died of cardiomyopathy, while her mother died of colon cancer. Her son and two sisters were diagnosed with FD.

Physical examination
At the last admission, the proband’s blood pressure was 99/65 mmHg. She was conscious, but presented with an appearance of weakness. Her tongue stuck out to the right. Her jugular vein was filling. Rales could be heard widely over both lung fields. The heart rate was 96 bpm. The intensity of the first heart sound was unequal. Systolic murmur (III/6) was identified in the apex of the heart. There was no edema in her lower limbs.

Laboratory examinations
Enzymatic measurement of α-GLA: The enzyme activity of α-GLA was reduced to only 1.0 nmol/h/mg protein in the son of the proband, while a normal range was observed in all other family members (Table 1).

Clinical and biochemical studies: All of the patients in this family, whether hemizygous or heterozygote, had left ventricular hypertrophy. All female family members (II3, II5, and II7) had atrial fibrillation, except the propositus granddaughter (IV-1), who did not undergo inspection due to being only 2 years old. The levels of troponin I were all increased, and the ejection fraction was generally lower in female than male patients (III-2). Female heterozygotes suffered more severe cardiovascular damage while the kidney damage occurred earlier in males than in female family members. Stroke was more common in women, possibly due to atrial fibrillation and older age. At the same time, cutaneous and neuralgia manifestations were present in males of the same lineage, suggesting a wider range of glycosphingolipid deposition in the hemizygote and more involved organs (Table 1, Figure 1-5).

Gene expression: (1) Whole exome sequencing results: In this family, we found a frameshift mutation (348delG:p.G116fs) according to the guidelines for mutation nomenclature recommended by the Human Genome Variation Society (www.hgvs.org/mutnomen). The mutation occurred in the guanine deletion at position 348 of the GLA gene, resulting in a series of changes in the code of the 116th amino acid and its downstream (Figure 6). This mutation causes a change in the GLA protein domain (Figure 7)[7,8], and (2) Conventional sequencing results: Sanger sequencing confirmed that the mutation occurred due to the guanine deletion in exon 2. Figure 8A shows the gene sequencing results of the proband (II-3). Adenine takes the place of guanine, thus causing a rearrangement of the subsequent amino acid sequence. The son of the proband (III-2) and the two sisters showed the same mutation (Figure 8B and C).
### Table 1 Clinical features

<table>
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<tr>
<th>Variables</th>
<th>II-3</th>
<th>II-5</th>
<th>II-7</th>
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<tr>
<td>Sex</td>
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<td>Male</td>
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ECG: Electrocardiogram; AF: Atrial fibrillation; SR: Sinus rhythm; LVPWD: Left ventricular posterior wall dimensions; IVST: Interventricular septal diastolic thickness; EF: Ejection fraction; hsTNI: High-sensitivity cardiac troponin I; NT-proBNP: N terminal pro B type natriuretic peptide.

Imaging examinations

CMR showed patchy enhancement of interventricular septum and left ventricular anterior wall hypertrophy. Delayed enhancement suggested the formation of a large number of fibrous scars in left ventricular hypertrophy (Figure 3).

FINAL DIAGNOSIS

The final diagnosis of the presented case was FD.

TREATMENT

The proband underwent atrial fibrillation radiofrequency ablation 7 years ago. Three
Fu A et al. A novel α-galactosidase A gene mutation

Figure 1 Family tree. The four-generation pedigree with the mutation p.G116fs in the GLA gene is shown. Roman numerals indicate generations; individuals within a generation are numbered from left to right. The proband (II-3) is denoted with an arrow. Oblique lines indicate patients who are already dead. Filled squares and circles indicate male and female patients, respectively. Black color represents patients with Fabry disease (FD), which were confirmed by genetic analysis. The proband’s father (I-2) suffered from cardiovascular disease and stroke. The proband (II-3) died of end-stage heart failure. The proband’s son (III-2) started enzyme replacement therapy in July 2020. The proband’s granddaughter (IV-1) was born before her father (III-2) was diagnosed with FD.

Figure 2 Electrocardiogram of the patient indicating atrial fibrillation rhythm. Pathological Q waves in inferior and lateral leads, and T wave inversion are shown.

years ago, she was given prednisone and tacrolimus because of edema and proteinuria. The son of the proband was given enzyme replacement therapy with agalsidase-β 65 mg (biweekly, intramuscularly) starting from July, 2020.

OUTCOME AND FOLLOW-UP

After 6 mo of ERT, serum creatinine in the son of the proband had no significant decrease. The pain in his skin was markedly relieved.

DISCUSSION

FD (MIM301500) was first described in 1898 by William Anderson and Johannes Fabry. After 65 years, Sweeley and Klionsky found an accumulation of a glycosphingolipid, globotriaosylceramide (Gb3), in patients with FD[9]. The incidence of FD in male newborns is 1/110000 to 1/4 million[10]. The main international databases for FD is the Fabry Outcome Survey in Europe, which currently lists 967 different GLA mutations in the human gene mutation database, including 671 missense/nonsense mutations[5].
A novel α-galactosidase A gene mutation

The GLA gene encoding α-Gal A is located on Xq22.1, with 7 exons and 12 kb in length[11]. Herein, we report a novel frameshift mutation in the GLA gene in four members of a family with classical FD phenotype, with early-onset signs in affected men. Genotype-phenotype correlation in FD is challenging. Many GLA mutations are family-specific; in some families, there are quite marked phenotype variations. In contrast, the disease manifestation may vary within patients carrying the same mutation[12]. Garman et al[13] discovered two types of α-GLA mutations that are responsible for the disease progression: Mutations near the active sites and mutations of buried residues far from the active sites. Mutations near the active sites have a higher pathogenic frequency and severe clinical phenotype, while mutations far from the enzyme active sites are relatively mild[13]. The structure of α-GLA is a homodimeric glycoprotein with each monomer composed of two domains. The first domain contains the active site and extends from residues 32 to 330, and the second domain is comprised of residues 331-429, burying much surface area within one monomer. Rickert et al[6] found that patients with active site or buried mutations showed a severe phenotype with multi-organ involvement and early disease manifestation. Patients with other mutations had a milder phenotype with less organ impairment and later disease onset. In addition, the α-GalA activity was lower in patients with active site or buried mutations than in those with other mutations while lyso-Gb3 levels were higher.
In the proband of our study, a frameshift mutation (348delG:p.G116fs) occurred due to the guanine deletion at position 348 of the GLA gene, resulting in a series of changes in the code of the 116th amino acid and its downstream, so that the GLA peptide chain was transformed into a completely different peptide sequence. Enzyme activity tests confirmed that the enzyme activity of the female members of the family was
Figure 8 Conventional sequencing results for four of the family members. (A) The proband (II-3), (B) Proband’s son (III-2), and (C) Proband’s two sisters (II-5, II-7) showing the same mutation; (D) Proband’s elder brother (II-1), eldest nephew (III-4), and the third niece (III-6) showing no mutant gene.

moderately decreased and that of the male members was extremely decreased.

The α-Gal activity in female subjects who carry a heterozygous pathogenic variant in the GLA gene, is subject to X chromosome inactivation, typically random, cell-dependent, often nonuniform across the silenced chromosome[14]. Likewise, it complicates correlations among the genetic variants, functional data, and organ involvement. Nevertheless, as a group, α-Gal activity is higher in female subjects with pathogenic GLA variants than in male subjects’ corresponding values. Consequently, up to one-third of X-chromosomal genes are expressed from both the active and inactive X chromosomes (Xa and Xi, respectively) in female cells, with the degree of “escape” from inactivation varying between genes and individuals[15], posing significant diagnostic challenges. In this study, the proband was a heterozygote but had classical characteristics such as heart failure and renal failure. Her sisters present nonclassical characteristics, whose manifestations are limited to cardiac involvement.

Clinically, FD diagnosis is primarily based on the clinical manifestation of multiple systems involving the brain, kidney, heart, and peripheral nerves, and also based on the comprehensive interrogation of family history. Patients may seek help from multiple medical specialists before a correct diagnosis is made, resulting in delayed treatment initiation[16]. Cardiac involvement is characterized by progressive cardiac hypertrophy, fibrosis, arrhythmias, heart failure, and sudden cardiac death. As myocardial fibrosis develops, the posterior and inferior LV wall can thin and become hypokinetic or akinetic[17]. Thinning of the LV posterior wall is a feature of FD related cardiomyopathy in the late stage. Further laboratory tests may include GLA activity test, pathological biopsy, and gene test, which are also considered the gold standard for diagnosis. Also, microscopic formation of typical onion-like osmiophilic inclusion bodies (such as myeloid corpuscle and zebra-corpuscle) in glomerular and tubular epithelial cell lysosomes is a typical pathological feature of lysosomal glycolipid aggregation, which is of great value in disease diagnosis. Early detection and treatment are crucial for achieving the best outcome.

Genetic testing, performed by whole-exome sequencing, and targeted analysis of the GLA gene could confirm clinical diagnosis. Nevertheless, the findings of a missense variant should not be considered an unequivocal validation of the diagnosis. Recently, a study examined 115 Japanese families with FD. No pathogenic mutations were identified in six families (5.2%). In total, 73 different disease-causing mutations were identified: 41 missense (56.2%), 11 nonsense (15.1%), four in-frame deletion (5.5%), 10 frameshift (13.7%), six splice site (8.2%), and one intronic (1.4%)[18]. As a result, many GLA variants of unknown significance (VOS) were identified. Therefore, the diagnosis
of FD should not over-rely on genetic testing, and both clinical manifestations and family history should be considered comprehensively[19].

The treatment of FD relies on specific and non-specific treatments. Non-specific treatment is used to deal with the involvement of various organs. In this family, all the women (II3, II5, and II7) developed atrial fibrillation and underwent radical ablation, and in one case (II5) left atrial appendage occlusion was performed. Specific treatments include ERT, which is currently approved to be marketed as a galactosidase-α and a galactosidase-β. In this study, the son of the proband had started ERT treatment, and the effect will be followed closely. The European Union also approved the molecular chaperone migalastat in 2016 for the long-term treatment of specific mutated FD in patients over the age of 16 years, which could increase endogenous α-Gal A activity in a prospective observational multicenter study[20]. Pre-treatment clinical assessment, continuous clinical monitoring, and establishment and improvement of disease database should be made during treatment.

Since FD is an X-linked genetic disorder, genetic counseling and prenatal diagnosis should also be performed for all patients. Here we report a female patient who had a son who was also diagnosed with FD. The son had a daughter and he definitely passed the abnormal X chromosome to her (with 1 abnormal X chromosome and 1 normal X chromosome). However, the daughter has a heterozygous GLA allele, which may have relatively mild clinical manifestations and still need to be followed closely.

CONCLUSION

In summary, our findings suggest that the novel mutation 348delG:p.G116fs may be associated with classical manifestations of FD. These new data can be helpful in the diagnosis of FD and increase clinical and molecular knowledge about the correlations between mutations in the GLA gene, enzyme activity, and clinical phenotype of FD.

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Cervical spondylotic myelopathy with syringomyelia presenting as hip Charcot neuroarthropathy: A case report and review of literature

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Author contributions: Lu Y and Ye GY were the patient's orthopedist, reviewed the literature and contributed to manuscript drafting; Shi CY reviewed the literature and contributed to manuscript drafting; Xiang JY, Li JB, and Gu HC analyzed and interpreted the imaging findings; Liu C performed the psychiatric analyses and interpretation and contributed to manuscript drafting; Lu Y and Ye GY were responsible for the revision of the manuscript for important intellectual content; all authors issued final approval for the version to be submitted.

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Abstract

BACKGROUND
Charcot neuroarthropathy (CN) is a systemic disease characterized by progressive bone loss and destruction, which is usually closely related to diabetes, HIV, etc. However, CN caused by syringomyelia accounts for only 5% of CN cases; the shoulder and elbow are most often involved, and the hip joint is rarely affected. As a rare factor, cervical spondylotic myelopathy (CSM) can be associated with syringomyelia, which is scarcely reported in the literature. Here, we present the first case report to date of CN of the hip caused by syringomyelia secondary to CSM.

CASE SUMMARY
We describe a 76-year-old male patient who was diagnosed with CSM due to neck pain and weakness of limbs 16 years ago. Four years ago, he noticed recurrent swelling of the right hip with pain and was diagnosed with degenerative arthritis. Recently, however, his symptoms gradually worsened, and because of progressive pain, destabilization and weakness of the right hip, he was admitted to our hospital. Through systematic physical, radiographic and laboratory examinations, we finally reached a diagnosis: CN of the right hip associated with syringomyelia secondary to CSM. After comprehensive evaluation of the patient's condition, we performed right total hip arthroplasty. During the follow-up, the patient felt well clinically and could walk independently with a knee brace.

CONCLUSION
We suggest a possible etiological association between CSM and syringomyelia,
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which may reflect a potential pathogenesis of CN. We encourage clinicians to actively carry out a detailed medical history and comprehensive physical and imaging examinations in patients with joint lesions, especially chronic shoulder neck pain, to rule out the possibility of this association, which plays a crucial role in the early diagnosis of CN. Arthroplasty may no longer be an absolute contraindication to surgical treatment of CN. Reasonable selection of the surgical strategy can markedly improve the clinical symptoms and quality of life of patients.

Key Words: Cervical spondylotic myelopathy; Syringomyelia; Hip; Charcot neuroarthropathy; Case report

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Core Tip: We report an unprecedented case of Charcot neuroarthropathy (CN) of the right hip associated with syringomyelia secondary to cervical spondylotic myelopathy (CSM). After comprehensive evaluation of the patient's condition, we performed right total hip arthroplasty. During the follow-up, the patient felt well clinically and could walk independently with a knee brace. We suggest a potential etiological association between CN and CSM complicated by syringomyelia, which reminds clinicians to actively carry out comprehensive physical and imaging examinations in patients with joint lesions, especially chronic shoulder neck pain, to rule out the possibility of this association. Detailed medical history acquisition and comprehensive examination play a crucial role in the early diagnosis of CN. Arthroplasty may no longer be an absolute contraindication to surgical treatment of CN. Reasonable selection of the surgical strategy can markedly improve the clinical symptoms and quality of life of patients.

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INTRODUCTION

Charcot neuroarthropathy (CN), or Charcot joint, is a systemic disease characterized by progressive bone loss and destruction of the joints and/or spine usually accompanied by dysfunctions of sensitive and autonomic nerves and is reported to be closely associated with diabetes mellitus, HIV, chronic alcoholism, end-stage renal disease, gigantism, intra-articular steroid injections, peripheral neuropathy, meningomyelocele, multiple sclerosis, myelodysplasia, leprosy, amyloidosis, and congenital pain insensitivity[1-5]. However, CN caused by syringomyelia accounts for approximately 5% of CN cases[4]. A retrospective study showed that syringomyelia-associated CN most often involves the shoulder (17 of 33, 50.0%) and elbow (17 of 33, 50.0%), while the hip joint is rarely involved (1 of 33, 2.94%)[5]. To date, a few cases of CN associated with syringomyelia have been reported, but a case report of CN of the hip caused by syringomyelia secondary to cervical spondylotic myelopathy (CSM) has never been published. Therefore, the purpose of this report is to discuss this rare pathological association and to provide new insights into the surgical treatment of CN.

CASE PRESENTATION

Chief complaints
A 76-year-old male patient presented to the Department of Orthopedics of our hospital complaining of worsening progressive right hip pain, a limp when walking and weakness of the right hip. He was admitted to our hospital.
History of present illness
The patient’s symptoms started four years ago with recurrent swelling of the right hip with mild pain, which had worsened in the last 48 h.

History of past illness
The patient initially presented with neck pain and weakness in all four limbs 16 years ago and was diagnosed with cervical spondylosis, which was treated conservatively.

Personal and family history
The patient had an unremarkable personal and family history.

Physical examination
The patient reported mild pain with flexion and extension of the cervical spine and activity limitation. Apparent swelling was observed in the left hip, and more than 110 mL of clear yellowish joint fluid was extracted. The active range of motion of the right hip was recorded as follows: flexion 90°, abduction 30°, internal rotation 20°, and external rotation 35°, with pain in all directions. The visual analog score was 6/10 points, and the Harris hip score (HHS) was 56 points. Neurologically, the sensation of pain and temperature in the upper and lower extremities was decreased, and proprioception and position sensation were normal. A pathologic reflex was not elicited. Both upper limbs and the right lower limb exhibited weakness with a muscle strength of 4/5, the muscle strength of the left lower limb was normal at 5/5, the right abductor had a muscle strength score of 5/5, and the modified Japanese Orthopedic Association score for CSM was 9/17 points. The bone mineral density (BMD) of the hip was 0.45 g/cm², and the T score was -2.9. Laboratory results were nonspecific.

Laboratory examinations
Blood biochemistry and urine analyses were normal. Electrocardiogram, chest X-ray and arterial blood gases were also normal. The BMD of the hip was 0.45 g/cm², and the T score was -2.9.

Imaging examinations
Magnetic resonance imaging (MRI) of the cervical spine showed cervical syringomyelia at C4, cervical disc herniation and spinal canal stenosis from the C3 to the C7 levels (Figure 1). Cervical computed tomography (CT) revealed destruction of the vertebral body at C4 and C5–7 vertebral body assimilation (Figure 2). Three-dimensional CT reconstruction, CT scans, and X-rays of the right hip joint showed joint space loss, articular surface collapse, and destructive changes in the acetabulum and femoral head (Figure 3A–C). T2W1 MRI of the right hip showed articular cartilage loss, degeneration of the joint, disordered soft tissue, and apparent joint fluid (Figure 3D).

FINAL DIAGNOSIS
The final diagnosis was CN of the hip associated with syringomyelia and CSM.
Figure 2 Computed tomography of the cervical spine. A: C4 vertebral body destruction; B: C5-7 vertebral body assimilation.

Figure 3 Right hip joint. A: Computed tomography (CT) three-dimensional reconstruction; B: CT; C: X-ray. Joint space loss, articular surface collapse, and destructive acetabulum and femoral head changes; D: Right hip magnetic resonance imaging, T2W1. Articular cartilage loss, joint degeneration, soft tissue disorder, and obvious joint fluid were observed.

TREATMENT

We performed total hip arthroplasty (THA) on the right hip (Figure 4) with a cement-type Lubinus anti-dislocation acetabular cup, D = 50 mm (Walde mar Link, Germany). We conducted pathological examinations on specimens of the right femoral head and synovial tissue collected intraoperatively. Tissue degeneration, calcification, small vascular proliferation and a multicore giant cell response were observed in some regions of femoral head tissue sections, and capillary hyperplasia with acute and chronic inflammatory cell infiltration, local tissue denaturation, and calcification were observed in synovial tissue sections (Figure 5). The pathologist considered that the pathological changes were consistent with CN.
Figure 4 Right hip X-ray film after total hip arthroplasty.

Figure 5 Pathological results. A: Right femoral head tissue sections during surgery. Tissue degeneration, calcification, small vascular proliferation and a multicore giant cell response in some regions; B: Synovial tissue sections of the right hip joint during the operation. Capillary hyperplasia was accompanied by infiltration of acute chronic inflammatory cells, local region tissue denaturation and calcification.

OUTCOME AND FOLLOW-UP

At the 6-mo follow-up, the patient felt clinically well, with no pain, joint fluid collection, or hip dislocation, and could walk independently with a knee brace. The right lower limb had a muscle strength score of 4/5, and the HHS score was 84 points. The 6-mo follow-up X-rays revealed that the acetabular inclination and anteversion were well maintained, without clinical manifestations of implant loosening compared with postoperative observations (Figure 6).

DISCUSSION

CN is considered a multifactorial disease that may lead to progressive bone resorption and destruction of the joints and further develop into joint instability, deformity, and structural collapse with or without pain[6,7]. The possible pathogenesis includes the following: (1) Inflammatory cascading reactions caused by excessive expression of proinflammatory cytokines lead to an increase in osteoclastogenesis; (2) Abnormal neurovascular reflexes at joints promote osteoclast activation, leading to osteopenia;
and (3) Painful sensory neuropathy and local temperature increases lead to the accumulation of microtrauma, resulting in joint dislocation and collapse[8,9]. Diabetes is considered the most common cause of CN and usually affects the foot and ankle joints[10]. However, syringomyelia-associated CN accounts for only approximately 5% of CN cases and easily causes upper limb CN[4,11], which most frequently involves the shoulder and elbow joints[3,12]. Only a few studies have reported that CN of the hip and knee joint is a rare complication of lumbar syringomyelia caused by epidural anesthetisa[13,14].

Syringomyelia is a chronic progressive condition in which cystic tubular cavitation of the spinal cord is commonly caused by disorders of normal cerebrospinal fluid (CSF) flow dynamics. The possible mechanisms of syringomyelia include: (1) CSF transfer from the fourth ventricle to the central canal[15,16]; and (2) CSF filtration through medullary channels through perivascular and interstitial metastasis[17]. While the etiology of syringomyelia is not completely understood, Chiari 1 malformation is generally considered the most significant etiology. Other possible causes include Chiari 2 malformation, myelomeningocele, tethered cord syndrome, hydrocephalus, infection, inflammatory conditions, trauma, extramedullary tumors, arachnoid cysts and spinal canal stenosis[18,19]. As a rare factor, CSM associated with syringomyelia is scarcely reported in the literature[20]. Four potential pathologic mechanisms have been considered for cervical syringomyelia secondary to CSM: Ischemia causing degeneration, microtrauma caused by persistent oppression, hydrodynamic changes in CSF, and the dynamic pincer effect[21]. In addition, enhanced blood flow signals in compressed segments of the cervical spinal cord suggest that dyskinesia of CSF movement may play a role in the process of lesion development[22,23]. The typical clinical manifestation of syringomyelia is weakening or loss of pain and temperature sensation, but proprioception and position sensation are maintained, which is known as "free anesthesia", with muscular atrophy and undernourishment at the level of lesions. With loss of the normal protective reflex, joints may suffer from unrecognized trauma or repeated injury, eventually leading to the occurrence of CN.

In this case, the patient had no significant previous medical history except for CSM diagnosed 16 years prior. According to the MRI results, we found that the patient had CSM and cervical syringomyelia at C4, without other special lesions. Therefore, we suspected that cervical syringomyelia at C4 was associated with long-term CSM, although this is an extremely rare pathological association. Meanwhile, a typical X-ray showed severe hip deterioration, bone loss, and destruction. Interestingly, we carried out systemic physical, laboratory and imaging examinations on the patient, which confirmed that the patient's blood glucose and laboratory results were normal, indicating no other systemic diseases. Therefore, we excluded the differential diagnoses of congenital pain insensitivity, infection and inflammatory joint disease. Finally, after discussion with neurologists and radiologists, the patient was diagnosed with CN of the right hip caused by cervical syringomyelia secondary to CSM.

Early CN is difficult to distinguish from other diseases due to a lack of characteristic clinical manifestations, typical X-ray findings, laboratory tests, and severe underlying neurological disease that can facilitate clinical diagnosis. The management goal for CN patients is early diagnosis and intervention to maintain joint and limb function and active treatment of primary underlying diseases. The surgical strategy for CN of the hip remains controversial.
In the past, THA was considered a contraindication in the absence of complete remission of the primary disease[24,25]. However, a recent study suggested that the clinical prognosis of patients undergoing primary THA for CN was significantly improved, including pain relief, mobility recovery, and joint stability enhancement. Notably, a higher risk of early complications, such as recurrent dislocation, femoral body loosening and periprosthetic fracture, is evident due to the loss of protective sensations and reflexes, osteopenia, and ligament and muscle weakness[26,27]. According to the study of Parvizi et al[28], in the largest series of TKA (non-THA) treatment for CN, the survival rate without mechanical failure within 8 years was 82.5%, the aseptic loosening rate was 5.3%, and the instability rate was 2.6%.

Therefore, adopting reasonable precautions to provide a fixed prosthesis and increase the stability of the hip joint is particularly important to avoid early complications, including: (1) The use of a larger diameter femoral head to reduce the risk of postoperative dislocation; (2) The use of a highly cross-linked polyethylene lining to reduce wear; (3) The use of 3D CT template software to produce femoral stems with appropriate anteverision and femoral offset; (4) The use of a CT navigation system to reproduce the preoperative plan as much as possible; (5) Judicious use of dual mobility constructs; and (6) Revision of the acetabular component and the use of multiple acetabular screws to enhance the firmness of the component[26,29,30]. In addition, we should adhere to conservative treatments during the perioperative period, such as weight-bearing, immobilization, passive stretching, physical therapy and nonsteroidal anti-inflammatory drugs[5].

In our case, the patient refused to undergo cervical decompression surgery. To relieve the pain in the hip joint and restore the motor function of the limbs, we performed right THA. In the preoperative evaluation, the patient suffered from osteoporosis and bone fragmentation and loss around the hip. We suspect that the mass and strength of bone around the hip joint cannot provide sufficient stability at the bone-implant interface if a biotype acetabular cup is adopted. Moreover, the cement-type prosthesis possesses a short curing time, and strong fixation is achieved immediately after curing. Two to three days postoperatively, the patient can begin to bear partial weight with support, which reduces perioperative complications. During the operation, to reduce the risk of dislocation, we used the Hardinge approach to preserve the external rotator muscle group, which can reduce hip joint stability impairment with close suturing of the joint tendon. The cement-type Lubinus anti-dislocation acetabular cup and a biologically fully coated biconical femoral stem were used to improve stability and promote bone ingrowth. After surgery, the patient’s symptoms improved, without complications such as recurrent dislocation, loosening of the femoral prosthesis, and fracture around the prosthesis.

CONCLUSION

We suggest a possible etiological association between CSM and syringomyelia, which may reflect a potential pathogenesis of CN. A differential diagnosis should be established for patients with joint swelling, pain and limited mobility, especially patients with chronic shoulder and neck pain. Imaging examinations should be actively performed to rule out the possibility of syringomyelia secondary to CSM. Therefore, the key to successful treatment of patients with CN is comprehensive medical history acquisition, examination and early diagnosis. In addition, arthroplasty may no longer be an absolute contraindication to surgical treatment of CN. Reasonable selection of the surgical strategy can markedly improve the clinical symptoms and quality of life of patients.

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Bullectomy used to treat a patient with pulmonary vesicles related to COVID-19: A case report

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Author contributions: Tang HX, Zhang L, and Wei YH conceived and designed the study; Wei YH and Li CS contributed to the literature search and data collection; Tian SF contributed to pathology examination and interpretation of the findings; Tang HX, Zhang L, Hu B, Zhou XF, and Lin J contributed to data interpretation; Tang HX, Zhang L, Mokadam NA, Zhu H, and Zhou XF contributed to the figures and writing of the report.

Informed consent statement: The patient has signed an informed consent. This study was reviewed and approved by the Medical Ethical Committee of Zhongnan Hospital of Wuhan University (Approval number 2020051).

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Abstract

BACKGROUND

The corona virus disease 2019 (COVID-19) has been a pandemic for more than one year and estimated to affect the whole world in the near future.

CASE SUMMARY

Here we reported that one COVID-19 patient with vesicles was treated by bullectomy. The patient’s perioperative laboratory tests were analyzed. The pathological findings of bullectomy were described and compared with those of common bulla cases.

CONCLUSION

This patient with vesicles underwent bullectomy and had a poor prognosis. He showed diffuse alveolar damage and extensive necrosis in bullectomy specimen.
We hope our report will be of interest for clinicians who will treat COVID-19 patients in the future.

**Key Words:** Corona Virus Disease 2019; Pulmonary vesicle; Surgical treatment; Case report

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**Core Tip:** We reported that one corona virus disease 2019 (COVID-19) patient with vesicles was treated by bullectomy. The patient’s perioperative laboratory tests were analyzed. The pathological findings of bullectomy were described and compared with those of common bulla cases. This patient with vesicles underwent bullectomy and had a poor prognosis. He showed diffuse alveolar damage and extensive necrosis in bullectomy specimen. We hope our report will be of interest for clinicians who will treat COVID-19 patients in the future.

**INTRODUCTION**

Pulmonary bullae are cavities more than one centimeter in diameter in the lung that form from structurally damaged lung tissue due to a variety of etiologies[1]. When nonoperative approaches for pulmonary bullae are ineffective, in some cases, surgical resection can be considered[2,3].

Corona virus disease 2019 (COVID-19) presents with a severe phenotype in as many as 26% of patients. Medical therapy is lacking, and many of these patients require intubation and even extracorporeal life support[4]. COVID-19 mainly attacks the lungs and other organs that express angiotensin-converting enzyme 2 receptors[5]. Patients with pneumonia who are infected with COVID-19 have been reported to develop pulmonary vesicles and tension pneumothorax with the use of ventilators. Pulmonary vesicles are defined as peripheral predominant consolidation patterns with internal round cystic changes[6]. Because of the high risk of health care worker transmission and the difficulty in performing an operation with full personal protective equipment, operations for patients infected with COVID-19 are quite difficult; thus, these operations are extremely rare. Recently, we performed a bullectomy under special circumstances as the last option to treat a patient.

**CASE PRESENTATION**

**Chief complaints**

Intermittent fever for 1 wk.

**History of present illness**

The patient suffered from chest tightness and chest pain due to infection with COVID-19 5 d ago. He urgently went to the local hospital to see a doctor. The perfect chest X-ray showed a right pneumothorax, and the patient’s dyspnea was progressively worsening. The local hospital gave an emergency tracheal intubation, connected to a ventilator to assist breathing, and transferred him to our intensive care unit for treatment.

**History of past illness**

Denies the history of diabetes, heart disease, etc. Denies the history of infectious diseases such as tuberculosis and hepatitis. Denies the history of food and drug
allergy. Denies the history of trauma surgery.

**Personal and family history**
Denies the family history of genetic disease.

**Physical examination**
T 38.3 °C, P 85 bpm, HR 20 bpm, diastolic blood pressure: 69 mmHg, systolic blood pressure: 116 mmHg, mentally clear, good spirits, no yellowing of skin and mucous membranes throughout the body, and superficial lymph nodes less than swollen. Pharyngeal is not congested, breath sounds in both lungs are clear, heart rhythm is uniform, no pathological murmurs are heard in each valve area. HR 85 bpm, the heart rhythm is uniform, no pathological murmur is heard in each valve area. Abdomen is soft, no tenderness and rebound pain, liver, spleen and ribs are not in reach, Murphy sign is negative, there is no percussion pain in the kidneys, no redness and swelling of the limbs and joints, and no edema of the lower limbs.

**Laboratory examinations**

**Imaging examinations**
The X-ray before bullectomy: Infection of both lungs, massive pneumothorax on the right side; The chest CT scan before bullectomy; Severe infection of both lungs, extensive bullae; The X-ray after bullectomy; The X-ray before bullectomy: Both lung infections, the right pneumothorax improved significantly.

**FINAL DIAGNOSIS**
Spontaneous pneumothorax, Respiratory failure, lung infection with COVID-19, Septic shock, ARDS.

**TREATMENT**
Received ECMO support, underwent a bullectomy.

**OUTCOME AND FOLLOW-UP**
This patient with vesicles underwent bullectomy and had a poor prognosis. He showed diffuse alveolar damage and extensive necrosis in bullectomy specimen.

**DISCUSSION**
The etiology of pulmonary bulla is complex and includes chronic obstructive pulmonary disease, emphysema, ventilator-related lung injury, and COVID-19[1]. Patients who have pulmonary vesicles shown on chest CT scans, and peripheral predominant consolidation patterns with internal round cystic changes[6], are more prone to pulmonary bullous formation. However, the influence of COVID-19 on pulmonary vesicles has not yet been reported.

Our study found that as alveolar structure destruction occurred quickly, the wall of the air cavity was significantly thicker than that of common pulmonary bulla (Figure 1G and H). In this process, the inflammatory storm is also an important factor.
Figure 1 Perioperative laboratory tests on patient underwent surgery. A: Perioperative blood routine results showed that COVID-19 patient with pulmonary vesicles showed higher rate of infections, fewer platelets, more neutrophils and fewer lymphocytes. B: After receiving surgery, the TLC and I/CLC in the patient’s circulating blood showed a "W-shaped" curve, NK cells and BLC showed an "M-shaped" curve, H/I LC showed a trend of increasing gradually, and cytokines (IL-2, 4, 6, 10, Interferon γ, TNF-α) showed an upward trend as a whole. C: The level of circulating estrogen was higher than the normal range, while the level of ACE was in the normal range. The COVID-19 patient with pulmonary vesicles showed varying degrees of liver and kidney damage, mild body temperature elevation, and large changes in fluid intake and output. D: 20 d before bullectomy, the chest CT scans of the patient showed changes in small vesicles in the lung. E: Chest X-ray,

Thus, we believe that the destruction of alveolar structure due to COVID-19 easily induces emphysema and then causes the formation of pulmonary vesicles. Although pulmonary vesicles are not exactly bulla, they can easily develop into them. The most effective approach to treat symptomatic pulmonary bulla is surgical resection[7], which is widely accepted by thoracic surgeons worldwide. Nevertheless, it was a difficult choice for treating COVID-19 patients with pulmonary vesicles. It is clear that when patients have tension pneumothorax, chest drainage tubes must be placed as soon as possible. In this study, one patient underwent bullectomy, with pulmonary vesicles induced by COVID-19 and tension pneumothorax. To our knowledge, this operation was the first bullectomy performed on a COVID-19 patient with both gross and histologic findings. Regrettably, the outcome of this patient was poor after undergoing bullectomy (Figure 1F).

This is also the first report on pathological findings of COVID-19 complicated by emphysematous bulla formation in the lung. Interactions of multiple factors, including diffuse alveolar damage overlapping with extensive necrosis, abundant neutrophils in lung tissue that can produce matrix metalloproteinase, and elevated levels of cytokines such as interleukins in the peripheral blood, may have led to bulla formation in this case.

CONCLUSION

In conclusion, COVID-19 may induce the formation of pulmonary vesicles, which have a thicker air cavity wall than common bulla. Considering ventilator-related lung injury, it is recommended to choose the ventilator mode and PEEP carefully. Based on the extensive destruction of lung tissue by COVID-19, the use of bullectomy is limited, only as a last resort and trial treatment if the patient accepts. More research is needed to explore the specific mechanisms of pulmonary vesicle formation to improve the efficacy of COVID-19 pneumonia treatment, especially in patients with severe COVID-19 with vesicles.

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Epibulbar osseous choristoma: Two case reports

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Abstract

BACKGROUND
Choristoma is a rare, benign, congenital proliferative tumor, with osseous choristoma being the rarest. Although the tumor is benign, effective identification is needed for its diagnosis and treatment. Here, we report the diagnosis and successful surgical treatment of two patients with osseous choristoma.

CASE SUMMARY
Two patients, a young female and young male patient, were found to have a mass on the ocular surface. The tumor presented on the superior temporal bulbar conjunctiva in the first patient and on the upper eyelid in the second patient. Ultrasound biomicroscopy detected a strong echo with clear boundaries covering the lower echo, and computed tomography examination revealed calcification. Both patients underwent surgery, and histopathological evaluation of the mass showed osseous choristoma. They were treated by excision and subsequently cured.

CONCLUSION
Osseous choristomas are usually asymptomatic. Our patients were cured immediately after surgery, suggesting that surgical treatment is an effective strategy.

Key Words: Osseous choristoma; Epibulbar choristoma; Prevelence; Treatment of choristoma; Case report
INTRODUCTION

Choristoma is a rare, benign, congenital proliferative tumor[1], which is defined as normal tissue that stops migrating during embryonic development and is located in an abnormal position. Epibulbar choristoma normally occurs sporadically and develops alone, or it may be associated with a variety of syndromes[2], such as Goldenhar syndrome, epidermal nevus syndrome, and encephalo-cranio cutaneous lipomatosis. Ocular choristomas can be classified as dermoid, dermolipoma, complex choristoma (choristomas with more than one tissue type), and single-tissue choristoma, among which osseous and complex choristomas are the rarest. The prevalence of epibulbar choristoma ranges from 1/10000 to 3/10000[2] and can occur at multiple sites, predominantly in the cornea, rectus muscle, and conjunctiva. In this study, we reviewed myoblastoma cases treated at our hospital since 2010 and provided reports and detailed preoperative, intraoperative, and postoperative lesion images, including gross and pathological images, of two patients with osseous choristoma.

CASE PRESENTATION

Chief complaints

Case 1: A 23-year-old woman with no obvious predisposing cause was found to have a soybean-sized mass above the outer sphere of her left eye and visited our hospital for more than 6 mo.

Case 2: A 31-year-old man presented with a mass in the right upper eyelid persisting for 1 mo.

History of present illness

Case 1: The patient was asymptomatic with no ocular pain or diplopia as well as no history of eye trauma or surgery.

Case 2: The patient was asymptomatic without any ocular pain or diplopia and had no history of eye trauma or surgery.

History of past illness

Case 1 and Case 2: The patients had no past illness.

Personal and family history

Case 1 and Case 2: The patients had no history of familial diseases.

Physical examination

Case 1: Pre-procedure examination of the patient showed a pale-white nodule with a 5 mm diameter on the superior temporal bulbar conjunctiva of the left eye. It presented with a hard texture, mild hyperemia, poorly defined boundary, irregular shape, and no tenderness. The nodule was closely adhered to the substrate and could not be moved. There was no eye protrusion, and eye movement was normal (Figure 1). No abnormalities were found in the anterior and posterior segments.

Case 2: A 5-mm mass was observed on the right upper eyelid. The skin showed
redness and swelling, and the mass protruded from the skin surface. The boundary was unclear, and there was no tenderness.

**Imaging examinations**

**Case 1:** Ultrasound biomicroscopy showed a strong elliptical echo in the superficial scleral layer under the bulbar conjunctiva at the superior temporal side, with a clear boundary, obscured inferior echo, and limited scope exploration (Figure 2). Computed tomography imaging indicated a massive calcification lesion of about 1.0 cm × 0.5 cm in the upper left part of the left eye conjunctiva, and the nodule appeared to be cartilaginous (Figure 3).

**FINAL DIAGNOSIS**

**Case 1**
Histopathological evaluation confirmed osseous choristoma of the superficial sclera (fibrous connective tissue and fat surrounding the oblate neoplasm; hard as bone; and after decalcification, the tumor tissue was found to be mature bone tissue, with multiple Hastelloy tubes and annular bone plates, and no other soft tissue). The patient was diagnosed with epibulbar osseous choristoma and was cured after surgical excision (Figure 4).

**Case 2**
Postoperative pathology confirmed osseous choristoma of the right upper eyelid. The tumor tissue was mainly composed of differentiated and mature bone and cartilage surrounded by a large number of proliferative collagen fibers.

**TREATMENT**

**Case 1**
We performed surgery to remove the neoplasm from the conjunctiva under local anesthesia; allo-scleral film was prepared to repair sclera. During the operation, the conjunctiva tissue on the surface was separated and the bone lesion with a diameter of 0.5 cm that was adhered to the scleral superficial tissue became visible. The neoplasm had a smooth surface and the sclera beneath was intact without pigment exposure, so it was then separated from the superficial sclera. Therefore, the capsule was sutured intermittently to reinforce the sclera. Postoperative suture removal was normal.

**Case 2**
Treatment involved surgery during which one piece of solid tissue was excised. The resected tissue was red and nodular, with a wide base and no adhesion to the surrounding tissue, and also the neoplasm had a smooth surface. So it was then separated and capsule of the eyelid was sutured intermittently. Postoperative suture removal was normal.
Figure 2 Preoperative ultrasound biomicroscopy image of the mass in the superior temporal quadrant of the left eye (case 1). A strong oval echo was observed in the superficial sclera under the bulbar conjunctiva, with a clear boundary obscuring the lower echo.

Figure 3 Preoperative computed tomography scan of the mass in the superior temporal quadrant of the left eye (case 1). Lumps of calcification were apparent in the upper left conjunctiva of the left eye.

OUTCOME AND FOLLOW-UP

Case 1
The patient was cured after the surgery. The patient needs to be followed 1 mo after operation. If there is no discomfort, the patient will be followed every half a year.

Case 2
Surgery was successful, and the patient was cured. The patient needs to be followed 1 mo after operation. If there are discomfort symptoms, the patient should see a doctor at any time.

DISCUSSION
The concept of osseous choristoma was first proposed in 1863[5]. Its etiology is unknown and is related to abnormal gene expression and mesenchymal development.
Figure 4 Hematoxylin-eosin staining after resection of the local tumor (magnification, 25 ×). A: Case 1. The pathology showed features of superficial scleral osteoblastoma: Flat and round tumors that were as hard as bone and surrounded by fibrous connective tissue and fat; B: Case 2. The pathology revealed features of osteoblastoma consisting of bone and cartilage tissue surrounded by numerous collagen fibers.

Trauma or infection stimulates the bone morphologic proteins, which leads to heterotopic ossification and accelerates the disease progression[3,4]. However, osseous choristoma does not have any malignant metastatic tendency and can be present at birth. It develops rapidly in early childhood and then gradually stabilizes and ceases growth[5], and it may eventually be detected due to symptoms such as foreign body sensation or conjunctival congestion in the later adolescent years.

At present, there is no unified conclusion on the relationship between the occurrence and development of osseous choristoma and sex. Although it has been reported that young women tend to have a high incidence[4-7], the association with sex was not significant due to the small number of cases[2]. Additionally, osseous choristoma is observed more frequently in the right eye than in the left eye, and its sites are mainly distributed in the conjunctiva, sclera, and ophthalmic muscle, with most of them located in the fascia of the superior temporal quadrant[8-10]. However, osseous choristoma occurring in the rectus muscle or eyelid is rare[6], and the frequency of these cases has not yet been statistically analyzed[1].

Since 2010, 296 cases of choristoma have been treated at our hospital, including 183 cases of dermoid cysts, 2 cases of osseous choristomas, 15 cases of osteoid lipomas, and 96 cases of dermoid tumors. The two cases of osseous choristoma, one female and one male patient, presented with a mass in the superficial sclera and eyelid, respectively. The prevalence of osseous choristoma in our hospital was 0.676%. Similar results were observed in a study conducted by Aldossary MM et al[2], in which among the 120 patients with myoblastoma of the ophthalmic surface, two had osteogenic myoblastoma, with a prevalence of 1.7%. Among the osseous choristoma cases in this study, one case was observed in a young woman, and it presented as a hard mass on the upper left temporal quadrant, which was in accordance with the previous reviews. CT imaging showed a high-density shadow, which was considered to be a dermoid tumor or lipoma, and a low-density focal area. Preoperative CT can be used to determine the properties and adhesion degree of the mass and the depth of the lesion resection. The treatment for osseous choristoma involves observation and surgical resection, with the surgical indications being foreign body sensation, irritative symptoms, and recurrent inflammation. In the study patients, the lesions were closely adhered to the sclera with poor activity, and surgical resection was performed for diagnostic and esthetic purposes[4].

The limitation of this case is that there are only two cases of epibulbar osseous choristoma, and the characteristics of osseous choristoma are not well summarized. In addition, the patients were not followed after surgery, so the postoperative outcome of the disease is unclear. Reviewing the previous literature, there are no large samples or long-term follow-up cases, so we suggest that the future study of osseous choristoma should increase the sample size to make statistical description of the primary sites, pathological features, prognosis and other aspects, so as to provide a clear diagnosis and outcome of the disease. Of course, special cases also deserve our attention.
CONCLUSION

We report two rare cases of osseous choristoma and their successful treatment. This study shows that clarifying the age, location, clinical manifestations, and CT findings of osseous choristoma can facilitate better diagnosis and guide further surgical treatment.

REFERENCES

Gastric submucosal lesion caused by an embedded fish bone: A case report

Jian Li, Qiu-Qiu Wang, Shuai Xue, Yan-Yan Zhang, Qin-Yu Xu, Xiao-Hong Zhang, Li Feng

Abstract

BACKGROUND
Submucosal tumors (SMTs) refer to elevated lesions that originate from the layers below the mucosa of the digestive tract, including the muscularis, submucosa and muscularis propria. With the development and application of endoscopy and endoscopic ultrasonography (EUS), the detection rate of SMTs has increased significantly in recent years. Various diseases can lead to SMTs. However, a foreign body embedded in the gastric antrum showing clinical manifestations of a SMT is rare.

CASE SUMMARY
We report the case of a 47-year-old woman, who presented with upper abdominal discomfort for one year, and was subsequently diagnosed with a gastric submucosal lesion caused by an embedded foreign body by EUS and computed tomography. Considering the size and potential complications of this lesion, endoscopic full-thickness resection was performed to achieve full resection in our endoscopy center. A fish bone was found in the lesion during the operation, and was successfully removed, and the defect was later closed with endoscopic purse-string sutures.

CONCLUSION
This case report highlights the management strategies of SMTs, the importance of being familiar with diagnostic methods related to submucosal lesions, and being able to conduct effective treatment when this rare condition is highly suspected.

Key Words: Submucosal tumors; Endoscopic full-thickness resection; Fish bone; Case report

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Core Tip: We present a patient who was hospitalized due to upper abdominal discomfort. After careful examination, the patient was diagnosed with a submucosal lesion caused by an embedded fish bone, and the lesion was subsequently removed by endoscopic full-thickness resection. This case highlights the management strategies for submucosal tumors, the importance of being familiar with diagnostic methods and being able to conduct effective treatment when this rare condition is highly suspected.

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DOI: https://dx.doi.org/10.12998/wjcc.v10.i3.1099

INTRODUCTION

Mistaken ingestion of a foreign body (FB) is common in children and adults. Being at the exploratory stage of development, children tend to put objects in their mouths [1, 2]. In adults, ingestion of a FB usually occurs in those who are diagnosed with psychiatric disorders and pica [3], and prisoners who try to escape from law enforcement during access to medical service [4]. An ingested FB can become lodged in any part of the gastrointestinal tract. Depending on the FB and lodged position, FB ingestion can cause serious complications such as obstruction, perforation and bleeding. Therefore, ingestion of a FB should be paid due attention in clinical practice.

The ingestion of a fish bone has been shown to be a common cause of a FB in the digestive tract in the emergency department [5]. In clinical settings, most FBs in the digestive tract can be successfully removed with the assistance of endoscopy. However, a few FBs may lodge in the digestive tract for a long time, which can lead to other diseases or complications. Submucosal lesions are caused by tumors that stem from the muscularis mucosa, submucosa, or muscularia propria. Submucosal lesions are frequently found in the gastrointestinal tract, especially in the stomach, as often as 1 in every 300 endoscopic examinations [6]. Here, we report a patient with a fish bone embedded in the gastric antrum who presented with the manifestations of a submucosal tumor (SMT).

CASE PRESENTATION

Chief complaints
A 47-year-old woman presented with upper abdominal discomfort for one year.

History of present illness
There were no obvious reasons for her abdominal pain and discomfort after meals. Her discomfort was usually relieved after 30 min rest. A fatty diet did not aggravate the development of her discomfort or radiate to her shoulders or back. No acid reflux, belching, hiccups or black stools were reported.

History of past illness
The patient had no previous noteworthy medical history.

Personal and family history
Family history was pertinent for her mother who was diagnosed with gastrointestinal stromal tumors aged 70 years.

Physical examination
The abdomen was soft and flat, with no spontaneous pain or tenderness. No positive results were found on physical examination.
Laboratory examinations
Biochemical and hematologic test results such as tumor markers, complete blood count, electrolyte levels and liver function revealed no abnormalities.

Imaging examinations
Gastroscopy and colonoscopy were performed in our endoscopy center after hospitalization. A submucosal lesion 11.1 mm in diameter was found in the gastric antrum by gastroscopy (Figure 1A). As this lesion originated from the submucosa layer, endoscopic ultrasonography (EUS) was carried out to further evaluate this lesion. The EUS results revealed a low echo of the submucosal mass-like lesion (7.2 mm × 11.1 mm) in the gastric antrum, which may have originated from the muscularis propria layer. It was also revealed that the mucosal layer of the lesion was mildly thickened; the boundaries and the serosa remained clear and basically continuous. In addition, a point-like hyperechoic image accompanied by a posterior sound shadow was detected by EUS (Figure 1B). An abdominal computed tomography (CT) scan was conducted to further confirm the relationship between the lesion and adjacent abdominal organs. Interestingly, the CT scan revealed a hyperdense linear structure in the gastric antrum wall, suggestive of a FB, which did not penetrate the serosa layer, and tissue edema, enlarged lymph nodes and exudation were not observed (Figure 1C).

FINAL DIAGNOSIS
Gastric submucosal lesion caused by an embedded fish bone.

TREATMENT
Endoscopic full-thickness resection (EFTR) was performed in order to achieve full resection. During the operation, a fish bone was found in the lesion (Figure 1D), and the lesion then was completely resected. The defect was subsequently closed with endoscopic purse-string sutures. An indwelling stomach tube was placed after endoscopic surgery. In order to prevent postoperative complications, intravenous nutrition, proton pump inhibitors and anti-infection drugs were also prescribed in this patient. The stomach tube was removed 48 h later and a liquid diet was prescribed for 72 h.

OUTCOME AND FOLLOW-UP
On the fourth day after surgery, the patient was in a stable condition and no discomfort or abnormalities were reported. She was then discharged from hospital. No discomfort was reported at the 30-d follow-up visit.

DISCUSSION
Submucosal tumors (SMTs) are elevated lesions which originate from the layers below the mucosa of the digestive tract, including the muscularis, submucosa, and muscularis propria[7]. The mechanism of SMTs involves abnormal hyperplasia in submucosal tissue or the muscle layer and genetic factors also play a critical role in this process. Patients with SMTs are often asymptomatic, and most SMTs are found during routine health screening. In recent years, due to the wide application of endoscopy and EUS in clinical practice, the detection rate of SMTs has increased. Although the exact incidence is unknown, the detection rate of SMTs by gastroscopy is 0.33%-0.76%[8]. According to their malignant potential, SMTs can be classified into those with malignant potential including glomus tumors, carcinoids and gastrointestinal stromal tumors (GISTs), and those without malignant potential, including mesenchymal tumors, lipomas, leiomyomas, schwannomas, desmoid tumors, duplication cysts, pancreatic rests, and giant cell tumors[9]. The histopathological types of SMTs are complicated, but most are benign tumors, and less than 15% of SMTs appear to have malignant potential[10]. Leiomyoma is the most common SMT in the esophagus, accounting for 2/3 of benign esophageal tumors[11]. The stomach is the most common...
Li J et al. Gastric submucosal lesion caused by fish bone

Figure 1 Findings from endoscopy and a computed tomography scan during the diagnostic process and endoscopic treatment. A: Endoscopy revealed an elevated lesion in the gastric antrum (blue arrow); B: Endoscopic ultrasonography showing a hypoechoic mass in the posterior wall of the gastric antrum (orange arrow); C: Abdominal computed tomography (CT) scan showing a hyperdense linear structure in the gastric antrum wall (blue arrow), CT value: 968 HU; D: During endoscopic surgery, an L-shape fish bone was removed from the lesion.

Gastric submucosal lesion caused by fish bone

Site for SMTs and the pathological types of stomach SMTs are even more complicated. GISTs, leiomyomas and pancreatic rests are also common in the stomach. Lipoma is the most common pathological type in the colon, while neuroendocrine tumors are most commonly found in the rectum[12]. However, the accidental ingestion of a fish bone mimicking a stomach SMT is rare. EUS is currently the most accurate diagnostic tool for evaluating SMTs in the digestive tract. It also plays an essential role in delineating histologic layers and providing key information for doctors when choosing therapeutic methods in patients with SMTs[13, 14]. Previous studies have shown that the sensitivity and specificity for distinguishing different types of SMTs are 64% and 80%[15], and for SMTs with a diameter less than 2 cm, EUS is superior to CT and magnetic resonance imaging (MRI)[16]. However, EUS has several technical deficiencies. By contrast, CT is an efficient and convenient tool for diagnosing FBs embedded in the gastrointestinal tract. Data from multiple studies demonstrate that the sensitivity and specificity of CT in identifying impacted fish bones were 90.9%-100% and 100%, respectively[17,18]. The CT images of a fish bone usually appear as a calcified structure[19], and a linear calcified structure was shown on the CT images in our patient (Figure 1C), which is consistent with a previous report [20]. Therefore, EUS combined with other imaging examinations such as CT or MRI are often used to enhance diagnostic accuracy. Furthermore, doctors can also obtain detailed information on the relationship between the tumor and surrounding blood vessels or organs using CT or MRI, which is helpful in planning therapeutic strategies.

Safe treatments to remove submucosal lesions caused by FBs are essential. Data from the European Society of Gastrointestinal Endoscopy clinical guideline indicated that approximately 80%-90% of ingested FBs can pass through the digestive tract spontaneously, and the remaining 10%-20% require endoscopic interventions[21]. A case from Brazil reported that an elevated lesion was found in the gastric antrum and
Table 1 Literature review of previous reports of submucosal lesions caused by foreign bodies

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Treatment</th>
<th>Chief complaint</th>
<th>Foreign body location</th>
<th>Foreign body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvalho et al[26], 2021</td>
<td>ESD</td>
<td>Unclear</td>
<td>Esophagus</td>
<td>Fish bone</td>
</tr>
<tr>
<td>Shan et al[23], 2019</td>
<td>Surgery</td>
<td>Abdominal pain</td>
<td>Stomach</td>
<td>Fish bone</td>
</tr>
<tr>
<td>Li et al[27], 2017</td>
<td>ESD</td>
<td>Abdominal pain</td>
<td>Stomach</td>
<td>Chicken bone</td>
</tr>
<tr>
<td>Yip et al[28], 2017</td>
<td>ESD</td>
<td>Odynophagia</td>
<td>Esophagus</td>
<td>Fish bone</td>
</tr>
<tr>
<td>Goh et al[22], 2017</td>
<td>Surgery</td>
<td>Routine examination</td>
<td>Stomach</td>
<td>Fish bone</td>
</tr>
<tr>
<td>Birk et al[24], 2014</td>
<td>Surgery</td>
<td>No symptoms</td>
<td>Stomach</td>
<td>Fish bone</td>
</tr>
<tr>
<td>Watanabe et al[20], 2014</td>
<td>Surgery</td>
<td>Abdominal pain</td>
<td>Stomach</td>
<td>Fish bone</td>
</tr>
<tr>
<td>Nagem et al[25], 2011</td>
<td>Regular follow-up</td>
<td>Throat pain</td>
<td>Esophagus</td>
<td>Fish bone</td>
</tr>
</tbody>
</table>

ESD: Endoscopic submucosal dissection.

mimicked a GIST. The lesion was immediately removed by exploratory laparotomy and a chicken bone was found in the lesion, which had penetrated the gastric wall and into the left lateral segment of the liver[22]. This suggests that FBs embedded in the digestive tract have the potential for perforation. In a few similar reports, endoscopic submucosal dissection (ESD) was used to remove FBs. As summarized in Table 1, treatment including ESD, surgery and regular follow-up varies depending on the patient’s condition[23-25]. With the development of novel endoscopic techniques, the removal of FBs is no longer restricted to surgery. Endoscopic surgery is a better option due to the advantages of fewer hospitalized days and less invasiveness. According to previous literature, ESD is a commonly used endoscopic intervention for the removal of FBs[26-28], which avoids the need for surgical exploration. However, when choosing endoscopic treatments, the status of patients and location of the FB should also be considered. In the present report, given the chronic abdominal discomfort in this patient and the potential for severe complications, we therefore performed EFTR for complete resection.

In summary, a gastric submucosal lesion caused by an embedded fish bone is uncommon in daily medical practice. Therefore, the possibility of embedded FBs should also be considered when trying to identify the cause of submucosal lesions or unexplained abdominal discomfort. The diagnosis of submucosal lesions mainly depends on EUS or CT. Traditionally, surgery is the main approach for the removal of an embedded fish bone if this FB cannot be observed under endoscopy. However, endoscopic surgery is becoming feasible with the maturity of novel techniques such as ESD and EFTR. During the follow-up period, EUS is superior to endoscopy due to its ability to delineate FB location, size and histologic layers. Moreover, EUS combined with other imaging data or abdominal examinations (CT/MRI) may also be necessary. Effective treatments are needed when this rare disease is highly suspected.

CONCLUSION

A submucosal lesion caused by an embedded fish bone is an exceptional condition. With the help of endoscopy, EUS and CT, the diagnosis of a FB is not difficult. However, difficulties arise when accurately locating the FB and conducting safe and effective treatment to remove it, especially in an urgent situation. EUS and CT are useful tools in the diagnosis of FBs in the gastrointestinal tract. At present, endoscopic surgery is the first-line treatment in this situation. This study demonstrates that being familiar with diagnostic methods and conducting effective treatment are essential when this rare condition is highly suspected.

ACKNOWLEDGEMENTS

We appreciate all the medical staff in our endoscopy center involved in the treatment of this patient.
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1998; 112: 360-364 [PMID: 9659498 DOI: 10.1017/s0022215100140460]


Metastasis to the thyroid gland from primary breast cancer presenting as diffuse goiter: A case report and review of literature

Wen Wen, Heng Jiang, Hsin-Yu Wen, Yu-Lan Peng

Abstract

BACKGROUND
Metastasis to the thyroid gland (TM) from primary breast cancer is uncommon and usually presents as thyroid nodules; however, diffuse goiter without thyroid nodules is the first sign of TM in rare cases. Skip metastases (SMs) to the lymph nodes in breast cancer, defined as discontiguous higher-level metastases in the absence of lower levels of contiguous metastases, have been reported in the contralateral cervical area of the primary tumor site in rare cases.

CASE SUMMARY
A 49-year-old previously healthy Chinese woman was diagnosed with right lateral invasive ductal carcinoma and underwent neoadjuvant chemotherapy treatment and bilateral mastectomy with axillary lymph node dissection. No malignancy of the left breast or axillary or distant metastases were identified preoperatively. However, enlarged left cervical lymph nodes were detected 36 mo after surgery, and rapidly enlarging thyroid glands without nodules were detected 42 mo after surgery. Fine-needle aspiration cytology was performed on the left cervical lymph nodes and left lobe of the thyroid, which were both revealed to contain metastases from the primary breast cancer. Additionally, the immunostaining profiles changed in the process of metastases. The patient was discharged with the NP (vinorelbine and cisplatin) regimen for subsequent treatment, and stable disease was determined when the curative effect was evaluated.

CONCLUSION
Diffuse goiter may be the first sign of TM, and enlarged lymph nodes in the
Contralateral cervical area may be SMs of primary breast cancer.

**Key Words:** Metastases to the thyroid gland; Diffuse goiter; Cervical lymph node recurrence; Breast cancer; Case report

**Core Tip:** This is a case report of metastasis to the thyroid gland (TM) from primary breast cancer presenting as diffuse goiter associated with skip metastases (SMs) to the contralateral cervical lymph nodes. The patient presented with a cervical mass and progressive neck swelling that were found to be metastases with altered immunostaining profiles upon fine-needle aspiration cytology. These findings indicate that rapidly occurring diffuse goiter without nodules may be the first sign of TM and that enlarged lymph nodes in the contralateral cervical area may be SMs of breast cancer. Raising awareness of these clinical presentations is helpful for the early detection of metastatic disease.

**INTRODUCTION**

Metastasis to the thyroid gland (TM) is uncommon, accounting for approximately 1.4% to 3% of all thyroid malignancies[1]. It was reported that metastases mostly arise from the lung (21.8%), followed by the gastrointestinal tract (18.2%), breast (14.5%), and kidney (12.7%), in a recent Chinese study[2]. Patients with TM usually present with symptoms of thyroid nodules, thyroiditis or neck swelling, dysphagia, dysphonia, and cough[3]. Diffuse goiter without thyroid nodules is rarely seen as the first manifestation of TM. Skip metastases (SMs) of breast cancer to the lymph nodes, defined as discontiguous higher-level lymphadenopathy in the absence of lower levels of contiguous lymphadenopathy, have rarely been reported. Chung et al[4] reported that SMs occurred in 2.6% of 1300 newly diagnosed invasive breast cancers, and only 6% of these SMs occurred in the contralateral lymph nodes. Here, we report a rare case of TM first presenting as rapidly occurring diffuse goiter without thyroid nodules associated with SMs to the contralateral cervical lymph nodes in a primary breast cancer patient.

**CASE PRESENTATION**

**Chief complaints**

A 53-year-old woman with a 4-year breast cancer history presented to the clinic with a cervical mass and progressive neck swelling without pain or airway pressure symptoms.

**History of present illness**

A 49-year-old Chinese woman was diagnosed with invasive ductal carcinoma (IDC) of the right breast with a chief complaint of palpable masses and right nipple discharge in October 2015 (Figure 1). The right axillary lymph nodes were also found to contain poorly differentiated metastatic breast carcinoma cells by fine-needle aspiration cytology (FNAC). No mass or enlarged lymph nodes were seen on the left side upon computed tomography (CT) scan or by ultrasound. The patient underwent preoperative neoadjuvant chemotherapy, with 3 cycles of the FEC (5-fluorouracil + epirubicin + cyclophosphamide) regimen and 3 cycles of the TG (vinorelbine + cisplatin) regimen (Figure 1). She was evaluated as having achieved partial remission...
after finishing chemotherapy. Subsequently, bilateral mastectomy was performed at the request of the patient in April 2016 (Figure 1). Surgical specimens showed T2N3 (12/27) M0 grade 3 IDC with ductal carcinoma in situ on the right side based on hematoxylin and eosin (HE) staining and immunohistochemistry (IHC). Right axillary and intercostal lymph node metastases were also observed microscopically. No carcinoma was found in the left breast or axillary lymph nodes. IHC staining suggested estrogen receptor (ER, strong positive), progesterone receptor (PR, moderate positive) and human epidermal growth factor receptor 2 (HER-2, 2+) positivity, and the monoclonal antibody Ki-67 index was 60%. After surgery, the patient received endocrine therapy with anastrozole and goserelin. Radiation therapy was administered in 25 fractions to the right breast.

In April 2019, the patient presented at the clinic with a cervical mass and intermittent neck discomfort without pain and airway pressure symptoms. CT revealed lymphadenopathy in the left cervical area and posterior mediastinum. Observation and follow-up were recommended. Two months later, the size of the mass had increased. Further investigation was performed by ultrasound, and levels IV and V left cervical nodal disease was confirmed. On CT with contrast, the patient was found to have left cervical and posterior mediastinum lymph node enlargement and suspected right scapula metastasis. No treatment was initiated per the patient’s decision, and she was scheduled to return to the clinic in 3 mo. In October 2019, the patient presented at her outpatient visit with progressive neck swelling that had persisted for 3 mo.

**History of past illness**

The patient had no history of any previous disease.
Personal and family history
The patient had no personal or familial risk factors for thyroid malignancies.

Physical examination
On physical examination, a hard fixed palpable nontender left lateral neck mass and enlarged thyroid gland were palpated.

Laboratory examinations
Tumor indicators revealed that the carcinoembryonic antigen was elevated at 3.92 μg/L and carbohydrate antigen 15-3 was elevated at 22.30 kU/L. The thyroid function analysis revealed mild hypothyroidism, with a thyroid stimulating hormone level of 5.61 mU/L and a free thyroxine level of 11.62 pmol/L. Blood analysis and inflammatory indicators were normal.

Imaging examinations
Multimodality ultrasound was performed to evaluate neck swelling, revealing homogeneous enlargement of the thyroid gland without nodules (measuring 2.5 cm × 5.0 cm × 2.2 cm in the right lobe, 2.5 cm × 5.0 cm × 2.0 cm in the left lobe, and 0.8 cm in the isthmus) and level II-VI left abnormally enlarged cervical lymph nodes (the largest measuring 2.7 cm × 2.8 cm × 2.3 cm with microcalcifications and heterogeneous high enhancement (Figure 2A). Fine-needle aspiration cytology (FNAC) of the enlarged thyroid and lymph nodes was conducted for diagnosis (Figure 2B).

Differential diagnosis
Hashimoto's thyroiditis, Grave's disease and primary/secondary thyroid malignancy were our initial differential diagnoses to explain the rapid growth of the thyroid gland.

FINAL DIAGNOSIS
The patient was ultimately diagnosed with TM from primary breast cancer and SMs to the contralateral cervical lymph nodes (Figure 3).

TREATMENT
Given the suspected bone metastasis and financial issues, the patient was discharged on the NP (vinorelbine and cisplatin) regimen (Figure 1).

OUTCOME AND FOLLOW-UP
To date, after 6 cycles of chemotherapy, the patient has remained clinically stable, and no recurrence at other sites has been detected or confirmed.

DISCUSSION
We performed a comprehensive literature search of the PubMed and Medline databases to identify studies of TM metastasis from breast cancer published from 2000 to 2020, and twenty-five articles were found. Detailed information about thyroid metastatic breast cancer was extracted from these articles (Table 1), and only descriptive analyses and literature reviews were found, given the low incidence of TM.

Metastasis to the thyroid gland is rare due to its rich blood supply; however, reports of TM have increased in recent years as a result of more sophisticated diagnostic methods, such as FNAC and proton emission tomography[5,6]. The characteristics of TM from breast cancer are listed in Table 1. We collected information regarding age, sex, histology of the primary tumor, other sites of recurrence, the time interval between primary diagnosis and TM, presentation of TM, treatment of metastasis, response to the treatment and follow-up for 45 women with TM of breast cancer from 2000 to 2020[6-30]. The development of TM does not seem to be age-related and mostly occurs in women. The time interval between primary and metastatic disease and the
Table 1 Characteristics of metastasis to the thyroid gland from primary breast cancer in reports from 2000 to 2020

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Study year</th>
<th>No of patients</th>
<th>Sex</th>
<th>Age</th>
<th>Primary tumor</th>
<th>Other recurrence</th>
<th>Time interval (mo)</th>
<th>Presentation</th>
<th>Treatment</th>
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LN: Lymph node.

prognosis of TM varies among the reports. In two patients, TMs were detected synchronously with the diagnosis of the primary cancer[17,21]. As shown in Table 1, TM has various clinical presentations. On physical examination, TM usually appears as a palpable mass or neck swelling, with or without dysphagia, hoarseness, dysphonia, pain and other symptoms, when thyroid metastasis is the first presentation of recurrent disease. In the reports that presented clinical information, most patients (90.1%) had thyroid nodules confirmed with ultrasound or CT, except one patient who had an enlarged thyroid with diffuse calcification[14] and
Thyroid metastases from breast cancer

one patient who presented with acute thyroiditis[25]. Only 5 out of 97 patients presented with diffuse goiter out of all primary cancer origins in a study at the Mayo Clinic[1]. Here, we report a rare case of TM presenting as diffuse goiter without thyroid nodules that had metastasized from primary breast carcinoma. This case report provides valuable information for clinicians, indicating that rapidly occurring diffuse goiter without other symptoms may be the first sign of TM in patients with malignant disease.

Thyroid metastasis usually occurs in patients with widespread metastatic disease, and the other sites of metastasis are primarily the bone and lung, according to the data shown in Table 1. Not all previously published studies contained information on other recurrence sites, and in 18 patients (41.9%, Table 1), the thyroid was the first and only site of recurrence. In the studies that contained histological information, primary breast cancer was mostly referred to as "invasive" carcinoma (85.7%), indicating that invasive carcinoma might be the most prevalent type of cancer to result in TM (Table 1). Among those reports, two patients had poorly differentiated adenocarcinoma as the primary disease[11], one patient had medullary carcinoma[11], one had metaplastic carcinoma[23], and one had mucinous carcinoma[7].

Patients with TMs generally have a poor prognosis[31,32]. Therapeutic choices for TM vary among reports, depending on the primary cancer origin, recurrence at other sites and the symptoms caused by TM. Surgical excision is considered the first choice for thyroid metastasis, and it has been reported that thyroidectomy improves the prognosis of patients[33]. Patients with multisite metastases are usually recommended for treatment with chemotherapeutic and endocrine approaches according to studies of metastatic breast cancer, but research on the effects of chemotherapy for thyroid metastasis is limited[34]. Among 30 patients, 16 were treated with chemotherapy, and 75% of them were clinically stable during follow-up (Table 1). It is believed that the biological behavior of primary cancer might be the primary influence on the prognosis of patients with TM[2]. Thus, therapeutic choices for TM patients should be determined individually and with multidisciplinary board discussion.

Figure 2 Ultrasound images of lymph node and thyroid. A: Contrast-enhanced ultrasound of the left cervical lymph node; B: Fine-needle aspiration cytology of the left lobe of the thyroid.
Another notable fact in this case report is that the patient had SMs in the contralateral cervical area of the primary tumor site, and no malignancy was previously found in the left breast or axillary region. SM to the lymph nodes in breast cancer is an important phenomenon, and it is critical to make the correct choice of surgical resection techniques and chemotherapies. It has been reported that only 6% of SMs occur on the contralateral side of the primary tumor site[4], with SMs accounting for 3.5% to 34.6% of metastatic lymph nodes[4,35,36]. Enlarged lymph nodes were detected before diffuse goiter. Aron et al[37] reported that the vast majority of metastases are able to remain dormant for a long period of time, referred to as metastatic dormancy. This suggests that thyroid metastasis probably occurred before the cervical lymph node changes and remained indolent and silent for a long period of time. It remains unknown whether the metastasis to the contralateral cervical lymph nodes originated from the thyroid metastasis or from the breast directly.

The IHC profiles of the patient changed during the process of cancer management. The immunostaining profiles of the core-tissue needle biopsy before NAC were ER (+++), PR (+++) and HER-2 (-), which changed to ER (+++), PR (+) and HER-2 (2+) in the surgical pathology results; finally, the cytology of enlarged lymph nodes and thyroid indicated triple negative breast cancer. Several studies have demonstrated that hormone receptor (ER and PR) status changes between initial core-tissue needle biopsy and surgical specimens obtained after chemotherapy and endocrine treatment. Tacca et al[38] reported that the positivity rate of HER-2 decreased from 42.0% to 32.1% after neoadjuvant chemotherapy, which could explain the conversion of HER-2 status between FNAC and surgery pathology results. A Chinese nationwide multicenter study showed that 37.7% of breast cancer patients have hormone receptor conversion in metastatic lesions, and patients with PR conversion had shorter overall survival times than patients whose PR remained positive ($P = 0.016$)[39]. This reveals that IHC profiles may change in the process of metastasis, which offers more information for
making precise individual treatment decisions.

CONCLUSION

Few studies on diffuse goiter without thyroid nodules and SMs in the contralateral cervical area have been published. This report illustrates that rapidly occurring diffuse goiter without thyroid nodules may be the first sign of TM and that enlarged lymph nodes in the contralateral cervical area may indicate SMs of primary breast cancer. This finding raises awareness of these clinical presentations, which would be helpful for the early detection of metastatic breast cancer. In addition, IHC profiles may change during the process of metastasis, which indicates that biomarker testing for the early detection of metastatic breast cancer. In addition, IHC profiles may change during the process of metastasis, which indicates that biomarker testing for metastatic breast disease may be crucial for clinical decision-making.

REFERENCES

New method to remove tibial intramedullary nail through original suprapatellar incision: A case report

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Author contributions: He M designed the case report; He M and Li J collected the data; He M analyzed the data and wrote the manuscript; All authors have read and approved the final manuscript.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report’s scientific quality classification
Grade A (Excellent): 0

Abstract

BACKGROUND
Since 2006, introducing a tibial intramedullary nail via the suprapatellar approach has been established; however, nail removal must be carried out using classic infrapatellar access, which can lead to complications. Here, we report a new method to remove the intramedullary nail through the original suprapatellar incision.

CASE SUMMARY
A 39-year-old man was hit by a vehicle in 2019. He was immobilized with a 10-mm × 330-mm tibial intramedullary nail via the suprapatellar approach due to left middle tibial fracture. Two years later, the patient requested for the implant to be removed. We used a new method to remove the tibial intramedullary nail through the original suprapatellar incision, and the operation went smoothly.

CONCLUSION
This case report indicates that suprapatellar access can be used to remove the intramedullary nail via the original incision without infrapatellar access, thus avoiding surgical complications.

Key Words: Suprapatellar approach; Nail removal; Infrapatellar access; Case report

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Core Tip: A 39-year-old man was immobilized with a 10-mm × 330-mm tibial intramedullary nail via the suprapatellar approach due to left middle tibial fracture. The end cap of the nail was purposely not inserted. Two years later, we used a novel method to remove the tibial intramedullary nail through the original suprapatellar incision.
INTRODUCTION

The advantages of intramedullary nail internal fixation, such as small trauma, central fixation, and closed reduction, are consistent with the biological osteosynthesis concept; thus, intramedullary nail internal fixation has become the gold-standard treatment for tibial shaft fracture[1].

Removal of the tibial intramedullary nail via the infrapatellar approach is simple, and it is performed under direct vision through the original incision. Thus, researchers tend to use the infrapatellar approach to remove the internal fixation inserted via the suprapatellar approach[2]; however, with this approach, new scars form, and the patellar ligament and the infrapatellar fat pad can become damaged[3].

The other option is to remove the tibial intramedullary nail via the suprapatellar approach. However, this method remains controversial because there are many difficulties in using the original incision to remove the internal fixation. In this paper, a quick and simple method of nail extraction via the original suprapatellar incision is proposed. To the best of our knowledge, this is the first report of this type of removal.

CASE PRESENTATION

Chief complaints
A 33-year-old man requested for the implant to be removed.

History of present illness
The patient who was hit by a car in 2019 had a history of multiple fractures. These fractures, including left mid-tibial fracture, were fixed with a 10-mm × 330-mm suprapellar tibial nail. The end cap of the nail was purposely not inserted. Two years later, the patient requested for the implant to be removed.

History of past illness
The patient had a history of internal fixation.

Personal and family history
The patient had no genetic or familial disease history.

Physical examination
Multiple surgical scars were visible on the left calf, and there was no sign of limited motion in the left knee joint.

Laboratory examinations
No abnormalities were observed on preoperative examination.

Imaging examinations
An X-ray examination showed that the broken end of the tibia had bony union (Figure 1).

FINAL DIAGNOSIS

The patient’s final diagnosis was bony union after multiple fractures.

TREATMENT

The patient was examined before surgery and had no contraindications. After
administering epidural anesthesia, the proximal locking nail was removed. Then, the knee was bent 30°, and a multi-holed guide pin sleeve was fine-tuned to allow a 2-mm guide needle to be accurately inserted into the cavity of the intramedullary nail with a depth of at least 2–3 cm via the original suprapatellar incision. The results were confirmed by intraoperative X-ray (Figure 2). A hollow jig was used to screw the end of the nail along the guide needle. This process accurately removed the bone on top of the nail without damaging surrounding structures, such as the meniscus and ligaments. After the jig was screwed into the end of the intramedullary nail and tightened, intraoperative fluoroscopy was used for confirmation (Figure 3). The proximal and distal locking nails were removed, and the intramedullary nail was retracted using a mallet (Figure 4).
Figure 3 A jig was screwed into the tail of the nail. A: A hollow jig was rotated along the guide needle and screwed into the tail of the nail; B: Antero-posterior X-ray view showing that the clamp was screwed into the end of the intramedullary nail and tightened; C: Lateral X-ray view showing that the clamp was screwed into the end of the intramedullary nail and tightened.

Figure 4 The intramedullary nail was struck out of the tibia using a mallet through the suprapatellar approach.

OUTCOME AND FOLLOW-UP
Two weeks postoperatively, the patient’s wound had healed well. At the 4-mo postoperative follow-up, the patient did not complain of pain in the left knee joint. The left knee could extend 0° and flex 120° (Figure 5). The Kujala score was 95 on the left knee.

DISCUSSION
Tibial intramedullary nail placement can be achieved by both suprapatellar and infrapatellar access. The suprapatellar approach has more advantages than the infrapatellar approach[4-8]; however, how best to remove the nail via the original suprapa-
When using the traditional method to remove the intramedullary nail through the original suprapatellar incision, it is necessary to first remove the bone above the intramedullary nail with a hollow drill, remove the end cap, and take out the end of the intramedullary nail and screw it into the target device to remove the intramedullary nail. Because the whole process is not performed under direct vision, the operation is difficult and time-consuming. The main difficulty lies in how to accurately remove the bone above the intramedullary nail. If care is not exercised, the meniscus and anterior and posterior cruciate ligaments can become damaged. The cap should then be safely removed without being lost in the joint space. Therefore, most surgeons have no choice but to use the infrapatellar access to remove the internal fixation, but this often leads to new scar formation. Moreover, surgical incision can also damage the saphenous nerve, the patellar ligament, and the infrapatellar fat pad, resulting in a high probability of postoperative knee pain.

A previous study has shown that the end cap of an intramedullary nail stops bony in-growth of tissue[9]. To facilitate the method presented in this paper, the end cap was not used in the initial nail placement, and bony in-growth of tissue enclosed the end of the nail. To remove the tibial intramedullary nail, a guide needle was inserted into the cavity of the intramedullary nail. The results were confirmed by intraoperative X-ray. A hollow jig was used to screw the end of the nail along the guide needle. This process accurately removed the bone on top of the nail. Thus, the difficulty in removing the nail via the original incision was greatly reduced. The whole process was easy, and no special tools were needed. Due to the use of a sleeve during surgery to protect important tissues within the joint, the likelihood of damage to the patellofemoral joint was greatly reduced.

However, this novel approach has some potential limitations that should be noted. First, removal of the bone above the intramedullary nail may lead to possible entry of large bone fragments into the articular cavity. Second, after intramedullary nail removal, the intramedullary content entering the joint cavity may lead to joint cavity extravasation and increase the risk of infection.

CONCLUSION

In this study, removal of the intramedullary nail via the original suprapatellar incision...
He M et al. Removal of tibial intramedullary nail

was simple and reliable, did not require special equipment, and did not require infrapatellar access, which reduced the likelihood of complications.

ACKNOWLEDGEMENTS

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REFERENCES

Recurrence of sigmoid colon cancer–derived anal metastasis: A case report and review of literature

Ling-Kang Meng, Dan Zhu, Yu Zhang, Yuan Fang, Wei-Zhen Liu, Xia-Qing Zhang, Yong Zhu

BACKGROUND
Distant metastasis of colorectal cancer to the anus is very rare, with only 30 related cases published in PubMed thus far. Therefore, recurrence of colorectal cancer derived anus metastases is rarely seen and less presented.

CASE SUMMARY
Here we report an 80-year-old male patient who underwent radical resection for sigmoid colon cancer in January 2010 and another surgery for anal fistula resection in December 2010. Postoperative pathology of the anal fistula revealed a metastatic moderately differentiated adenocarcinoma. The patient subsequently received chemotherapy and radiotherapy. In May 2020, after the patient reported symptoms of anal swelling and pain, computed tomography and magnetic resonance imaging revealed a perianal abscess. Perianal mass biopsy was performed, and the postoperative pathological diagnosis was metastatic moderately differentiated adenocarcinoma.

CONCLUSION
This case highlights that there is a risk of recurrence of anal metastasis of colorectal cancer even after 10 years of follow-up. We also reviewed the literature and discuss potential mechanisms for anal metastasis of colorectal cancer, thus providing some suggestions for treatment of these cases.

Key Words: Sigmoid colon cancer; Colorectal cancer; Anal metastasis; Recurrence; Case report

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Core Tip: Metastasis of colorectal cancer to the anus is very rare. We describe a patient who had a local anal metastatic recurrence after chemotherapy and local anal radiotherapy. This case highlights that there is a risk of recurrence of anal metastasis of colorectal cancer even after 10 years of follow-up.

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INTRODUCTION

The incidence of colorectal cancer is 38.7 per 100000 and 50%-60% of patients develop distant metastases with the liver being the most common site of involvement[1]. The most common seeding metastatic site of colorectal cancer is the anastomosis[2]. In contrast, metastasis at the anus is rare, with only 30 cases published in PubMed thus far. Due to the limited number of cases and insufficient information for anal metastasis of colorectal cancer, the diagnosis of such patients is difficult. In addition, there is currently no standard treatment and postoperative management strategy for anus metastasis of colorectal cancer. In most cases, patients receive surgical treatment and some are also treated with radiotherapy or chemotherapy. Patients who receive surgical treatment typically exhibit a good prognosis with a low recurrence rate. We reviewed and analyzed the relevant literature to provide more information to help clinicians better recognize and treat similar cases in the future.

CASE PRESENTATION

Chief complaints
In May 2020, an 80-year-old man presented with symptoms including anal swelling and pain.

History of present illness
Patient’s symptoms started a month ago with recurrent episodes of anal swelling and pain, as well as blood in the stool and diarrhea.

History of past illness
The patient went to hospital for colonoscopy due to repeated blood in stool in January 2010. A mass in sigmoid was found and pathology showed moderately differentiated adenocarcinoma. Subsequently, he underwent an open radical resection of the sigmoid colon in January 2010. Postoperative pathological examination showed moderately differentiated adenocarcinoma, pT3N1M0, with invasion to the serosal layer; the margin was free and 1 of 29 Lymph nodes was positive. In December 2010, the patient complained of anal swelling and pain and subsequently underwent anal fistula resection. Postoperative pathology revealed moderately differentiated adenocarcinoma and the margin was free. In December 2010, the patient began 6 cycles of chemotherapy with the FOLFIRI regimen and one course of local anal radiotherapy (45 Gy in 25 fractions). In September 2019, he was admitted to the Department of Hematology for four rounds of Azacytidine chemotherapy for myelodysplastic syndrome (MDS).

Personal and family history
The patient did not have any history of anal disease. His family history was unremarkable.

Physical examination
Our physical examination found an approximate 3 cm × 3 cm perianal ring-shaped mass with obvious tenderness.
**Laboratory examinations**
Blood count shows lymphocyte count $1.03 \times 10^9/L$, red blood cell count $3.52 \times 10^{12}/L$, hemoglobin 114 g/L and albumin 33.2 g/L. Blood tests for cancer-associated markers revealed the carcinoembryonic antigen (CEA) of 5.95 ng/mL and carbohydrate antigen 199 (CA199) of 20.59 U/mL. Fecal occult blood test was positive.

**Imaging examinations**
Colonoscopy did not detect any mass or abnormality. Computed tomography found low-density shadows on the posterior edge of the anal canal. Magnetic resonance imaging further confirmed that the 22.8 mm $\times$ 24.2 mm lesion went through the external sphincter. The internal fistula was located at 6 o’clock on the posterior edge of the anal canal; the external fistula was at the left side of the buttocks; and the subcutaneous soft tissue signal of the buttocks was increased (Figure 1). Biopsy test of the anal mass was performed by resecting the most obvious swollen part at the lithotomy position. Postoperative pathology of this soft and poorly structured tissue showed moderately differentiated adenocarcinoma with large amounts of necrotic tissue that was positive for cytokeratin 20 (CK20) and negative for cytokeratin 7 (CK7) (Figure 2).

**FINAL DIAGNOSIS**
Based on pathology as well as the patient’s history, the final diagnosis was metastatic anal cancer derived from sigmoid colon cancer.

**TREATMENT**
The patient underwent a biopsy test of the anal mass.

**OUTCOME AND FOLLOW-UP**
We planned to perform abdominoperineal resection (APR) after chemotherapy for MDS in another hospital. However, the patient died due to MDS in November 2020.

**DISCUSSION**
The most common distant metastasis site of colorectal cancer is the liver [3]. Regarding implantation metastases, the most common ones are observed at anastomoses and biopsy sites, and some studies have reported metastases at fistulas and hemorrhoids [2, 4-9]. Metastasis of sigmoid colon cancer to the anus is very rare [10], and so far, the underlying mechanism remains unknown. One possible explanation for these metastases is that improper operation during surgery may cause tumor cells to fall off and relocate, but in general, tumor cells do not easily implant to intact mucosa. However, the intestinal mucosa could possibly be damaged when surgical instruments or fingers are used to expand the anus during surgery. In this case, damaged intestinal mucosa might become an adhesion target for tumor cells, which would then colonize and begin to proliferate [5, 6, 8, 11]. This phenomenon has been observed in mouse models. For example, Hubens et al [12] observed that mice with damaged intestinal mucosal develop gut tumors after colorectal cancer cells perfuse into the colon, while no mice with intact intestinal mucosa showed tumor growth. Another possible explanation is that tumor cells were already implanted into the existing fistula before resection of the primary tumor. Occasionally, clinical symptoms appear when the tumor grows to a sufficient size [13]. In addition, one study reported the same DNA aneuploid cell line in sigmoid colon tumors and perianal tumors [14]. These findings supported a potential metastasis mechanism of tumor cells migrating from the colon to anus. More cases and studies are warranted to better elucidate the underlying mechanisms.

Diagnostic criteria have not been established in metastatic anal cancer. First, colorectal cancer cases with first symptoms as anal fistula and perianal abscess should be excluded [3]. Additionally, diagnosis of metastatic anal cancer should include
Figure 1 Imaging documented the anal mass (see orange arrows). A: Low-density shadows on the posterior edge of the anal canal in computed tomography; B and C: magnetic resonance imaging showed increased signal in anal tissue.

Figure 2 Pathology of anal mass. A: Histology showed moderately differentiated adenocarcinoma, as orange arrow marked; B and C: Pathological staining for CK7 (B) and CK20 (C), with orange arrows marking negative and positive staining.

primary tumors in the colon with five exclusion criteria for primary anal fistula cancer: (1) More than 10 years of history of anal fistula; (2) Induration and severe pain at the anal fistula; (3) Mucus secretion; (4) Internal opening in the anus and anal recess; and (5) No tumor on the cranial side of the anal fistula[15]. More importantly, immunohistochemical staining of CK7 and CK20 biomarkers is usually used to confirm the presence of a metastatic tumor. Anal tissue shows strong positive expression only for CK7, while colorectal tumor tissue shows positive CK20 expression[16,17]. Immunohistochemical analyses of the tumor in the current case were CK20 positive and CK7 negative, consistent with our diagnosis as metastatic anal cancer.

We further reviewed previous publications of these cases. Guiss[18] published the first case report of sigmoid colon cancer implanted anal fistula in 1954. We retrieved 25 papers from PubMed describing a total of 30 cases of colorectal cancer metastasis to the anus (Table 1). Among the 30 patients, there was only one female, and the mean patient age was 60.2 yr. Seventeen patients (56.7%) had a history of anal disease. Most patients complained of anal abscess and induration as first symptoms. All primary tumors were located in or below the descending colon; 13 tumors (43.3%) were located in the colon, 12 tumors (40%) were at the junction of the rectum and sigmoid colon, and the remaining 5 tumors (16.7%) were in the rectum. This location information may support the idea that seeding metastasis, instead of hematogenous or lymphatic was more likely the cause of anal metastasis formation. All 30 patients underwent radical
<table>
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<td></td>
<td></td>
<td>Perianal abscess</td>
<td>10 yr</td>
<td>LR</td>
<td>MDA</td>
<td>No</td>
<td>5/death due to MLD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M: Male; F: Female; NS: Not specified; AF: Anal fistula; DC: Descending colon; SC: Sigmoid colon; RS: The junction of the rectum and sigmoid colon; R: Rectum; APR: Abdominoperineal resection; SCR: Sigmoid colon resection; AR: Anterior resection; LAR: Low anterior resection; DCR: Descending colon resection; LR: Local resection; BVI: Blood vessel invasion; WDA: Well differentiated adenocarcinoma; MDA: Moderately differentiated adenocarcinoma; NA: Not available.

Primary tumor resection; 16 cases (53.3%) had synchronous metastases and the rest 14 cases (46.7%) had metachronous metastases at approximately 8.5 mo post-surgery. Due to the limited number of cases, there is currently no standard treatment method.
for colorectal cancer–derived anus metastasis. Surgery is still the most common treatment method. Among the 30 cases, 13 patients (43.3%) received APR surgery, 15 patients (50%) underwent additional local lesion resection after radical colorectal surgery, and 3 patients (10%) did not undergo surgery because of extensive tumor metastasis or disapproval of the surgical plan. Overall, postoperative pathology was mostly moderately or well-differentiated adenocarcinoma. Notably, only 11 patients received radiotherapy and chemotherapy during the perioperative period. However, the prognosis of most patients was good. The average follow-up time for patients was 29.9 mo. Only one patient died 10 mo after surgery from extensive peritoneal metastasis[19].

Compared with the previously reported cases, our cases show some unique characteristics and findings. The patient received radiotherapy and chemotherapy after resection of local anal metastatic lesions in 2010, but recurrence of local anal tumor still occurred 10 years later. However, this patient needed chemotherapy for MDS with ring sideroblasts and with multilineage dysplasia (RS-MLD), so only a perianal mass biopsy was performed to confirm the diagnosis. Although APR surgery was planned after the chemotherapy, the patient still required management for RS-MLD and died 5 mo later.

The main surgical treatment options are APR and local resection. Although APR is more effective in reducing the risk of residual tumor cells, the life quality of patients is relatively poor. Therefore, we suggest that local resection should be considered first to ensure that patients have a better quality of life after surgery when the anus tumor does not aggressively grow. In addition, Ikeda et al[5] indicated that tumors should be treated first when the patient exhibits other anal diseases. Otherwise, it is possible that the tumor cells may easily implant on the anal wound and cause anal recurrence. Regarding perioperative radiotherapy and chemotherapy, a retrospective study of metastatic anal cancer patients from 1950 to 2011 found that the combination of preoperative or postoperative radiotherapy, chemotherapy and radical surgical resection provided patients with better survival compared with patients receiving surgeries only[20]. At present, there is no standard postoperative follow-up management guideline, so we should extend the postoperative follow-up time for such patients to detect the disease and provide treatment in a timely manner.

The current patient reported no anal disease before the first radical surgery. Although without immune-histological result, histological features of this anal mass were moderately differentiated gland cancer, similar to primary tumor in sigmoid colon. Considering the anatomical structure of the colon and anus and combined with the patient’s medical history, we therefore believe that the lesion was derived from sigmoid colon tumor cells. Since any shed tumor cells would not be implanted on the intact intestinal mucosa, as discussed above, we assume that this may be from stapler use that damaged the anal mucosa during the operation. The patient showed a relapse at the anus, and the colonoscopy showed no tumor in the colon. Moreover, immunohistological results showed the tumor was derived from colon, so it was possible that a small amount of tumor cells had remained in the anus. Moreover, chemotherapy for MDS for 4 mo potentially impaired the patient’s immune system, causing any remaining tumor cells to proliferate.

Norgren et al[11] and Tranchart et al[21] also reported cases of recurrence of local scars in the anus caused by the use of staplers and retractors during operation. Therefore, surgeons should be aware of the importance of protecting the mucosa during surgical procedures, for example during staple use and retraction. Another study reported the presence of tumor cells in washing solution after rectal washing during surgery. Therefore, sterile water or 5% povidone-iodine and other cytotoxic solutions may be useful to wash the surgical area to reduce the numbers of any remaining tumor cells and prevent local recurrence[22,23]. A close follow-up around the anus after surgery is also recommended.

**CONCLUSION**

Metastasis of colorectal cancer to the anus is very rare. The clinical symptoms are similar to benign anal diseases like perianal abscesses and anal fistula, which makes the diagnosis of metastasis of colorectal cancer to the anus more difficult. Currently, pathological examination and staining of CK7 and CK20 markers can contribute to diagnosis of anal metastases. In addition, surgeons should pay attention to protecting the normal mucosa during operation to reduce the possibility of implant metastasis caused by iatrogenic injury. During surgery, surgical area irrigation with cytotoxic
solution is also recommended to reduce the number of remaining tumor cells. For patients with anal metastasis, the follow-up time after surgery should be extended. Accumulating more clinical data is necessary to establish treatment and postoperative management standards for colorectal cancer-derived anal metastasis.

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Mycoplasma hominis meningitis after operative neurosurgery: A case report and review of literature

Nian-Long Yang, Xiao Cai, Qing Que, Hua Zhao, Kai-Long Zhang, Sheng Lv

Abstract

BACKGROUND

Mycoplasma hominis (M. hominis), which causes central nervous system infections in adults, is very rare. It is also relatively difficult to culture mycoplasma and culturing requires special media, resulting in a high rate of clinical underdiagnosis. Therefore, clinicians often treat patients based on their own experience before obtaining pathogenic results and may ignore infections with atypical pathogens, thus delaying the diagnosis and treatment of patients and increasing the length of hospital stay and costs.

CASE SUMMARY

A 44-year-old man presented to the hospital complaining of recurrent dizziness for 1 year, which had worsened in the last week. After admission, brain magnetic resonance imaging (MRI) revealed a 7.0 cm × 6.0 cm × 6.1 cm lesion at the skull base, which was irregular in shape and had a midline shift to the left. Based on imaging findings, meningioma was our primary consideration. After lesion resection, the patient had persistent fever and a diagnosis of suppurative meningitis based on cerebrospinal fluid (CSF) examination. The patient was treated with the highest level of antibiotics (meropenem and linezolid), but the response was ineffective. Finally, M. hominis was detected by next-generation metagenomic sequencing (mNGS) in the CSF. Therefore, we changed the antibiotics to moxifloxacin 0.4 g daily combined with doxycycline 0.1 g twice a day for 2 wk, and the patient had a normal temperature the next day.

CONCLUSION

Mycoplasma meningitis after neurosurgery is rare. We can use mNGS to detect M. hominis in the CSF and then provide targeted treatment.
Mycoplasma hominis meningitis after neurosurgery

Key Words: Neurosurgery; Central nervous system infections; Meningitis; Mycoplasma hominis; Next-generation sequencing; Case report

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Core Tip: Mycoplasma meningitis after neurosurgery is relatively rare. Intracranial infections with atypical pathogens are difficult to identify. Because Mycoplasma hominis (M. hominis) has no cell wall, it cannot be observed by Gram staining. Moreover, the difficulty of culturing M. hominis increases the challenge of clinical detection and often delays treatment. Next-generation metagenomic sequencing can be used to identify the pathogen in the early stage of the disease.

INTRODUCTION

Mycoplasma hominis (M. hominis) is a common colonizer in the microflora of the genitourinary tract of many sexually active adolescent females. M. hominis can be found in the cervical or vaginal secretions of up to 50% of healthy women[1]. At present, it has been demonstrated that pathogenic M. hominis is mainly distributed in the oropharynx and urogenital tract[2]. M. hominis is associated with certain diseases of parturient women, their fetuses and newborns, but it is rare for M. hominis to cause central nervous system infections in adults. Because M. hominis has no cell wall, it cannot be observed by Gram staining. Moreover, the difficulty of culturing M. hominis increases the challenge of clinical detection and often delays treatment. Here, we report a case of M. hominis infection secondary to craniocerebral surgery detected by next-generation metagenomic sequencing (mNGS). We also reviewed relevant literature to analyze the clinical features, diagnosis and treatment methods of central nervous system infections caused by M. hominis to deepen the understanding of this type of infection among clinicians and improve the diagnosis and treatment options.

CASE PRESENTATION

Chief complaints

A 44-year-old man presented to our hospital complaining of worsening dizziness.

History of present illness

One year before admission, the patient suffered from repeated episodes of dizziness without blurred vision, nausea, vomiting or limb dysfunction. However, the symptom did not cause alarm. A week ago, his dizziness worsened, and he presented to the hospital.

History of past illness

Healthy, with no specific diseases.

Physical examination

Physical examination upon admission showed that the patient had no nystagmus, no neck rigidity, normal muscle strength and muscular tension of the limbs, and negative pathological signs.

Laboratory examinations

On admission, the patient's examination results were completely normal, including
leukocyte count, hypersensitive C-reactive protein, procalcitonin, electrolytes, liver and kidney function tests and coagulation function tests. On the third postoperative day, the leukocyte count was $14.8 \times 10^9$/L (reference range: 4-10 × $10^9$/L), and the neutrophil count (NEUT%) was 89.5% (reference range: 40%-75%). The cerebrospinal fluid (CSF) examination showed $62.9 \times 10^6$ white blood cell (WBC)/μL, with a protein level of 8036 mg/L, glucose level of 3.8 mmol/L and chloride ion concentration of 139 mmol/L.

**Imaging examinations**
The brain magnetic resonance imaging (MRI) examination revealed a massive mass outside the right anterior and middle cranial base. The main body of the lesion was in the middle cranial base with an irregular shape and a size of approximately 7.0 cm × 6.0 cm × 6.1 cm. The right ventricle and cerebral peduncle were compressed, and the midline was shifted to the left (Figure 1). On postoperative day 10, we reviewed the brain MRI and excluded a brain abscess (Figure 2).

**FINAL DIAGNOSIS**
The initial diagnosis on admission was intracranial space-occupying meningioma. Meningioma, *M. hominis* meningitis and pulmonary infection were diagnosed postoperatively.

**TREATMENT**
The patient was admitted to the hospital, and preoperative examinations were completed. The patient underwent intracranial tumor resection on May 4, 2020. The operation lasted approximately 9 h, and the intraoperative bleeding volume was 2000 mL. Preoperative and postoperative cefathiamidine was used to prevent infection. On the second day after surgery, the patient was conscious. The muscle strength of the left limb was approximately grade 3, whereas the muscle strength of the right limb was normal. The patient was extubated successfully on postoperative day 3. Also, on postoperative day 3, the patient developed fever with a temperature of 38.3°C. Laboratory studies revealed that the leukocyte count was $14.8 \times 10^9$/L (reference range: 4-10 × $10^9$/L), and the NEUT% was 89.5% (reference range: 40%-75%). Then, we changed the antibiotic to cefoperazone-sulbactam. However, the patient's temperature continued to increase. At this time, we found that the patient had neck rigidity. Thus, we performed a lumbar puncture. The CSF examination showed a WBC level of $62.9 \times 10^3$ WBC/μL, protein level of 8036 mg/L, glucose level of 3.8 mmol/L and chloride ion concentration of 139 mmol/L. Blood cultures drawn on postoperative day 3 revealed *Staphylococcus* infection. The antibiotics were changed to meropenem and norvancomycin on postoperative day 6, and a brain abscess was excluded by brain MRI (Figure 2). *M. hominis* was detected in the CSF by mNGS on postoperative day 12. At that time, we believed that *M. hominis* meningitis was rare, the possibility of mycoplasma intracranial infection was low, and the possibility of contamination was high. Thus, we did not adjust the treatment plan. Afterwards, the patient was treated with linezolid and levofloxacin successively, but the body temperature still fluctuated between 38 °C and 39 °C. Just when we were at a loss, we discussed and developed a treatment plan with the neurosurgeons, infectious disease specialists, and hematologists and decided to use special media to culture the CSF for mycoplasma. We also reviewed the literature on *M. hominis* meningitis. A total of 19 studies published from inception to the end of June 2020 were retrieved, including 11 cases of *M. hominis* brain abscess, 6 cases of meningitis and 2 cases of spinal cord abscess (Table 1). Finally, *M. hominis* was cultured from the CSF, which confirmed the mNGS results. We finally changed the antibiotic to moxifloxacin combined with doxycycline on postoperative day 18. The patient's temperature returned to normal on the second day after adjustment of the treatment plan, and the patient was later discharged from the hospital (Figure 3).

**OUTCOME AND FOLLOW-UP**
At follow-up 1 year later, the muscle strength of the patient's left limb had returned to

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**Table 1**

<table>
<thead>
<tr>
<th>M. hominis Meningitis</th>
<th>11 cases of M. hominis brain abscess</th>
<th>6 cases of meningitis</th>
<th>2 cases of spinal cord abscess</th>
</tr>
</thead>
</table>

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*WJCC*  |  |  |  |  |  |
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Year published</th>
<th>Country</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>History</th>
<th>Preoperative diagnosis</th>
<th>Risk factors</th>
<th>Clinical symptoms</th>
<th>DM</th>
<th>Specimens</th>
<th>IM</th>
<th>Antibiotics used after diagnosis</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paine et al [15]</td>
<td>1950</td>
<td>USA</td>
<td>M</td>
<td>20</td>
<td>No</td>
<td>Eyeball trauma</td>
<td>Head trauma</td>
<td>Fever, headache, neck stiffness</td>
<td>C</td>
<td>P</td>
<td>BA</td>
<td>St</td>
<td>Cure</td>
</tr>
<tr>
<td>Payan et al [16]</td>
<td>1981</td>
<td>USA</td>
<td>M</td>
<td>29</td>
<td>No</td>
<td>Subdural hematoma, brain contusion</td>
<td>Motor vehicle accident</td>
<td>Fever, disturbance of consciousness</td>
<td>C</td>
<td>P</td>
<td>BA</td>
<td>Te+Er</td>
<td>Cure</td>
</tr>
<tr>
<td>McMahon et al [17]</td>
<td>1990</td>
<td>USA</td>
<td>M</td>
<td>76</td>
<td>Hypertension</td>
<td>Subarachnoid hemorrhage</td>
<td>Urethral catheterization</td>
<td>Fever, disturbance of consciousness</td>
<td>C</td>
<td>CSF</td>
<td>Meningitis</td>
<td>-</td>
<td>Death</td>
</tr>
<tr>
<td>Kersten et al [18]</td>
<td>1995</td>
<td>USA</td>
<td>M</td>
<td>20</td>
<td>No</td>
<td>Eye contusion, hematoma of frontotemporal lobe</td>
<td>Motor vehicle accident, head trauma, hormone therapy</td>
<td>Fever, right eye swelling</td>
<td>C</td>
<td>P</td>
<td>Right eye abscess, BA</td>
<td>Dox + Cli</td>
<td>Cure</td>
</tr>
<tr>
<td>Zheng et al [19]</td>
<td>1997</td>
<td>USA</td>
<td>F</td>
<td>22</td>
<td>No</td>
<td>Right frontal lobe cerebral hemorrhage</td>
<td>Vaginal delivery</td>
<td>Fever, left-sided weakness</td>
<td>ELISA</td>
<td>P</td>
<td>BA</td>
<td>-</td>
<td>Cure</td>
</tr>
<tr>
<td>Cohen et al [20]</td>
<td>1997</td>
<td>USA</td>
<td>F</td>
<td>18</td>
<td>No</td>
<td>Subdural hemorrhage, ventricular hemorrhage</td>
<td>Motor vehicle accident</td>
<td>Fever</td>
<td>C</td>
<td>CSF</td>
<td>Meningitis</td>
<td>Dox + Cip + Ery</td>
<td>Cure</td>
</tr>
<tr>
<td>House et al [21]</td>
<td>2003</td>
<td>USA</td>
<td>F</td>
<td>40</td>
<td>No</td>
<td>Cavernous hemangioma of the right frontal lobe</td>
<td>Perineal ulcers</td>
<td>Fever, nausea, limb dysfunction</td>
<td>C + NGS</td>
<td>P</td>
<td>BA</td>
<td>Cip + Met</td>
<td>Cure</td>
</tr>
<tr>
<td>Kupila et al [7]</td>
<td>2006</td>
<td>Finland</td>
<td>M</td>
<td>40</td>
<td>No</td>
<td>Scalp laceration</td>
<td>Head trauma, cystoscopy, catheterization</td>
<td>Disturbance of consciousness</td>
<td>NGS</td>
<td>P</td>
<td>BA</td>
<td>Te</td>
<td>Cure</td>
</tr>
<tr>
<td>McCarthy et al [22]</td>
<td>2008</td>
<td>Australia</td>
<td>M</td>
<td>48</td>
<td>No</td>
<td>Intracranial colloid cyst</td>
<td>Surgical infection</td>
<td>Fever, disturbance of consciousness</td>
<td>NGS + C</td>
<td>Subdural empyema, bone flap</td>
<td>BA</td>
<td>Gat</td>
<td>Cure</td>
</tr>
<tr>
<td>Al Masalma et al [23]</td>
<td>2011</td>
<td>USA</td>
<td>F</td>
<td>41</td>
<td>No</td>
<td>Spontaneous abortion</td>
<td>Dilatation and curettage</td>
<td>Disturbance of consciousness</td>
<td>NGS</td>
<td>P</td>
<td>BA</td>
<td>Dox</td>
<td>Cure</td>
</tr>
<tr>
<td>Lee et al [24]</td>
<td>2012</td>
<td>Netherlands</td>
<td>F</td>
<td>48</td>
<td>No</td>
<td>Subarachnoid hemorrhage</td>
<td>Ventricular drainage tube</td>
<td>Fever</td>
<td>NGS + C</td>
<td>CSF</td>
<td>Meningitis</td>
<td>Mox</td>
<td>Cure</td>
</tr>
<tr>
<td>Sato et al [25]</td>
<td>2012</td>
<td>Japan</td>
<td>M</td>
<td>26</td>
<td>Hypogammaglobulinemia</td>
<td>Arthritis</td>
<td>Hypogammaglobulinemia</td>
<td>Joint swelling and pain, headache</td>
<td>C+NGS</td>
<td>CSF, joint effusion, blood</td>
<td>Meningitis</td>
<td>-</td>
<td>Death</td>
</tr>
<tr>
<td>Henao-Martinez et al [26]</td>
<td>2012</td>
<td>USA</td>
<td>M</td>
<td>40</td>
<td>No</td>
<td>Right subdural hematoma, subarachnoid</td>
<td>Head trauma</td>
<td>Fever</td>
<td>C+NGS</td>
<td>Brain debridement tissue</td>
<td>BA</td>
<td>Dox</td>
<td>Cure</td>
</tr>
</tbody>
</table>
DISCUSSION

Intracranial infection is a common complication after neurosurgery with a reported incidence of less than 10% and a high incidence at 3 to 7 d postoperatively. Infection is mainly caused by Gram-positive bacteria, which can manifest as subdural empyema, brain abscess, ventriculitis, or meningoencephalitis[3,4]. In recent years, the epidemiology of pathogenic bacteria causing intracranial infections after neurosurgery has changed. Gram-negative bacteria exhibit an obvious increasing trend, and multidrug-resistant or extensively drug-resistant Acinetobacter baumannii also exhibits a gradually increasing trend[5]. Intracranial infection with M. hominis is common in neonates but rare in adults after craniocerebral surgery. Current studies have found that cerebrospinal fluid leakage, ventricular drainage, multiple operations, surgical incision infection, and long operation time (greater than 4 h) are independent risk factors for intracranial infection after craniocerebral surgery[6]. There are three main sources of intracranial infection with mycoplasma: direct contamination during hemorrhage, cerebral contusion, or meningitis after neurosurgery.
trauma, direct contamination during surgery, or bacteremia caused by urogenital tract manipulation secondary to brain site infection. Mycoplasma contains surface proteins that promote cell adhesion and can spread to other sites, leading to infection when the mucosa is damaged, such as with instrument manipulation, surgery, and trauma[1]. Although the results of urine culture were negative many times in this patient, the urinary catheter was continuously indwelling after surgery. Because the urinary tract is a common site of mycoplasma, the possibility of intracranial infection caused by the urinary tract could not be excluded in this patient. Earlier, Kupila et al[7] reported a case of brain abscess with M. hominis secondary to cystoscopy and an indwelling catheter. In this case, the risk of secondary intracranial infection after surgery was significantly increased due to the large tumor volume, long operation time, greater volume of intraoperative bleeding, and presence of a postoperative extradural drainage tube. The patient developed fever on postoperative day 3, and Staphylococcus was detected in blood cultures. Early empirical coverage of Gram-positive bacteria was performed, but the treatment was ineffective. During treatment, we reviewed the relevant domestic and international literature. There have been a few reports on M. hominis infection in adults after craniocerebral surgery. In addition, we lacked clinical experience, so the treatment for M. hominis was delayed. Fortunately, the patient was finally cured and discharged.

At present, mycoplasma culture is the main method for detection of mycoplasma in domestic medical institutions, and this process mainly uses liquid medium for direct culture with simultaneous drug sensitivity tests. Mycoplasma releases ammonia gas by decomposing arginine, resulting in pH changes in the liquid medium and thus a change in the color of the indicator to infer the culture result. Because cholesterol is an

Figure 1 Magnetic resonance imaging scan of the brain. T1- (A) and T2-weighted images (B) showed a large extracerebral mass at the right anterior, middle and posterior cranial base (orange arrow).

Figure 2 Magnetic resonance imaging scan of the brain on postoperative day 10. T1- (A) and T2-weighted imaging (B) did not reveal an abscess in the surgical area.
important component of the cell membrane of mycoplasma and mycoplasma itself does not have the ability to synthesize it, animal serum must be added to the culture medium in vitro to provide cholesterol components. Therefore, the liquid medium must contain arginine and cholesterol. If the solid culture method is adopted, the specimen is cultured in a CO\(_2\) environment for 24-48 h after inoculation and characteristic “fried egg-like” colonies can be observed under the microscope. Due to the uncertainty of the factors leading to pH changes in liquid media, false-positive results may occur. Therefore, the liquid culture method can be combined with the solid culture method in clinical practice to improve the mycoplasma detection rate. The possibility of mycoplasma infection was not considered during the culture of the CSF specimen of this patient, and no special medium was used. Thus, the results of repeated culture were negative. After the mNGS test results suggested \(M.\) hominis, we cultured the CSF again using special medium, and the results confirmed the intracranial infection caused by \(M.\) hominis. Most of the cases we reviewed were diagnosed by mNGS, which not only directly sequences the genomes of samples but also identifies a variety of unknown pathogens in the samples. Compared with traditional culture methods, mNGS requires less time and is more efficient[8]. Long et al[9] showed that, compared with blood cultures, mNGS had a higher sensitivity and pathogen detection rate (30.77% vs 12.82%). Currently, the conserved region of 16S rRNA is the main gene sequence used for the construction of primers. Studies have found that the application of 16S rRNA by real-time reverse transcription PCR (qRT-PCR) can further improve the positive rate of specimen detection and eliminate false-positives[10].

Because mycoplasmas lack a cell wall, they are resistant to β-lactam and glycopeptide antibiotics that act on the cell wall. Tetracyclines that interfere with protein synthesis are commonly used to treat mycoplasmas, which are also sensitive to quinolones that inhibit DNA replication. \(M.\) hominis is typically resistant to macrolides and aminoglycosides. In the cases reviewed, 9 patients were switched to tetracycline antibiotics after the pathogen was confirmed as \(M.\) hominis, and all the patients were cured. In patients with meningitis caused by \(M.\) hominis, if doxycycline treatment fails, clindamycin or fluoroquinolones may be used instead[11]. In the treatment of this patient, levofloxacin was used in the early stages, but the treatment effect was not ideal. After the combined application of moxifloxacin and doxycycline, the patient’s body temperature and infection indices gradually improved. \(M.\) hominis was most sensitive to doxycycline and minocycline but more resistant to erythromycin, norfloxacin and clarithromycin[12]. Although some studies have shown that the drug resistance rate of levofloxacin to mycoplasma has exhibited a declining trend in recent
years, the drug resistance rate of *M. hominis* is approximately 23.08%[13]. However, Zhang *et al*[14] used PCR to amplify drug-resistant genes and found that the drug resistance rate of *M. hominis* to levofloxacin reached 87.9% due to ParC S911 and ParC K144R gene variation. Therefore, doxycycline remains the drug of choice for the treatment of *M. hominis*.

**CONCLUSION**

*M. hominis* infection after craniocerebral surgery in adults is rare, but it can be clearly diagnosed by special culture or mNGS. The clinical prognosis is generally good when treated with targeted anti-infection therapy.

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