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REVIEW

Role of infrapatellar fat pad in pathological process of knee osteoarthritis: Future applications in treatment

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Abstract

It has been found that obese people have a higher proportion in suffering from osteoarthritis (OA), not only in the weight-bearing joints like knee and hip joints, even in non-weight-bearing joints such as hand joints. One of the reasons is because the large amount of adipose tissue secretes some factors, which can promote the occurrence of arthritis. As an important structure of the knee joint, the infrapatellar fat pad (IPFP) is actually a piece of adipose tissue. The aim of this review is to offer a comprehensive view of the anatomy and physiological characteristics of IPFP and its relationship with the pathological process of OA, indicating the important function of IPFP in OA. At the same time, with the development of adipose derived stem cells in the treatment of OA, owing to its special advantages, the IPFP is becoming a kind of important, minimally invasive fat stem cell source, providing a new approach for the treatment of OA. We hope that this review will offer an overview of all published data regarding the IPFP and will indicate novel directions for future research.

Key words: Infrapatellar fat pad; Osteoarthritis; Human mesenchymal stem cells

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Core tip: The infrapatellar fat pad (IPFP) is a piece of adipose tissue, however, due to its special position in the knee joint and the anatomical and physiological characteristics, IPFP plays important roles in the pathological process of osteoarthritis (OA). Meanwhile, the IPFP is becoming a kind of important, minimally invasive fat stem cell source, which can treat OA. We herein widely review the pertinent literature, summarize and analyze the IPFP related vascular and nerve supply, the dual role of IPFP, and its future application prospects.

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INTRODUCTION

Traditionally speaking, what we said osteoarthritis (OA) always refer to the large joint OA, such as knee OA (KOA) and hip OA. This disease mainly affected women aged over 65, especially obese people and those with previous injuries[1]. OA can be considered as a disease that changes not just cartilage but the entire joint organ, including the subchondral bone, menisci, ligaments, periarticular muscle, capsule, and synovium[2]. Although most OA cases are caused by mechanically factors that injury the joint tissues, OA is generally recognized as a multifactorial process such as age, heredity, obesity, and nutritional factors[3,4]. Recent studies indicated the increased risk of hand OA in obese patients, leading people to focus on the systemic inflammatory mediators secreted by adipose tissue, such as cytokines, interleukins (ILs), growth factors, and adipokines in knee OA[5]. Meanwhile, it was found that weight loss can significantly reduce the knee pain in OA patients[6]. And it is worth mentioning that OA has been defined as a local inflammatory disease[7].

The infrapatellar fat pad (IPFP), which is located intra-articularly and extrasynovially in the knee joint, is abundant in adipose tissue[8]. Considering the IPFP location and the role of inflammatory mediators in the OA process, few researchers are now paying attention on the effect of IPFP in the physiopathology of OA and its new application prospect in this disease. This review is therefore to provide a brief update on the role of IPFP in the process of OA and the innovative therapeutic strategies using IPFP nowadays.

ANATOMIC AND BIOLOGICAL CHARACTERISTICS OF IPFP

The IPFP was first described by Hoffa as "Hoffas fat pad" and "Hoffas disease" in 1904[9]. It is an intra-articular and extrasynovial inclusion, covered by synovial membrane[8,10]. On the transverse section, the IPFP is located between the patellar retinacula and patellar tendon anteriorly and the trochlear surface of the femur posteriorly; on the sagittal section, it is located inferiorly to the patella and anteriorly to the femoral condyle and intercondylar notch. The IPFP is composed of adipocytes and adipose connective tissues containing collagen that is embedded in an amorphous ground substance encompassing glycosaminoglycans. It can be divided into two portions, inner and outer tissues[11]. The inner tissue is the core of the pad with hard pillow-like adipose tissue with cushioning properties, whereas the outer tissue is a soft adipose tissue surrounding the inner tissue. It was described that the inner tissue may undergo a compressive load and the outer tissue may undergo a tensional load. The IPFP has the space-filling properties in the joint cavity, which implies an essential role in joint function such as secreting synovial fluid[12], promoting lubrication[13], and shock absorption.

The periphery of the IPFP is highly vascularized, but the center is poorly vascularized. The blood of IPFP is supplied by two vertical arteries, which are connected by two to three horizontal arteries[14] (Figure 1). The primary blood supply originates from the synovial membrane. The IPFP is also richly innervated and contains lymphatic vessels. Bohnsack *et al*[15] and Witoński *et al*[16] found significant distribution of substance-P (SP) nerves inside the IPFP. As a neurotransmitter, SP is released from primary afferent nerve endings and exists in the central, autonomous, and peripheral nerve systems. Besides pain mediation, SP also plays an important role in chronic inflammatory conditions[17]. This neurogenous inflammation is hypothesized to attribute to anterior knee pain. As reported, the nerves of the IPFP are originating from the posterior articular branch of the posterior tibial nerve[18]. However, a study aiming at knee joint innervation described the innervations of the IPFP in detail[19]. The anteromedial portion of the IPFP is innervated by branches of the saphenous, tibial, and obturator nerves and the nerve to vastus medialis, while the anterolateral portion is supplied by branches from the nerve to vastus lateralis as well as the tibial, recurrent peroneal, and common peroneal nerves. The major component

of the IPFP is adipose tissue, which can secrete proinflammatory cytokines and growth factors[20]; therefore, the IPFP could be believed to play a role in joint inflammation. As reported, the IPFP could participate in OA synovial inflammation and the severe inflammation in the IPFP was associated with severe pain in OA[21,22].

DUAL ROLE OF IPFP IN KOA

The IPFP itself can not only secrete large amounts of inflammatory cytokines, adipokines, and growth factors, but also respond to the local inflammatory environment in the joint. A cross-talk between the IPFP and the joint exists (Figure 2). Many scholars believe that the IPFP plays an important role in KOA, but these results are not completely consistent[23].

Positive effect of IPFP in KOA

Since the IPFP is located between articular cartilage and bone surface, it may reduce the knee load and protect the knee joint under physiological conditions or in the early stage of KOA[24]. It has been reported that the IPFP can improve the distribution of joint fluid in joints by increasing synovial area and reduce the friction during exercise[25]. In 2014, Pan *et al*[24] conducted a cohort study of 1100 community populations. They found that the maximum area of female IPFP was negatively correlated with the degree of medial tibial plateau and femoral cartilage damage, and the WOMAC score of knee pain at rest through multivariate analysis. For every 1 cm^2 increase in the area of the IPFP, the score of female knee pain at rest decreased by 0.86 points after 2.6 years. However, the maximum area of female IPFP had nothing to do with the degree of cartilage damage of the lateral tibial plateau and the WOMAC score when walking or going up and down stairs. The above association is not seen in men. It can be seen that the IPFP is at least associated with pain symptoms in women with KOA and has protective effects on cartilage.

Han *et al*[26] conducted a cross-sectional survey of 977 community populations in 2014. The result showed that the area of the IPFP was significantly positively correlated with age, height, and articular cartilage volume. However, the area of the IPFP had nothing to do with body mass index (BMI), although BMI was recognized as a risk factor for KOA. They also found that the area of the IPFP was negatively correlated with osteophytes and subchondral bone marrow edema, and these two indicators were associated with anterior knee pain and cartilage degradation, and were the most common lesions of the subchondral bone in KOA. In addition, some researchers have found that the volume of the IPFP was positively correlated with age though it was not related to the BMI of healthy people and patients with KOA, indicating that changes in body metabolism do not necessarily affect the IPFP, and that the IPFP might not be an inflammatory factor in the early stages of KOA[27]. Therefore, the IPFP may play a protective role in the early stages of KOA.

The IPFP may protect the knee joint by secreting protective biochemical factors. Some researchers have found that lipid-mediated lipoxygen A4 levels in IPFP-derived fat-regulating mediators were higher in healthy people, which can prevent cartilage degradation in the knee[28,29]. In addition, leptin secreted by the IPFP can promote the production of articular cartilage proteoglycan and type II collagen, stimulate the synthesis of insulin-like growth factor-1 and transforming growth factor-β, enhance chondrocyte proliferation, and thus protect against the pathogenesis of KOA[30-34]. What's more, IPFP-derived mesenchymal stem cells (MSCs) have a greater chondroitin effect than bone marrow-derived MSCs, and it can also block the secretion of proinflammatory mediators in synovial and chondrocytes of OA patients[35,36]. A randomized controlled trial in 2015 also showed that mixed transplantation of the IPFP and type I collagen scaffold in the animal model of meniscus injury has better meniscus repair than simple type I collagen scaffold transplantation, which can relieve the formation of KOA to a certain extent[37].

Another important reason may be that the IPFP has the effect of relieving shock, since abnormal biomechanical load plays an important role in the occurrence and development of KOA[38]. Meanwhile, the IPFP may also increase the stability of the knee joint, like the patellar ligament, so as to prevent the occurrence of KOA.

Negative effect of IPFP in KOA

The IPFP is in close contact with the synovial layer and articular cartilage, producing cytokines locally in the joint cavity[39]. In patients with KOA, the IPFP can secrete higher levels of inflammatory factors and adipokines than subcutaneous fat, such as IL-6, lipase, adiponectin, and visfatin, which can promote the pathological process of KOA[40].

Obesity state, indeed, is described as a chronic active inflammatory condition[41], as

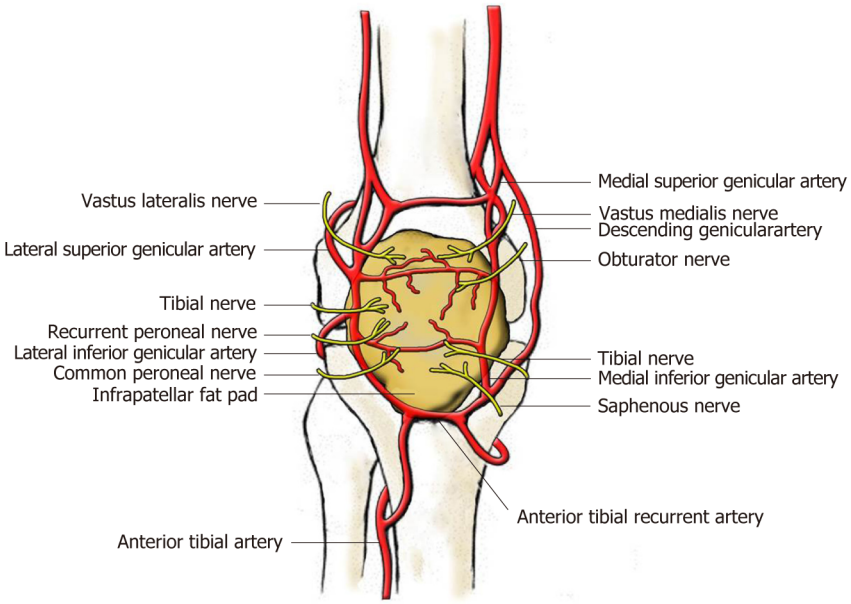

Figure 1 Blood and nerve supply of the infrapatellar fat pad (view from the front).

manifested by increased levels of the inflammatory markers C-reactive protein and IL-6 in the systemic circulation of obese individuals. Obesity has been demonstrated as an important risk factor for the incidence of OA, not only because of mechanically overweight load but also metabolic effects. KOA is the most common OA[42]. Increased weight in women could elevate the risk for KOA[43], while weight loss may reduce the risk for developing symptomatic KOA among adults[44] and reducing BMI could bring healthy benefits[45]. The overload of obese people allows an easy understanding for the mechanical pathogenesis of OA. However, the metabolic factor was observed in the development of OA in non-weight-bearing joints, such as hand OA in overweight or high BMI people. Adipokines secreted by adipose tissue may be involved in the development of OA[5,46]. In 2014, Ballegaard *et al*[22] found that KOOS pain scores in obese patients with KOA were significantly positively correlated with magnetic resonance imaging (MRI) inflammatory signal variables in the IPFP, so inflammatory IPFP in obese patients with KOA may cause knee pain. A cross-sectional study by Cowan in 2015 also showed that patellofemoral arthritis patients had greater IPFP volume on MRI compared with healthy knees, and the volume of IPFP was also positively correlated with KOOS pain scores[47]. The reduction of joint space in patients with KOA can lead to inflammation of the IPFP, which can increase the secretion of synovial inflammatory factors on the surface of femoral condyle while causing swelling of the patellar ligament[48,49].

In addition, some studies hold the opposite view that leptin exerts a proinflammatory role, even though leptin secreted by the IPFP has a protective effect on joints. Leptin level was higher in synovial fluid and serum of OA patients compared with controls[50,51] and had a positive correlation with severity of OA[52]. An *in vitro* study showed that leptin could increase matrix metallopreteinase (MMP) production in human osteoarthritic cartilage and correlated with MMP-1 and MMP-3 in OA synovial fluid[53]. Leptin could also increase the gene expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5[33] and production of NO, PGE2, IL-6, and IL-8[54]. Therefore, inflammatory IPFP may also have a negative effect in the pathogenesis of KOA.

ROLE OF IPFP IN ANTERIOR KNEE PAIN

In patients with KOA, anterior knee pain is a very important complaint of patients, and it is also a problem that patients want to solve urgently. Benjamin *et al*[55] and Eivazi *et al*[56] had proposed the conception of an "enthesis organ", including the structures like ligament, tendon, or joint capsule attached to bone. The IPFP is one of the most important components of the enthesis organ in the anterior knee region. It is always considered as a potential pathogenic factor of knee pain, especially anterior knee pain. The IPFP pressure varies when the knee motions[57] and hyperpression would induce chronic hyperplasia of the IPFP[58]. However, the mechanical

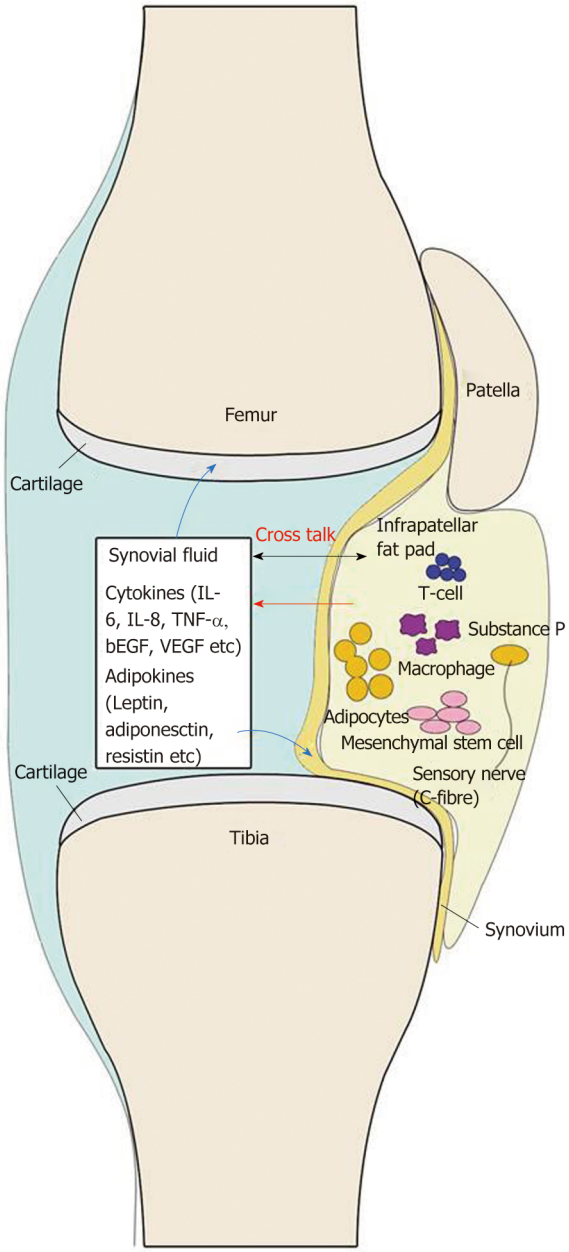

Figure 2 Current view of the infrapatellar fat pad and its interaction with other joint tissues. The cells in the infrapatellar fat pad (IPFP) secrete cytokines, adipokines, and other factors to the synovial fluid, and then these factors react on the cells in the IPFP, synovium, and cartilage.

hypertrophy or swelling is not the main cause of anterior knee pain. Inflammation is the onset of any pain. The inflammatory mediators increased in the IPFP in patients with anterior knee pain[59]. On the other hand, chronic hypertrophy of the IPFP and concomitant soft tissue impingement lead to ischemia, induce the abnormal distribution of SP, and ultimately result in chronic neurogenic tissue inflammation[60,61]. Zhang *et al*[62] showed that the signal intensity change of the IPFP on MRI was closely related to the fluctuation of knee pain symptoms in patients with KOA. However, excision of the IPFP did not improve knee joint function, range of motion, or symptoms of anterior knee pain in patients with KOA, and the exact relationship between the IPFP and knee pain before KOA has not been elucidated[63].

FUTURE APPLICATION OF IPFP IN TISSUE ENGINEERING

Human MSCs have been identified to be multipotent and can be isolated from a large number of adult tissues such as bone marrow, adipose tissue, and umbilical cord

blood. MSCs[64,65]. They have tissue-regenerative properties that exert potent immunomodulatory, antiapoptotic, antifibrotic, and anti-inflammatory effects[35,66,67]. MSCs from the IPFP have been proved to possess significant chondrogenic potential and could provide a clinically feasible source of chondroprogenitor cells[68,69]. MSCs from the IPFP are found to be therapeutic for a one-step surgical procedure to regenerate cartilage tissue[70] and improve symptoms of KOA by intra-articular injection[71]. The extraction procedure of MSCs derived from bone marrow, however, is invasive[72]. In this respect, MSCs isolated from adipose tissue is less invasive and MSCs derived from intra-articular joint tissues are more phenotypically similar to chondrocytes[73]. In the presence of chondrogenic media, fat pad-derived MSCs produce Types I, II, and VI collagen. Type VI collagen, which also presents in articular cartilage, plays an important role in the interaction between chondrocytes and the extracellular matrix[74]. Furthermore, Luo *et al*[69,75] demonstrated that IPFP-derived stem cells could produce a structure and spatial composition mimicking those of native articular cartilage[75]. Prabhakar *et al*[76] isolated progenitor cells from the IPFP, expanded, and then seeded them onto a mechanically stable biodegradable polymer film. After culturing the cells for 28 d, they found the self-assembled tissue rich in sulfated glycosaminoglycan and collagen which had the potential to be implanted into defect sites as a potential treatment for cartilage defect regeneration. Therefore, benefiting from its location, minimal invasiveness, and chondrogenic potential, the IPFP could be used for treating OA *via* repairing damaged cartilage.

DISCUSSION

In this review, we discuss the role of the IPFP in the disease process of KOA and its application prospect in the treatment of this disease. OA has been regarded as a degenerative joint disease with alteration of articular cartilage. Inflammation is recognized as contributing to the symptoms and progression of OA. Obesity is nowadays considered as a chronic low-grade inflammatory status which is related to the release of inflammatory mediators by adipose tissue. The IPFP is indeed a form of adipose tissue and enlarges with age[27]. Due to its close location with articular cartilage and the synovium, it can be speculated with much relationship with OA and synovitis.

Adipocytes, preadipocytes, macrophages, fibroblasts, and other cells in the IPFP release inflammatory mediators such as adipokines and cytokines growth factors that influence cartilage degeneration and synovitis. With the characteristics of minimal invasiveness and multipotentiality, MSCs are now being isolated from the IPFP to provide novel therapies for KOA.

However, further studies should be performed to investigate the precise role of the IPFP in KOA. The role of adipokines in pain should be investigated. In addition, whether adipokines exert their effect on nociceptors in joints remain to be studied. Besides the effect of mediators released by the IPFP on cartilage, tissues such as the synovium and bone marrow are also involved in KOA, so it is also pertinent to explore the effect of mediators on these tissues.

In conclusion, we consider that the IPFP plays an important role in the physiopathology of KOA. Further attention should be focused on the IPFP to explore its new application in KOA.

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MINIREVIEWS

Application of Newcastle disease virus in the treatment of colorectal cancer

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Abstract

Colorectal cancer (CRC) is one of the main reasons of tumor-related deaths worldwide. At present, the main treatment is surgery, but the results are unsatisfactory, and the prognosis is poor. The majority of patients die due to liver or lung metastasis or recurrence. In recent years, great progress has been made in the field of tumor gene therapy, providing a new treatment for combating CRC. As oncolytic viruses selectively replicate almost exclusively in the cytoplasm of tumor cells and do not require integration into the host genome, they are safer, more effective and more attractive as oncolytic agents. Newcastle disease virus (NDV) is a natural RNA oncolytic virus. After NDV selectively infects tumor cells, the immune response induced by NDV's envelope protein and intracellular factors can effectively kill the tumor without affecting normal cells. Reverse genetic techniques make NDV a vector for gene therapy. Arming the virus by inserting various exogenous genes or using NDV in combination with immunotherapy can also improve the anti-CRC capacity of NDV, and good results have been achieved in animal models and clinical treatment trials. This article reviews the molecular biological characteristics and oncolytic mechanism of NDV and discusses *in vitro* and *in vivo* experiments on NDV anti-CRC capacity and clinical treatment. In conclusion, NDV is an excellent candidate for cancer treatment, but more preclinical studies and clinical trials are needed to ensure its safety and efficacy.

Key words: Newcastle disease virus; Exogenous gene; Armed virus; Oncolytic therapy; Colorectal cancer

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Core tip: At present, the main treatment for colorectal cancer are surgical treatment and chemotherapy, but the majority of patients die due to liver or lung metastasis or recurrence. Therefore, there is an urgent need to find more effective treatment strategies to reduce mortality. Newcastle disease virus can selectively infect tumor cells and can also improve the ability of Newcastle disease virus to resist colorectal cancer by constructing an autologous tumor vaccine. Clinical treatment tests have shown a good therapeutic effect and its potential to become a new treatment for colorectal cancer.

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INTRODUCTION

Colorectal cancer (CRC) is one of the main causes of global cancer death, and its incidence continues to rise[1]. At present, surgery and chemotherapy are still the main treatments for CRC. About one-third of patients with CRC will have metastatic tumors of the liver or lung, making their 5-year overall survival rate only 50%[2]. Similar to many other malignancies, CRC is a heterogeneous disease, therefore optimizing treatment and reducing related mortality is the main challenge. In the current treatment of tumors, in addition to traditional surgery, radiotherapy and chemotherapy, immunotherapy has become a very hot field, which mainly includes oncolytic virus[3], tumor vaccine[4], targeted therapy[5], immune cell therapy[6] and immunological checkpoint inhibitors[7]. With the development of genetic engineering and the application of molecular biology, the treatment of CRC with oncolytic virus has been rapidly developed in recent decades, and genetically modified viruses have been used to evaluate the efficacy of anti-CRC *in vitro* and *in vivo*.

NEWCASTLE DISEASE VIRUS (NDV)

Molecular biological characteristics of NDV

NDV is a highly infectious avian pathogen[8] that is an avian paramyxovirus type I virus, and a member of the genus *Avulavirus* in the family Paramyxoviridae[9]. NDV is a bilayered, lipid-coated RNA virus of approximately 100-300 nm with a predominantly spherical morphology. The genome of NDV is a nonsegmented negative-sense, single-stranded RNA [ssRNA(-)] molecule consisting of 15186 nucleotides containing six open reading frames encoding six structural proteins: nuclear protein (NP), phosphoprotein (P), large polymerase protein (L), matrix protein (M), hemagglutinin-neuraminidase (HN) and fusion protein (F). Among its structural proteins, NP, P and L combine with the viral RNA to form the ribonucleoprotein complex, which is responsible for replication of the virus[10] (Figure 1). M comprises a layer underneath the viral membrane that is involved in the assembly and budding of the virus. HN and F are present as oligomers, which together with the lipid bilayer membrane of the host constitute the outer envelope of the virus and are involved in entry of the virus into a cell. F is typically present as the inactive polypeptide F0; cleavage produces the mature membrane-anchored F1 and the membrane-distal F2 domain, resulting in an infectious virus[11].

The HN protein of NDV can trigger a conformational change in the F protein through receptor sialic acid-mediated endocytosis to release the fusion peptide and promote fusion of the virus with the cell membrane and allow the ribonucleoprotein complex to enter the cytoplasm of a host cell[12]. The genome replicates in the cytoplasm[13]: (1) The genomic ssRNA(-) is transcribed into messenger RNA in the cytoplasm for translation into different structural proteins; (2) The antigenome copy, or ssRNA(+), is used as the template for viral genome amplification, and subsequent budding releases virus progeny[14]; and (3) HN can scavenge sialic acid residues and promote spread of the virus in infected tissues. Selective replication of the virus results in host cell lysis only in tumor cells[15,16,17] with replication that is 10000 times

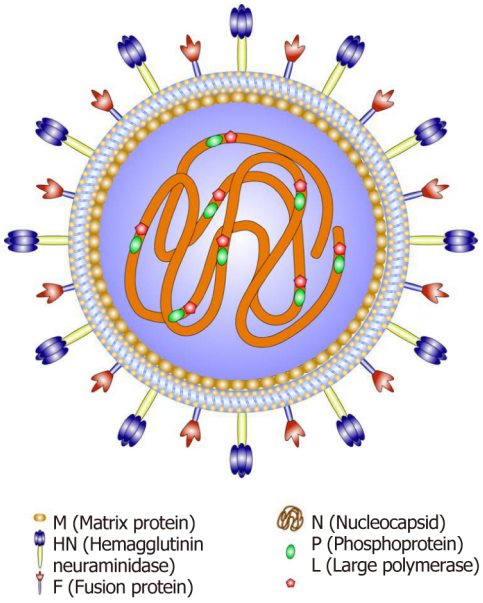

Figure 1 Molecular structure of Newcastle disease virus. Newcastle disease virus contains nuclear protein, P, L protein, M, HN and F. Among these proteins, NP, P and L combine with the viral RNA to form the ribonucleoprotein complex.

faster than that in most normal human cells. Viral replication is terminated *via* the defense mechanisms of interferon (IFN) in normal cells[18]. In contrast, tumor cells usually have a weak type I IFN response and are also less sensitive to type I IFN receptor-mediated signaling; therefore, use of NDV in cancer patients is safe. The frameshift variant protein V is formed during transcription of the P gene. V participates in the antiviral reaction in avian cells, whereby the inhibition of IFN induction by NDV is suppressed by reduced stimulation of IFN-β through degradation of signal transduction and activator of transcription 1 *via* interactions with avian cell proteins[19]. This response occurs because the immune escape mechanism functions only in birds and not in mammalian cells. Therefore, it appears that the V protein reduces the range of NDV hosts.

NDVs are usually divided into three types according to pathogenicity and virulence: Velogenic strains (virulent strains, strongly toxic), mesogenic strains (poisonous strains, moderately toxic), or lentogenic strains (attenuated strains, poorly toxic or nontoxic)[20]. These viruses are also divided into two groups based on the degree of their influence on tumors: (1) Oncolytic strains that form syncytia in tumor cells and have viral oncolytic activity either *in vitro* or *in vivo*; and (2) Nononcolytic strains that inhibit tumor growth and can increase survival, though their killing effect on human tumor cells is still unclear. In general, the site of F protein cleavage mainly determines the virulence of NDV. The F-cleavage site of velogenic and mesogenic strains usually has the polybasic amino acid structure $_{112}$R/G/KR-Q/KK/RR↓F$_{117}$, which is recognized and cut by Folin-like proteases (RXK/RR), inducing deadly respiratory diseases (such as chicken mites) in birds[21]; examples include MTH-68/H, PV701 and Beaudette C. In contrast, lentogenic strains have the single amino acid motif $_{112}$GR/KQGR↓L$_{117}$, which is cleaved by extracellular trypsin-like proteases; thus, replication is limited to specific tissues, and these strains are currently used. For the production of vaccines[22], such as NDV-LaSota and NDV-HUJ, Heiden *et al*[23] constructed a recombinant attenuated strain of NDV clone-30. When the F-cleavage site was altered, the intracerebral pathogenicity index was enhanced 1.2-fold. Overall, NDV virulence is related to the site of F cleavage and is regulated by multiple factors.

Although NDV is dangerous to many birds, its pathogenicity in humans is weak. Furthermore, most people are seropositive for NDV, and thus the immunogenicity that NDV may cause can be ignored. Under natural conditions, highly virulent NDV strains may infect humans but cause only mild fever, cough and other flu-like symptoms.

The oncolytic mechanism of NDV

The cells of various human tumors, such as liver cancer[24], glioblastoma[25] and lymphoma[26], have been shown to be sensitive to NDV. In addition, because NDV RNA transcription and translation are not related to cell proliferation, the virus can target tumor stem cells, dormant tumor cells, and X-ray-irradiated vaccine tumor

cells[27].

The oncolytic mechanism of NDV mainly include the following aspects. First, the virus selectively infects and replicates in tumor cells. Second, indirect effects of the innate and adaptive immune responses of the host immune system act against the virus, involving natural killer (NK) cells and cytotoxic T lymphocytes targeting the antigen[28,29]. Third, the envelope protein also participates in the oncolytic effect. Fourth, the apoptotic pathway promotes the oncolytic effect. Fifth, the virus induces immunogenic death, necrosis and autophagy (Figure 2).

The NDV envelope protein participates in oncolytic effects

Studies have found that a variety of proteins of NDV can impact infected cells, and protein sequence characterization also revealed that M, L and F have a BH-3 domain that shows homology with the proapoptotic factor Bcl-2[30]. Among these proteins, HN is an important immunogenic protein and virulence factor. Indeed, it has been reported that despite weaker efficacy than the parental NDV AF2240 strain, HN gene expression alone was able to induce apoptosis in human breast cancer MCF-7 cells[31]. HN induces release of type I IFN from human peripheral blood mononuclear cells and up-regulates expression of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)[32]. He *et al*[33] constructed a novel oncolytic adenovirus containing the human telomerase reverse transcriptase (hTERT) promoter and expressing the HN protein of NDV (Ad-hTERTp-E1a-HN) that selectively inhibits esophageal cancer EC-109 cells and inhibits tumor growth in mice. In addition, studies have indicated that the M protein of NDV AF2240 strain binds to Bax through its BH-3 domain to promote the transfer of Bax from the cytoplasm to the mitochondrial membrane, thereby activating the intrinsic apoptotic pathway[34].

The IFN system affects oncolytic activity

NDV stimulates the body's immune system to produce a variety of cytokines with anti-tumor activity, such as various IFNs or TNF. Type I IFN exerts a direct antitumor effect by targeting tumor cells and tumor stem cells and can indirectly stimulate immune system activity to assist in tumor killing. The recombinant NDV obtained by inserting the influenza virus NS1 gene, which antagonizes the IFN system in mammalian cells, into the NDV Hitchner-B1 genome promoted apoptosis in human tumor cell lines and the B16 melanoma mouse model with enhanced oncolytic effects[35].

An apoptotic pathway is involved in the NDV oncolytic effect

Some studies have shown that the oncolytic effect of NDV is related to the various apoptotic pathways of cells, and the apoptosis induced by NDV requires viral replication and expression of apoptotic proteins.

The exogenous apoptosis pathway is mainly mediated by the death receptor Fas and its ligands FasL and TRAIL. For example, due to overexpression of Fas, the recombinant attenuated strain rNDV-B1/Fas exhibited enhanced oncolytic ability *in vitro* and *in vivo*, which resulted in an earlier and stronger apoptotic response in infected cells[36]. Compared to the wild-type virus, Bai's recombinant virus, LaSota-TRAIL[37], increased expression of TRAIL by 3-fold. In a mouse experiment, the LaSota-TRAIL group displayed significantly increased survival and decreased tumor recurrence. Other studies have indicated that due to overexpression of the inhibitor of apoptosis protein Livin in tissues of stage III melanoma, advanced melanoma is more susceptible to the NDV-HUJ virus than is an early-stage tumor[38]. Up-regulation of the apoptosis inhibitory protein survivin prolongs the viability of human breast cancer-resistant cells infected with the NDV AF2240 strain and increases viral protein synthesis as well as viral replication[39].

NDV causes immunogenic cell death

One immunotherapy mechanism of oncolytic viruses is immunogenic cell death (ICD)[40]. The manner in which NDV induces ICD in tumor cells includes immunogenic apoptosis, necrosis and pyroptosis, with termination of protein synthesis and subsequent exposure to calreticulin, heat shock proteins and the viral proteins HN and F.

Under endoplasmic reticulum stress, accumulation of unfolded proteins or misfolded proteins in the endoplasmic reticulum can cause the unfolded protein response, a specific response in NDV-infected cells. Activation of the unfolded protein response triggers caspase 12-induced cell death. Moreover, Cheng *et al*[41] demonstrated that the structural proteins NP and P induce autophagy through the endoplasmic reticulum stress pathway.

The corresponding pattern recognition receptors of innate immune cells include cytoplasmic RIG-1, PKR, TLR and NKp46. The pathogen-associated molecular pattern

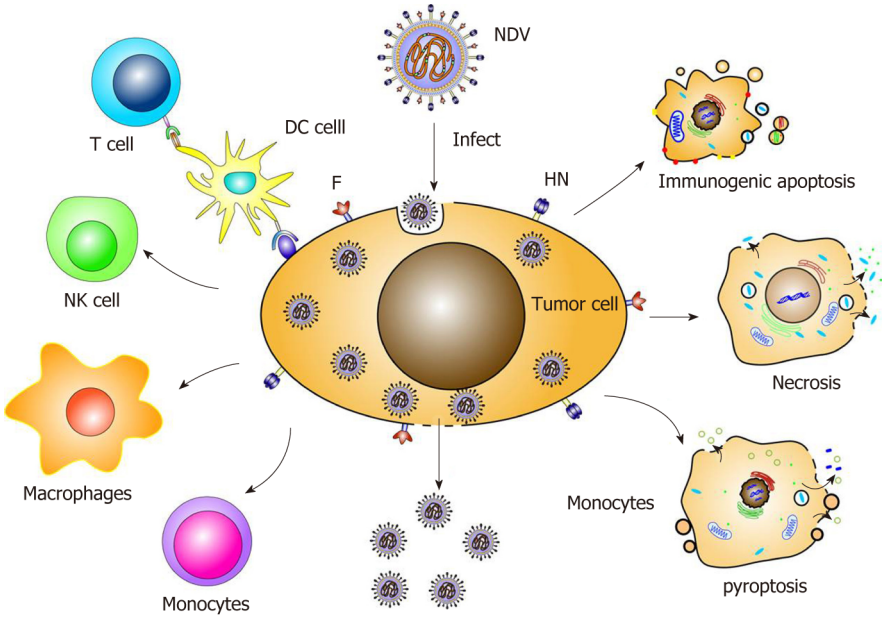

Figure 2 The main anti-tumor mechanisms and consequences of NDV infection of tumor cells. NDV enters the cytoplasm of tumor cells by endocytosis, stimulates the host's innate immune response, activates NK cells, monocytes and macrophages and promotes acquired immunity. The antigen presentation of tumor cells is enhanced. NDV can cause the immunogenic cell death of tumor cells *via* immunogenic apoptosis, necrosis and pyroptosis. HN: Hemagglutinin-neuraminidase; NDV: Newcastle disease virus; NK: Natural killer.

of NDV involves the virus 5′-adenosine triphosphate leader RNA, dsRNA and HN protein. Pattern recognition receptors trigger a variety of immune responses, including induction of type I IFN responses, promotion of immune cell activation and release of immune factors. In a mouse *in situ* glioma model, NDV virus therapy and molecules such as calreticulin, heat shock proteins and high mobility group protein-1 were found to induce ICD, stimulating specific immune T cells. Additionally, interaction between RIG-1 and RNA simultaneously activated type I IFN and induced IL-1β production. The double-stranded RNA of NDV induces expression of TLR-3, FN-α and heat shock proteins, which promotes tumor cell apoptosis and inhibits tumor growth by enhancing the immune system response. NKp46 of NK cells recognizes viral HN proteins and transmits cytotoxicity-inducing signals, increasing production of IFN-α and TRAIL. After NDV infection of monocytes and NK cells, IL-2, IFN-γ, GM-CSF and TNF-α are produced to promote tumor-killing activity through TRAIL[42,43].

Viral infection of tumors can promote an immunosuppressive environment by inducing immune cytokines and chemokines (RANTES and IP-10)[44]. Although cytokines and chemokines recruit and activate neutrophils, NK cells, macrophages and $CD4^+$ and $CD8^+$ T lymphocytes, contributing to viral clearance, these molecules also alter immunosuppression. HN molecules on the surface of infected tumor cells increase the cell adhesion strength of lymphocytes and T cells for T cell costimulation. As the first responder of innate immunity, neutrophils cause the release of the chemokines CXCL1, CCL2 and CXCL10, thereby mediating immunogenic cell death[45].

NDV induces programmed cell death, necrosis and autophagy

After NDV infection of cells, the accumulation of HN and F proteins on the host cell surface promotes the formation of cell syncytia and lead to cell-to-cell fusion, which ultimately triggers necrosis, syncytium disintegration, content release and an inflammatory response. Cell necrosis is also activated by caspase 8 through the cellular TLR and the TNF family.

The HN and F proteins of NDV rapidly induce syncytium formation and initiate stable autophagy fluxes in lung adenocarcinoma cells (A549), synergistically inducing autophagosome fusion with lysosomes for cell degradation[46]. Autophagy is beneficial for viral replication in the early stages of NDV infection of tumor cells and lengthens the cell life cycle by regulating apoptosis. Mitochondrial autophagy has been reported to promote oncolytic NDV replication by breaking the intrinsic apoptosis regulation pathway in lung adenocarcinoma cells[47]. Several ongoing trials are evaluating the impact of autophagy on human tumor therapy[48]. For example, Hu *et al*[49] found that

NDV-infected U251 cells can promote autophagy to degrade lung cancer cells. The autophagy regulators chloroquine and rapamycin significantly enhanced the oncolytic effect of NDV on A549 cells in mice[50]. This finding provides a new idea for exploring the antitumor strategy utilized by autophagy regulators and NDV.

Extracellular matrix molecules in solid tumor tissues affect cell migration and invasion

NDV treatment in different metastatic tumor models has revealed a blockade effect on tumor migration and invasion. Studies have shown that the NDV strain AF2240 can reduce the migration ability of breast cancer cells by directly inducing a decrease in cell proliferation[51]. In addition, oral squamous cell carcinoma was infected with the NDV D90 strain, and a correlation between apoptosis induction and cell migration was observed[52]. Studies have shown that simultaneous injection of NDV, extracellular matrix-degrading enzymes, collagenase and heparanase into a tumor can increase spread of the virus in tumor tissue, thereby enhancing the oncolytic effect[53]. However, because proteolytic enzymes are involved in tumor metastasis and their inhibitors have been used to suppress metastatic tumors, it is not known whether application of virus loading of a matrix metalloproteinase is feasible in the clinic.

NDV IN THE TREATMENT OF CRC *IN VITRO* AND IN MICE

The NDV strains currently used in mouse experimental models and clinical tests include pathogenic (MTH-68/H, Ulster and PV701) and nonpathogenic (Hitchner-B1, LaSota, 73-T and HUJ) strains[54]. NDV has been shown to have potent anti-tumor effects against colon tumors[55], hepatocellular carcinoma[56] and melanoma[57].

The appeal of viral vectors is related to their broad host cell range and high expression levels of foreign genes. Typically, viral vectors with a DNA or RNA genome can be loaded with foreign genes of different lengths; for example, most vectors allow insertion of 6-8 kb, including extensive therapeutic genes[58]. Recently, reverse genetics technology has been applied to help produce recombinant NDV (rNDV) from nonsegmented negative-strand RNA-cloned cDNA to enhance oncolysis[59], as follows: Mutating the F gene[60]; inserting a gene encoding a cytokine, such as IL-2[61], IL-15[62] or IL-7[63], to enhance the immunostimulatory effect; simultaneous insertion of two cytokines synergistically to increase antitumor effects[64]; and inserting a bispecific antibody consisting of a single-chain variable region that can simultaneously target virus and immune cells. Concerning T cells, the targets can be CD3, CD25 and CD28, whereas the target of NDV can be F or HN[65] (Figure 3).

Chia *et al*[66] incubated with human NDV strain AF2240 *in vitro* with human CRC cell lines (SW620, ddd-1, Dks8, HCT116p53+/+, HCT116p53-/- and HT29). Cell death occurred in 70-90% of cells after 96 hours of culture, providing a basis for *in vivo* experiments. In one study, a nude mouse model of human colon cancer SW620 cells was established, and the anti-tumor effect was studied by intratumoral and intravenous injection using NDV Mukteshwar strain. The results showed that tumor growth in mice was inhibited by 43% and 40%, respectively, and the survival time was prolonged[55].

In the subcutaneous model of BALB/c mice bearing CT26 colon cancer, NDV Ulster strain was injected in and around the tumor after tumor inoculation, and the tumor was completely relieved by the 40th day. The long-term survival rate was 70%[67]. When NDV was used for local treatment of liver metastases of MTH-68/HCT26 colon cancer cells, it was observed that tumor growth was significantly inhibited, the survival time of mice was prolonged, and there was no toxic side effect on mice[68]. Ockert *et al*[69] constructed a tumor-bearing mouse model of human colon cancer (SW620, HT29 and MM17387) and injected intratumoral and intraperitoneal injections with NDV 73-T strain. The results showed that multi-dose NDV injection significantly inhibited tumor growth in mice compared with single dose, and the inhibition rate reached 77-96%.

Vigil *et al*[70] treated the constructed CT26 colon cancer mouse model with the NDV recombinant strain rNDV/F3aa-IL-2 expressing IL-2. Compared with the wild-type control virus, the recombinant virus can significantly reduce the tumor growth of the tumor-bearing mice and prolong the survival time, so that the condition of most mice is sustained. Yamaki *et al*[71] established CRC multifocal liver metastasis model of rats or multifocal lung metastasis model using the expression of mutant (L289A) new town of fusion protein of soluble tumor cystic stomatitis virus carrier (types) [rVSV NDV/F (L289A)][72] for hepatic artery and local drug delivery. The pulmonary metastasis models did not show long-term survival. However, in the mice liver

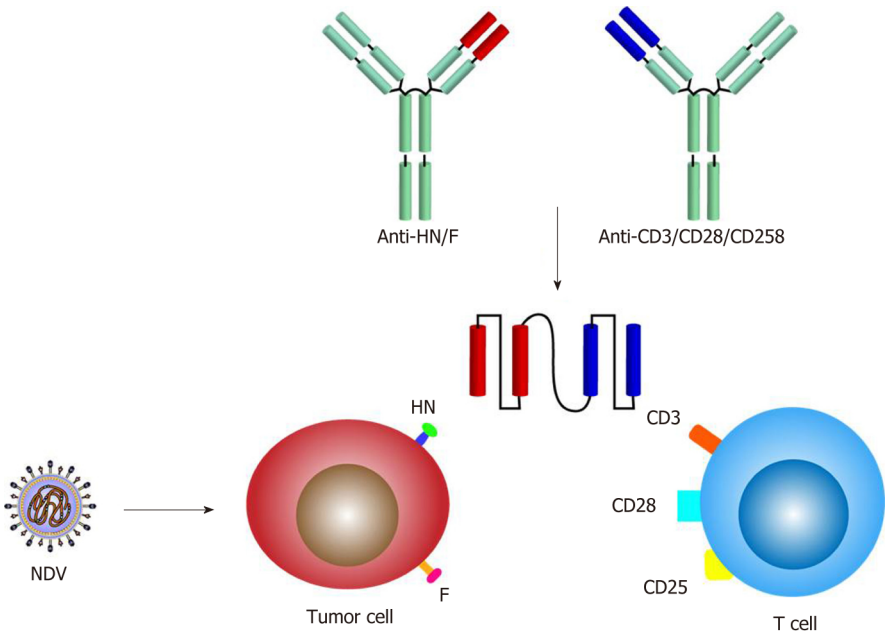

Figure 3 Bispecific antibodies consisting of single-chain variable regions can be simultaneously targeted to viruses and immune cells. When NDV infects tumor cells, bispecific antibodies can target F or HN on the surface of tumor cells and can simultaneously target CD3, CD25 or CD28 on the surface of immune T cells. F: Fusion protein; HN: Hemagglutinin-neuraminidase; NDV: Newcastle disease virus.

metastasis model, four of the seven rats survived for more than 100 d. This result provided an effective basis for the follow-up clinical trials.

CLINICAL APPLICATION OF NDV AGAINST CRC

The application of NDV tumor therapy has entered the clinical phase (phases I, II and III)[73], and three methods are generally used in clinical trials: (1) Injection of infectious virus alone; (2) Injection of intact tumor cells infected with NDV; (3) Injection of the protein lysate of NDV-infected tumor cells; and (4) Combined use. As NDV infection of tumor cells can lead to enhanced immunogenicity, an autologous tumor vaccine (ATV-NDV) can be constructed using the tumor cells of a patient[74], and clinically postoperative activity-specific immunotherapy has been performed for patients with CRC (Table 1).

Some phase I-IV clinical trials based on NDV vectors have yielded encouraging results. For the first time, Bohle *et al*[75] treated 16 patients with colon cancer after tumor resection with a live, nontoxic NDV-modified tumor cell vaccine. Of the 16 patients, 12 patients had an enhanced specific anti-tumor response. In a phase I clinical trial, 20 patients with CRC were treated with ATV-NDV-specific immunotherapy, with the exception of four patients with mild fever and no serious side effects. Among them, 16 patients produced an active specific immune response to the vaccine, which provided effectiveness for subsequent clinical trials[76].

Liebrich *et al*[77] isolated tumor cells from human primary CRC and mixed vaccine with NDV nonlytic strain Ulster and used it for vaccination in 23 patients with colorectal liver metastasis. The results showed the active immune response in the patient is increased and can be further used in related tests. In a phase II clinical trial, 23 patients with liver metastases from CRC were completely resected after a consecutive 3 mo of ATV-NDV vaccination. At least 18 mo follow-up showed a 61% tumor recurrence in vaccinated patients compared with 87% of patients who underwent surgery alone[78]. In a prospective randomized phase III trial, 50 patients with CRC hepatic metastases were randomized into an experimental group and a control group. The experimental group received six doses of ATV-NDV. After approximately 10 years of follow-up, no differences in primary and secondary endpoints were detected in the total patient group. However, the experimental subgroup (13 patients with colon cancer) showed significant advantages in terms of overall survival. The vaccination appeared to help prolong overall survival and metastasis-free survival[79].

In a phase III trial, Liang *et al*[80] used ATV-NDV to treat 335 patients with stage I-IV

Table 1 Summary of clinical studies using NDV in CRC

Ref.	Type of NDV	Aim of the study	Tumor type	Number of subjects	Outcomes
Bohle *et al*[75]	ATV-NDV	Phase I clinical trial	Colon cancer	16 patients	2 patients exhibited an enhanced specific anti-tumor response
Lehner *et al*[76]	ATV-NDV	Phase I clinical trial	CRC	20 patients	16 patients produced an active, specific immune response
Liebrich *et al*[77]	ATV-NDV	Phase II clinical trial	CRC	23 patients	The active immune response in the patients was increased
Schlag *et al*[78]	ATV-NDV	Phase II clinical trial	CRC	23 patients	A 61% tumor recurrence rate was observed in vaccinated patients compared with 87% of patients treated with surgery alone
Schulze *et al*[79]	ATV-NDV	Phase III clinical trial	CRC	50 patients	Advantages in terms of overall survival in subgroup; ATV-NDV appears to be beneficial prolonging overall survival and metastasis-free survival
Liang *et al*[80]	ATV-NDV	Phase III clinical trial	CRC	567 patients	Average survival and median survival of the immunotherapy group (310 patients) were higher than those of the control group (257 patients)
Schirrmacher *et al*[82]	ATV-NDV and bsHN-CD28	Phase I clinical trial	CRC	14 patients	4 patients experienced a partial diminishment of metastases

ATV: Autologous tumor vaccine; CRC: Colorectal cancer; NDV: Newcastle disease virus.

CRC. The average survival and median survival of the immunotherapy group (310 patients) were higher than those of the control group. The other 25 patients with advanced disease had a 1-year survival rate of 96%. The total effective rate of immunotherapy was 24%. After NDV vaccine immunotherapy, the number of NK cells increased, and the immune function improved significantly. In patients with stage IV colon cancer, tumor memory T cells (MTCs) may induce tumor-associated antigens either spontaneously or upon vaccination with an antitumor vaccine, which may constitute a potential mechanism in those patients with long-term survival[81].

Multiple immunizations of colon cancer patients with ATV-NDV using CD3 or CD28 on HN or F and T cells simultaneously targeting the virus have been shown to be clinically effective and improve long-term survival[82]. Attachment of the NDV-specific single-chain antibody anti-HNxanti-CD28 (bsHN-CD28) to ATV-NDV enhances T cell costimulatory signals. After using ATV-NDV-bsHN-CD28 to treat 14 cases of advanced CRC not surgically treatable in the phase I clinical trial, no tumor-reactive blood circulation MTCs were detected in any of the patients before vaccination; however, all patients exhibited the immune response of tumor-reactive MTCs at least once during five vaccinations. Among them, four patients experienced partial diminishment of metastases[83].

NDV-based treatment causes only mild pus-like symptoms, and these side effects are temporary and well tolerated; even high doses *via* intravenous administration did not cause significant toxicity. Although rNDV treatment may lead to some side effects, the response is negligible when compared to the therapeutic effect. When a patient is initially desensitized with a lower dose, the subsequent maximum tolerated dose may be increased by a factor of ten[84].

DISCUSSION

The multiple preclinical data and clinical trials with oncolytic NDV clearly demonstrate its efficacy for CRC. However, there are several questions that need to be

resolved .One of the key issues in oncolytic therapy is targeted delivery[85]. Moreover, the extracellular matrix and other barriers in solid tumors may interfere with and slow viral transmission, thereby reducing oncolysis.

In addition, due to the induction of neutralizing antibodies, the oncolysis effect of the virus after repeated viral administration may decrease over time[86]. To overcome the potential neutralizing effect of the antibody on the virus, an aptamer was used to block the antibody, thereby preventing neutralization of the virus. Furthermore, treatment with a paramyxovirus mesenchymal stem cell vector protects the virus against neutralizing antibodies with efficient transfer of the virus to the tumor.

In terms of safety and treatment outcomes, improvement is needed for the application of NDV. For example, NDV 73T damages normal cells while killing tumor cells. In clinical use, NDV may also pose safety problems for medical personnel and the environment because NDV virulent strains and poisonous strains can cause fatal respiratory and neurological diseases in poultry. Furthermore, insertion of an exogenous gene into the NDV genome has pros and cons: On the one hand, exogenous genes may enhance the anti-tumor effect of NDV; on the other hand, insertion of foreign genes may affect replication of the virus.

CONCLUSION

In 2005, the Chinese government approved the first transgenic oncolytic type 5 adenovirus (Adeno-5; H101) virus for the treatment of tumors; in 2013, the United States Food and Drug Administration affirmed the use of herpes simplex virus type 1 (HSV1; T-Vec). Thus, oncolytic viruses have become a new generation of biosafety agents. Among these agents, NDV meets many conditions for new drugs used in the human body: Selective potent oncolytic activity; a strong type I IFN reaction and a wide range of immunostimulatory effects. NDV directly destroys cancer cells, induces ICD and activates DC1- and Th1-directed antitumor immune responses, resulting in effective destruction of cancer cells and the development of tumor-associated MTCs after onset, which provides long-term protective activity to prolong survival.

Driven by successful animal experiments and clinical trials, the strategy of introducing, integrating and improving NDV anti-CRC therapy has been widely explored. The development of reverse genetics technology allows NDV to be employed as a vector for the integration of therapeutic genes. In the future, we envision multimodal combination therapy as the ideal option for treating complex diseases, such as tumors. Indeed, by combining oncolytic NDV with hyperthermia, therapeutic transgenes, dendritic cells, T cells, bispecific antibodies or NDV-based vaccines (*e.g.*, ATV-NDV), multimodal combination therapy is expected to be a good application prospect. However, more research is needed to determine the preclinical and clinical effects of NDV to verify its safety and efficacy in CRC therapy.

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ORIGINAL ARTICLE

Basic Study

Reduced microRNA-451 expression in eutopic endometrium contributes to the pathogenesis of endometriosis

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Author contributions: Gao S performed the majority of the experiments and analyzed the data; Liu S and Deng P performed the molecular investigations; Wang DB conceived and designed the experiments; Gao S and Gao ZM wrote the manuscript.

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Abstract

BACKGROUND

Endometriosis (EMs) is a chronic and recurrent, but benign, disease in women of reproductive age, and EMs patients have a high risk of developing gynecological tumors and autoimmune disorders. The etiology of EMs is not clear. Certain genetic markers in the eutopic endometrium are key in the pathogenesis of EMs. MicroRNAs (miRNAs) are implicated in several biological processes, such as cell proliferation, differentiation, and apoptosis. MiR-451 is interesting, as it acts as a tumor suppressor and is relevant to the poor prognosis of cancers.

AIM

To evaluate the expression levels and role of miR-451 in the eutopic endometrium and predict possible targets of miR-451 and related signaling pathways.

METHODS

Quantitative real-time polymerase chain reaction was used to evaluate miR-451 expression in cultured cell lines as well as in pathologic tissues from 40 patients with EMs and 20 donors with no history of the disease (controls). Cell Counting Kit-8 and flow cytometric assays were performed to determine cell proliferation and survival rates after transfection with miR-451 mimics and siRNAs. MiR-451 targets were predicted using miRDB and miRcode target-predicting databases.

RESULTS

We observed lower miR-451 levels in eutopic endometrial tissues from patients with EMs than in control tissues, and this difference was not related to the American Society for Reproductive Medicine stage. Ectopic overexpression of

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miR-451 in eutopic cells induced apoptosis and inhibited cell proliferation. SiRNA-mediated miR-451 knockdown reversed these effects. Using miRDB and miRcode, we identified 12 potential miR-451 target genes. We hypothesize that the expression of *YWHAZ*, *OSR1*, *TTN*, and *CDKN2D* may be regulated by miR-451 and be involved in disease pathogenesis.

CONCLUSION
Reduced miR-451 expression in the eutopic endometrium contributes to the pathogenesis of EMs by promoting cell proliferation and reducing apoptosis. Thus, miR-451 is a novel biomarker for EMs. *YWHAZ*, *OSR1*, *TTN*, and *CDKN2D* are potential target genes of miR-451 and may have key roles in this disease.

Key words: Endometriosis; miR-451; Proliferation; Apoptosis; Pathogenesis

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Core tip: Despite the high prevalence of endometriosis (EMs), its etiology is unclear. This study focuses on the expression of miR-451 in patients diagnosed with EMs. We report miR-451 as a novel biomarker of EMs as it is downregulated in the eutopic endometrium. *YWHAZ*, *OSR1*, *TTN*, and *CDKN2D* were identified as potential target genes of miR-451 that may have important roles in disease pathogenesis. We believe that our study contributes significantly to the literature because it suggests a novel biomarker for EMs that may facilitate the early diagnosis of the disease without the need for invasive methods such as laparoscopic examination.

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INTRODUCTION

Endometriosis (EMs) is a chronic and recurrent, but benign, disease in women of reproductive age, with a morbidity of approximately 10%. It is characterized by the presence of functional endometrial glands and stroma outside the uterine cavity[1,2]. Typical symptoms of EMs include cyclic pelvic pain, dysmenorrhea, dyspareunia, and infertility. Previous studies have reported that EMs patients have a high risk of developing gynecological tumors and autoimmune disorders[3,4]. Thus, EMs can cause severe psychological and physiological harm to those affected by it and imposes a substantial social burden[5,6].

Despite its high prevalence and incapacitating symptoms, the etiology of EMs is not clear. Evidence suggests that it is a multifactorial disease. Retrograde menstruation, immune system disorders, and genetic and environmental factors have been proposed as susceptibility factors for EMs[7-9]. The susceptibility factor of retrograde menstruation proposed by Sampson is the most widely accepted[10]. However, almost all women of reproductive age exhibit some degree of retrograde menstruation, and only 10% to 15% suffer from EMs[11,12]. Recently, more evidence has emerged to support the theory that genetic changes in the eutopic endometrium may be the key molecular events in the pathogenesis of EMs[13].

MicroRNAs (miRNAs) are short noncoding RNA molecules that regulate genetic expression post-transcriptionally and are implicated in several biological processes, such as cell proliferation, differentiation, and apoptosis[14,15]. Some miRNAs have been reported to be abnormally expressed in reproductive cancers[12,16,17], and miR-451 is of particular interest, as it acts as a tumor suppressor and is relevant to the poor prognosis of cancers. Aberrant miR-451 expression has been shown in eutopic and ectopic endometrial tissues; however, data regarding differences in miR-451 expression in the eutopic endometrium from healthy patients and those with EMs remain inconclusive[18,19].

In our study, we examined miR-451 expression in the eutopic endometrium of women with and without EMs and evaluated the role of miR-451 in cell proliferation.

Finally, we predicted possible targets of miR-451 and the related signaling pathways.

MATERIALS AND METHODS

Tissue collection

Pathologic tissues were collected from patients with grade III cervical intraepithelial neoplasia, including 40 with EMs and 20 without. All 60 subjects underwent total hysterectomy at the Shengjing Hospital of China Medical University between 2009 and 2010. The EMs group included 2, 6, 20, and 12 cases at American Society for Reproductive Medicine (ASRM) stage I, II, III, and IV, respectively, of the disease. None of the patients had a history of endocrine, immune, or metabolic disorders and none had received any hormonal or antibiotic treatments within 3 mo prior to surgery.

Cell lines and culture

The ectopic and eutopic endometrial tissues were digested overnight at 37 °C with Dispase IV for 70 min and Dispase II for 50 min (Sigma, United States). After filtration through 100 and 400 mesh nylon screens, the obtained primary cells were rinsed in PBS and then cultured for 24 h in DMEM/F12 medium supplemented with 15% fetal bovine serum and antibiotics at 37 °C in an atmosphere containing 5% CO_2.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from the EMs tissues using the TRIzol reagent. cDNA was synthesized from miR-451 using a TaqMan® miRNA Reverse Transcription Kit and used in a 1:5 dilution ratio for qRT-PCR, which was performed, using an miRNA Assays kit and Universal Master Mix following the kit protocols (Applied Biosystems). U6 was used as the endogenous control. Conditions of reverse transcription were as follows: 16 °C for 30 min, 42 °C for 30 min, 85 °C for 5 min, and then holding at 4 °C. Conditions for qRT-PCR were as follows: enzyme activation at 95 °C for 10 min followed by 40 cycles of denaturation for 15 s at 95 °C and annealing and extension for 60 s at 60 °C. All experimental samples were run in triplicate and each qRT-PCR reaction was repeated at least two times. MiR-451 expression levels were calculated and analyzed using the $2^{-\Delta\Delta Ct}$ relative quantitation method.

Transfection

Using Lipofectamine™ 2000 reagent, miR-451 mimics and miR-451 inhibitors were transfected into EMs cells and normal endometrial cells, respectively. The oligonucleotide sequence of the miR-451 mimic is 5′-AAA CCG UUA CCA UUA CUG AGUU-3′, and its NC sequence is 5′-UUC UCC GAA CGU GUC ACG UTT-3′. The oligonucleotide sequence of the miR-451 inhibitor is 5′-AAC UCA GUA AUG GUA ACG GUUU-3′, and the sequence of the scrambled siRNA is 5′-CAG UAC UUU UGU GUA GUA CAA-3′. In addition, cells transfected with or without the empty vector were used as the control groups. All cells were incubated at 37 °C in an atmosphere containing 5% CO_2 for 24 to 96 h post transfection.

Cell proliferation analysis

Cellular proliferation analysis was performed using the Cell Counting Kit-8 (CCK-8) assay. After transfection with miR-451 mimics/inhibitors for 24h, 48h, 72h, and 96 h, 2 × 10^3 cells were added to 96-well plates and incubated overnight at 37 °C in an atmosphere containing 5% CO_2. Then, 10 μL of CCK-8 was added to each well (Beyotime Biotechnology). The cells were incubated for another 4 h at 37 °C in an atmosphere containing 5% CO_2, and then cell viability was determined by measuring the optical density at 450 nm.

Flow cytometry to assess apoptosis

Annexin V-FITC/PI double-staining assays were performed for analysis of apoptosis. Cells were collected and suspended in PBS 24 h after transfection. Cells were then stained in 500 μL of binding buffer with 5 μL of each of annexin V-FITC and PI (KeyGen Biotech), incubated in the dark at room temperature for 5-15 min, and subjected to flow cytometric analysis to assess cellular apoptosis within 1 h.

Prediction of target genes and microarray data

Using miRDB (http://mirdb.org/miRDB/index.html) and miRcode (http://www.mircode.org/), we predicted the target genes of miR-451. Expression levels of the identified targeted genes were determined by analyzing the GSE7846 gene profile from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). This dataset includes the expression data of endometrial cells derived from patients with

EMs (ectopic group) and without EMs (normal group). We screened the differentially expressed genes with a *P*-value < 0.01 and an adjusted *P*-value < 0.01 between the EMs and control groups.

Statistical analysis

Data are expressed as the mean ± SEM. Statistical comparisons between groups were determined using the *t*-test and χ^2 test. Statistical significance was defined as $P < 0.05$. Analyses were performed using the R 3.4.2 and SPSS 22.0 software.

Ethics approval and informed consent

This study was approved by the China Medical University Research Ethics Committee according to the Helsinki Declaration, and written informed consent was obtained from each study participant.

RESULTS

MiR-451 expression is reduced in eutopic tissues and cell lines derived from EMs patients compared to the controls

qRT-PCR was performed to quantitatively analyze the expression levels of miR-451 in eutopic tissues from the EMs and control groups. As shown in Figure 1A, we observed a significant reduction in miR-451 expression in the EMs group compared to the control group (EMs, 0.22 ± 0.06; control, 1.12 ± 0.11, $P < 0.01$). Consistent with the tissue results, miR-451 expression in cells was significantly lower in the EMs group than in the control group (Figure 1B). The correlation of miR-451 expression levels with ASRM stage was then analyzed, as shown in Table 1. No significant association was found between miR-451 expression and ASRM stage ($P > 0.05$).

MiR-451 mimic inhibits cell proliferation and induces apoptosis in EMs eutopic cells

MiR-451 levels in eutopic cells transfected with miR-451 mimic were higher than those in the non-transfected control and scrambled mimic oligomer groups (miR-451 mimic, 1.33 ± 0.28; control, 0.25 ± 0.06; scrambled, 0.32 ± 0.09, $P < 0.01$) (Figure 2A). CCK-8 assay results showed that transfection with miR-451 mimic suppressed the proliferation rate of EMs cells (Figure 2B). To investigate whether the reduced cell proliferation resulted from apoptosis, we evaluated the effect of miR-451 mimic on cellular apoptosis using flow cytometry. MiR-451 mimic induced early apoptosis in a larger number of cells compared to scrambled oligonucleotides, and this difference was statistically significant ($P < 0.01$) (Figure 2C and 2D). Thus, overexpression of miR-451 in EMs cells induces apoptosis and inhibits cell proliferation.

Transfection of miR-451 siRNA into control eutopic cells promotes cell proliferation and inhibits cell apoptosis

As shown in Figure 3A, miR-451 expression was significantly attenuated in the siRNA-transfected group compared to the non-transfected and scrambled mimic oligomer groups (miR-451 siRNA, 0.41 ± 0.14; control, 1.23 ± 0.08; scrambled, 1.06 ± 0.06, $P < 0.01$). Additionally, the proliferation ability of miR-451 siRNA-transfected cells was greater than that of the other two groups (Figure 3B). We used flow cytometric assay to evaluate the effect of miR-451 siRNA transfection on apoptosis. Our results showed that the proportion of early apoptotic cells was significantly lower in the miR-451 siRNA group compared to that in the scrambled group ($P < 0.01$) (Figure 3C and 3D). These results indicate that, in eutopic cells, miR-451 reduces apoptosis and increases cell proliferation.

Prediction of miR-451 target genes

Using the miRDB and miRcode miRNA target prediction databases, we identified a total of 12 genes targeted by miR-451, namely, *OSR1, MEX3C, CUX2, ZNF644, TBC1D9B, DCAF5, CDKN2B, TTN, YWHAZ, CDKN2D, EIF2AK3*, and *TBX1* (Figure 4A). As shown in Figure 4B, among the targeted genes, the expression levels of *OSR1*, *YWHAZ*, *TTN*, and *CDKN2D* were significantly different between the two groups according to *GSE7846* ($P < 0.05$, adj. $P < 0.05$). The logFC values of *OSR1*, *YWHAZ*, *TTN*, and *CDKN2D* were 0.76, 0.43, 0.33, and 0.63, respectively. Furthermore, according to the pathway analysis data in the Kyoto Encyclopedia of Genes and Genomes, *YWHAZ* and *CDKN2D* may have important roles in the cell cycle in EMs (Figure 4C).

DISCUSSION

Table 1 Comparison of miR-451 expression in patients at different american society for reproductive medicine stages

ASRM stage	Cases (*n*)	MiR-451 level ($2^{-\Delta\Delta CT}$)	*P*-value
I	2	0.21	> 0.05
II	6	0.16	> 0.05
III	20	0.27	> 0.05
IV	12	0.19	> 0.05

ASRM: American Society for Reproductive Medicine.

In this study, qRT-PCR analysis of eutopic endometrial tissues and cells showed that miR-451 was significantly downregulated in patients with EMs compared to normal controls (P = 0.011). Although we did not observe a significant association between miR-451 expression and the ASRM stage of EMs, ectopic overexpression of miR-451 in eutopic cells in EMs was shown to be associated with reduced cell proliferation and increased apoptosis. Conversely, siRNA-mediated knockdown of miR-451 promoted the proliferation and reduced the apoptosis of eutopic cells.

The "eutopic endometrium determinism" theory suggests that the occurrence of EMs is mainly dependent on the characteristics of eutopic endometrial lesions, and retrograde menstruation may act as a precipitating factor. Thus, genetic dysregulation in the endometrium is crucial in the pathogenesis of EMs. Identifying differentially expressed genes between patients with and without EMs would serve as a minimally invasive method to diagnose EMs and evaluate the risk of recurrence. For example, Mahdian *et al*[20] reported that *MIF*, *CD74*, and *COX-2* are essential in inflammation and endometrium reconstruction during the menstrual cycle, and increased expression of these genes is a molecular biomarker for the development and pathophysiology of EMs. In addition, Sapkota *et al*[21] also identified five novel loci (*CCDC170*, *FN1*, *SYNE1*, *ESR1*, and *FSHB*) and nineteen independent single nucleotide polymorphisms that are significantly associated with the risk of EMs.

Furthermore, miRNAs regulate the expression of target genes and key cellular processes in EMs. In 2009, Burney *et al*[14] reported the downregulation of the miR-9 and miR-34 miRNA families in eutopic cells in the setting of EMs, and this downregulation is closely related to progesterone resistance in early secretory endometrium. Laudanski *et al*[22] showed that miR-483-5p and miR-629-3p are downregulated in EMs, and this is associated with inflammation. Moreover, miR-21 was shown to be significantly upregulated in severe EMs (stage III/IV) compared to mild EMs (stage I/II)[23]. Notably, miR-451 has been established as a tumor-suppressor gene in gastric, colorectal, bladder, and non-small cell lung carcinomas[24], and it was also shown to be downregulated in ovarian cancer compared to its concurrent EMs[25]. In addition, Nothnick *et al*[26] showed that deficiency of miR-451 regulates fibrinogen alpha chain and reduces endometrial implantation in a mouse model. Similarly, we found that miR-451 was downregulated in the eutopic endometrium in EMs compared to normal controls in studies involving both tissues and cells.

Using miRNA target-predicting databases, we identified 12 potential target genes of miR-451 and analyzed their expression levels according to the *GSE7846* dataset. Finally, a total of four genes, *YWHAZ*, *OSR1*, *TTN*, and *CDKN2D*, were selected for further analysis. Among these target genes of miR-451, *YWHAZ* has previously been shown to be overexpressed in tissues in EMs[19,27]. Joshi *et al*[19] reported that miR-451 regulates *YWHAZ* expression and promotes proliferation of eutopic cells in baboons with EMs[19]. However, the roles of *OSR1*, *TTN*, and *CDKN2D* in EMs have not been reported until now. Published reports suggest that *OSR1* inhibits proliferation and induces cellular apoptosis by acting on the WNK and NF-κB pathways, and *OSR1* is dramatically downregulated in several carcinomas[28-30]. In addition, *CDKN2D* has been shown to be involved in carcinogenesis and has been identified in gynecological cancers. This gene may be regulated by miR-451 in esophageal carcinoma cell lines[31]. Thus, our study provides several novel therapeutic targets for EMs.

Notably, most studies on EMs have only focused on identifying differences between ectopic lesions and eutopic endometrium. For example, Graham *et al*[18] reported that miR-451 is overexpressed in ectopic lesions compared to eutopic lesions and reduces cell survival by regulating *MIF*. In this study, we found significant differences in miR-451 expression in the eutopic endometrium of patients with and without EMs, which effectively supports the "eutopic endometrium determinism" theory. Furthermore, we identified four potential target genes of miR-451 by bioinformatics analysis and analyzed their downstream pathways.

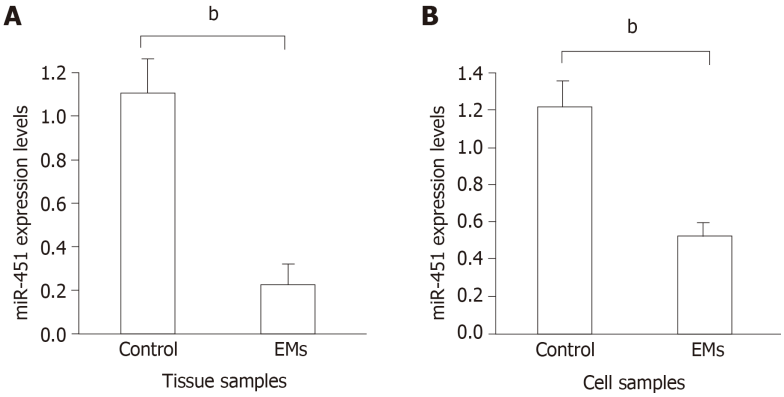

Figure 1 Expression of miR-451 in eutopic tissues and cell lines. A: MiR-451 expression in eutopic tissues from endometriosis (EMs) and control groups was quantified using quantitative real-time PCR. Data are expressed as $2^{-\Delta\Delta Ct}$ (mean ± SE, $n = 4$). $^{b}P < 0.01$ *vs* control; B: Expression of miR-451 was compared between EMs and control cell lines. Data are expressed as $2^{-\Delta\Delta Ct}$ (mean ± SEM, $n = 4$). $^{b}P < 0.01$ *vs* control.

Our study has two limitations. First, the number of included patients was relatively small. Second, the *in silico*-predicted targets of miR-451 need to be validated through experiments, such as 3′-UTR luciferase reporter assays. However, we believe that our results indicate a novel role of miR-451 in EMs and support several potential biomarkers in the form of miR-451 targets that may be used for future clinical diagnosis and therapy of this disease.

In conclusion, miR-451 is a novel biomarker for EMs and is downregulated in the eutopic endometrium. *YWHAZ*, *OSR1*, *TTN*, and *CDKN2D* are potential target genes of miR-451 and may have important roles in the pathogenesis of EMs.

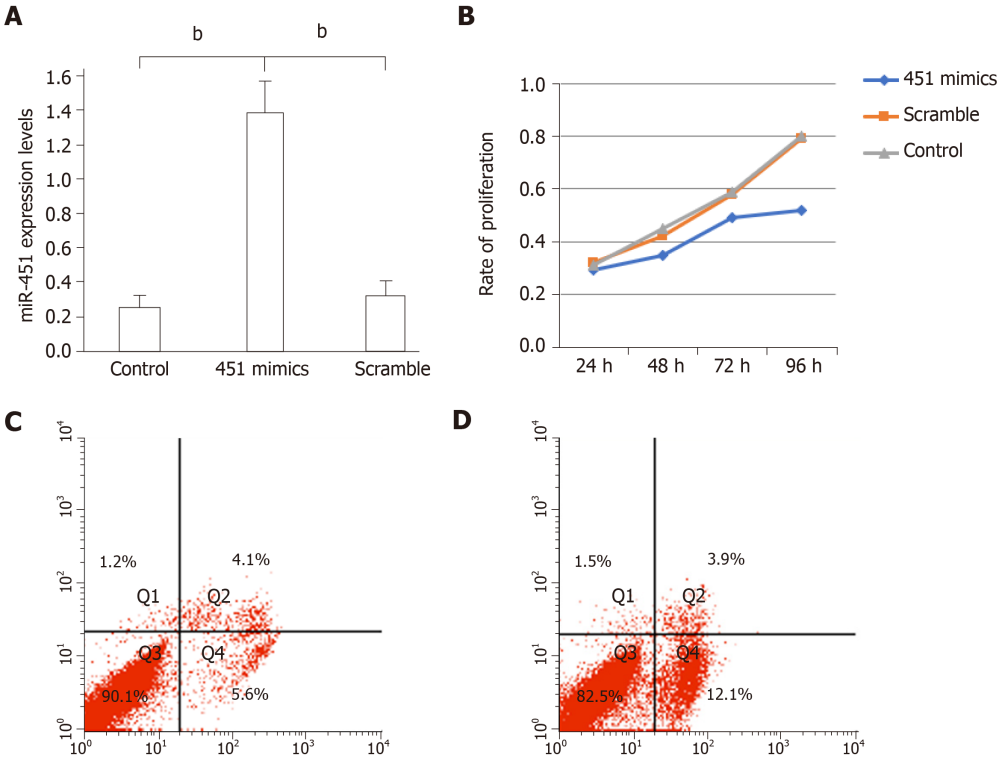

Figure 2 Transfection with miR-451 mimic inhibits cell proliferation by inducing the apoptosis of eutopic cells in endometriosis. A: MiR-451 expression was significantly increased after transfection with miR-451 mimic. Data are expressed as $2^{-\Delta\Delta Ct}$ (mean ± SEM, $n = 4$). $^{b}P < 0.01$ *vs* control and scrambled; B: Cell Counting Kit-8 assays revealed a lower proliferation rate of cells transfected with miR-451 mimic compared to cells in the control and scrambled groups ($^{a}P < 0.05$); C and D: Flow cytometric analysis of apoptosis in cells transfected with scrambled siRNA and miR-451 mimic, respectively. Cells are divided into four sections: Q1: Annexin V-FITC- PI+ represents mechanical error; Q2: Annexin V-FITC+ PI+ represents late apoptotic or necrotic cells; Q3: Annexin V-FITC- PI- represents non-apoptotic cells; Q4: Annexin V-FITC+ PI- represents early apoptotic cells.

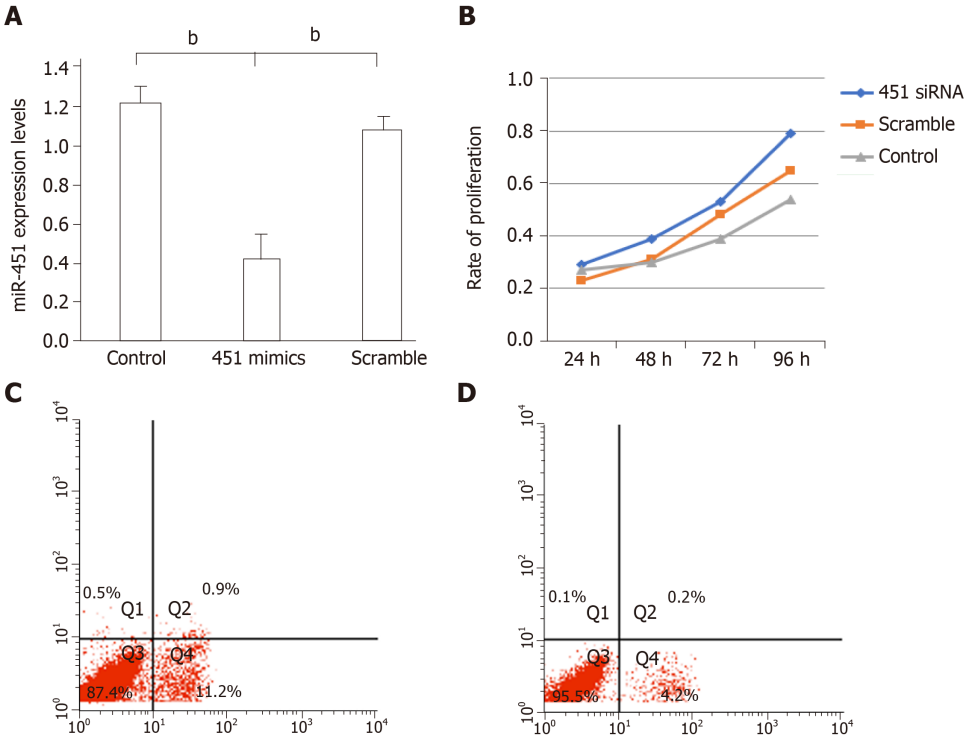

Figure 3 Transfection with miR-451 siRNA decreases apoptosis and promotes cell proliferation in control eutopic cells. A: MiR-451 expression was significantly inhibited after transfection with miR-451 siRNA. Data are expressed as $2^{-\Delta\Delta Ct}$ (mean ± SEM, $n = 4$). $^{b}P < 0.01$ *vs* control and scrambled; B: Cell Counting Kit-8 assays revealed that the proliferation rate of cells transfected with miR-451 siRNA was higher than those of cells in the control and scrambled groups ($^{a}P < 0.05$); C and D: Flow cytometric analysis of apoptosis in cells transfected with scrambled siRNA and miR-451, respectively.

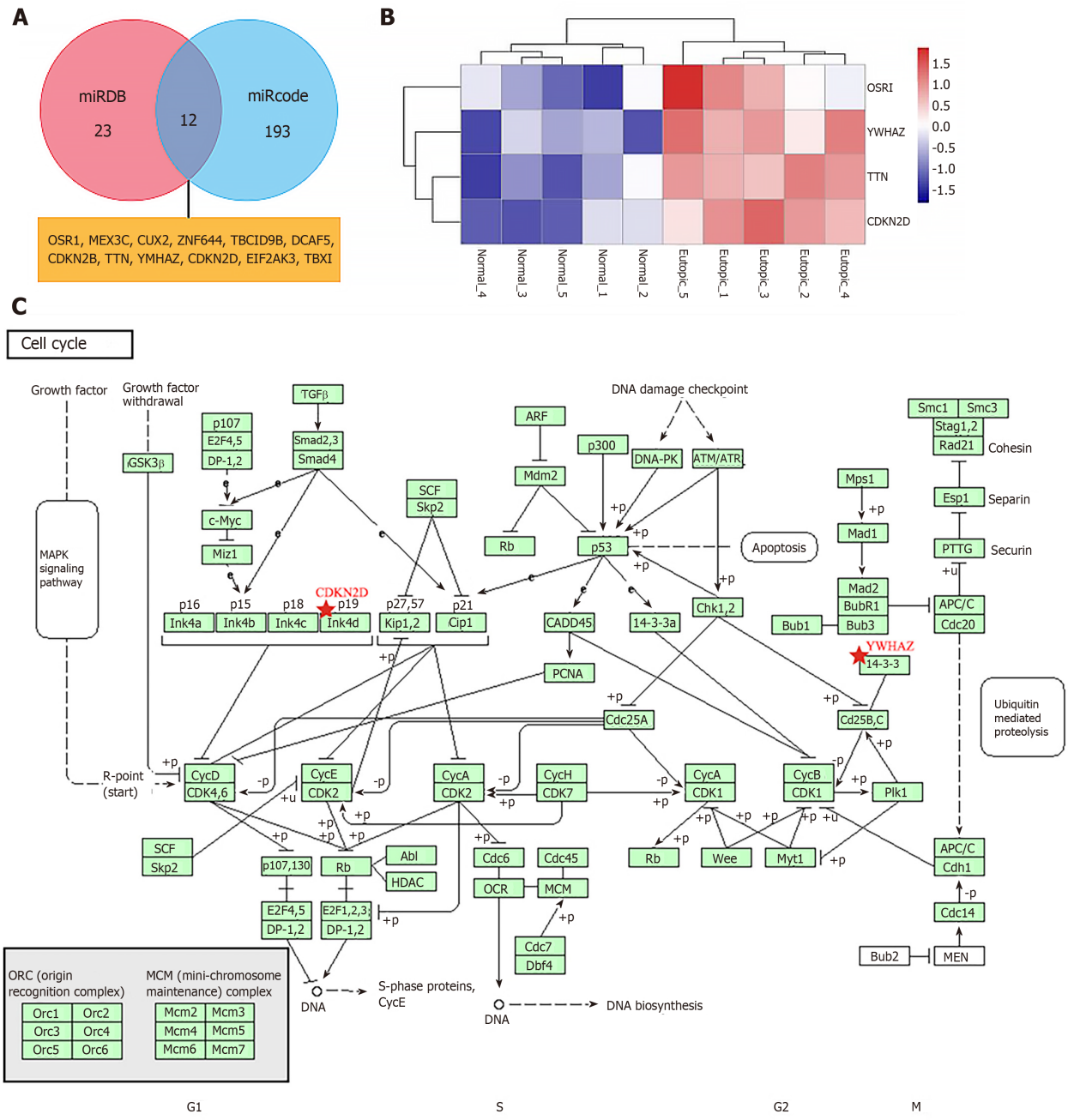

Figure 4 Results of bioinformatics analysis of miR-451 target genes according to the GSE7846 dataset. A: Potential target genes of miR-451 predicted based on miRDB and miRcode databases; B: Heatmap of expression levels of *OSR1*, *YWHAZ*, *TTN*, and *CDKN2D*; C: Role of *YWHAZ* and *CDKN2D* in the cell cycle according to Kyoto Encyclopedia of Genes and Genomes pathway analysis.

ARTICLE HIGHLIGHTS

Research background

Despite the high prevalence of endometriosis (EMs), its etiology is unclear.

Research motivation

MiR-451 acts as a tumor suppressor and is relevant to the poor prognosis of cancers.

Research objectives

To evaluate the expression levels and role of miR-451 in the eutopic endometrium and predict possible targets of miR-451 and related signaling pathways.

Research methods

Quantitative real-time PCR was used to evaluate miR-451 expression. Cell Counting Kit-8 and flow cytometric assays were performed to determine cell proliferation and survival rates.

Research results
MiR-451 was downregulated in the eutopic endometrium and related with EMs cell proliferation and apoptosis. *YWHAZ*, *OSR1*, *TTN*, and *CDKN2D* were identified as potential target genes of miR-451.

Research conclusions
Reduced miR-451 expression in the eutopic endometrium contributes to the pathogenesis of EMs by promoting cell proliferation and reducing apoptosis.

Research perspectives
MiR-451 is a novel biomarker for EMs. *YWHAZ*, *OSR1*, *TTN*, and *CDKN2D* are potential target genes of miR-451 and may have key roles in this disease.

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ORIGINAL ARTICLE

Case Control Study

Application of self-care based on full-course individualized health education in patients with chronic heart failure and its influencing factors

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Author contributions: Sun J, Zhang ZW, Ma YX, and Liu W designed this work, collected and interpreted the data, and drafted the manuscript; Wang CY designed this work, critically revised the manuscript, and performed overall supervision; all authors contributed to the final approval and accountability for the manuscript.

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Abstract

BACKGROUND

The treatment of heart failure not only needs to relieve the clinical symptoms and improve the quality of life for patients but also needs to select scientific and reasonable ways to prevent or delay the progression of the disease, thus reducing the mortality and hospitalization rate. Although the previous regimen can effectively relieve symptoms in the early stage of treatment, long-term use may cause adverse events, such as arrhythmia, and even increase mortality. Therefore, conventional treatment cannot meet the actual health needs of patients, and scientific nursing intervention is very necessary.

AIM

To investigate the application of self-care based on full-course individualized health education (FCIHE) and its influencing factors in patients with chronic heart failure (CHF).

METHODS

We enrolled CHF patients who were admitted to our center between September 2015 and June 2016 and divided them into an intervention group (n = 50) and control group (n = 50) using a random number table. Routine nursing care was applied to the control group, and FCIHE was offered to the intervention group. The self-care behavior, 6-min walking distance (6MWD), and 36-item short form health survey (SF-36) scores were compared between the two groups. The influencing factors of the self-care were also analyzed.

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RESULTS

The 6MWD was not significantly different between the two groups at admission ($P > 0.05$); however, at 3 and 6 mo after discharge, 6MWD was significantly increased, and it was significantly longer in the intervention group ($P < 0.05$). The scores for self-care behavior showed no significant difference at admission between the two groups ($P > 0.05$); however, at 3 and 6 mo after discharge, the total scores for self-care maintenance, management, confidence, and behavior of the intervention group were significantly higher than those of the control group ($P < 0.05$). There were no significant differences in the SF-36 scores at admission ($P > 0.05$); however, at 3 mo and 6 mo after discharge, the scores for all eight subscales, including physical functioning, role limitations due to physical problems, bodily pain, general health perceptions, vitality, social functioning, role-limitations due to emotional problems, and mental health, were significantly higher in the intervention group ($P < 0.05$). As shown by logistic regression analysis, the influencing factors of self-care mainly included age, cardiac function class, and education background (odds ratio > 1; all $P < 0.05$).

CONCLUSION

FCIHE improved self-care behavior and cardiac function in CHF patients. Age, cardiac function, and education level affected the implementation of self-care among CHF patients.

Key words: Full-course individualized health education; Chronic heart failure; Self-care; Influencing factors

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Core tip: Chronic heart failure is usually the end stage of most cardiovascular diseases, with high prevalence, high mortality, and high readmission rates. Individualized health education can effectively improve the self-care behavior of patients with chronic heart failure, improve the heart function of patients, and effectively provide scientific, professional, and individualized health guidance for patients. Good self-care awareness provides important guidance for the development of self-care programs for patients with chronic heart failure.

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INTRODUCTION

Chronic heart failure (CHF) is the end stage of most cardiovascular diseases. As one of the serious public health concerns worldwide, it drastically undermines the quality of life of patients due to its high prevalence, high mortality, and high readmission rate[1]. Studies have shown that appropriate self-care behavior among CHF patients helps to maintain physical stability and improve their ability to manage diseases[2]. However, the self-care behavior among CHF patients is far from satisfactory, as reflected in low medication compliance, poor knowledge of symptom management, and lack of awareness in diseases. The full-course disease management model refers to a model that efficiently integrates the existing limited resources for disease management[3]. Up to now the full-course management model has been applied in the management of patients with chronic disease and/or mental disorders, with good effectiveness. In the present study, based on the full-course disease management model and the individual features of patients, we applied full-course individualized health education (FCIHE) in CHF patients, providing patients with scientific, professional, and tailored health guidance and helping them learn and adopt the concept of self-care behavior, in an attempt to provide new guidance for the development of self-care programs for CHF patients.

MATERIALS AND METHODS

General data

This study is a randomized controlled trial. The CHF patients who were admitted to our center between September 2015 and June 2016 were selected by convenience sampling. Inclusion criteria were: (1) Meeting the diagnostic criteria of CHF released by the American Heart Association[4]; (2) New York Heart Association (NYHA) class II or above; and (3) Being conscious and able to communicate with researchers and participating in the study voluntarily. The exclusion criteria included: (1) Cognition or learning problems; (2) Could not take care of themselves; (3) Serious comorbidities or complications; and (4) Had participated in other self-care-related research. A total of 100 eligible patients with CHF were enrolled according to the inclusion/exclusion criteria, and all of them signed the informed consent forms. These patients were equally divided into the intervention group and control group (50 patients each) using a random number table. The baseline data including cardiac function (evaluated with NYHA classification), hospital stay, and age were matched between these two groups ($P > 0.05$) (Table 1).

Interventional method

Patients in the control group received routine nursing care including routine nursing during hospitalization, guidance at discharge (including the delivery of a health education manual), and telephone follow-up. The telephone follow-up was performed by a cardiology nurse 2 wk after discharge, and the follow-up content included recent health conditions (*e.g*., asthma and edema) and routine health guidance (exercise, drug use, and diets). Patients in the intervention group received FCIHE, as described below.

Setting up a research team

The self-care multidisciplinary research team for CHF patients was established, consisting of two cardiology nurses, two cardiologists, one clinical pharmacist, one rehabilitation therapist, and one nutritionist. Among them, two cardiology nurses had long been engaged in cardiac nursing and had been independently responsible for patient management in the cardiology ward, with extensive experience in the nursing and health education of CHF patients. In this study, they were responsible for self-care guidance throughout the study. Two cardiologists had extensive clinical experience and were responsible for assisting patient management. The clinical pharmacist, rehabilitation therapist, and nutritionist provided professional guidance during the development of the FCIHE plan for each patient. Led by the cardiology nurses, the whole team jointly established disease/health management plans for patients during hospital stay and after discharge, with an attempt to improve patients' self-care abilities and ultimately improve their quality of life.

Content of the intervention

During hospital stay: On the day of admission, the patient's disease condition, awareness of disease, family status, education background, and learning ability were evaluated to establish a health record. According to the evaluation results, the research team formulated an in-hospital health education plan for each patient to define the daily health education content; mainly covering the primary disease-related knowledge, disease pathogenesis mechanism, treatment plan and goals, medication guidance, exercise, nutrition guidance, and prevention of acute attack. A clear-cut intervention program was listed in an EXCEL form, allowing the patients to participate in the self-care process while receiving nursing and initially adopt a concept of self-care behavior. After being aware of their disease and its treatment, the patients were expected to understand the importance of self-care and increase their self-care ability and confidence through scientific and professional health guidance. With the guidance of the team members, the patients developed a self-care plan and implemented it. The nursing staff spent 3 d to help patients correct their self-care plan and monitored its implementation. Then, the patient was required to complete self-care independently, and their daily medication, diet, and exercise plans were modularized and digitized in the form of a daily schedule, which was inspected by the nursing staff the next day in hospital or by family members after discharge.

Continued intervention after discharge: The cardiology nurses managed the whole process. Through the internet-based medical platform, the cardiology nurses answered questions online from 09:00-17:00 h daily. The caregivers assisted in co-managing patients and supervising the patients to perform self-care in a reasonable way. Hospital nursing staff prepared a weekly health education program, with a new theme every week, covering information on medication, exercise, and nutrition. The

Table 1 Comparison of baseline data between the two groups

Group	Male / female	Age in yr	NYHA class			Hospital stay in d	Body mass index as kg/m²	Education background		
			II	III	IV			Middle school and under	High school or technical school	Junior college, university and above
Intervention group, *n* = 50	31/19	68.21 ± 4.69	17	24	9	15.63 ± 3.21	22.78 ± 3.94	38	9	3
Control group, *n* = 50	29/21	68.57 ± 4.12	18	25	7	15.21 ± 3.05	23.03 ± 4.11	35	10	5
χ^2/t value	0.167	0.408	0.299			0.671	0.310	0.457		
P value	0.683	0.684	0.861			0.504	0.757	0.499		

NYHA: New York Heart Association.

messages were delivered to patients through an APP platform or WeChat public account. However, a detailed self-care plan was still developed and implemented by patients themselves. The nursing staff performed weekly telephone follow-up within 3 mo after discharge, and then once every 2 wk after 3-4 mo, and once every month after 5–6 mo, so as to learn the current health status of the patients and provide corresponding health guidance and self-care correction. The research team members met every 2 mo, and patients or their families were encouraged to participate in the meetings; during which the difficulties encountered in the latest interventions were shared and countermeasures proposed. The meeting results and consensus were published online to inform patients who did not participate.

Upgraded patient self-care: After a patient had performed self-care for 3 mo, the nursing staff contacted the patient by telephone or WeChat, inquiring and recording the patient's latest care and recent schedule. The patients were informed that the CHF Patient Club meetings were arranged at the end of the 4th, 5th, and 6th mo after discharge. Five to 10 patients were invited as guests to participate in each meeting, so as to fully mobilize the enthusiasm of patients in self-care. In addition, their experience in self-care and disease control encouraged more new patients to implement self-care.

Evaluation indicators

Baseline data were collected at admission, and these data were reviewed at the 3rd and 6th mo after discharge, so as to fully learn the physical condition of the patients after the intervention. Meanwhile, disease consultation was provided by cardiologists. All data were collected and verified on site by two investigators, so as to ensure the accuracy and completeness of the information.

Six-minute walking distance: Six-minute walking distance (6MWD) is an important indicator for evaluating cardiac function. A four-stage evaluation method can be used. However, since cardiac function gradually worsens with the progression of HF, it is difficult to observe directly the improvement in cardiac function using this staging method. Therefore, the walking distance was directly used for the evaluation in this study.

Self-care of heart failure index[5]: Self-care of heart failure index is composed of 22 items divided into three subscales: Self-care maintenance (10 items), self-care management (six items), and self-care confidence (six items). Among them, the self-care maintenance applies the Likert scale (rated as 1 = never or rarely; 2 = sometimes; 3 = usually; and 4 = daily or always). The self-care management is mainly used to measure the patient's ability in symptom recognition and implementation and evaluation of treatment. Symptom recognition has one item, evaluated with a 0–4 scale, reflecting the speed of a patient identifying their symptoms. Treatment implementation has four items, evaluated with a Likert 1–4 scale (rated as 1 = not likely; 2 = somewhat likely; 3 = likely; and 4 = very likely). Evaluation of treatment has one item, evaluated with a Likert 0–5 scale (0 = I did not try anything; 1 = not sure; 2 = somewhat sure; 3 = sure; and 4 = very sure). Self-care confidence contains six items, evaluated with a Likert 1–4 scale (1 = not confident; 2 = somewhat confident; 3

= very confident; and 4 = extremely confident). The following format was used to calculate the standard scores of all three subscales: [(actual score – minimum possible score)/(maximum possible score – minimum score)] × 100%. A threshold value of 70 was used to evaluate whether the patients' self-care behavior met the criteria. Cronbach's α coefficients for these three subscales were 0.656, 0.736, and 0.869, respectively, and the overall Cronbach's α coefficient of the scale was 0.853[6].

36-item short form health survey: The 36-item short form health survey (SF-36) questionnaire revised by the Medical College of Zhejiang University was used in the survey. It contained eight subscales, including physical functioning, role limitations due to physical problems, bodily pain, general health perceptions, vitality, social functioning, role-limitations due to emotional problems, and mental health. Scores range from 0 to 100. The higher the score, the better the quality of life of patients. The split-half reliability of the SF-36 was 0.920, and the Cronbach's α was 0.880.

Analysis of the influencing factors of patient self-care: With patient self-care as a dependent variable and age, cardiac function class, and education background as independent variables, multivariate logistic regression analysis was performed to evaluate the influencing factors of patient self-care.

Statistical analysis

The data were double-checked by two researchers and before inputting into the SPSS 22.0 software (Armonk, NY, United States) for statistical analysis. The numerical data are presented as (n, %) and compared by χ^2 test, and the measurement data are represented as (mean ± standard deviation) and compared with t test at a test level $\alpha = 0.05$. $P < 0.05$ was regarded as statistically significant. The analysis of influencing factors was based on multivariate logistic regression analysis.

RESULTS

6MWD at different time points

6MWD was not significantly different between the two groups at admission ($P > 0.05$). Three and 6 mo after discharge, however, 6MWD was significantly increased, and it was significantly longer in the intervention than control group ($P < 0.05$) (Table 2).

Self-care behavior scores at different time points

The scores for self-care behavior showed no significant difference at admission between these two groups ($P > 0.05$). However, at 3 and 6 mo after discharge, the total scores of self-care maintenance, management, confidence, and behavior of the intervention group were significantly higher than those of the control group ($P < 0.05$) (Table 3).

SF-36 scores at different time points

There were no significant differences in the SF-36 scores at admission ($P > 0.05$). However, at 3 and 6 mo after discharge, the scores of all eight subscales, including physical functioning, role limitations due to physical problems, bodily pain, general health perceptions, vitality, social functioning, role-limitations due to emotional problems, and mental health were significantly higher in the intervention than control group ($P < 0.05$) (Table 4).

Influencing factors of patient self-care

As shown by logistic regression analysis, the influencing factors of self-care mainly included age, cardiac function class, and education background [odds ratio >1; all $P < 0.05$] (Table 5).

DISCUSSION

Connotation and advantages of FCIHE

FCIHE at the nursing level is designed to offer dynamic monitoring of the disease condition and the healthcare needs of patients and then provide tailored health guidance according to their actual situation. In our current study, individualized self-care education covering medication, exercise, and nutrition was provided during hospital stay according to the patients' treatment protocol, disease awareness, and management status. After the patients were discharged, continued guidance on disease/health management was offered by cardiology nurses, so as to help the

Table 2 Comparison of 6-min walking distance between the two groups at different time points

Groups	At admission	3 mo after discharge	6 mo after discharge	F value	*P*-value
Intervention group, n = 50	126.48 ± 30.23	280.56 ± 38.84	401.52 ± 35.68	776.592	0.000
Control group, n = 50	124.12 ± 28.17	207.35 ± 36.27	265.42 ± 20.53	301.032	0.000
t value	0.404	9.741	23.378	-	-
P-value	0.687	0.000	0.000	-	-

patients learn their disease conditions, establish a self-care concept, and better carry out self-management of CHF.

Along with the improved living standards and changed lifestyles in China, the prevalence of CHF has declined but remains high, which brings a heavy burden to patients, their families, and society. Due to impaired heart function, the patients have decreased exercise endurance and are more susceptible to symptoms such as fatigue, palpitation, and dyspnea, which seriously affect the patients' activities. The daily activities of patients with severely impaired heart function are restrained, markedly undermining their quality of life[7]. With the application of FCIHE in our study, a multidisciplinary research team with members including cardiologists and cardiology nurses, clinical pharmacists, nutritionists, and rehabilitation therapists was established, and a comprehensive and scientific health education program was developed. In addition, comprehensive evaluation was immediately performed at admission, along with in-hospital intervention and continuous intervention after discharge. The individualization and continuity of health education was guaranteed, which, to a certain extent, ensured the seamless transition from hospital to family.

FCIHE helps to improve cardiac function in CHF patients

In the present study, 6MWD at admission was not significantly different between the two groups (P > 0.05); however, at 3 and 6 mo after discharge, 6MWD was significantly longer than at admission, and it was significantly longer in the intervention than control group, suggesting FCIHE improved NYHA class. 6MWD is mainly used to evaluate cardiac function in patients with moderate to severe cardiopulmonary diseases. It is not only a key observation indicator in clinical trials but also an important predictor of survival rate. It can adequately reflect the actual status of cardiac function. In our current study, FCIHE offered full-time dynamic individualized health guidance, which increased the patients' knowledge and awareness of their disease and improved self-care behavior; as a result, their NYHA class was improved. Many other studies[8-14] have also demonstrated that good self-management can improve cardiac function and quality of life.

FCIHE helps to improve self-care behavior

In this study, the scores of self-care behavior at admission showed no significant difference between these two groups (P > 0.05). Three and 6 mo after discharge, however, the scores of self-care maintenance, management, confidence, and behavior in the intervention group were significantly higher than those in the control group (all P < 0.05). This suggested that patients' self-care behavior score increased over time, and FCIHE improved self-care behavior by providing guidance on self-care. Tavazzi *et al*[15] had similar findings[15-17]. Analysis of the changes in self-care maintenance, management, and confidence and behavior scores is more conducive to overall evaluation of patients' self-care behavior, and change in self-care behavior is closely associated with the content of FCIHE. Gielen *et al*[18] reported that most of the traditional self-care guidance for CHF patients focuses on health education at discharge, with uniform and non-tailored content, which is solely delivered by medical staff and passively received by patients. Thus, the effectiveness of health education is limited[18-20]. During FCIHE, comprehensive evaluation is performed at admission, followed by the creation of a health archive. A personalized health education program during hospitalization was formulated according to the patients' treatment plan and self-care status, with an attempt to enable the patients to be aware of their disease condition and self-care status, understand the importance of self-care, and establish a concept of self-care[21-23]. After discharge, the cardiology nurses provided continuous guidance through internet medical platforms and telephone follow-up; during which the importance of self-care was reiterated and scientific and reasonable guidance was provided. In addition, patients were urged to take self-care measures through telephone follow-up[24]. The main caregivers were included in the collaborative patient management, which was more comprehensive and efficient and improved the patients' self-care behavior and ability and confidence in disease

Table 3 Comparison of self-care behavior scores between the two groups at different time points

Items	Time point	Intervention group, *n* = 50	Control group, *n* = 50	*t* value	*P*-value
Self-care maintenance	At admission	28.14 ± 8.25	27.53 ± 7.86	0.379	0.706
	3 mo after discharge	32.76 ± 7.43	28.64 ± 8.72	2.543	0.013
	6 mo after discharge	38.21 ± 7.43	30.04 ± 8.22	5.214	0.000
Self-care management	At admission	48.78 ± 10.73	46.58 ± 9.08	1.107	0.271
	3 mo after discharge	52.48 ± 9.16	49.20 ± 6.27	2.089	0.039
	6 mo after discharge	55.47 ± 12.08	50.76 ± 8.13	2.287	0.024
Self-care confidence	At admission	32.72 ± 11.65	30.90 ± 10.74	0.812	0.419
	3 mo after discharge	38.12 ± 10.04	33.79 ± 11.18	2.038	0.044
	6 mo after discharge	43.75 ± 14.37	36.30 ± 10.44	2.966	0.004
Total score	At admission	109.64 ± 24.26	105.01 ± 20.12	1.039	0.302
	3 mo after discharge	123.36 ± 21.88	111.63 ± 18.37	2.903	0.005
	6 mo after discharge	137.43 ± 18.74	117.10 ± 19.05	5.380	0.000

management.

FCIHE helps to improve quality of life

As shown in our study, there was no significant difference in the SF-36 score between these two groups at admission ($P > 0.05$). Three and 6 mo after discharge, the quality of life score was also significantly higher in the intervention than in the control group. The SF-36 scores accurately reflect the quality of life from eight subscales including physical functioning, role limitations due to physical problems, bodily pain, general health perceptions, vitality, social functioning, role-limitations due to emotional problems, and mental health. As shown by the SF-36 scores, FCIHE significantly improved the quality of life of our patients, which might be explained by the fact that patients in the intervention group gradually changed from passive nursing to active self-care after implementation of FCIHE, which accurately covered the patients' daily drug use, diet, and exercise behavior and played an active role in regulating body status and controlling disease conditions[25-27]. In addition, Segan *et al*[28] reported that FCIHE established a progressive closed-loop nursing mode of self-care – guided care – self-care, which helped patients to optimize continuously their care measures during self-care and thus markedly improved their quality of life[28,29].

Influencing factors of self-care

Age, cardiac function, and education level affected the implementation of self-care among our patients, directly or indirectly. Although it has been reported that age is not a useful factor for predicting self-care behavior, our current study found that age was an influential factor[30,31]. In fact, old patients with CHF usually have a longer disease course, and HF itself has certain specific symptoms. These patients often experience repeated and painful dyspnea and edema at night, which reminds patients to follow strictly medication instructions and control their diet properly, which helps to optimize their self-care behavior. A higher NYHA functional class is associated with more severe disease, which leads to more stringent restrictions on physical activities[32,33]. Slightly increased activity may cause dyspnea and fatigue, which also limits the patients' normal self-care behavior. CHF patients with higher education level typically have higher income and are more able to follow the dietary plan and take drugs on time[34,35]. Self-care is a long process and patients often need time to learn it, and patients with higher education level are more likely to receive information on scientific diet and exercise, as well as related treatment plans, and they can understand the importance of treatment adherence. As a result, patients with high education background can benefit more from self-care.

In conclusion, CHF is the end stage of most heart diseases, with poor prognosis and high mortality. Scientific and rational treatment and self-care are the key factors to improve quality of life and prognosis. Currently, self-care among CHF patients is far from satisfactory. Poor knowledge of symptom recognition and treatment and lack of confidence in treatment have restricted the implementation of self-care. FCIHE offers professional, scientific, and multifaceted self-care guidance through tailored health education during hospitalization and continuous follow-up after discharge, which is conducive with optimizing patient self-care behavior, increasing their confidence in disease treatment, and improving quality of life. FCIHE has the following advantages. (1) The full-course dynamic management helps the medical staff to learn the patient's

Table 4 Comparison of SF-36 scores between the two groups

Items	Time point	Intervention group, *n* = 50	Control group, *n* = 50	*t* value	*P*-value
Physical functioning	At admission	68.74 ± 15.33	68.78 ± 16.02	1.276	0.205
	3 mo after discharge	75.26 ± 10.58	71.34 ± 8.17	2.074	0.041
	6 mo after discharge	81.79 ± 9.55	75.27 ± 13.28	2.819	0.006
Physical role	At admission	27.58 ± 9.14	28.01 ± 9.23	0.234	0.815
	3 mo after discharge	43.64 ± 11.25	31.97 ± 10.82	5.287	0.000
	6 mo after discharge	51.94 ± 12.03	38.79 ± 11.16	5.667	0.000
Bodily pain	At admission	49.87 ± 13.26	50.17 ± 11.46	0.121	0.904
	3 mo after discharge	61.28 ± 11.97	53.88 ± 12.31	3.047	0.003
	6 mo after discharge	78.19 ± 12.35	56.87 ± 10.98	9.123	0.000
Emotional functioning	At admission	50.17 ± 13.33	50.20 ± 12.65	0.012	0.991
	3 mo after discharge	63.94 ± 11.81	54.08 ± 13.70	3.855	0.000
	6 mo after discharge	72.08 ± 13.49	57.84 ± 13.22	5.331	0.000
Vitality	At admission	54.33 ± 10.58	54.42 ± 11.38	0.041	0.967
	3 mo after discharge	61.24 ± 9.25	57.28 ± 9.97	2.059	0.042
	6 mo after discharge	65.37 ± 9.79	59.88 ± 11.43	2.579	0.011
Social functioning	At admission	51.87 ± 13.24	51.90 ± 12.77	0.012	0.991
	3 mo after discharge	57.42 ± 11.21	53.26 ± 9.61	1.992	0.049
	6 mo after discharge	63.08 ± 10.97	57.81 ± 10.95	2.404	0.018
Mental health	At admission	61.25 ± 11.44	61.37 ± 10.97	0.054	0.957
	3 mo after discharge	71.79 ± 10.58	64.33 ± 12.57	3.211	0.002
	6 mo after discharge	78.63 ± 11.52	68.19 ± 11.43	4.549	0.000
General health	At admission	54.72 ± 10.69	54.65 ± 11.73	0.031	0.975
	3 mo after discharge	61.33 ± 12.37	56.87 ± 9.29	2.039	0.044
	6 mo after discharge	66.87 ± 10.61	58.92 ± 13.64	3.253	0.002

medical condition and self-care needs in a more comprehensive and real-time manner. According to the real situation of the patients, the health guidance programs can be adjusted timeously; (2) the full-course participation of medical staff in the health education of patients helps to establish a good doctor–patient relationship and thus increase adherence to treatment and care protocols; and (3) participation of multidisciplinary teams can facilitate healthcare professional development and increase their ability to serve patients. However, our study had some limitations. All the cases selected were from the same hospital, and the intervention duration was short (only 6 mo after discharge). Therefore, the effectiveness of the intervention needs to be verified further in multicenter, long-term studies.

Table 5 Influencing factors of self-care behaviors

Risk factors	Regression coefficient	Standard error	*P*-value	OR value	95%CI
Age	2.284	0.972	0.019	9.816	1.461–65.965
NYHA class	2.533	1.086	0.020	12.591	1.498-105.801
Education background	2.217	0.249	0.000	9.180	5.634-14.955
Constant	−5.329	2.208	0.003	0.002	–

NYHA: New York Heart Association.

ARTICLE HIGHLIGHTS

Research background

Chronic heart failure (CHF) disease, as a chronic disease, has certain progressive characteristics. Although conventional medication alone can delay the progression of the disease, it often fails to achieve full recovery. Therefore, the current clinical practice is that it can be treated with drugs, but it cannot be cured. This also shows that the addition of scientific nursing intervention on the basis of treatment has important auxiliary significance. The self-care based on full-course individualized health education is a comprehensive nursing measure born in this context. It can make scientific, professional, and individualized health guidance for the patients throughout the whole process, so that patients can form a positive self-care behavior concept, develop a sense of self-care and enables themselves to delay further the disease progression through daily intervention, thus contributing to the improvement of life quality.

Research motivation

At present, there is no unified comprehensive nursing intervention for long-term service of patients with CHF. Although some reports have introduced some nursing interventions, the pertinence and self-participation are not strong, and it is difficult to significantly improve the actual condition of patients. The motivation of this study is to support better the patient's clinical treatment process through self-care based on full-course individualized health education and integrate the nursing intervention into the clinical treatment process, so as to exert more obvious therapeutic effects, help control the condition, and improve the state of life of the patients.

Research objectives

The goal of this study is to explore the application of a new type of nursing intervention - self-care based on full-course individualized health education - in patients with CHF in order to extend this nursing intervention to the clinical treatment of hospitals at all levels. At present, the core concept of initial nursing intervention has been formed. If it is expanded and promoted in depth, it can make a positive contribution to the prognosis and survival of patients with CHF.

Research methods

A total of 100 patients with CHF in the Cardiology Department were selected for the study. The patients in the control group received the existing CHF routine nursing in our hospital. The patients in the intervention group received the self-care based on full-course individualized health education. After 6 mo of follow-up, 6-min walking distance, self-care behavior score, and SF-36 score were compared, and the influencing factors of patient self-care were analyzed.

Research results

The study found that after applying the self-care based on full-course individualized health education, the patient's 6-min walking distance was longer, and the self-care behavior score and SF-36 score were better. At the same time, logistic regression analysis found that the factors affecting patients' self-care mainly include age, cardiac function classification, and education level. Therefore, the clinical application of self-care based on full-course individualized health education can better improve the overall life quality and clinical prognosis of patients. At the same time, scientific interventions based on age, cardiac function classification, and cultural level are targeted to promote better the patient's self-care effect. However, how to implement it in detail is one of the problems to be solved in the future.

Research conclusions

The new findings of this study are mainly that the self-care based on full-course individualized health education can better assist clinical treatment and can achieve therapeutic effects similar to treatment by intervening in the overall behavior of patients. The new theory proposed in this study is mainly the construction and content exploration of self-care based on full-course individualized health education, which is expected to provide more clearly theoretical ideas for future nursing interventions. The appropriate summary of the current knowledge provided by this study is that self-care based on full-course individualized health education has an important role and has a positive impact on patients. The original insight is that the self-care based on full-course individualized health education can achieve a certain level of nursing intervention, which may be similar to conventional care, or better. The new hypothesis is that the self-care based on

full-course individualized health education may be a new type of care for patients with CHF. The new method proposed in this study is mainly the formulation and implementation of the self-care based on full-course individualized health education. The new phenomenon found in this study was that the effect of self-care based on full-course individualized health education was better than expected, and it also contributed to the improvement of patients' quality of life. The hypothesis confirmed through experiments in this study is that the self-care based on full-course individualized health education has a better clinical prognosis for patients. The implications of this study for clinical practice in the future is that the content of self-care based on full-course individualized health education can be further optimized or expanded, so as to enrich better the theoretical knowledge system and ultimately better serve CHF patients.

Research perspectives

From this study, we can learn that the self-care based on full-course individualized health education is conducive to the patient's condition symptom control and quality of life. A limitation of the study is that the sample size is relatively small. Future research directions include attempts to expand sample size, conduct multi-center research, and further extend follow-up time while expanding the care content system. The best method for the future research is to conduct a stratified study of large sample sizes or a simultaneous study of multi-level medical institutions to obtain more accurate results and conclusions.

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ORIGINAL ARTICLE

Retrospective Study

Predicting surgical site infections using a novel nomogram in patients with hepatocelluar carcinoma undergoing hepatectomy

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Abstract

BACKGROUND

Surgical site infections (SSI) remain a major cause of morbidity after hepatectomy for hepatocellular carcinoma (HCC).

AIM

To identify the risk factors associated with SSI, and develop a nomogram to predict SSI among patients undergoing hepatectomy.

METHODS

We retrospectively reviewed the data of patients diagnosed with HCC undergoing hepatectomy at two academic institutions in China, and evaluated the occurrence of SSI. Independent risk factors for SSI were identified using univariate and multivariate analyses. Based on these independent risk factors, a nomogram was established using the data of patients in the first institution, and was validated using data from an external independent cohort from the second institution.

RESULTS

The nomogram was established using data from 309 patients, whereas the validation cohort used data from 331 patients. The operation duration, serum albumin level, repeat hepatectomy, and ASA score were identified as independent risk factors. The concordance index (C-index) of the nomogram for

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SSI prediction in the training cohort was 0.86; this nomogram also performed well in the external validation cohort, with a C-index of 0.84. Accordingly, we stratified patients into three groups, with a distinct risk range based on the nomogram prediction, to guide clinical practice.

CONCLUSION
Our novel nomogram offers good preoperative prediction for SSIs in patients undergoing hepatectomy.

Key words: Surgical site infection; Nomogram; Hepatectomy; Risk factors

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Core tip: Surgical site infections (SSI) remain a major cause of morbidity among patients undergoing liver resection. The aim of this study was to establish a nomogram to predict SSI in patients who underwent hepatectomy for hepatocellular carcinoma. A total of 309 patients were used to develop the prediction model based on identified risk factors, and 331 patients were used as an external validation cohort. The prediction model showed better performance comparing to National Nosocomial Infection surveillance risk index both in training and validation cohorts. This nomogram integrating information of medical history, liver function, performance status, and intra-operative risk may have a potential for helping surgeons identify the patients with increased risk of SSI in clinical practice.

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INTRODUCTION

Over the last few decades, with the advances in operative techniques and intensive perioperative management, the perioperative mortality rate after hepatectomy has been markedly reduced[1,2]. However, the relatively high morbidity rate among these cases is still a problem. Nosocomial infections remain a major cause of morbidity among patients undergoing liver resection, and surgical site infections (SSI) reportedly account for > 50% of infectious complications after surgery[3-7]. In particular, in patients undergoing hepatectomy for hepatocellular carcinoma (HCC), SSI have a significant impact on morbidity, mortality, prolonged hospitalization, costs, and long-term oncology outcomes[8]. Hence, SSI prevention has been considered a top priority for improving perioperative outcomes. Previous studies suggest that many factors can influence SSIs in patients undergoing hepatectomy, including age, overweight status, liver function, hepatolithiasis, hypoalbuminemia, anemia, diabetes, repeat hepatectomy, operating time, intraoperative blood loss and transfusion, postoperative bile leakage, and prolonged drainage[3,5,7,9-14]. However, some of these factors remain controversial.

To identify patients with an increased risk of SSI and improve their perioperative outcomes, several prediction models have been developed, such as the Nosocomial Infection Control index and the National Nosocomial Infection Surveillance (NNIS) risk index proposed by Centers for Diseases Control and Prevention (CDC)[15,16]. However, these models have been developed using data from a wide range of patients undergoing various surgical procedures with different disease conditions. Hence, the applicability of these prediction models is limited in patients undergoing hepatectomy for HCC, whose conditions are usually worse as compared to patients undergoing other surgical procedures. The identification of actual risk factors for SSI after hepatectomy for HCC and development of effective forecasting models to screen out patients at high risk of SSI are vital for improving individual clinical decision making and the perioperative morbidity rate.

A nomogram is a convenient and widely applicable tool for surgeons to predict the

risk of adverse events and prognosis of patients. Several prediction models have been developed to predict the survival rates and postoperative complications of patients undergoing hepatectomy[17-19]. However, only few studies have focused on developing prediction models for SSI. In the present study, we aimed to investigate the risk factors for SSI after hepatectomy for HCC, and develop a prediction model for SSI by analyzing clinical data from a consecutive series of patients undergoing hepatectomy at our institution and validate the prediction model in an external cohort.

METERIALS AND METHODS

Patient cohort and data collection

The data of 640 patients with HCC who underwent attempted curative liver resection were retrospectively collected from two academic institutions in China. Patients from the Second Affiliated Hospital of Zhejiang University School of Medicine were used as the training cohort (n = 309), whereas patients from Eastern Hepatobiliary Surgery Hospital were used as the validation cohort (n = 331). These two institutions are high-volume centers for liver cancer surgery. Only patients who underwent hepatectomy (R0 or R1 resection) and had histopathologically confirmed HCC according to the European Association for the Study of the Liver (EASL) criteria were included[20]. The inclusion criteria were: (1) Age between 18 and 85 years; (2) Patients with resectable HCC scheduled to undergo hepatectomy; and (3) Liver function classified as Child-Pugh A or B. Patients who underwent hepatectomy with biliary reconstruction or concomitant organ resection, such as colorectal resection, were excluded. This study was approved by the ethics committees of all involved hospitals. Written informed consent was obtained from patients for the use of their clinical data for research.

Patient management

The records of all patients were reviewed preoperatively by experienced surgeons and radiologists to determine whether the planned procedures were appropriate based on the extent of progression, liver function, and general condition of the patients. Resectability and disease progression were preoperatively evaluated according to the imaging studies. Liver function was assessed using liver biochemistry tests, the Child-Pugh grade, and the indocyanine green retention rate at 15 min. Anatomic hepatectomy was performed whenever possible, and partial hepatectomy was conducted in patients with limited liver reserve and tumor at a specific location. Prophylactic antibiotics (a first-generation cephalosporin) were administered 30 min before skin incision, every 3 h during the surgery, and twice daily for 2 d after the surgery, according to the CDC guidelines. Drains were routinely placed in the right subphrenic space, foramen of Winslow, or along the cut surface of the liver, based on the type of hepatectomy, and were connected to a closed drainage system. Tubes were routinely removed when there were no signs of bile leakage, hemorrhage, or infection on postoperative day 3 or 4.

Data collection and outcomes definition

The reviewed data of patients undergoing hepatectomy included gender, age, body mass index (BMI), activities of daily living (ADL), American Society of Anesthesiologists (ASA) physical status, history of smoking and alcohol abuse, etiology, comorbidity, hepatitis, cirrhosis, portal hypertension, Child-Pugh classification, laboratory test, repeat hepatectomy, and surgical information. SSI were diagnosed according to the criteria of the NNIS[21]. For the assessment of SSI, all postoperative data of the patients relevant to SSI were reviewed (*e.g.*, fever, postoperative radiologic examination, laboratory tests, characteristics of drainage fluid, culture of fluid and tissue from the surgical site, interventional radiologic procedures, reoperation, and organ failure). Perioperative death was defined as death of patients within 30 days after surgery. The Clavien-Dindo classification was used to assess the severity of SSIs. The ASA score was determined by experienced anesthesiologists before surgery[22]. The NNIS risk index was calculated based on the following criteria: ASA > 2, wound class (contaminated, dirty, or infected), and operation duration above the 75th percentile, wherein each present risk factor added 1 point to the total score[16].

Statistical analysis

Continuous variables are expressed as median and interquartile range, whereas categorical variables are expressed as frequency and proportion. The risk factors for SSI were identified using univariate and multivariate analyses with SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, United States). The categorical variables were analyzed using the chi-square test or Fisher's exact test, when appropriate.

Continuous variables were analyzed using the Mann-Whitney *U* test, whereas logistic regression analysis was performed for multivariate analysis; for these analyses, odds ratios (ORs) and their 95% confidence intervals (CIs) were reported. A nomogram was formulated based on the results of multivariate logistic regression analysis, using the rms package in R, version 3.2.1 (http://www.r-project.org/). Points are added across independent variables according to the nomogram to derive the total points, which are converted to predicted probabilities. The performance of the nomogram was measured using the concordance index (C-index), and was assessed in the validation cohort against that in the training cohort. A P-value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

A total of 640 patients met the enrollment criteria and were included in the study (mean age, 54.9 years; 87.8% of males); all the patients underwent hepatectomy for HCC. The patients from the first institution were used as the training cohort (n = 301) and those from the second institution were used as the validation cohort (n = 331). No significant differences were observed in the baseline characteristics of the patients between the two cohorts (Table 1). Among all the patients, the incidence rates of overall, incisional, and organ/space SSI in the training cohort were 10.6% (n = 33), 7.4% (n = 23), and 5.2% (n = 16), respectively. The incidence rates of overall, incisional, and organ/space SSI in the validation cohort were 11.4% (n = 37), 7.1% (n = 26), and 4.2% (n = 14), respectively. The mean postoperative stay duration was 9.58 (range, 3–45) and 9.76 (range, 3–56) days in the training and validation cohorts, respectively. The 30-d mortality rate was 0.7% in the training cohort, with 1 death due to sepsis and 1 death due to liver failure, whereas the 30-d mortality rate was 0.6% in the validation cohort, with 1 death due to postoperative hemorrhage and 1 death due to intra-abdominal infection.

Univariate and multivariate analyses of risk factors for overall SSI

The pre-operative and intra-operative variables in the training cohort were evaluated using univariate analysis and logistic regression to identify the factors associated with SSI. In particular, six variables were found to be significantly associated with the occurrence of SSI on univariate analysis, including ASA score, major resection, intraoperative blood loss, duration of operation, repeat hepatectomy, and serum albumin level (Table 2). Thereafter, logistic regression identified three pre-operative variables (serum albumin level, repeat hepatectomy, and ASA score) and one intra-operative variable (duration of operation) as independent predictors of overall SSI (Table 2).

Development and validation of a predictive nomogram

We developed a nomogram to predict SSI in patients after hepatectomy for HCC by integrating the four factors identified during the multivariate analysis (Figure 1). Each factor was assigned a weighted number of points. The total number of points for each patient was calculated using the nomogram, and was associated with an estimated probability for SSI. For example, a patient with 46 g/L serum albumin (12.5 points) and ASA physical status classified as 2 (0 point) underwent a 3-hour surgery (19.8 points), including initial resection for HCC (0 point). The total score for this patient was 32.3, indicating a 4.6% probability of developing SSI.

The C-index of the nomogram for predicting SSI was 0.86 for the training cohort and 0.84 for the validation cohort (Figure 2A and 2C). Moreover, the calibration curve indicated adequate consistency between predictions using the nomogram and the actual observed outcome in both cohorts (Figure 2B and 2D).

We compared the nomogram with the NNIS risk index in both the training and validation cohorts, and our nomogram showed better prediction accuracy (Figure 3).

Risk groups based on the nomogram

We stratified the patients of the entire cohort into three groups with a distinct risk of SSI, based on the predicted risk distribution using the nomogram. The predicted mean risk of the low-risk group was 7.46% (total points < 70; predicted rate, < 10%), of the intermediate-risk group was 21.42% (total points, 70–104; predicted rate, 10%–50%), and of the high-risk group was 71.07% (total points > 104; predicted rate, > 50%). The observed incidences of SSI differed significantly between the three groups, and were close to the predicted SSI rate (Table 3).

Table 1 Baseline characteristics of the cohorts

	Training cohort (*n* = 309)		Validation cohort (*n* = 331)		*P*-value
	No. of patients	Percent	No. of patients	Percent	
Gender					0.197
Male	266	86.1	296	89.4	
Female	43	13.9	35	10.6	
Age, yr					0.095
Median	56		55		
IQR	48-63		48-61		
BMI, kg/m^2					0.111
Median	22.7		23.0		
IQR	20.6-24.8		21.1-27.2		
ASA score					0.395
1	61	19.7	80	24.2	
2	210	68.0	214	64.6	
3	38	12.3	37	11.2	
Smoking					0.241
Yes	136	44.0	161	48.6	
No	173	56.0	170	51.4	
Alcohol consumption					0.140
Yes	159	51.5	151	45.6	
No	150	48.5	180	54.4	
Diabetes					0.616
Yes	29	9.4	35	10.6	
No	280	90.6	396	89.4	
Etiology					0.204
Hepatitis B	227	73.5	243	73.4	
Hepatitis C	12	3.9	7	2.1	
Child-Pugh grade					0.562
A	295	95.5	319	96.4	
B and C	14	4.5	12	3.6	
TNM stage					0.201
I	148	47.9	139	42.0	
II	114	36.9	125	37.8	
IIIa	17	5.5	34	10.2	
IIIb	28	9.1	30	9.1	
IVa	2	0.6	3	0.9	
IVb	0	0	0	0	
Cirrhosis					0.130
Yes	218	70.6	215	65.0	
No	91	29.4	116	35.0	
AFP, ng/mL					0.876
Median	65.8		74.8		
IQR	8.7-479.8		8.4-754.2		
ALT, U/L					0.126
Elevated	109	35.3	98	29.6	
Normal	200	64.7	232	70.4	
AST, U/L					0.219
Elevated	139	44.9	133	40.2	
Normal	170	55.1	198	59.8	
Albumin, g/L					0.316
Median	40.6		40.1		
IQR	36.7-43.9		37.5-42.7		

TB, μmol/L					0.410
Median	14.2		14.7		
IQR	11.3-18.6		11.6-18.4		
INR					0.792
Median	1.04		1.04		
IQR	0.98-1.10		0.98-1.10		
Hb, g/L					0.504
Median	145		147		
IQR	132-156		135-155		
PLT, 10^9/L					0.141
Median	142		138		
IQR	106-192		102-178		
ICG15%					0.775
Median	5.4		5.8		
IQR	3.0-8.6		2.8-8.5		
History of RFA					0.228
Yes	13	4.2	21	6.3	
No	296	95.8	310	93.7	
History of TACE					0.584
Yes	8	2.6	11	3.3	
No	301	97.4	320	96.7	
Repeat hepatectomy					0.868
Yes	48	15.6	53	16.0	
No	261	84.4	278	84.0	
Type of hepatectomy					0.109
Major	81	35.2	69	20.8	
Minor	228	64.8	262	79.2	
Duration of surgery, min					0.351
Median	237		223		
IQR	184-302		183-292		
Blood Loss, mL					0.215
Median	160		220		
IQR	80-400		110-330		
Intraoperative blood transfusion					0.305
Yes	63	20.4	57	17.2	
No	246	79.6	274	82.8	
Surgical site infection					0.840
Yes	33	10.7	37	11.2	
Grade II	21	6.8	23	6.9	
Grade IIIa	7	2.3	9	2.7	
Grade IIIb	2	0.6	1	0.3	
Grade IVa	2	0.6	2	0.6	
Grade IVb	0	0	1	0.3	
Grade V	1	0.3	1	0.3	
No	276	89.3	294	88.8	
Hospital stay, d					0.323
Median	8		8		
IQR	6-11		6-12		

BMI: Body mass index; ADL: Activities of daily living; AFP: Alpha-protein; TB: Total bilirubin; INR: International normalized ratio; Hb: Hemoglobin; PLT: Platelets; SSI: Surgical site infection; RFA: Radiofrequency ablation; TACE: Transcatheter arterial chemoembolization.

DISCUSSION

SSI have been defined as infections associated with surgical procedures. According to data published by the CDC and Healthcare Infection Control Practices Advisory

Table 2 Univariate and multivariate analyses of risk factors for surgical site infections in the training cohort

	Univariate analysis			Multivariate analysis		
	OR	95%CI	*P*-value	OR	95%CI	*P*-value
Gender	0.600	0.278-1.297	0.192			
Age, yr	0.975	0.941-1.010	0.282			
BMI, kg/m^2	1.004	0.894-1.129	0.930			
ASA score > 2	2.674	1.346-5.315	0.006	4.518	1.528-13.360	0.006
Smoking	0.862	0.499-1.653	0.654			
Alcohol consumption	1.002	0.526-1.911	0.994			
Diabetes	0.966	0.314-2.970	0.951			
Child-Pugh grade	1.044	0.840-1.298	0.655			
TNM stage	1.174	0.873-1.416	0.247			
Cirrhosis	0.960	0.476-1.935	0.909			
Elevated ALT, U/L	1.193	0.618-2.303	0.600			
Elevated AST, U/L	1.299	0.682-2.476	0.425			
Albumin, g/L	0.789	0.721-0.836	0.001	0.900	0.817-0.992	0.033
TB, μmol/L	0.989	0.968-1.011	0.071			
Hb, g/L	1.007	0.987-1.027	0.145			
History of RFA	1.554	0.329-7.337	0.575			
History of TACE	1.201	0.143-9.071	0.866			
Repeat hepatectomy	2.364	1.203-4.646	0.013	3.859	1.435-10.381	0.007
Type of hepatectomy	2.649	1.406-4.994	0.002	1.277	0.506-3.228	0.605
Duration of surgery, min	1.009	1.006-1.013	< 0.001	1.011	1.007-1.015	< 0.001
Blood loss, mL	1.001	1.000-1.002	0.002	1.001	0.999-1.002	0.329
Intraoperative blood transfusion	1.464	0.717-2.991	0.299			

BMI: Body Mass Index; TB: Total bilirubin; Hb: Haemoglobin; SSI: Surgical site infections; RFA: Radiofrequency ablation; TACE: Transcatheter arterial chemoembolization.

Committee (HICPAC) in 2017, the incidence of SSI after bile duct, liver, and pancreatic operations was 3.6%[23]. In previous reports, the SSI rate after hepatectomy ranged from 2.1% to 14.5%[4,7,10,24]. Although it might be impossible to completely reduce the SSI rate to 0%, surgeons currently foster an environment with zero tolerance to SSI. Nevertheless, the reduction of the SSI rate after hepatectomy remains a major challenge.

In the present study, we aimed to establish a forecasting model that could identify patients with an increased risk of SSI immediately after surgery, thus offering surgeons the opportunity to reduce the SSI rate by modifying the treatment in advance. To our knowledge, this is the first study to develop a nomogram for predicting SSI in patients undergoing hepatectomy for HCC. Furthermore, the nomogram was verified using an external cohort to assess the reliability and practicability of the forecasting model. Here, we discuss the following four risk factors for SSI identified in the present study: Serum albumin level, repeat hepatectomy, ASA score, and duration of operation.

We found that patients who underwent repeat hepatectomy were more likely to develop SSIs, consistent with previous studies[25-28]. One explanation for this finding is that, to ensure minimum trauma for patients, the original incision site was usually considered as the optimal operation approach for repeat hepatectomy. This may lead to delayed wound healing and increased risk for SSI due to the presence of scar tissue, which may also be associated with hypo-perfusion of the surgical site. Scar removal may help increase perfusion of the surgical wound; however, this approach may lead to higher tension at the surgical site, which may also contribute to poor wound healing. In addition, this finding may be related to the higher bile leak rate in patients undergoing repeat hepatectomy. Sadamori *et al*[6] reported that repeat hepatectomy was an independent risk factor for bile leak and SSI after surgery[6]. The damage and latent stricture of the hepatic duct induced by radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), or initial surgery may be the main cause of increased bile leak rate and SSI rate in patients undergoing repeat hepatectomy. We

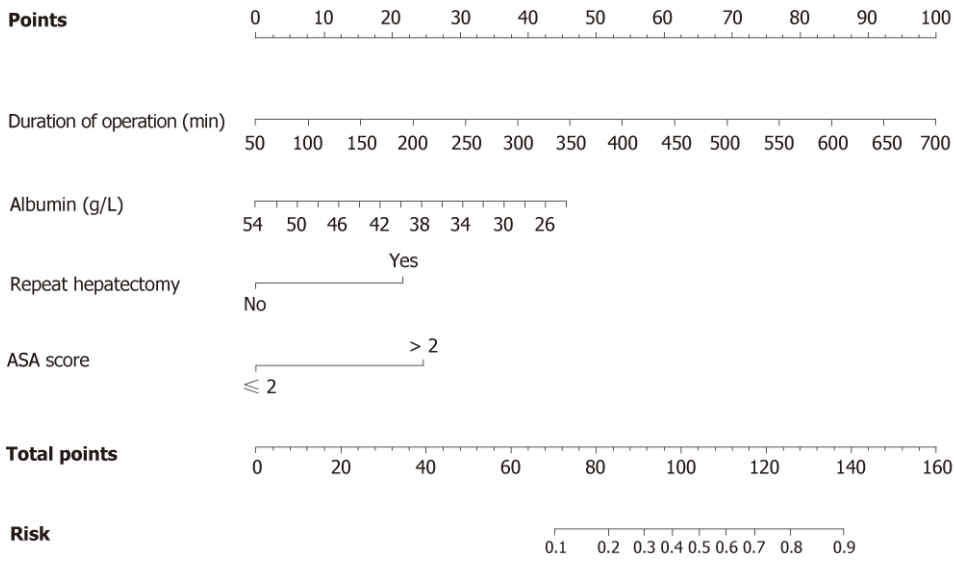

Figure 1 Nomogram predicting the probability of surgical site infection occurrence in patients undergoing hepatectomy.

only included pre- and intra-operative predictors in our study to establish an advanced prediction model, thus aiming to provide surgeons with opportunities to reduce the SSI rate. However, given the significant association between SSI and postoperative factors such as bile leakage, ascites, and prolonged drainage, we believe that surgeons should be alert to the increased risk of SSI in certain patients in clinical practice, especially those who develop bile leakage regardless of whether they have a relatively low nomogram score.

Preoperative hypoalbuminemia is frequently observed in patients undergoing hepatectomy. Several studies have suggested that hypoalbuminemia has a great predictive value for the mortality and morbidity rates in this population[5,13,25]. As a marker of malnutrition and liver dysfunction, hypoalbuminemia was the only laboratory value identified as an independent risk factor for SSI in our study. This finding may be related to impaired tissue healing caused by decreased collagen synthesis and granulation tissue formation at the surgical site[29,30]. Another possible explanation could be that hypoalbuminemia may cause tissue edema, which could subsequently lead to hypo-perfusion of the surgical site. Furthermore, fluid collection at the surgical site due to decreased serum osmolality provides a medium for bacterial propagation[31]. Moskovitz *et al*[32] reported that the use of specialized enteral diets enriched with specific immunonutrients can improve the perioperative outcomes of malnourished patients undergoing gastrointestinal surgery. Thus, we believe that hypoalbuminemia patients may benefit from additional nutrition support and more intensive perioperative care.

We observed that a high ASA score and prolonged surgery were significantly associated with the occurrence of SSI, in line with previous studies[5,6,13]. Moreover, these two predictors have been found to be associated with postoperative complications in a wide range of patients with different disease conditions. Patients with a higher ASA score were more likely to have concomitant diseases and a worse performance status. Prolonged surgery reflects a more complicated surgical procedure and increased surgeon fatigue, which could lead to additional technical errors. In cases with a prolonged operating time, the incision and tissue are exposed to the environment for a longer duration, which could contribute to an increased risk of bacterial contamination. In fact, the experience of the surgical team plays an important role in determining the duration of surgery. In the present study, all procedures were performed by experienced surgeons with well-trained support staff at high-volume centers. However, due to the varying conditions of different surgical teams, the magnitude of this association may change in different centers.

The four factors finally included in the nomogram are easily available, and the predicted probability of SSI can be calculated immediately after surgery through this method. Moreover, we categorized patients into three groups based on their distinct risk of developing an SSI to better guide clinical practice. For patients in the intermediate- and high-risk groups, we suggest that intensive monitoring of the patient's condition after surgery should be implemented, and prolonged postoperative antibiotic administration and upgraded antibiotic agents should be considered.

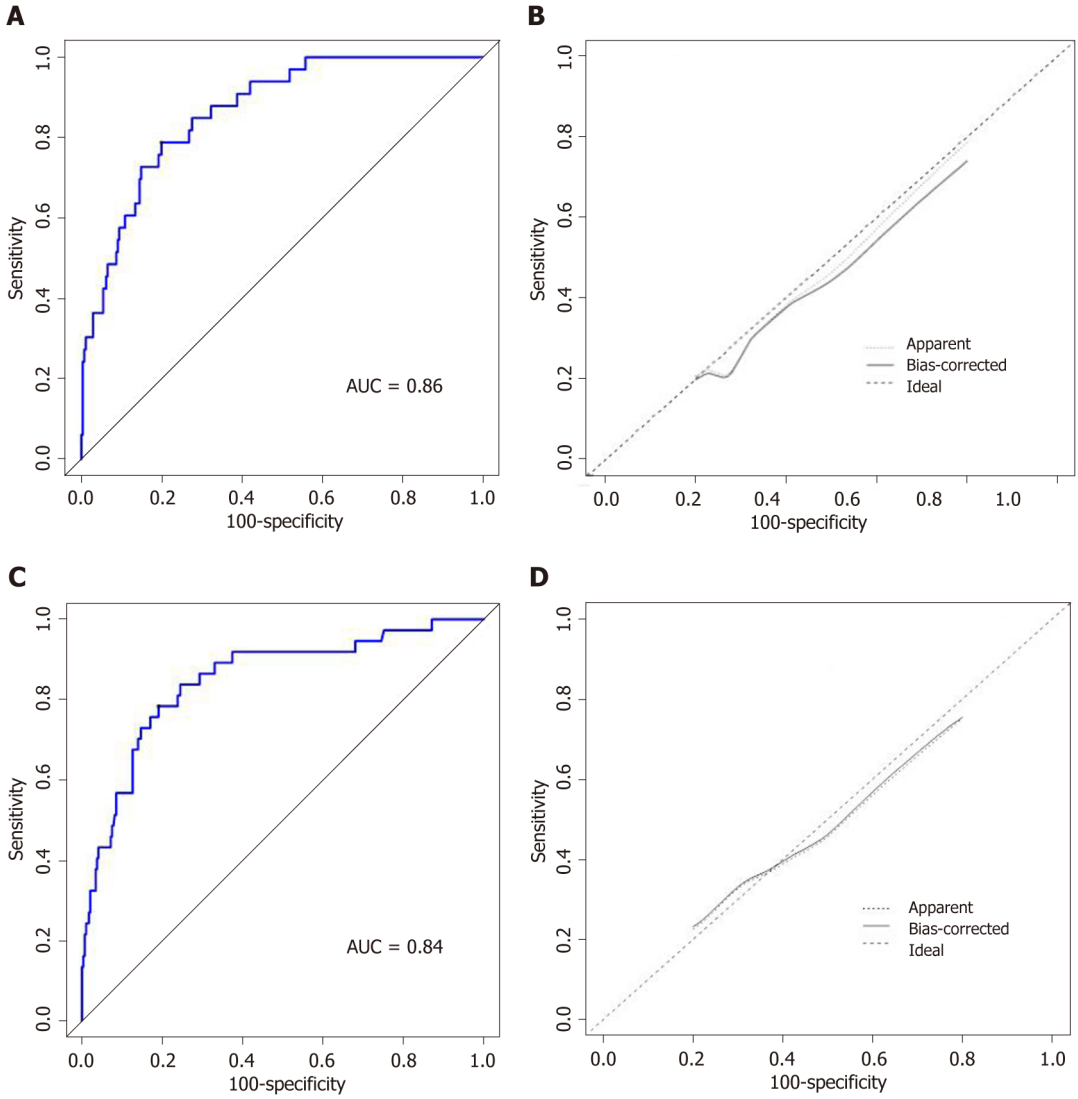

Figure 2 Receiver operating characteristic curve of nomogram prediction in the training cohort (AUC = 0.86) (A) and in the training cohort (AUC = 0.84) (B) Calibration curve of nomogram prediction in the training cohort (C) and validation cohort (D). ROC: Receiver operating characteristic; AUC: Area under the ROC curve.

In the present study, the area under the ROC curve for the nomogram was significantly higher than that for the NNIS risk index (0.85 *vs* 0.75 for the whole population). Our nomogram appears to indicate a higher accuracy for predicting SSI, as compared to the NNIS risk index. One reason for this finding is that the NNIS risk index stratifies the SSI risk using three equally weighted factors: ASA > 2, wound class, and operation duration. However, the wound classification does not apply in our population as all the surgeries included in our study were clean. In addition, our prediction model integrated the information of hepatic surgery history and liver function, which were significantly associated with SSI in our population. Furthermore, the NNIS risk index was developed using a wide range of patients, whereas our prediction model was established only using patients who underwent hepatectomy for HCC. The increased relevance could explain the better performance of our prediction model in this population.

Nevertheless, the present study has certain limitations. First, the possibility of selection bias should be considered because of the retrospective nature of our study. The rate of major hepatectomy was relatively low, which could have contributed to the low SSI and mortality in our cohorts. Some of the intraoperative parameters such as body temperature, administration of inspired oxygen, and exact volume replacement during the surgery were difficult to obtain retrospectively, even though previous studies have found that these parameters may be associated with SSI occurrence[33-35]. Although most patients were managed according to the CDC guidelines, incomplete records may still lead to bias. Moreover, we included only 640 patients in our study. Although this cohort is statistically adequate for developing and

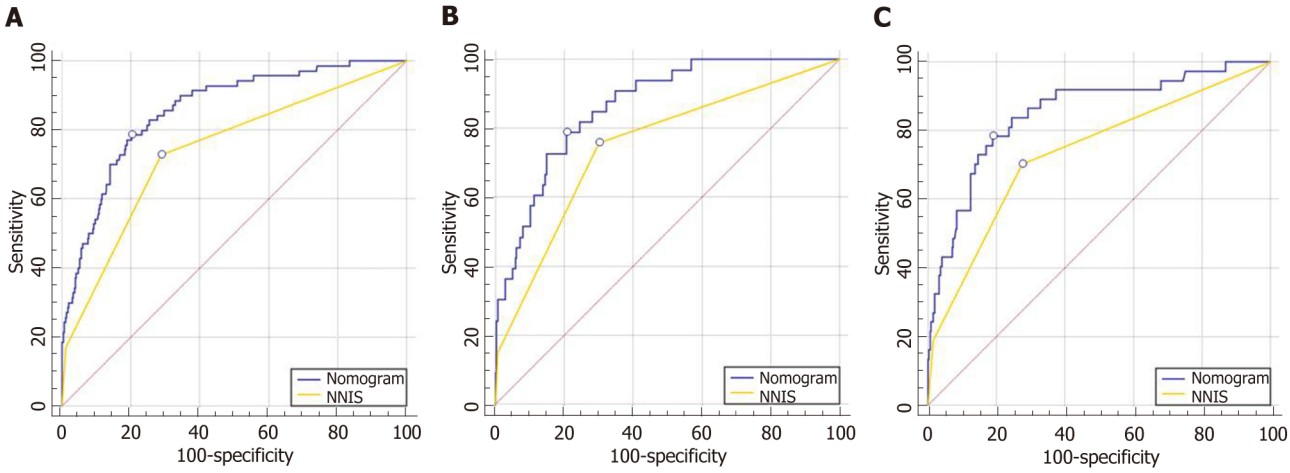

Figure 3 Receiver operating characteristic curve of prediction with the nomogram and the NNIS risk index. The area under the ROC curve (AUC) for the NNIS risk index was 0.75 for the entire population, 0.75 for the training cohort, and 0.73 for the validation cohort. The AUC for the nomogram was 0.86 for the training cohort, 0.84 for the validation cohort, and 0.85 for the entire population.

validating a prediction model, it is still a relatively small cohort compared to that in other studies. Furthermore, the nomogram was developed and validated using data only from two high-volume centers in China, and the ability of this model to predict SSIs in a wider population should be tested in additional institutions.

In conclusion, we have developed the first forecasting model to predict SSI in patients undergoing hepatectomy for HCC. This nomogram is able to stratify patients into three groups with distinct risks of SSI, and performs well on external validation. However, the reliability and applicability of the nomogram need to be validated in additional centers in the future.

Table 3 Risk groups based on the predicted nomogram

Group	Total points	Predicted risk	Predicted mean risk (95%CI)	Observed rate
Low-risk	< 70	< 10%	7.46 (7.34-7.60)	2.8% (12/429)
Intermediate-risk	70-104	10%-50%	21.42 (20.01-22.83)	21.6% (40/185)
High-risk	> 104	> 50%	71.07 (65.84-76.29)	69.2% (18/26)

ARTICLE HIGHLIGHTS

Research background

Surgical site infections (SSI) reportedly account for > 50% of infectious complications after hepatectomy for hepatocellular carcinoma (HCC). It has a significant impact on morbidity, mortality, prolonged hospitalization, costs, and even long-term oncology outcomes. Hence, SSI prevention has been considered a top priority for improving perioperative outcomes. Previous studies suggest that many factors can influence SSIs in patients undergoing hepatectomy. However, some of these factors remain controversial.

Research motivation

Models to identify the patients with an increased risk of developing SSI are limited. National Nosocomial Infection surveillance (NNIS) risk index was developed using data from a wide range of patients undergoing various surgical procedures with different disease conditions. Hence, the applicability of NNIS is limited in patients undergoing hepatectomy for HCC. To develop an effective forecasting model to screen out patients at high risk of SSI is vital for improving individual clinical decision making and the perioperative morbidity rate.

Research objectives

In this study, we aimed to investigate the risk factors for SSI after hepatectomy for HCC, and develop a prediction nomogram for SSI by analyzing clinical data from a consecutive series of patients undergoing hepatectomy at our institution and validate the prediction model in an external cohort.

Research methods

The data of 640 patients with HCC who underwent attempted curative liver resection were retrospectively collected from two academic institutions in China. The records of all patients were reviewed. We identified the independent predictors of SSI using multivariate logistic regression analysis. Then, a nomogram was formulated based on the identified factors, using the rms package in R, version 3.2.1 (http://www.r-project.org/). The performance of prediction model was assessed using an external cohort from the second hospital.

Research results

The logistic regression identified three pre-operative variables (serum albumin level, repeat hepatectomy, and ASA score) and one intra-operative variable (duration of operation) as independent predictors of overall SSI. We developed a nomogram to predict SSI in patients after hepatectomy for HCC by integrating the four factors identified. Our nomogram showed better prediction accuracy compared to the NNIS risk index. Finally, we stratified the patients of the entire cohort into three groups with a distinct risk of SSI, based on the predicted risk distribution using the nomogram.

Research conclusions

Our nomogram appears to indicate a higher accuracy for predicting SSI, as compared to the NNIS risk index. Our prediction model integrated the information of hepatic surgery history and liver function, which were significantly associated with SSI in our population. The NNIS risk index was developed using a wide range of patients, whereas our prediction model was established only using patients who underwent hepatectomy for HCC. The increased relevance could explain the better performance of our prediction model in this population.

Research perspectives

This nomogram based on identified factors is able to stratify patients into three groups with distinct risks of SSI, and performs well on external validation. We primarily focused on the preoperative and intra-operative predictors because we aimed to develop a prediction model to identify suitable patients for enhanced recovery after surgery at a relatively early time point. In the future, we will assess the performance of this model among a diverse population of patients.

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ORIGINAL ARTICLE

Retrospective Study

Serological investigation of IgG and IgE antibodies against food antigens in patients with inflammatory bowel disease

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Author contributions: Wang HY and Zhang HJ designed the study; Li Y, Jiao CH, Zhao XJ, Li XT, Lu MJ and Mao XQ collected specimens; Wang HY and Li Y performed the research; Wang HY and Li JJ analyzed the data; Wang HY wrote this manuscript; Zhang HJ supervised the report.

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Data sharing statement: No

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Abstract

BACKGROUND

Food antigens have been shown to participate in the etiopathogenesis of inflammatory bowel disease (IBD), but their clinical value in IBD is still unclear.

AIM

To analyze the levels of specific immunoglobulin G (IgG) and E (IgE) antibodies against food antigens in IBD patients and to determine their clinical value in the pathogenesis of IBD.

METHODS

We performed a retrospective study based on patients who visited the First Affiliated Hospital of Nanjing Medical University between August 2016 and January 2018. A total of 137 IBD patients, including 40 patients with ulcerative colitis (UC) and 97 patients with Crohn's disease (CD), and 50 healthy controls (HCs), were recruited. Serum food-specific IgG antibodies were detected by semi-quantitative enzyme-linked immunosorbent assay, and serum food-specific IgE antibodies were measured by Western blot. The value of food-specific IgG antibodies was compared among different groups, and potent factors related to these antibodies were explored by binary logistic regression.

RESULTS

Food-specific IgG antibodies were detected in 57.5% of UC patients, in 90.72% of CD patients and in 42% of HCs. A significantly high prevalence and titer of food-specific IgG antibodies were observed in CD patients compared to UC patients and HCs. The number of IgG-positive foods was greater in CD and UC patients

additional data are available.

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than in HCs (CD *vs* HCs, $P = 0.000$; UC *vs* HCs, $P = 0.029$). The top five food antigens that caused positive specific IgG antibodies in CD patients were tomato (80.68%), corn (69.32%), egg (63.64%), rice (61.36%), and soybean (46.59%). The foods that caused positive specific IgG antibodies in UC patients were egg (60.87%), corn (47.83%), tomato (47.83%), rice (26.09%), and soybean (21.74%). Significantly higher levels of total food-specific IgG were detected in IBD patients treated with anti-TNFα therapy compared to patients receiving steroids and immunosuppressants (anti-TNFα *vs* steroids, $P = 0.000$; anti-TNFα *vs* immunosuppressants, $P = 0.000$; anti-TNFα *vs* steroids + immunosuppressants, $P = 0.003$). A decrease in food-specific IgG levels was detected in IBD patients after receiving anti-TNFα therapy ($P = 0.007$). Patients who smoked and CD patients were prone to developing serum food-specific IgG antibodies [Smoke: OR (95%CI): 17.6 (1.91-162.26), $P = 0.011$; CD patients: OR (95%CI): 12.48 (3.45-45.09), $P = 0.000$]. There was no difference in the prevalence of food-specific IgE antibodies among CD patients (57.1%), UC patients (65.2%) and HCs (60%) ($P = 0.831$).

CONCLUSION
CD patients have a higher prevalence of food-specific IgG antibodies than UC patients and HCs. IBD patients are prone to rice, corn, tomato and soybean intolerance. Smoking may be a risk factor in the occurrence of food-specific IgG antibodies. Food-specific IgG antibodies may be a potential method in the diagnosis and management of food intolerance in IBD.

Key words: Inflammatory bowel disease; Food-specific immunoglobulin G; Food intolerance

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Core tip: Food antigens have been indicated to participate in the etiopathogenesis of inflammatory bowel disease. However, their value is disputable, as some studies found that food immunoglobulin G (IgG) and E (IgE) antibodies can be expressed in healthy individuals. This study analyzed the levels of specific IgG and IgE antibodies against food antigens in inflammatory bowel disease patients and found that Crohn's disease patients not only have higher prevalence of food-specific IgG, but also intolerance against rice, corn, tomato and soybean.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disease of the gastrointestinal tract, which includes ulcerative colitis (UC) and Crohn's disease (CD). Increasing evidence indicates that IBD results from an abnormal mucosal immune system triggered by environmental factors[1,2].

Various environmental factors such as environmental pollution, smoking, stress and foods[3-6] are thought to induce or aggravate IBD. Of these factors, diet is considered to contribute to the course of IBD[7]. A multicenter case-control study in Japan found that sweets, fats, including monounsaturated and polyunsaturated fatty acids, soil, fish and shellfish were positively associated with IBD risk[8]. Another study showed that increased consumption of alcohol or red meat was associated with an increased probability of relapse in UC patients[9]. Two large prospective cohort studies in Japan and Europe verified that high consumption of n-6 polyunsaturated fatty acids may aggravate IBD by altering the fatty acid composition of the cell membrane and immunomodulating leukotrienes and prostaglandins[10,11]. Studies have shown that food intolerance may be involved in this process[12]. Some food antigens are

thought to be involved in the formation and development of human chronic intestinal inflammatory diseases in genetically susceptible patients[13,14].

Food allergy and food intolerance are two types of adverse reactions to food. Food allergy is typically mediated by immunoglobulin E (IgE) antibodies, which produce an immediate and sometimes life-threatening response, the most well-known being type 1 food allergy to peanuts or shellfish. The immune system mounts an attack against normally harmless food ingredients. In contrast, food intolerance is mediated by immunoglobulin G (IgG) antibodies, and the underlying mechanism may be the stimulation of neutrophils or other cells in the innate immune system, creating a complex clinical course. Food intolerance significantly contributes to IBD patients' symptoms. The advantages of removing certain foods from the daily diet have been a focus in recent studies[15-17]. It is thought that classic food intolerance is caused by food allergies based on IgE-mediated antibody responses; however, immediate allergic reactions are rare in IBD[18,19]. Therefore, a delayed immune response mediated by IgG antibodies following exposure to a particular antigen may account for adverse food reactions in IBD[20]. However, this mechanism is debatable, as some studies found that food IgG antibodies can be expressed in healthy individuals[21-26]. The purpose of this study was to analyze the levels of IgG and IgE antibodies against food antigens in IBD patients and determine their clinical value in IBD pathogenesis.

MATERIALS AND METHODS

Subjects

We performed a retrospective study using blood samples obtained from patients who visited the First Affiliated Hospital of Nanjing Medical University between August 2016 and January 2018. According to the consensus on the diagnosis of IBD drawn up by European Crohn's and Colitis Organization[27,28], each patient met the diagnostic criteria for CD or UC. We excluded patients who had been diagnosed with ischemic bowel disease, radiation enteritis, cardio-cerebral vascular diseases, infectious diseases, cancer or received surgery within 3 months. Healthy controls (HCs) were chosen from our Physical Examination Center to represent the general population. Finally, a total of 137 IBD patients, including 40 patients with UC and 97 patients with CD, and 50 HCs were enrolled in this study. Written informed consent was obtained from all patients. The Ethics Committee of the First Affiliated Hospital of Nanjing Medical University approved the study and the consent procedure.

Serum IgG/IgE assay

Serum IgG antibodies to 14 unique food antigens (chicken, beef, codfish, egg, crab, shrimp, milk, pork, rice, corn, mushroom, wheat, soybean and tomato) were assessed using enzyme-linked immunosorbent assay according to the manufacturer's protocol (Biomerica Inc., Irvine, CA, United States). The IgG concentration was classified into the following four grades: negative (Grade 0, less than 50 U/mL), mild sensitivity (Grade +1, 50-100 U/mL), moderate sensitivity (Grade +2, 100-200 U/mL) and high sensitivity (Grade +3, > 200 U/mL). IgE-specific antibodies to food allergens (chicken egg white, cow's milk, peanut, beef, mutton, shrimp, crab, marine fish including gadus, lobster, scallop, and freshwater fish including trout, weever, carp) were examined by western blotting according to the manufacturer's instructions (EUROIMMUN Medizinische Labordiagnostika AG, Germany). An IgE concentration less than 0.35 kU/L was considered negative. Levels of 0.35-3.5 kU/L, 3.5-17.5 kU/L and > 17.5 kU/L were classified as mild specific antibody concentration, moderate specific antibody concentration with obvious clinical symptoms, and high specific antibody concentration, respectively.

Statistical analysis

The data were analyzed using SPSS software (version 21.0). Enumeration data were analyzed by the Chi-squared test. Continuous numerical variables were expressed as the mean ± SD. Comparisons between groups were performed using the Student's *t* test or ANOVA test, as appropriate. Correlations among variables were analyzed by binary logistic regression. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the patients

The demographic and clinical characteristics of the 50 HCs, 40 UC patients and 97 CD patients are summarized in Table 1. The average age in the UC, CD and HC groups

was similar, the mean disease course in the UC and CD group was similar. The percentage of rectum, left-sided and entire colon type in UC patients was 25%, 17.5%, and 57.5%, respectively; 76.29% of CD patients had ileal or ileal-colon lesions, and 30.93% of CD patients had undergone IBD-related surgery. In total, 61.86% of CD patients and 75% of UC patients were in the active stage. In addition, 61.86% of CD patients and 30% of UC patients received treatment with steroids, immunosuppressive agents, or anti-TNFα; and 18.56% of CD patients received enteral nutrition.

Proportion of serum food-specific IgG in UC and CD patients

The positive rate of food-specific IgG in UC patients, CD patients and HCs was 57.5%, 90.72% and 42%, respectively. CD patients showed higher positive rate of food-specific IgG than HCs (P = 0.000). However, there was no significant difference between UC patients and HCs (P = 0.144) (Figure 1A). The number of IgG-positive food items was higher in UC and CD patients than in HCs (UC *vs* HCs, P = 0.029; CD *vs* HCs, P = 0.000; CD *vs* UC, P = 0.006) (Figure 1B). CD patients showed positive IgG against an average of 3.8 foods [range 1-8; 95% confidence interval (CI): 3.41-4.20], while UC patients and HCs showed positive IgG against an average of 2.56 foods (range 1-8; 95%CI: 1.73-3.39) and 1.57 foods (range 1-3; 95%CI 1.26–1.87), respectively. The average levels of total serum IgG in CD patients, UC patients and HCs were 138.6 ± 75.65, 115.6 ± 80.11, and 105.9 ± 52.3 U/mL, respectively. The average levels of total serum IgG in CD patients were significantly different from those in UC patients and HCs (CD *vs* UC, P = 0.03; CD *vs* HCs, P = 0.017), while there was no significant difference between UC patients and HCs (P = 0.554) (Figure 1C). The seropositive rate of moderate and high sensitivity was 39.13% in UC patients, 84.09% in CD patients and 71.43% in HCs, and the CD group had higher sensitivity to specific food allergens than the UC group (P = 0.000).

Distribution spectrum of food-specific IgG to 14 unique food allergens in UC and CD patients

In CD patients, the top nine food allergens causing positive serum IgG were tomato (80.68%), corn (69.32%), egg (63.64%) rice (61.36%), soybean (46.59%), milk (19.32%), wheat (17.65%), codfish (13.64%), and mushroom (3.4%). In UC patients, the nine main food allergens causing positive serum IgG were egg (60.87%), corn (47.83%), tomato (47.83%), rice (26.09%), soybean (21.74%), milk (21.34%), codfish (17.39%), wheat (8.7%) and mushroom (4.35%). The food-specific IgG detected in HCs was due to egg (66.7%), milk (28.6%), corn (19%), soybean (14.3%), mushroom (14.3%), rice (4.8%), and tomato (4.8%) (Figure 2), similar to the findings in a previous report[29]. In the present study, CD patients were more sensitive to tomato, corn, rice, soybean, wheat and codfish, while UC patients were more sensitive to tomato, corn and rice (Table 2). CD patients had significantly higher levels of food-specific IgG than UC patients and HCs against rice (CD *vs* HCs, P = 0.000; CD *vs* UC, P = 0.01), soybean (CD *vs* HCs, P = 0.001; CD *vs* UC, P = 0.015), corn (CD *vs* HCs, P = 0.000; CD *vs* UC, P = 0.013), wheat (CD *vs* HCs, P = 0.012; CD *vs* UC, P = 0.016), tomato (CD *vs* HCs, P = 0.001; CD *vs* UC, P = 0.201), and egg (CD *vs* HCs, P = 0.054; CD *vs* UC, P = 0.021). No significant differences were observed for beef (P = 0.148), chicken (P = 0.429), crab (P = 0.385), shrimp (P = 0.164), codfish (P = 0.812), pork (P = 0.496), milk (P = 0.452) or mushroom (P = 0.122) specific IgG compared with HCs. No significant differences in food-specific IgG to 14 dietary antigens were observed between UC patients and HCs (Figure 3).

Relationship between food-specific IgG and disease location

The disease location of CD and UC patients was divided into subgroups according to the Montreal classification. The positive rates of food-specific IgG among subgroups of UC or CD patients were not significantly different.

Association between food-specific IgG and disease activity

The Crohn's Disease Activity Index (CDAI) was used to evaluate CD patients' disease activity, and the Mayo score was used to assess UC patients' disease activity. Both CD and UC patients were divided into positive and negative food-specific IgG subgroups, with no marked statistical difference (CDAI: Food-specific IgG+ *vs* food-specific IgG-, P = 0.27; Mayo: food-specific IgG+ *vs* food-specific IgG-, P = 0.58). It was found that the group with more than three positive allergens had a higher CDAI score compared with the group with two positive allergens (P = 0.033). The Mayo score in the group with multiple positive allergens was higher than that in the group with a single positive allergen (2 positive *vs* 1 positive, P = 0.024; ≥ 3 positive *vs* 1 positive, P = 0.046). We also divided CD and UC patients into three subgroups according to the degree of seropositivity. There were no significant differences among the three

Table 1 Clinical characteristics of all subjects, *n* (%)

Clinicopathological features	UC, *n* = 40	CD, *n* = 97	HCs, *n* = 50
Male	26 (65)	69 (71.13)	30 (60)
Female	14 (35)	28 (28.87)	20 (40)
Age in yr, mean ± SD	34.22 ± 12.50	33.51 ± 11.77	36.2 ± 10.73
Duration of disease in yr, mean, 95%CI	3.69 (2.12-4.86)	2.80 (2.01-3.54)	/
Disease activity			
Remission	10 (25)	37 (38.14)	/
Mild	6 (15)	36 (37.11)	/
Moderate	17 (42.5)	23 (23.71)	/
Severe	7 (17.5)	1 (1.04)	/
Age at diagnosis in yr			
A1, ≤ 16	/	6 (6.19)	/
A2, 17-40	/	67 (69.07)	/
A3, > 40	/	24 (24.74)	/
Disease location			
L1, terminal ileum	/	41 (42.27)	/
L2, colon	/	17 (17.53)	/
L3, ileocolon	/	33 (34.02)	/
L1+L4	/	3 (3.09)	/
L3+L4	/	3 (3.09)	/
E1, rectum	10 (25)		/
E2, left-sided colon	7 (17.5)		/
E3, entire colon	23 (57.5)		/
Disease behaviour			
B1, non-stricturing, non-penetrating		58 (59.79)	/
B2, stricturing		32 (32.98)	/
B3, penetrating		6 (6.19)	/
B2 + B3		1 (1.04)	/
CDAI, mean ± SD		153.96 ± 80.22	
Mayo, mean ± SD	6.52 ± 3.24		
Smoker	10 (25)	8 (8.25)	/
Perianal diseases	1 (2.5)	5 (5.15)	/
Medications			
5-ASA	32 (80)	31 (31.96)	/
Sulfasalazine	2 (5)	/	/
Steroids	10 (25)	9 (9.28)	/
Immunosuppressive agents	2 (5)	19 (19.59)	/
Anti-TNF	/	32 (32.99)	/
Enteral nutrition	/	18 (18.56)	
No meds	4 (10)	/	/
Surgery, IBD-related	2 (5)	30 (30.93)	/

E1, E2, E3: Disease location of UC using the Montreal Classification; A1, A2, A3: Age at diagnosis; L1, L2, L3: Disease location; B1, B2, B3, B2 + B3: Disease behavior of CD using the Montreal Classification; UC: Ulcerative colitis; CD: Crohn's disease; HCs: Healthy controls; CDAI: Crohn's Disease Activity Index; IBD: Inflammatory bowel disease.

subgroups (Figure 4).

Relationship between food-specific IgG and clinical characteristics

The food-specific IgG-positive group had higher levels of inflammatory biomarkers, such as leukocyte count, platelet count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) compared with the food-specific IgG-negative group, although there was no significant difference between the groups (Table 3).

The association between demographic and other factors with food-specific IgG is shown in Table 4. Subjects who smoked were prone to developing serum food-related

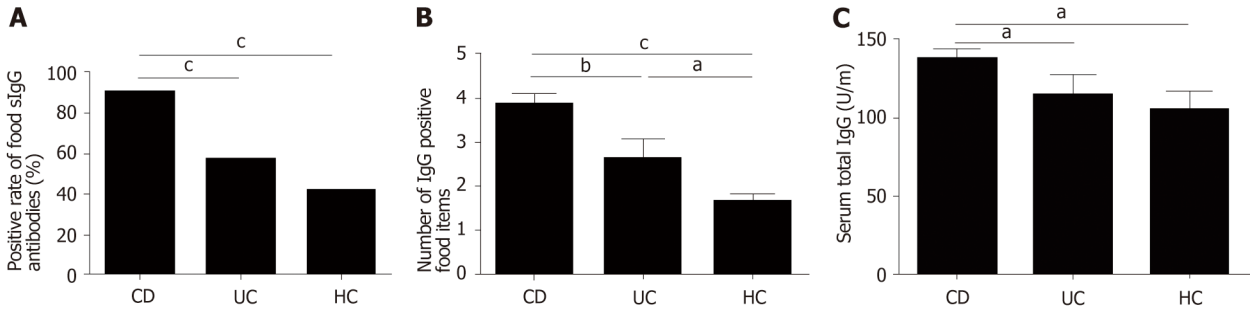

Figure 1 High sensitivity to food antigens in inflammatory bowel disease patients. A: Positive rate of food-specific IgG antibodies in CD patients, UC patients and HCs. Statistics: Chi-squared test; B: The number of IgG-positive food items in CD patients, UC patients and HCs; C: Comparison of the total serum IgG levels in CD patients, UC patients and HCs. Statistics: ANOVA), $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. UC: Ulcerative colitis; CD: Crohn's disease; HCs: Healthy controls; ANOVA: One-way analysis of variance.

IgG antibodies [OR (95%CI): 17.6 (1.91-162.26); $P = 0.011$]. CD patients were more predisposed to food intolerance than UC patients [OR (95%CI): 12.48 (3.45-45.09); $P = 0.000$] (Table 4).

Anti-TNFα was an effective biological agent in IBD patients. Higher total food IgG levels were found in patients treated with anti-TNFα compared to patients treated with steroids or immunosuppressants (anti-TNFα *vs* steroids, $P = 0.013$; anti-TNFα *vs* immunosuppressants, $P = 0.000$) (Figure 5A). However, we found a decrease in food-specific IgG levels in IBD patients after introducing anti-TNFα (before anti-TNFα *vs* after anti-TNFα, $P = 0.009$) (Figure 5B).

Prevalence of serum specific IgE to antigens

It was shown that 65.2% of CD patients, 57.1% of UC patients and 60% of HCs had specific IgE antibodies; and 62.5%, 12.5%, and 25% of UC patients and 26.67%, 30%, and 43.33% of CD patients were sensitive to one, two, or three or more allergens, respectively. These values were 25%, 33.33%, and 41.67% in HCs. The seropositive rate of moderate and high sensitivity was 50% in UC patients, 56.67% in CD patients and 71.43% in HCs. The differences among the three groups in the occurrence of IgE positivity were not statistically significant ($P = 0.831$). The average levels of total serum IgE in subjects with CD, UC and HCs were 3.68 ± 6.62, 0.61 ± 0.17, and 0.90 ± 0.68 kU/L, respectively. There was no significant difference among CD patients, UC patients and HCs (CD *vs* HCs, $P = 0.202$; UC *vs* HCs, $P = 0.933$; CD *vs* UC, $P = 0.316$) (Figure 6).

Proportion of serum food-specific IgE antibodies

In this study, 16.67% of HCs had food-specific IgE against marine fish in contrast to 46.67% of CD patients and 25% of UC patients. In addition, 20% of CD patients had IgE to beef in contrast to only 8.33% of HCs. This was even more pronounced in terms of IgE antibodies to egg white, with 6.67% of CD patients and 12.5% of UC patients showing IgE antibodies, while HCs showed no egg white IgE antibodies (Figure 7).

DISCUSSION

Immune tolerance to exogenous antigens, including commensal bacteria and food proteins is a pivotal regulation mechanism for maintaining intestinal homeostasis. A breakdown in immune tolerance to exogenous antigens is considered to play a role in the initiation and development of chronic inflammation in IBD[30]. Some IBD patients have shown an improvement in symptoms following exclusion diets[31-33]. Previous studies have investigated the potential role of serum IgG/IgE in food intolerance/allergy[34,35], and a thorough study of exclusion diet according to food IgG/IgE tests has been conducted[36,37]. However, the reliability and clinical practicability of food-specific IgG/IgE detection remain controversial, although it is a potential method for the diagnosis of food intolerance/allergy.

In the present study, CD patients had high levels of serum food-specific IgG antibodies compared to UC patients and HCs. Most (90.72%) of the CD patients had higher levels of IgG antibodies, similar to previous findings[17]. Both CD and UC patients also had more IgG-positive food items and higher sensitivity. Similar results were reported by Cai *et al*[17], who found that IBD patients were sensitive to multiple food antigens. Food allergy was once thought to play a part in the progression of

Table 2 Percentage of positive food-specific IgG antibodies against 14 food items in ulcerative colitis patients, Crohn's disease patients, and healthy controls

	CD	UC	HCs
Beef	0	0	0
Chicken	2.27	0	0
Codfish	13.64[a]	17.39	4.8
Corn	69.32[c]	47.83[a]	19
Crab	0	0	0
Egg	63.64	60.87	66.7
Mushroom	3.4	4.35	14.3
Milk	19.32	21.34	28.6
Pork	1.14	0	0
Rice	61.36[c]	26.09[a]	4.8
Shrimp	1.14	4.21	0
Soybean	46.59[c]	21.74	14.3
Tomato	80.68[c]	47.83[c]	4.8
Wheat	17.05[b]	8.7	0

Statistics: Chi-squared test
[a]$P < 0.05$,
[b]$P < 0.01$,
[c]$P < 0.001$. UC: Ulcerative colitis; CD: Crohn's disease; HCs: Healthy controls.

IBD[38]. However, food-specific IgE was not found in CD patients in the studies by Huber *et al*[39] and Bartůnková *et al*[40]. In the present study, 57.1% of UC patients, 65.2% of CD patients, and 60% of HCs had detectable specific IgE to different food antigens, but these values were not statistically significant.

A study showed that the common food allergens that caused positive IgG in CD patients were egg (73.3%), rice (56.7%), corn (56.7%), tomato (46.7%) and soybean (43.3%). In addition, the frequent food allergens in UC patients were egg (81.0%), rice (14.3%), corn (14.3%), tomato (9.5%) and milk (9.5%). The corresponding food allergens in HCs were egg (69.3%), milk (14.8%), and crab (14.8%)[17]. In the present study, the most common food-specific IgG antibodies detected in CD patients were against tomato in 80.68% of patients, followed by corn in 69.32%, egg in 63.64%, rice in 61.36%, soybean in 46.59%, milk in 19.32%, wheat in 17.65%, and codfish in 13.64% of patients. The most common food-specific IgG antibodies detected in UC patients were egg (60.87%), corn (47.83%), tomato (47.83%), rice (26.09%), soybean (21.74%), milk (21.34%), codfish (17.39%), and wheat (8.7%). The most common food-specific IgG antibodies detected in HCs were egg (66.7%), milk (28.6%), and corn (19%). Collectively, these data suggest that both IBD patients and HCs showed a high level of egg-specific IgG antibodies, while IBD patients may be prone to rice, corn, tomato and soybean intolerance. Foods such as rice, wheat, corn, soybean, and tomato are traditional products and some of the most commonly used ingredients in China. As such, most Chinese people are frequently exposed to these intestinal antigens. No marked changes were observed in beef, shrimp, crab, chicken, pork, or mushroom specific IgG between CD patients and HCs. This may be because CD patients subconsciously avoid triggering foods (*e.g.*, beef, shrimp, crab, chicken, pork, and mushroom) to alleviate the antibody response.

Food-specific IgG antibodies are often discovered in IBD patients whose small intestine is affected, which might be related to lactose malabsorption[17,41]. A survey showed that IgG-positive IBD patients had higher mean levels of ESR and high sensitivity-CRP and had severe disease activity[26]. In our study, there was no significant difference in the CDAI and Mayo score between the positive and negative IgG antibody subgroups. The multiple positive allergens group had a higher CDAI/Mayo score compared with the single positive allergen group. In addition, the food-specific IgG-positive group had higher levels of inflammatory biomarkers, such as ESR and CRP, leukocyte count, and platelet count compared to the food-specific IgG-negative group, although no significant differences were observed.

In some studies, it was reported that food intolerance was more common in females than in males[42-45]. In this study, females were 2.07-fold more likely than males to develop food intolerance, but this difference was not statistically significant. Some studies have demonstrated that female hormones exert a pro-inflammatory effect,

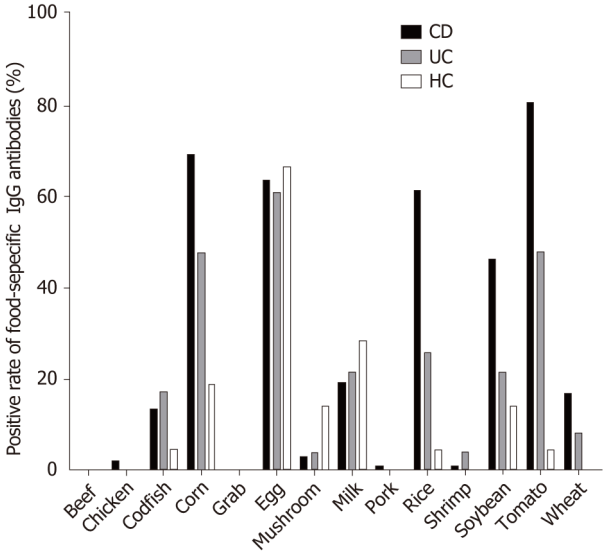

Figure 2 Distribution of positive IgG to food allergens in CD patients, UC patients and HCs. UC: Ulcerative colitis; CD: Crohn's disease; HCs: Healthy controls.

while testosterone inhibits the pro-inflammatory process, such as histamine release and mast cell degranulation[46]. Some investigations have reported that the elderly population may be more likely to develop food allergies than younger individuals[47]. However, other studies have revealed that the younger age group had higher levels of food-specific IgG compared with older people[45,48], which is similar to the results in the present study. Maturation of intestinal mucosa with increasing age may influence food intolerance resulting in differential immune responses at different ages. This study also demonstrated that CD patients were more predisposed to food intolerance than UC patients. Smoking was found to be a risk factor for food intolerance in IBD patients. The etiology and pathogenesis of smoking in IBD is not yet entirely clear due to the complex chemical composition of tobacco. Potential mechanisms worth considering include gene expression changes relevant to immune responses and the citrullination of various proteins, which then influence the three-dimensional structure of proteins in such a way that the altered proteins subsequently act as antigens[49].

Anti-TNFα, a biological agent, has been confirmed to be effective in the treatment of IBD patients. Our study showed higher levels of total food-specific IgG in IBD patients receiving anti-TNFα therapy compared to patients receiving treatment with steroids or immunosuppressants. A decrease in total food-specific IgG levels in IBD patients was observed after the introduction of anti-TNFα therapy. Anti-TNFα treatment can result in long-term remission and mucosal healing. Intestinal barrier repair and prolonged inflammation suppression may improve intestinal barrier dysfunction, which prevents food antigens from entering the circulation leading to the production of food-specific IgG[45].

The present study has several limitations. Firstly, we only tested common food-specific IgG antibodies and did not measure food-specific IgG to nuts, fruits or food ingredients. Secondly, we did not conduct a follow-up study to determine whether excluding IgG-positive foods had an effect on IBD patients. Thirdly, children were not included in our study. Finally, our sample size was small; therefore, larger cohort studies should be conducted to confirm our results and to reveal the possible underlying mechanism.

In conclusion, the level of food-specific IgG is higher in CD patients than in UC patients and HCs. IBD patients may be prone to rice, corn, tomato and soybean intolerance. Although the mechanism of food intolerance in IBD is still unclear, we believe that food-specific IgG antibodies may provide a clinical benefit for IBD patients *via* diet restriction. The role of food-specific IgG in food intolerance should be investigated in future studies.

Table 3 Comparison of laboratory results between positive and negative food-specific IgG subgroups in inflammatory bowel disease patients

Laboratory results	sIgG (+)	sIgG (-)	*P*-value
	n = 111	*n* = 26	
WBC, × 10^9/L	6.56 ± 2.57	6.26 ± 2.17	0.62
Hb, g/L	121.59 ± 23.72	123.71 ± 29.27	0.77
HCT	38.45 ± 6.38	37.36 ± 6.14	0.83
PLT, × 10^9/L	280.75 ± 110.31	233.65 ± 86.34	0.13
CRP, mg/L	17.27 ± 6.64	11.17 ± 4.84	0.13
ESR, mm/H	21.49 ± 19.88	20.12 ± 19.40	0.64
ALB, g/L	37.08 ± 5.24	40.21 ± 5.23	0.16

Data are expressed as mean ± SD: Student's *t* test. No statistical significance ($P > 0.05$). WBC: White blood cell; Hb: Hemoglobin; PLT: Platelet; CRP: C-Reactive protein; ESR: erythrocyte sedimentation rate; HCT: Hematocrit; ALB: Albumin.

Table 4 Correlation between food-specific IgG and clinical parameters

Parameter	Odds ratio	95%CI	*P*-value
Disease type, UC/CD	12.48	3.45-45.09	0.000[c]
Gender, male/female	2.07	0.62-6.88	0.237
Age, < 40 yr/≥ 40 yr	0.44	0.14-1.41	0.167
Smoker	17.6	1.91-162.26	0.011[a]

UC: Ulcerative colitis; CD: Crohn's disease; 95%CI: 95% Confidence interval. Statistics: Binary logistic regression,
[a]$P < 0.05$,
[c]$P < 0.001$. UC: Ulcerative colitis; CD: Crohn's disease; IBD: Inflammatory bowel disease

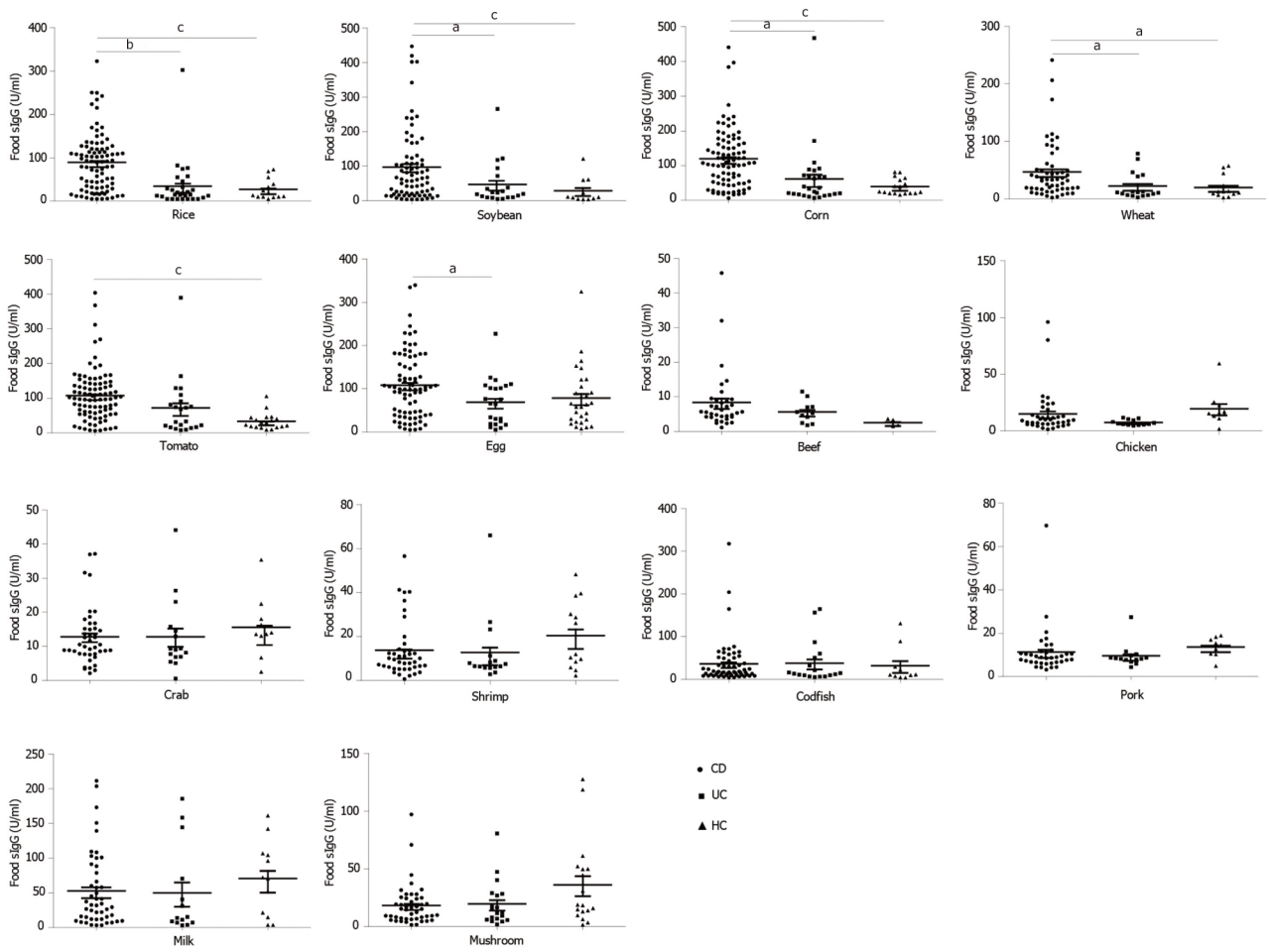

Figure 3 The levels of food-specific IgG against 14 common daily foods in CD patients, UC patients and HCs. Statistics: ANOVA, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. UC: Ulcerative colitis; CD: Crohn's disease; HCs: Healthy controls; ANOVA: One-way analysis of variance.

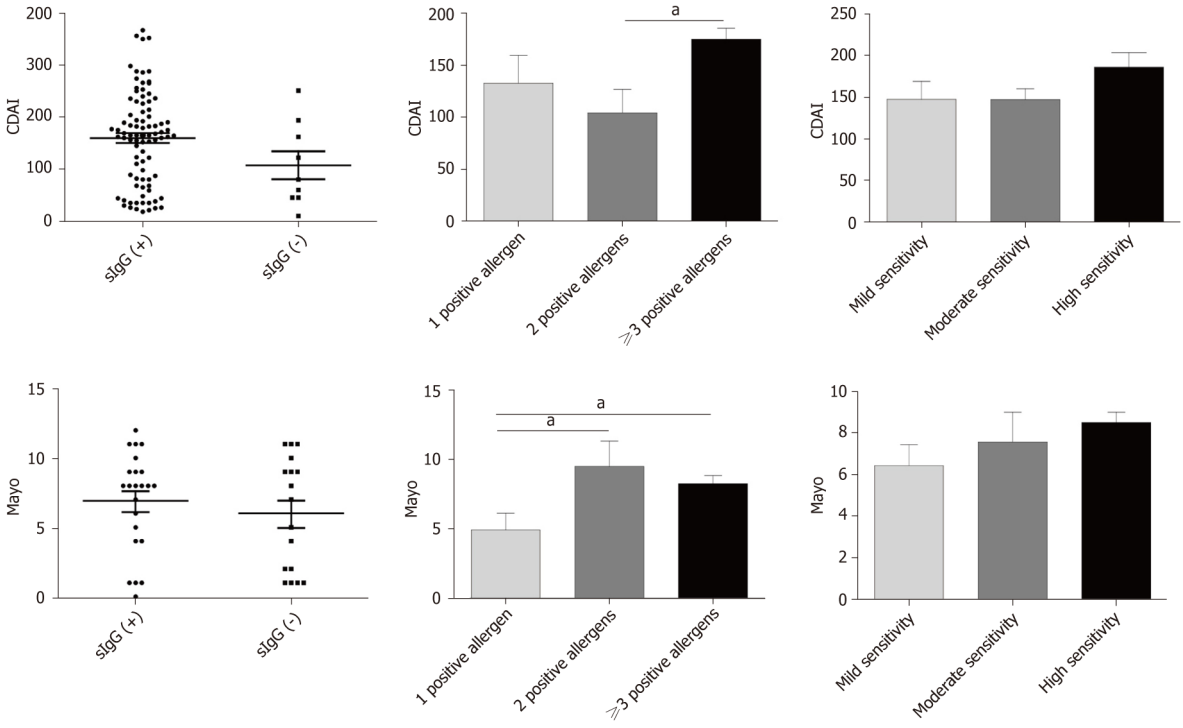

Figure 4 Association between food-specific IgG antibodies and disease activity. Data are shown as mean ± SD by ANOVA, $^{a}P < 0.05$. ; ANOVA: One-way analysis of variance.

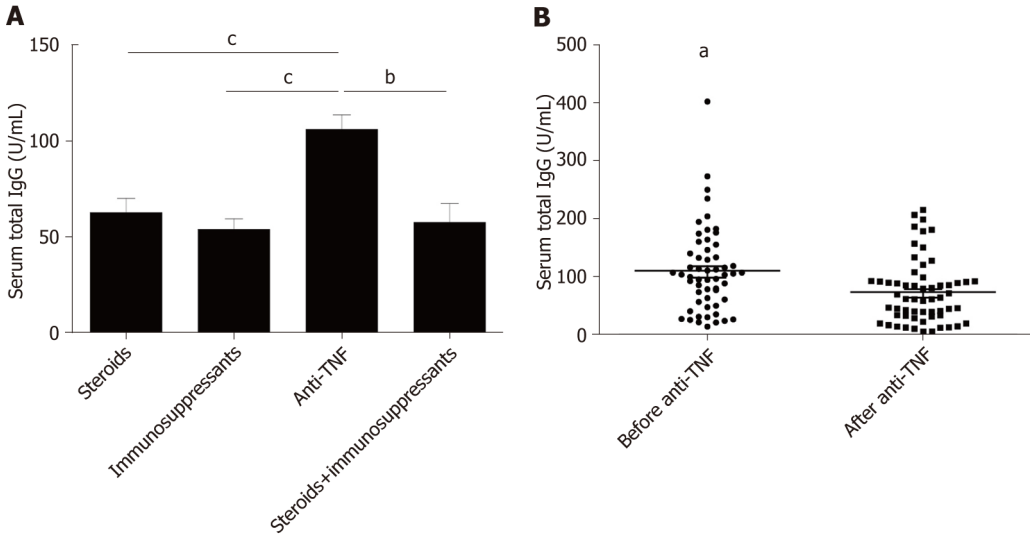

Figure 5 Serum food-specific IgG concentrations in patients treated with different medications and comparison of food-specific IgGs for inflammatory bowel disease patients before and after Infliximab treatment. A: Serum food-specific IgG concentrations in patients treated with different medications. Statistics: ANOVA; B: Comparison of Food-specific IgGs for inflammatory bowel disease patients before and after Infliximab treatment. Statistics: paired-samples *t* test, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. ANOVA: One-way analysis of variance.

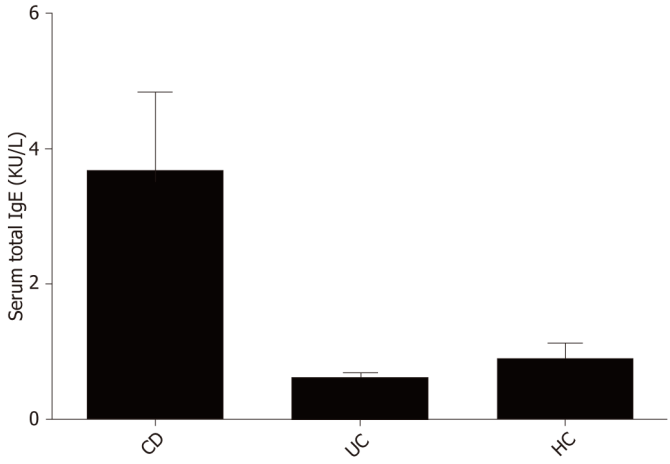

Figure 6 Comparison of total serum IgE levels between CD patients, UC patients and HCs. Statistics: ANOVA. UC: Ulcerative colitis; CD: Crohn's disease; HCs: Healthy controls; ANOVA: One-way analysis of variance.

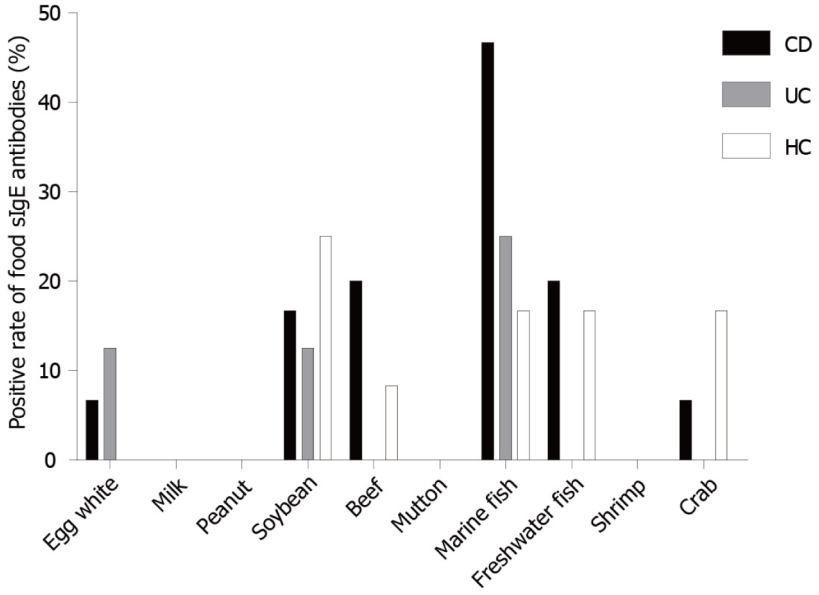

Figure 7 Distribution of positive IgE to food allergens in CD patients, UC patients and HCs. UC: Ulcerative colitis; CD: Crohn's disease; HCs: Healthy controls.

ARTICLE HIGHLIGHTS

Some food antigens have been considered to be involved in the processes of formation and development of human chronic intestinal inflammatory diseases. Food allergy and food intolerance are two types of adverse reactions to food. Food allergy is typically mediated by IgE antibodies. In contrast, food intolerance is mediated by IgG antibodies. However, this mechanism is disputable, as some studies found that food IgG and IgE antibodies can be expressed in healthy individuals. The purpose of this study was to analyze the levels of immunoglobulin G (IgG) and E (IgE) antibodies against food antigens in inflammatory bowel disease (IBD) patients and explore their clinical value in IBD pathogenesis.

Research background

IBD is a chronic relapsing inflammatory disease of the gastrointestinal tract, which includes ulcerative colitis (UC) and Crohn's disease (CD). Increasing evidence indicates that IBD results from an abnormal mucosal immune system triggered by environmental factors. Of these factors, food antigens have been considered to involve in the processes of formation and development of IBD. Food allergy and food intolerance are two types of adverse reaction to food. Food allergy is typically mediated by IgE antibodies. In contrast, food intolerance is mediated by IgG antibodies. However, this mechanism is disputable, as some studies found that food IgG and IgE antibodies can be expressed in healthy individuals.

Research motivation

Food antigens have been suggested to participate in the etiopathogenesis of IBD. The advantages from removing certain foods from daily diet was focused on in recent studies. A number of IBD patients suffer from food intolerances, and they show an improvement of well-being by avoiding specific nutritive components. Previous studies have either researched on the potential involvement of various IgG/IgE subclasses in food intolerance/allergy. Although testing for the presence of food-specific IgG/IgEs has been regarded as a potential tool for the diagnosis of food intolerance/allergy, the accuracy and clinical utility of such testing remain unknown.

Research objectives

The purpose of this study was to analyze the levels of IgG and IgE antibodies against food antigens in IBD patients and explore the clinical value in the pathogenesis of IBD.

Research methods

A total of 137 IBD patients, including 40 patients with UC and 97 patients with CD, and 50 healthy controls (HCs) were enrolled in this study. Blood samples were obtained from patients who visited the First Affiliated Hospital of Nanjing Medical University between August 2016 and January 2018. Serum IgG antibodies to 14 unique food antigens were assessed using enzyme-linked immunosorbent assay. IgE-specific antibodies to food allergens were examined by Western blot.

Research results

CD patients had a higher prevalence of food-specific IgG compared to UC patients. CD patients were more sensitive to tomato, corn, rice, soybean, wheat and codfish, while UC patients were more sensitive to tomato, corn and rice. Significantly higher levels of total food-specific IgG were

detected in IBD patients treated with anti-TNFα therapy compared to patients receiving steroids or immunosuppressants. A decrease in food-specific IgG levels was detected in IBD patients after receiving anti-TNFα therapy. Smokers and CD patients were prone to developing serum food-specific IgG antibodies.

Research conclusions

The prevalence of food-specific IgG is higher in CD patients than in UC patients and HCs. IBD patients may be prone to rice, corn, tomato and soybean intolerance.

Research perspectives

Food-specific IgG antibodies may provide a clinical benefit for IBD patients *via* diet restriction. In the future, the role of food-specific IgG in food intolerance should be further investigated.

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ORIGINAL ARTICLE

Retrospective Study

Incidence of infectious complications is associated with a high mortality in patients with hepatitis B virus-related acute-on-chronic liver failure

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Abstract

BACKGROUND

In China, hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) is the most common liver failure characterized by serious clinical syndromes of liver decompensation with a very high mortality. Bacterial and/or fungal infections are the most common complications that are associated with high short-term mortality. Bacterial translocation from the intestine, impaired hepatic clearance, and immune paralysis of circulating immune cells are thought to contribute to infectious complications in liver failure. The control of bacterial and fungal infections is the key to improving HBV-ACLF outcomes. Active prevention, early diagnosis, and timely treatment of bacterial and fungal infections are essential for treating HBV-ACLF.

AIM

To investigate the frequency and role of bacterial and fungal infections in patients with HBV-ACLF.

METHODS

Patients with HBV-ACLF hospitalized at Taihe Hospital, Hubei University of Medicine from January 2014 to December 2017 were retrospectively enrolled. Patient-related information was retrieved from the hospital case database, including general information, blood biochemistry, complications, *etc*. According to the occurrence of secondary infection or not, the patients were divided into an

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.

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infection group and a non-infection group. The sites, types, and incidences of bacterial and fungal infections and the influence of infections on the prognosis of HBV-ACLF were statistically analyzed. The risk factors for infections were assessed by unconditional logistic regression.

RESULTS

There were 174 cases of HBV-ACLF that met the enrollment criteria, of which 114 (65.52%) were diagnosed with infectious complications. Infections occurred in the abdominal cavity (87 cases), respiratory tract (51 cases), urinary tract (18 cases), and biliary tract (10 cases). Patients with infectious complications had a significantly higher 28-d mortality (70.18%, 80/114) than those without (40.00%, 24/60) (70.18% *vs* 40.00%, $P < 0.05$). And patients with infectious complications had a much higher incidence of non-infectious complications (54.39%, 62/114) (54.39% *vs* 15.00%, $P < 0.05$), leading to an extremely high 28-d mortality of 88.71% (55/62) ($P < 0.05$). The grade of liver failure, period of hospital stay ≥ 30 d, age ≥ 45 years, and percentage of neutrophils > 70% were identified as risk factors for infectious complications.

CONCLUSION

The high incidence of infectious complications in patients with HBV-ACLF is associated with severity and deterioration of the disease and may contribute to the extremely high mortality of these patients.

Key words: Hepatitis B; Acute-on-chronic liver failure; Bacterial infection; Fungal infection; Prognosis

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Core tip: Hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) is the most common type of liver failure with a high mortality and complications. Bacterial and/or fungal infections are the most common complications of liver failure. The aim of this study was to investigate the frequency and role of bacterial and fungal infections in patients with HBV-ACLF. A total of 174 patients with HBV-ACLF were retrospectively analyzed. Patients with infectious complications had a significantly higher mortality (70.18%) than those without (40.00%, 24/60). In conclusion, infections can significantly increase the mortality rate of liver failure.

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INTRODUCTION

Liver failure is a severe form of liver damage caused by a variety of factors, manifested as destroyed liver anatomical structure, function failure, and occurrence of serious clinical syndromes of liver decompensation, with mortality rates as high as 50%-80%[1]. In China, the most common cause of liver failure is hepatitis virus infections, among which hepatitis B virus (HBV) is the most prevalent, and acute-on-chronic liver failure (ACLF) is the major clinical subtype[2]. The mechanism of ACLF is very complex and far from elucidated, although the "three-strike" theory is widely accepted[3]. HBV-related ACLF (HBV-ACLF) is often complicated by hepatic encephalopathy, hepatorenal syndrome, gastrointestinal hemorrhage, and bacterial and/or fungal infections[4], among which bacterial and/or fungal infections are the most common complications of liver failure. The rate of bacterial and fungal infections in liver failure patients can be as high as 81.2%[5]. Bacterial translocation from the intestine, impaired hepatic clearance, and immune paralysis of circulating immune cells are thought to contribute to infectious complications in liver failure[6].

Bacterial or fungal infections and liver failure are mutually causative and

interactive. Infection-induced sepsis is a common cause of ACLF. Due to impaired immunity and hypoproteinemia, patients with middle- or late-stage liver failure are susceptible to bacterial and fungal infections[7]. Thus, bacterial and/or fungal infections are a trigger as well as a complication of liver failure. Since infections play such an important role in the occurrence, development, and prognosis of liver failure, the prevention, early diagnosis, and treatment of infections are indispensable in the management of liver failure. Preventing and controlling bacterial and/or fungal infections is extremely challenging due to the increasing incidence of antibiotic resistance and the diversification of multidrug-resistant bacteria[8].

In this study, 203 patients with HBV-ACLF were retrospectively analyzed. The aim of the study was to investigate the relationship between bacterial and/or fungal infections and prognosis. The characteristics of infection sites, infection types, and factors related to the development of infections in liver failure were also investigated.

MATERIALS AND METHODS

Patients

This retrospective study enrolled HBV-ACLF patients admitted to the Department of Infectious Diseases, Taihe Hospital, Shiyan, China, from January 2014 to December 2017. Diagnostic criteria for chronic hepatitis B were based on the Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update)[9]. ACLF was defined according to Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver 2014[10]. Diagnosis of infections was made in accordance with the CDC definitions for nosocomial infections, 1988[11]. The diagnostic criteria for fungal infection were based on the EORTC/MSG study[12]. Sepsis and septic shock were defined according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[13]. The exclusion of patients was based on the following criteria: (1) Co-infections with hepatitis A virus (HAV), hepatitis C virus (HCV), hepatitis D virus, hepatitis E virus (HEV), or human immunodeficiency virus; and (2) Liver failure complicated by alcoholic, drug-induced, or autoimmune liver diseases. This study met the ethical requirements and was approved by the Ethical Committee of Taihe Hospital.

Data collection

General information and clinical data were collected from the patient database of Taihe Hospital, including (1) Age and gender; (2) Biochemical indicators: G test, GM test, blood lactic acid, serum procalcitonin, C-reactive protein, erythrocyte sedimentation rate, alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl transferase, total bilirubin (TBIL), albumin (ALB), and creatinine; (3) White blood cell (WBC) count, percentage of neutrophils, and platelet count; (4) Coagulation function indicators: prothrombin activity, prothrombin time (PT), standardized international ratio; and (5) Complications of HBV-ACLF: bacterial and fungal infections, hepatic encephalopathy, hepatorenal syndrome, and gastrointestinal hemorrhage. Patients who left the hospital in an extremely critical condition or died were considered as dead.

Definitions related to infections and HBV-ACLF

Spontaneous bacterial peritonitis was defined as polymorphonuclear cell count in ascitic fluid ≥ 250/mm^3, with no other known cause of infection. Infectious diarrhea was consistent with one of the following: acute diarrhea, fecal routine microscopic examination showing the presence of white blood cells (≥ 10/high power field); acute diarrhea, with fever, nausea, vomiting, abdominal pain, *etc*.; acute diarrhea occurring more than three times a day for two consecutive days or more than five times a day; pathogen-related evidence can be detected. Urinary tract infection was defined as abnormal urinary sediment (> 10 leukocytes/field) with positive urine Gram stain or culture in a symptomatic patient. Soft and skin tissue infections were considered when the skin had purulent secretions, pustules, furuncles, *etc*.; the patient had local pain or tenderness, local redness, or fever, and the pathogen was positive. Lower respiratory tract infections were considered when imaging findings suggested that pulmonary infiltrative lesions were associated with at least one respiratory symptoms, such as cough, sputum, dyspnea, and chest pain, with at least one finding on auscultation (rales or crepitations), or one sign of infection (core body temperature > 38 °C or < 36 °C with shivering or leukocyte count > 10000/mm^3 or < 4000/mm^3) in the absence of antibiotic use. Spontaneous bacteremia was defined as positive blood cultures without a known source of infection. Unproven infection was considered when fever and leukocytosis required antibiotic treatment without any identifiable

source[5,8,11].

Diagnostic criteria for fungal infections were the following. Invasive candidiasis was defined as isolation of *Candida* spp in one or more blood cultures (candidaemia) or from normally sterile body fluids. Invasive aspergillosis was defined as detection of *Aspergillus* by direct examination and/or culture of respiratory samples in the presence of radiological imaging compatible with lung infection[12].

Sepsis was defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock was defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities were associated with a greater risk of mortality than with sepsis alone[13].

Score models and ACLF grade

Model for End-Stage Liver Disease (MELD) score, MELD-sodium (MELD-Na) score, integrated MELD (iMELD) score, Child-Turcotte-Pugh (CTP) score, albumin-bilirubin (ALBI) score, and the ACLF grading system were used as described in the previous literature[2,14]. CTP score is the cumulative result of the scores for five items (ascites, hepatic encephalopathy, TBIL, Alb, and PT extension time), with 1-3 points for each item and a maximum of 15 points. The equation for MELD score was as follows: 3.8 LN [TBIL (mg/dL)] + 11.2 LN (INR) + 9.6 LN [Cr [mg/dL)] + 6.4 × cause (0 for cholestatic or alcoholic liver diseases and 1 for all others). The equation for MELD-Na score was as follows: MELD + 1.59 × (135 - Na), wherein Na is 135 mmol/L if Na > 135 mmol/L and 120 mmol/L if Na < 120 mmol/L. The equation for iMELD score is as follows: MELD + (0.3 × Age) - (0.7 × Na) + 100. The equation for ALBI score is as follows: [$\log_{10}$TBIL (μmol/L) × 0.66] + [Alb (g/L) × -0.085].

ACLF grade 1 (ACLF-1) was defined as the presence of renal single organ failure, nervous system failure with renal damage, or other single organ failure with renal/neural damage; ACLF grade 2 (ACLF-2) as the presence of two organ failures; ACLF grade 3 (ACLF-3) as the presence of three organ failures and above[1].

Statistical analysis

Data were analyzed using SPSS 23.0 statistical software (Chicago, IL, United States). Normally distributed variables are expressed the mean ± standard deviation and were compared between two groups using the *t*-test. Non-normally distributed variables are presented as medians (interquartile range), and comparisons between two groups were performed using the Mann-Whitney U test. Count data are described as rates or percentages, and the χ^2 test or Fisher's exact probability test were applied for group comparisons. Unconditional logistic regression was used to analyze infection-related factors. The area under the receiver operating characteristic curve was used to assess the predictive power of the factors for the incidence of infections, and the cut-off value of the continuous variable was calculated. $P < 0.05$ was considered statistically significant.

RESULTS

Patient inclusion

Two hundred and three patients with HBV-ACLF met the inclusion criteria, and 29 patients were excluded based on the exclusion criteria (15 patients with HAV, HCV, or HEV infection; 9 with drug-induced liver disease; 3 with alcoholic liver disease; and 2 with autoimmune liver disease). Thus, 174 patients were finally included, including 114 with infectious complications and 60 without (Figure 1). Almost all infections were nosocomial infections (84.21%, 96/114), and few patients presented infections on admission (15.79%, 18/114).

Baseline characteristics

Among the 174 HBV-ACLF patients, there were 138 males and 36 females, with an average age of 47.44 ± 11.49 years (range, 22-76 years). A total of 181 bacterial or fungal infection events occurred in the 114 patients with infections. The ACLF patients with infectious complications were dominated by female and elderly patients compared with those without infectious complications ($P < 0.05$) (Table 1). All 114 patients with infections met the diagnostic criteria for sepsis, among whom 10 (8.77%, 10/114) presented septic shock.

Sample culture and pathogen identification

A total of 188 samples were collected and cultured, among which 39 were positive. The positive rates for bacterial or fungal culture in ascites, sputum, blood, urine, and throat swabs were 4.76% (3/63), 52.27% (23/44), 7.69% (3/39), 21.05% (4/19), and 50% (5/10), respectively. The 10 stool samples and 2 bone marrow samples were all

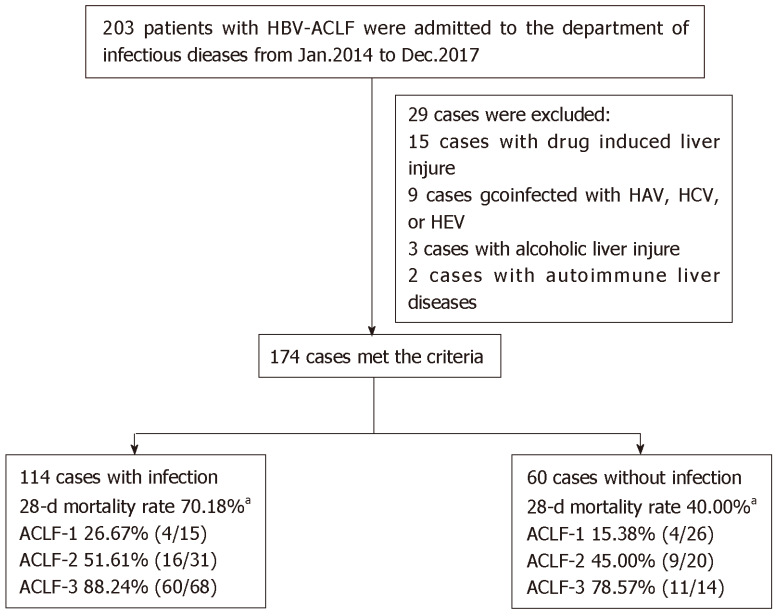

Figure 1 The recruitment and inclusion of patients with hepatitis B virus-related acute-on-chronic liver failure. [a]*P* < 0.05 compared with those without infections. HBV-ACLF: Hepatitis B virus-related acute-on-chronic liver failure; HAV: Hepatitis A virus; HCV: Hepatitis C virus; HEV: Hepatitis E virus; ACLF: Acute-on-chronic liver failure.

negative, while the only secretion sample was positive (Table 2).

A total of 41 strains were isolated from the 188 samples, with 21 bacteria (8 Gram-positive, 13 Gram-negative) and 20 fungi. There were 14 (66.67%, 14/21) strains of *Enterobacter* and *Enterococcus* out of the 21 bacteria, among which *Enterobacter* was the most frequently detected (52.38%, 11/21) and dominant among the Gram-negative bacteria (84.62%, 11/13). Among the 21 bacteria strains, multidrug resistant bacteria were isolated from 11 (52.38%) samples. The 20 fungi identified included 8 yeast-like fungi, 3 *Candida albicans*, 3 *Candida tropicalis*, 4 *Candida parapsilosis*, 1 *Aspergillus fumigatus*, and 1 *Candida lusitaniae* (Table 3).

Infection sites

There were 181 infections among the 114 HBV-ACLF patients with infections, among whom 64 (56.14%) presented one infection site and 50 (43.86%) patients had two or more infection sites. Among the patients with two or more infection sites, 37 (32.46%) were infected at two sites, 9 (7.89%) at three, and 4 (3.51%) at four. The most common infection site was the abdominal cavity (87 cases), followed by the respiratory tract (51 cases), urinary tract (18 cases), biliary tract (10 cases), intestinal tract (4 cases), oral cavity (4 cases), blood (3 cases), skin and soft tissue (3 cases), and venous catheter site (1 case).

HBV-ACLF patients with infectious complications show a high incidence of non-infectious complications

Among the 174 HBV-ACLF patients, 71 (40.8%, 71/174) presented non-infectious complications (hepatic encephalopathy, hepatorenal syndrome, and gastrointestinal hemorrhage), with a total of 58 (58.59%) hepatic encephalopathy cases, 26 (26.26%) hepatorenal syndrome cases, and 15 (15.15%) gastrointestinal hemorrhage cases. There were significantly higher frequencies of non-infectious complications in HBV-ACLF patients with infections than in those without, including hepatic encephalopathy (56.82% *vs* 13.33%, $P < 0.001$), hepatorenal syndrome (27.27% *vs* 18.18%, $P < 0.01$), and gastrointestinal hemorrhage (15.91% *vs* 9.09%, $P < 0.05$) (Table 1).

Risk factors for infectious complications in patients with HBV-ACLF

Among the 174 HBV-ACLF patients, 114 presented infections. Various indexes, such as gender, age, serum HBV DNA load, severity of liver failure, ALT, AST, TBIL, ALB, period of hospital stay, PT, WBC, and neutrophil percentage, were analyzed to determine their roles in infectious complications. The cut-off values for the associated indexes were established by unconditional logistic regression, which showed that age ≥ 45 years, middle- and late-stage of ACLF, AST < 538.5 U/L, ALT < 493.5 U/L, TBIL ≥ 348.35 mol/L, WBC > 10 G/L, neutrophil percentage > 70%, hospital stay ≥ 30 d,

Table 1 Baseline characteristics of hepatitis B virus-related acute-on-chronic liver failure patients with and without infections

	Total (*n* = 174)	Infection (*n* = 114)	No infection (*n* = 60)	$t/\chi^2/z$	*P*-value
Age (yr)	47.44 ± 11.49	49.28 ± 11.32	43.95 ± 11.10	2.972	0.003
Male, *n* (%)	138 (79.31)	82 (71.93)	56 (93.33)	10.975	0.001
Hepatic encephalopathy	25 (14.37)	23 (20.18)	2 (3.33)	9.063	0.002
Hepatorenal syndrome	11 (6.32)	10 (8.77)	1 (1.67)	3.351	0.100
Gastrointestinal hemorrhage	11 (6.32)	10 (8.77)	1 (1.67)	3.351	0.100
ACLF-1	41 (23.56)	15 (13.16)	26 (43.33)	19.874	0.000
ACLF-2	51 (29.31)	31 (27.19)	20 (33.33)	0.715	0.398
ACLF-3	82 (47.13)	68 (59.65)	14 (23.33)	20.806	0.000
INR	2.09 (1.07)	2.215 (1.24)	1.835 (0.695)	-3.193	0.001
ALB (g/L)	32.38 ± 5.31	31.42 ± 4.63	34.197 ± 6.06	-3.110	0.002
Cr (μmol/L)	55.65 (35.425)	57.20 (41.175)	55.00 (25.525)	-1.604	0.109
Na (mmol/L)	138.20 (6.52)	137.25 (7.95)	139.1 (5.325)	-2.752	0.006
WBC (G/L)	5.375 (3.623)	6.32 (4.07)	4.64 (3.085)	-3.655	0.000
NE%	69.85 (16.55)	72.95 (13.875)	63.15 (12.95)	-4.300	0.000
ALT (U/L)	379.50 (842.25)	305.5 (608.75)	793.5 (867.5)	-4.050	0.000
AST (U/L)	260.50 (591)	224.5 (342.5)	599.0 (858.25)	-4.001	0.000
TBIL (μmol/L)	260.16 ± 128.63	277.2 ± 134.7	227.8 ± 110.13	2.599	0.010
PT (S)	23.30 (11.325)	25.20 (14.3)	21.15 (7.15)	-2.956	0.003
MELD	20.58 ± 8.52	22.33 ± 8.92	17.25 ± 6.58	4.267	0.000
MELD-Na	22.51 ± 10.44	25.09 ± 11.14	17.6 ± 6.7	5.525	0.000
iMELD	38.81 ± 10.87	41.74 ± 11.38	33.23 ± 7.05	6.076	0.000
ALBI	-1.205 ± 0.53	-1.103 ± 0.45	-1.399 ± 0.613	3.293	0.001
CTP	11.00 (2)	12.00 (2)	10.00 (2.75)	-4.896	0.000

TBIL: Total bilirubin; PT: Prothrombin time; WBC: White blood cells; NE%: Percentage of neutrophils; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin. INR: Standardized international ratio; Cr: Creatinine; Na: Sodium; CTP: Child-Turcotte-Pugh; MELD: Model for End-Stage Liver Disease; MELD-Na: MELD-sodium; iMELD: Integrated MELD; ALBI: Albumin-bilirubin; ACLF-1: Acute-on-chronic liver failure grade 1; ACLF-2: Acute-on-chronic liver failure grade 2; ACLF-3: Acute-on-chronic liver failure grade 3.

ALB ≤ 33.1 g/L, and PT ≥ 27.55 s were all risk factors for bacterial and/or fungal infections (Figure 2, Table 4).

Infectious complications accelerate the progression of HBV-ACLF

In patients with infections, TBIL was much higher at admission than that in patients without infections, and the elevated TBIL was sustained during the hospitalization without any obvious reduction. TBIL in patients without infections was mildly increased at week 1 of hospitalization and steadily decreased from week 2 to week 4 or at discharge. The difference in TBIL between the above two groups was statistically significant at admission, week 2, and week 4 ($P < 0.05$) (Figure 3A). Similarly, PT in patients with infections was much higher at admission than in patients without infections ($P < 0.05$) and remained at a high-level during hospitalization, while PT in patients without infections gradually decreased. PT differed significantly between patients with and without infections at admission, week 1, week 2, and week 4 ($P < 0.05$, Figure 3B).

Infectious complications lead to extremely critical situations and a high mortality in patients with HBV-ACLF

The scores of MELD, MELD-Na, iMELD, CTP, and ALBI were all much higher for patients with infections than for patients without ($P < 0.01$) (Table 1). Further investigation showed that the higher the scores of MELD, MELD-Na, iMELD, CTP, and ALBI, the higher the incidence of infections ($P < 0.05$) (Table 5). A comparison of HBV-ACLF levels between patients with or without infections yielded the following results: ACLF-1 13.16% *vs* 43.33%, ACLF-2 27.19% *vs* 33.33%, and ACLF-3 59.65% *vs* 23.33%, which implied that ACLF patients with infections presented higher ACLF grades than those without.

There were 104 (59.77%) deaths among the 174 HBV-ACLF patients. The mortality among patients with infections was markedly increased (70.18%, 80/114) compared with those without (40%, 24/60) ($P < 0.05$). Two or more infection sites further raised

Table 2 Positivity rate among 188 cultured samples

	Samples	Positive samples	Positive rate (%)
Ascites	63	3	4.76
Sputum	44	23	52.27
Blood	39	3	7.69
Urine	19	4	21.05
Throat swab	10	5	50
Stool	10	0	0
Bone marrow	2	0	0
Other secretion	1	1	100
Total	188	39	20.74

the mortality compared with one infection site (80% *vs* 62.5%, $P < 0.05$). Among all 174 patients, 71 had non-infectious complications, with a mortality of 88.73% (63/71). Among the 114 patients with infections, 62 had non-infectious complications, with a mortality of 88.71% (55/62), which was remarkably higher than that in infected patients without non-infectious complications (48.09%, 25/52) ($P < 0.05$). The mortality of the 114 sepsis patients was 70.18% (80/114), among whom 10 septic shock patients were all dead (100%).

DISCUSSION

This study showed that among HBV-ACLF patients, the incidences of bacterial and fungal infection and in-hospital 28-d mortality were 65.5% and 59.77%, respectively. The mortality of patients with infections was significantly higher than that of patients without (70.18% *vs* 40%). Infections at multiple sites markedly raised mortality compared with an infection at a single site. The above findings are consistent with previous reports[15-17]. In this study, the most common infection site was the abdominal cavity, followed by the respiratory tract, urinary system, and biliary tract, which is basically in accordance with results from other studies[18-20]. Thus, surveillance for signs of infection in the abdominal cavity, lungs, and urinary system and repeated ascites tests, urine tests, and chest imaging examinations are necessary for early identification of bacterial or fungal infections.

Among the 188 samples, only 20.94% were positive by culture, which implied that it is very hard to characterize the pathogen of infection in HBV-ACLF patients. Thus, the timing of sampling and bedside culture is essential to improve the detection rate. Among the positive samples, Gram-negative bacteria accounted for 61% of all detected bacteria and were mostly *Enterobacter*, which was associated with the bacterial translocation from the intestine[21-23]. Thus, antibiotics with a high sensitivity for *Enterobacter* can be applied for the empiric therapy. The finding that 11 of 21 (52.38%) bacterial strains were multidrug-resistant is noteworthy since multidrug-resistant bacteria are difficult to control and lead to a high mortality. *Candida* was the most frequently isolated fungus among the 20 fungal samples, followed by yeast-like fungi. *Aspergillus fumigatus* was isolated in one sample. The lung lesion of this specific patient progressed rapidly, complicated by the occurrences of respiratory failure and hepatic encephalopathy in the short term, and the patient died after 24 d of hospitalization. According to the related literature[24-26], secondary fungal infection is often induced by "double infection", with nosocomial infection as the most common acquisition type, which leads to an extremely critical situation and high mortality. Risk factors for secondary fungal infection include long-term use of broad-spectrum antibiotics, long hospital stays, use of glucocorticoids, and invasive procedures[27,28]. Immune function failure in the middle and late stages of liver failure is another important factor for secondary fungal infection. Thus, oral care and regular screening for fungi in sputum, throat swabs, urine, and stools are very important for patients with ACLF, and timely intervention should be applied when early signs of fungal infection are evidenced.

The susceptibility of HBV-ACLF patients to bacterial and fungal infections is complex and involves multiple risk factors. Based on the results of this study, age ≥ 45 years, advanced stages of ACLF, AST < 538.5 U/L, ALT < 493.5 U/L, TBIL ≥ 348.35 μmol/L, WBC > 10 G/L, neutrophil percentage > 70%, hospital stay ≥ 30 d, ALB ≤ 33.1 g/L, and PT ≥ 27.55 s were identified as risk factors related to infection,

Table 3 Distribution of bacteria and fungi and source of specimens

	Total	constituent ratio (%)	Source of specimens (*n* = 41)					
			Ascites	Sputum	Blood	Urine	Throat swab	Other secretion
Escherichia coli	3	7.32	2	0	0	1	0	0
Enterococcus faecium	2	4.88	1	0	0	1	0	0
Klebsiella pneumoniae	4	9.76	0	4	0	0	0	0
Acinetobacter baumannii	2	4.88	0	2	0	0	0	0
Enterobacter aerogenes	3	7.32	0	2	0	0	1	0
Staphylococcus aureus	2	4.88	0	1	1	0	0	0
Klebsiella ozaenae	1	2.44	0	1	0	0	0	0
Enterococcus faecalis	1	2.44	0	0	1	0	0	0
Streptococcus haemolyticus	1	2.44	0	0	1	0	0	0
Streptococcus agalactiae	1	2.44	0	0	0	1	0	0
Staphylococcus haemolyticus	1	2.44	0	0	0	0	0	1
yeast-like fungi	8	19.51	0	6	0	1	1	0
Candida	11	26.83	0	8	0	0	3	0
Aspergillus fumigatus	1	2.44	0	1	0	0	0	0

consistent with previous reports[29,30]. Other risk factors mentioned in the literature include invasive procedures, use of antibacterial drugs, and comorbid illness (*e.g.*, diabetes)[31,32]. In this study, the incidence of non-infectious complications as well as mortality were significantly increased in ACLF patients with infections compared with those without, indicating that effective control of bacterial and fungal infections may improve the outcomes of HBV-ACLF.

Various score models have been proposed to predict the prognosis of liver failure[14,33]. In this study, MELD, MELD-Na, iMELD, CTP, and ALBI scores were all markedly higher in HBV-ACLF patients with infections than in their counterparts without infections. Increasing scores were correlated with increasing infection incidence and high mortality, providing more evidence that bacterial and fungal infections will accelerate the progression of HBV-ACLF and lead to a poor prognosis.

The levels of TBIL and PT were mildly decreased in HBV-ACLF patients with infections at week 1, supposedly due to the comprehensive measures implemented, such as an artificial liver support system[34,35] and antiviral treatment. The levels of TBIL and PT remained high or even increased thereafter until week 4. By contrast, in the patients without infections, TBIL and PT were mildly elevated at the first week. Most of these patients were in the early stage of ACLF when admitted, and the elevation of TBIL and PT reflected the progression of ACLF. After that, the levels of TBIL and PT steadily declined. These results support the notion that bacterial or fungal infections will significantly prolong the hospital stay.

In summary, HBV-ACLF patients are susceptible to bacterial and fungal infections characterized by multiplicity of infection sites and diversity of pathogens, dominated by the Gram-negative bacillus *Enterobacter*. Once complicated with bacterial and/or fungal infections, HBV-ACLF patients have a high incidence of non-infectious complications, resulting in an advanced grade of ACLF and high mortality. Thus, the control of bacterial and fungal infections is pivotal for improving the outcomes of HBV-ACLF, and active prevention, early diagnosis, and timely treatment of bacterial and fungal infections are indispensable for the treatment of HBV-ACLF. As a retrospective single-center study with a relatively small sample size, the profiling of bacterial and fungal infections might be incomplete, and there may be bias in the frequencies of infectious complications and related mortality of HBV-ACLF. A prospective, multi-center, cohort study is needed to further characterize bacterial and fungal infections in HBV-ACLF patients.

Table 4 Risk factors for the development of bacterial and/or fungal infections

		Total	Secondary infectionn (%)	χ^2	OR (95%CI)	*P*-value
Gender	Female	36	32 (88.89)	9.263	5.46 (1.83, 16.31)	0.002
	Male	138	82 (59.42)			
Age (yr)	≥ 45	107	80 (74.77)	10.214	2.88 (1.51, 5.49)	0.001
	< 45	67	34 (50.75)			
HBVDNA (IU/mL)	$< 4.575 \times 10^5$	79	59 (74.68)	6.281	2.32 (1.20, 4.46)	0.012
	$\geq 4.575 \times 10^5$	95	55 (57.89)			
Stage of liver failure	Middle and late	131	102 (77.9)	30.459	9.09 (4.15, 19.89)	0.000
	Early	43	12 (27.91)			
ACLF grade	ACLF-1	41	15 (36.59)	18.117	5.05 (2.40, 10.64)	0.000
	ACLF-(2, 3)	133	99 (74.44)			
ALT (U/L)	< 493.5	97	75 (78.95)	15.273	3.71 (1.92, 7.17)	0.000
	≥ 493.5	77	39 (50.65)			
AST (U/L)	<538.5	120	92 (76.67)	19.847	4.78 (2.40, 9.51)	0.000
	≥ 538.5	54	22 (40.74)			
TBIL (μmol/mL)	≥ 348.35	40	34 (85)	7.925	3.83 (1.50, 9.73)	0.005
	< 348.35	134	80 (59.7)			
WBC (G/L)	≥ 10	24	24 (100)	7.099	15.73 (2.07, 119.45)	0.008
	< 10	150	90 (60)			
NE%	> 70	86	71 (82.56)	20.283	4.95 (2.47, 9.94)	0.000
	≤ 70	88	43 (48.86)			
Hospital stay (d)	≥ 30	48	40 (83.33)	9.312	1.45 (0.66, 3.17)	0.003
	< 30	126	74 (58.73)			
ALB (g/L)	≤ 33.1	101	76 (75.25)	9.812	2.80 (1.47, 5.33)	0.002
	> 33.1	73	38 (52.05)			
PT (s)	≥ 27.55	58	48 (82.76)	10.685	3.64 (1.68, 7.89)	0.001
	< 27.55	116	66 (56.89)			

TBIL: Total bilirubin; PT: Prothrombin time; WBC: White blood cells; NE%: Percentage of neutrophils; ALT: Alanine aminotransferase; ALB: Albumin. AST: Aspartate aminotransferase; ACLF grade: Acute-on-chronic liver failure grade; ACLF-1: Acute-on-chronic liver failure grade 1; ACLF-(2, 3): Acute-on-chronic liver failure grades 2 and 3.

Table 5 Correlation of grades of acute-on-chronic liver failure and liver failure scores with bacterial and/or fungal infections

	Total, *n*	Infection, *n*(%)	No infection *n* (%)	Corelation coefficient	Sig	χ^2	*P*-value
ACLF grade	174	114	60	0.365	0.000	26.697	0.000
ACLF-1	41	15 (36.59)	26 (63.41)				
ACLF-2	51	31 (60.78)	20 (39.22)				
ACLF-3	82	68 (82.93)	14 (17.07)				
MELD	174	114	60	0.285	0.001	15.421	0.001
< 20	87	46 (52.87)	41 (47.13)				
20-29	62	45 (72.58)	17 (27.42)				
30-39	21	19 (90.48)	2 (9.52)				
≥ 40	4	4 (100)	0				
MELD-Na	174	114	60	0.294	0.001	16.475	0.001
< 25	121	69 (57.02)	52 (42.98)				
25-34	31	23 (74.19)	8 (25.81)				
35-44	17	17 (100)	0				
≥ 45	5	5 (100)	0				
iMELD	174	114	60	0.368	0.000	27.181	0.000
< 30	32	15 (46.88)	17 (53.12)				
30-39	74	39 (52.7)	35 (47.3)				
40-49	41	34 (82.93)	7 (17.07)				

50-59	20	19 (95)	1 (5)				
≥ 60	7	7 (100)	0				
ALBI	174	114	60	0.215	0.015	8.454	0.015
≤ -2.60	4	1 (25)	3 (75)				
-2.60~-1.39	49	26 (53.06)	23 (46.94)				
> -1.39	121	87 (71.9)	34 (28.1)				
CTP	174	114	60	0.257	0.000	12.301	0.000
5-9	33	13 (39.4)	20 (60.6)				
≥ 10	141	101 (71.63)	40 (28.37)				

CTP: Child-Turcotte-Pugh; MELD: Model for End-Stage Liver Disease; MELD-Na: MELD-sodium; iMELD: Integrated MELD; ALBI: Albumin-bilirubin; ACLF grade: Acute-on-chronic liver failure grade; ACLF-1: Acute-on-chronic liver failure grade 1; ACLF-2: Acute-on-chronic liver failure grade 2; ACLF-3: Acute-on-chronic liver failure grade 3.

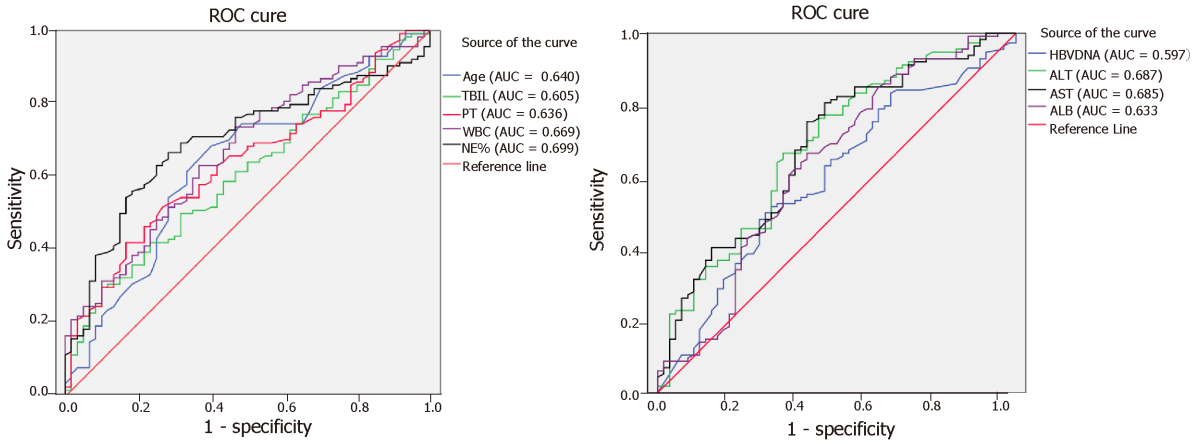

Figure 2 Receiver operating characteristic curve for assessing risk factors for bacterial and/or fungal infections in patients with hepatitis B virus-related acute-on-chronic liver failure. ROC: Receiver operating characteristic; HBV-ACLF: Hepatitis B virus-related acute-on-chronic liver failure; TBIL: Total bilirubin; PT: Prothrombin time; WBC: White blood cell; NE%: Percentage of neutrophils; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin.

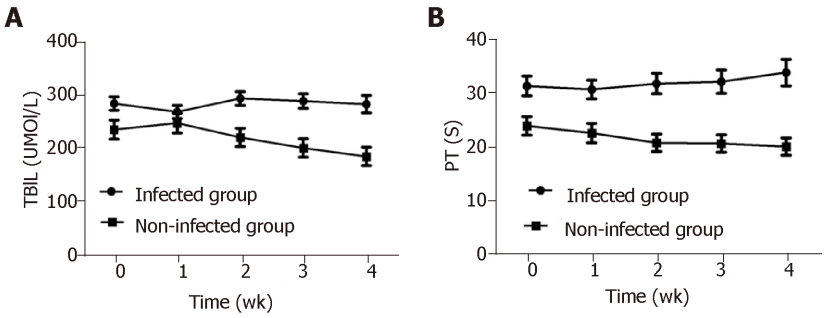

Figure 3 Kinetics of serum total bilirubin and prothrombin time in hepatitis B virus-related acute-on-chronic liver failure patients during hospital stay. A and B: Serum total bilirubin (A) and prothrombin time (B) in hepatitis B virus-related acute-on-chronic liver failure patients at admission, week 1, week 2, and week 4. TBIL: Total bilirubin; PT: Prothrombin time.

ARTICLE HIGHLIGHTS

Research background

Bacterial and/or fungal infections are a trigger as well as a complication of liver failure, since patients with middle- or late-stage liver failure are susceptible to bacterial and fungal infections, and infection-induced sepsis is a common cause of acute-on-chronic liver failure (ACLF), while the risk factors which predispose to infections are not clear.

Research motivation
Infections are important causes of mortality in liver failure. However, the type of infection, the site of infections, predictors of infection, and their impact on outcomes in patients with hepatitis B virus-related ACLF (HBV-ACLF) are not fully elucidated. Establishing a model for predicting secondary infections in liver failure may be vital for clinical management of HBV-ACLF.

Research objectives
To investigate the influence of secondary infections on the progression of the disease and the related factors of secondary infections in patients with HBV-ACLF, and to elucidate the relationship between the infections in HBV-ACLF and the prognosis of the disease.

Research methods
Patients with HBV-ACLF at Taihe Hospital of Hubei University of Medicine from January 2014 to December 2017 were retrospectively enrolled. General information and clinical data were collected from the patient database of Taihe Hospital. The infection sites, complications, infection types, and infection rate and the influence of infections on the prognosis of HBV-ACLF were analyzed. SPSS23.0 software was used for statistical analyses. Unconditional logistic regression was used to analyze infection-related factors. The area under the receiver operating characteristic curve was used to assess the predictive power of the factors for the incidence of infections.

Research results
HBV-ACLF was susceptible to secondary infections, which were characterized by multiple sites and multiple strains. The pathogens of bacterial infection were mostly from *Enterobacter*, and the detection rate of pathogens was low. Patients with infectious complications had a significantly higher 28-d mortality (70.18%) than those without (40.00%, 24/60), and patients with infectious complications had a much higher incidence of non-infectious complications (54.39%, 62/114), leading to an extremely high mortality of 88.71% (55/62). The grade of liver failure, period of hospital stay ≥ 30 d, age ≥ 45 years, and percentage of neutrophils > 70% were identified as risk factors for infection complications.

Research conclusions
The high incidence of infection complications in patients with HBV-ACLF is associated with the severity and deterioration of the disease and may contribute to the extremely high mortality of these patients. Prevention of the occurrence of infections and early diagnosis and timely treatment of infections are indispensable for the treatment of HBV-ACLF.

Research perspectives
As a retrospective study, there are limitations like relatively small number of cases and imperfect follow-up data. Especially, the long-term survival rate and related biochemical indicators are not well tracked. In the future, prospective, multi-center, large-sample cohort studies are needed.

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ORIGINAL ARTICLE

Clinical Trials Study

R/S ratio in lead II, and the prognostic significance of red cell distribution width in acute coronary syndrome

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Clinical trial registration statement: This registration policy applies to prospective, randomized, controlled trials only.

Informed consent statement: Written informed consent was not necessary because the study was performed retrospectively by screening patient files.

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Abstract

BACKGROUND

In spite of developing medical technologies to discover the etiopathogenesis of diseases and developments in the treatment of coronary artery disease, acute coronary syndromes (ACS) continue to be the main cause of mortality and morbidity worldwide. New cardiac biomarkers and techniques are needed to help provide rapid diagnosis in order to evaluate risk in coronary artery patients.

AIM

To evaluate the effects of R to S ratio (RSR) in the electrocardiograph of patients with ACS, from the point of the arising complication after myocardial infarction (MI), to three-vessel disease (TVD) and mortality.

METHODS

The data of 1,296 patients with ACS, who presented to the emergency department of our hospital with chest pain between January 2014 and December 2018 and were admitted to the cardiology clinic, were retrospectively included in this cross-sectional cohort study. Patients with an RSR value less than I were assigned to group I, while those with an RSR value greater than I were assigned to group II.

RESULTS

In our study, 466 (35.9%) of the 1,296 patients, 357 (38.3%) in group 1 and 109 (29.9%) in group 2, were female, with a mean age of 61.56 ± 9.42. ST-elevation MI 573 (44.2%), unstable angina (UA) 502 (38.7%) and non ST-elevation MI 220 (17%) were more prevalent in group I. Acute anterior MI 263 (20.3) in group I, and acute inferior MI 184 (14.2) in group II was higher. Ischemic heart failure was the most common complication. In group II, the red cell distribution width (RDW) was 15.42 ± 1.82, the gensini score was 48.39 ± 36.44, the left ventricular ejection

revised according to the CONSORT 2010 Statement.

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fraction was 41.17 ± 10.41, the TVD was 111 (8.5), and the mortality rate was 72 (5.6), which was significantly higher than group I RDW; in MI with ST and non-ST-elevation, in TVD, mortality and complications were high and low in UA. In single and multivariate regression analyses, the variables were associated with ACS risk.

CONCLUSION
RSR levels may be an auxiliary predictive value in ACS in terms of complications developing after MI, TVD, and mortality.

Key words: Acute coronary syndrome; Emergency department; R/S ratio; Red cell distribution width

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Core tip: This study was obtained from the data of 1,296 patients with acute coronary syndrome who presented to the emergency department with chest pain between January 2014 and December 2018. In the R to S ratio (RSR) > 1 group, the left ventricular ejection fraction was lower, while the gensini scores and troponin values in the 0, 6th, and 12nd hours were significantly higher. The RDW value was high in the group with RSR < 1. In the group with RSR > 1, complications that occur after anterior myocardial infarction, three-vessel disease and mortality were high, and the prognosis was worse. The most common acute anterior myocardial infarction was observed.

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INTRODUCTION

Coronary artery disease (CAD) includes a group of diseases that can have reduced incidence when risk factors are controlled. CAD is the leading cause of death in men and women. CAD increases with age, and affects people in their most productive years[1]. CAD may lead to death from a clinically unclear disease. For this reason, early diagnosis and rapid planning of treatment are of great importance in CAD. Each 30-min delay between the onset of symptoms and reperfusion therapy increases mortality[2]. Percutaneous coronary intervention is a method of revascularization that can be applied in patients with acute coronary syndrome (ACS). It has a positive effect on mortality reduction in high-risk patients[3].

While the electrical axis is calculated on a healthy human electrocardiogram (ECG), the left ventricle is three times larger than the right ventricle. A depolarization waveform that mainly moves backward and to the left is seen on the electrocardiography tracing, and large R waves consequently take shape in leads I, II, III and aVF[4]. Right ventricular mass is more dominant than left ventricular mass in the right heart growth. Due to the right ventricle being more dominant, the depolarization wave moves forward and to the right in the ECG tracing. As a result, deep S waves are observed in leads I, II, III and aVF[5]. Additionally, the average electrical axis can be changed in the intraventricular conduction system blocks[6].

There are many studies that can give early warning for CAD, with the exception of cardiac troponins (cTn). One of them is the erythrocyte distribution width (RDW), and a measure of the heterogeneity of circulating erythrocytes. There is a strong relationship between CAD and high RDW, but the underlying mechanism is still unclear. In the studies conducted, the neurohumoral system is activated in people with CAD and heart failure. As the mediators increase in circulation, the erythropoiesis process is accelerated, and the RDW is eventually increased[7-9].

In previous studies, we have investigated the relationship between ACS, RDW and cTns, but we have not been able to detect a study based on lead II derivation in the literature according to the R to S ratio (RSR) > 1 and RSR < 1. In our study, we aimed to evaluate ST-elevation myocardial infarction (STEMI) subgroups [acute inferior myocardial infarction (AIMI), acute anterior myocardial infarction (AAMI)], non-

STEMI (NSTEMI) and high-risk unstable angina (UA) groups; in terms of RSR, cTn I, complications following acute myocardial infarction (AMI), three-vessel disease (TVD) and mortality.

MATERIALS AND METHODS

Study design and population

We retrospectively obtained the results of 1296 patients (466 females, 830 males, mean age 61.56 ± 9.42 years; distribution 26-82 years) who presented to the emergency department (ED) between January 2014 and December 2018 for chest pain, and were admitted to the Cardiology Clinic with the pre-diagnosis of ACS. The patients excluded from the study included those whose biochemistry and hemograms were not studied, 12-lead ECG was not performed during the ED, angiographies and echocardiographies were not performed after hospitalization, and in whom sepsis and septic shock, pulmonary thromboembolism, pericarditis and myocarditis, blunt chest traumas, all malignancy types, chronic kidney failure, cerebrovascular diseases, toxic hepatitis, chronic liver diseases, and cTn were not examined.

The patients were high-risk UA patients according to the AIMI, AAMI, and NSTEMI group, which are the subgroups of STEMI, and the Braunwald classification[10]. AIMI, inferolateral MI, inferoposterior MI, posterior MI, and right ventricular MI were evaluated in the AIMI subgroup; septal MI, anterior MI, lateral MI, high lateral MI, and diffuse anterior wall MI were evaluated in the AAMI subgroup. The patients were referred to group I for RSRs less than one, and to group II for RSRs greater than one in the lead II. These groups were compared in terms of age, gender, cTn I, TVD, gensini score, and mortality. cTn Is were recorded as troponin I, II, III by being repeated at the 0^{th}, 6^{th}, and 12^{th} h after admission to the ED.

The patients who had chest pain and/or discomfort lasting at least 30 min, and ECG with STEMI according to 2013 ACCF/AHA guidelines, were included in the study[11]. UA/NSTEMI is defined according to the criteria of the 2014 AHA/ACC Guideline for the Management of Patients With NSTE-ACS. All of the patients were checked with Transthoracic Echocardiography (TTE) to look for whether focal wall motion abnormalities were present. A Philips Epiq 7 Ultrasound Machine was used for TTE in this study.

The demographic, clinical, and laboratory data from the date of presenting to the ED due to ACS, including the RSR and cTn I levels, were assessed by reviewing the hospital's medical records.

Hemogram was measured using a Beckman Coulter Automated CBC Analyzer (Beckman Coulter, Inc., Fullerton, CA, United States).

Blood was analysed with the Cobas 6000 (C6000-Core, Cobas c-501 series, Hitachi, Roche, United States). The hemogram and biochemistry results were studied between 45-60 min.

Cardiac biomarker analysis

Venous blood samples from the antecubital veins of patients were obtained to measure the serum levels of cTn I. cTn I, STAT Elecsys and Cobas e 411 Hitachi Roche analysers were used to measure cTn I levels. The cTn I levels of patients were measured at the 0^{th}, 6^{th} and 12^{th} h.

Electrocardiography

Twelve-lead ECG was performed at the bedside with Cardiofax ECG-9132K (Nihon Kohden, Tokyo, Japan) when the patient was admitted to the ED.

Angiographic analysis

Angiographic evaluations were performed by two experienced cardiologists who were blinded to the study. Discrepancies were solved by consensus. The extent and severity of CAD were assessed by the gensini score[12].

Gensini scoring system

The gensini score was calculated by multiplying the severity coefficient, which was assigned to each coronary stenosis according to the degree of luminal narrowing (reductions of 25%, 50%, 75% 90%, 99%, and complete occlusion were given gensini scores of 1, 2, 4, 8, 16, and 32, respectively), by the coefficient identified based on the functional importance of the myocardial area supplied by that segment: The left main coronary artery, 5; the proximal segment of the left anterior descending coronary artery, 2.5; the mid segment of the left anterior descending coronary artery, 1.5; the apical segment of the left anterior descending coronary artery, 1; the first diagonal branch, 1; the second diagonal branch, 0.5; the proximal segment of the circumflex

artery, 2.5 (if right coronary artery dominancy exists, 3.5); the distal segment of the circumflex artery, 1 (if dominant, 2); the obtuse marginal branch, 1; the posterolateral branch, 0.5; the proximal segment of the right coronary artery, 1; the mid segment of the right coronary artery, 1; the distal segment of the right coronary artery, 1; and the posterior descending artery, 1[12].

All patients were given written informed consent, and the study was approved by the Ethics Committee of the Cumhuriyet University, Faculty of Medicine.

The study was conducted by following the Declaration of Helsinki for Human Research, and was approved by the institutional review board.

Statistical analysis

The data obtained from this study were analysed using the SPSS 15.0 (SPSS, Inc, Chicago, IL) software package. The Shapiro-Wilk's test was used while analysing the normal distribution of the variables based on their unit numbers. While analysing the differences between groups, the independent samples *t*-test was used for the normally distributed variables, while the Mann Whitney *U* and Kruskal Wallis-H tests were used for the non-normally distributed variables. In the case of significant differences in the Kruskal Wallis-H test, the groups with differences were determined by using the Post-Hoc Multiple Comparison Test. The χ^2 analysis was carried out while analysing the correlations between the groups of nominal variables. The Fisher's Exact Test was used when the expected values in the cells of the 2 × 2 tables did not have sufficient volume, and Spearman correlation analysis was carried out in the R × C tables with the help of Monte Carlo Simulation. We used univariate analysis to quantify the association of variables with the development of RSR. The variables found to be statistically significant in the univariate analysis were used in a multivariate Cox proportional hazards model with forwarding stepwise method to determine the independent prognostic factor for the development of RSR. In addition, the Friedman's two-way ANOVA test was used for the data with regard to the time difference between groups. A significance level of 0.05 was used while interpreting the results; *P* values less than 0.05 were considered as statistically significant.

RESULTS

The clinical and demographic characteristics of the patients are listed in (Table 1).

In the chi-square analysis with the variables according to the RSR groups of ACS, UA, NSTEMI, and STEMI were more common in group I for both males and females. AIMI was more common in group II, and AAMI was more common in group I. However, TVD and mortality were more common and statistically significant in group II ($P < 0.05$, Table 2).

In the chi-square analysis of ACS groups according to the variables, ischemic heart failure, ventricular tachycardia, and acute pulmonary oedema were more common in AAMI. Atrioventricular block was more common in AIMI. TVD and mortality were observed to be more common in AAMI ($P < 0.05$, Table 3).

In the chi-square analysis of ACS, according to the blocked major coronary artery in terms of complications, ischemic heart failure and ventricular tachycardia were most commonly detected in the left anterior descending (LAD = L2), atrioventricular block was most commonly detected in the right coronary artery, pericardial effusion and cardiac tamponade were most commonly detected in the LAD, and acute pulmonary oedema was most commonly detected in the circumflex artery. TVD and mortality were most commonly found in the LAD ($P < 0.05$, Table 4). RDW; STEMI, NSTEMI, TVD, post-AMI complications and group I were high.

The univariate and multivariate Cox regression analysis of the variables between the RSR groups were statistically significant ($P < 0.05$, Table 5).

DISCUSSION

In the literature, Davies *et al*[13] 1959 and Evans *et al*[14] 1966 looked to the mortality and prognosis effects of S on leads II and III. Bär *et al*[15] tried to determine the prognosis of RSR on leads V1 and V2 in 1984. However, we have not been able to detect a study with RSR in the literature based on lead II derivation. On applications for the Emergency Service Department, we aimed to correlate the RSR groups on lead II derivation with TVD, mortality and the complications that may occur after ACS.

Lead II derivation includes both blocks and non-block R and S waves. Also, lead II derivation is the potential difference between right arm and left leg that is showed on ECG. Lead II is equal to the total voltage of leads I and III. For this reason, it has the

Table 1 Baseline characteristics of acute coronary syndrome study patients

	All patients	Patients with		z	*P*-value
		RSR > 1	RSR < 1		
Baseline characteristics					
Age, mean ± SD, yr	61.56 ± 9.42	60.9 ± 11.36	62.22 ± 10.40	-0.706	0.001
Sex, Male/Female	830/466	255/109	575/357		0.002
Laboratory finding					
TG, mg/dL	141.69 ± 81.30	139.30 ± 75.97	143.71 ± 85.52	-0.195	0.232
CHOL, mg/dL	173.71 ± 72.40	173.00 ± 70.72	174.30 ± 74.18	-0.156	0.286
HDL, mg/dL	34.44 ± 9.13	34.14 ± 9.23	34.44 ± 9.14	-0.057	0.398
LDL, mg/dL	108.75 ± 50.88	108.97 ± 50.91	108.56 ± 50.87	-0.404	0.065
LVEF, %	52.29 ± 14.27	41.17 ± 10.41	54.30 ± 11.48	-6.704	0.001
GS	37.88 ± 36.7	48.39 ± 36.44	29.74 ± 37.56	-7.549	0.001
cTn 1, ng/mL	1.86 ± 3.92	1.19 ± 1.84	0.42 ± 0.81	-7.148	0.001
cTn 2	5.02 ± 4.61	5.92 ± 6.12	2.43 ± 3.81	-8.101	0.001
cTn 3	11.81 ± 12.31	9.49 ± 12.74	4.57 ± 7.72	-7.361	0.001
BS, mg/dL	137.31 ± 60.49	136.94 ± 64.19	135.02 ± 63.44	-0.718	0.526
WBC, 10^3/μL	10.17 ± 3.31	9.89 ± 3.25	10.64 ± 3.82	-0.382	0.247
MCV, fL	88.23 ± 7.32	88.92 ± 7.42	88.48 ± 7.60	-0.249	0.642
MCH, pg	29.31 ± 2.31	29.43 ± 2.41	29.62 ± 2.38	-0.262	0.342
MCHC, g/dL	32.92 ± 0.81	32.96 ± 0.79	32.63 ± 0.72	-0.593	0.329
RDW, %	14.48 ± 2.12	15.42 ± 1.82	14.92 ± 2.48	-7.254	0.001
MPV, fL	8.53 ± 1.36	10.48 ± 3.47	8.96 ± 2.24	-2.823	0.019
Neu, %	6.25 ± 3.56	7.42 ± 1.44	6.82 ± 2.31	-14.762	0.001
Lymph, %	2.13 ± 1.12	2.32 ± 1.21	2.02 ± 1.19	-0.667	0.422

TG: Triglycerides; CHOL: Cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; RSR: R to S ratio; LVEF: Left ventricular ejection fraction; GS: Gensini score; cTn: Troponin; BS: Blood sugar; WBC: White blood cell; MCV; Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; MPV: Mean platelet volume; Neu: Neutrophil; Lymph: Lymphocyte. $P < 0.05$.

feature of being the clearest seen derivation on all ECG waves. It is used to evaluate atrial and ventricular hypertrophies in the diagnosis of cardiac rhythm and pathology. It shows lower wall ischemia and possible circumflex artery lesions in coronary ischemic pathologies. For these reasons, we preferred lead II derivation in our study.

In patients with ACS, it is possible to identify patients who are at risk with serial troponin measurements. It has been shown that the risk of cardiac complications has a high prognostic value in patients with high levels of cardiac troponin. cTns have an important role in the diagnosis, prognosis and treatment of ACS. CTn is important in separating UA from NSTEMI, and verifying the diagnosis. High cTn I values are important in the diagnosis of UA for patients with ACS who have cardiac risk and whose CK-MB levels are normal[16-18]. In the TIMI 18 study, patients with a cTn I level higher than the 99th percentile had a three-fold higher risk of having a MI or erythrocyte MI than those with a cTn I level of < 0.1 ng/mL[19]. James *et al*[20] reported that in 7,115 patients diagnosed with NSTEMI-ACS, the risk of mortality was low in patients with cTn-T levels < 0.01 μg /L.

In our study, cTn I values in groups 2, 6, and 12 were significantly higher than in group 1. The number of cases was low, but the gensini score indicating the prevalence of coronary artery was high. As a result, three vessel disease was common, and the left ventricular ejection fraction was low. As a result, in group II, high cTn I, post-AMI complications, TVD, prognosis and mortality were higher. The values in group II were at the highest level in the AIMI and AAMI subgroups, but lower in the NSTEMI group, and less in UA with less inflammation. In group I, three vessel disease was low, and the result was a low gensini score, and thus the left ventricular ejection fraction was high. Patients' clinical, prognosis and mortality were better than group II. These variables were evaluated in univariate regression analyses, and it was found to be significant after multivariate regression analyses. In addition, cTnI, AMI postoperative complications, TVD and mortality correlations were positively

Table 2 χ^2 analysis of acute coronary syndrome according to R to S ratio variables

		Patients with		χ^2	*P*-value
		RSR > 1, *n* (%)	RSR < 1, *n* (%)		
Sex, Male/Female		255/109 (19.7/8.4)	575/357 (44.4/27.5)	6.6	0.001
Diagnosis	UA	78 (6.9)	424 (32.7)	284.12	0.001
	AIMI	184 (14.2)	65 (5.0)		
	AAMI	61 (4.7)	263 (20.3)		
	NSTEMI	40 (3.1)	180 (13.9)		
Complication	No	114 (8.9)	524 (40.5)	89.23	0.001
	IHF	151 (11.7)	251 (19.4)		
	VT	16 (1.2)	66 (5.1)		
	AV Block	43 (3.1)	21 (1.7)		
	PE/CT	9 (0.7)	18 (1.3)		
	APE	37 (2.5)	46 (3.9)		
Tree-vessel Disease	No	253 (19.6)	733 (56.5)	10.03	0.001
	Yes	111 (8.5)	199 (15.3)		
Mortality	No	292 (22.5)	845 (65.2)	24.39	0.001
	Yes	72 (5.6)	87 (6.7)		

UA: Unstable angina; AIMI: Acute inferior myocardial infarction; AAMI: Acute anterior myocardial infarction; NSTEMI: Non-ST elevation MI; IHF: Ischemic heart failure; VT: Ventricular tachycardia; AV: Atrioventricular; PE: Pericardial effusion; CT: Cardiac tamponade; APE: Acute pulmonary oedema. $P < 0.05$.

correlated. It is important to determine low troponin values and small increases in these levels accurately and consistently. We are of the opinion that it is extremely useful to determine the critical threshold level for follow-up and treatment in the diagnosis and prognosis of patients with ACS.

Although it is known that there is a relationship between CAD and RDW, the underlying pathophysiological mechanism is still unclear. The first of the pathophysiological mechanisms suggested that inflammation, which has an important role in the atherosclerotic process, causes the release of cytokines into the circulation and increases RDW levels[21-23]. Secondly, mediators resulting from increased neurohumoral activity during ACS stimulate erythropoiesis and increase RDW levels[24]. They found that RDW is an independent risk factor for hospital and long-term mortality in patients with ACS[25-27]. Warwick *et al*[27] also emphasized that RDW is an important risk in long-term mortality in patients undergoing coronary artery bypass surgery. In their study, Lippi *et al*[28] suggested that high RDW should be used together with cardiac markers in patients admitted to the ED. Tenekecioglu *et al*[29] found that RDW was higher in patients diagnosed with NSTEMI than patients diagnosed with UA. They showed that RDW showed a positive correlation with cTn I. Therefore, they suggested that RDW could be a useful parameter in the ED.

In our study, we found a close relationship between ACS and RDW. We found that high RDW values may be an independent marker of ACS with positive troponin. RDW can be used both as a guide in the diagnosis of ACS and in prognosis. In addition, RDW was associated with morbidity and mortality in acute heart failure, CAD and AMI. In group II, RDW values were highest in the STEMI group, followed by UA following NSTEMI. In addition, complications after AMI were significantly higher in TVD and mortality. In group I, these values were lower than group II. RDW was strongly correlated with univariete and multivariate regression analysis, mortality, TVD and RSR groups. Therefore, patients with asymptomatic atherosclerosis, and therefore with high RDW levels prior to ACS, may experience faster myocardial damage and increased troponin exposure to AMI. We think that it is a useful parameter that can be used with RDW and cTn in patients admitted to the emergency room due to CAD and ACS.

In this study, we found that cTn I, GS, LVEF, TVD and mortality levels increased in patients with ACS according to RSR > 1 and < 1 in leads II. We found that the level of cardiac troponin is higher in cases where myocardial involvement like UA is less than the other groups and STEMI, and that the level of cTn I is higher than in NSTEMI. In group II, cTn I values in CAD patients correlate with the degree of myocardial involvement, and suggest that it increases due to inflammatory events occurring

Table 3 χ^2 analysis of acute coronary syndrome according to diagnostic variables

Diagnosis		UA, *n* (%)	AIMI, *n* (%)	AAMI, *n* (%)	NSTEMI, *n* (%)	χ^2	*P*-value
Complication	No	388 (29.8)	102 (7.8)	76 (5.9)	74 (5.7)	384.01	0.001
	IHF	72 (5.5)	76 (5.9)	176 (13.6)	78 (6.0)		
	VT	11 (0.8)	8 (0.6)	41 (3.2)	22 (1.7)		
	AV Block	9 (0.7)	47 (3.6)	4 (0.3)	4 (0.3)		
	PE/CT	9 (0.7)	5 (0.4)	6 (0.5)	7 (0.5)		
	APE	14 (1.1)	12 (0.9)	45 (3.5)	12 (0.9)		
TVD	No	497 (38.3)	193 (14.9)	156 (12.9)	139 (10.7)	293.61	0.001
	Yes	5 (0.4)	56 (4.3)	192 (14.8)	58 (4.4)		
Mortality	No	485 (37.4)	207 (16)	280 (21.6)	165 (12.7)	56.73	0.001
	Yes	17 (1.3)	42 (3.2)	68 (5.2)	32 (2.5)		

UA: Unstable angina; AIMI: Acute inferior myocardial infarction; AAMI: Acute anterior myocardial infarction; NSTEMI: Non-ST elevation MI; IHF: Ischemic heart failure; VT: Ventricular tachycardia; AV: Atrioventricular; PE: Pericardial effusion; CT: Cardiac tamponade; APE: Acute pulmonary oedema; TVD: Tree-vessel disease.

during AMI. The number of cases in group II was poor. RDW, mortality, TVD, AIMI and GS were high in this group. Although the number of cases in group I was high, the number of complications was low, and the prognosis was good because GS was lower and LVEF was higher.

Study limitation

The most important constraints were the retrospective nature of the study, the strengths to reach the results, and the single-centre study. Additionally, we could not access the drug use history, secondary life and CAD risk factors that may affect the prognosis of patients. The main limitation was that ECG and cTn data could not be reached again after admission to ED and hospitalization.

CONCLUSION

We think that RSR and high RDW levels may be a prognostic factor in terms of complications after AMI, such as cTn I, TVD and mortality, and that RSR effects in ACS patients are an open subject to research.

Table 4 χ^2 analysis of acute coronary syndrome according to blocked major coronary artery variables

	Complication						TVD		Mortality	
	No, *n* (%)	IHF, *n* (%)	VT, *n* (%)	AV Block, *n* (%)	PE/CT, *n* (%)	APE, *n* (%)	No, *n* (%)	Yes, *n* (%)	No, *n* (%)	Yes, *n* (%)
RCA	128 (9.8)	74 (5.7)	11 (0.8)	24 (1.8)	6 (0.4)	5 (0.4)	165 (12.7)	184 (14.2)	243 (20)	37 (2.8)
R1	23 (1.8)	8 (0.7)	0 (0)	6 (0.4)	0 (0)	1 (0.1)	26 (2)	47 (3.6)	26 (2)	4 (0.3)
R2	32 (2.5)	12 (0.9)	0 (0)	3 (0.2)	1 (0.1)	0 (0)	32 (2.5)	45 (3.4)	36 (2.7)	1 (0.1)
R3	34 (2.6)	15 (1.1)	1 (0.1)	4 (0.3)	0 (0)	1 (0.1)	46 (3.5)	23 (1.8)	45 (3.5)	4 (0.3)
R4	18 (1.4)	11 (0.8)	0 (0)	1 (0.1)	1 (0.1)	5 (0.4)	29 (2.2)	11 (0.8)	33 (2.5)	7 (0.5)
L	74 (5.7)	6 (0.4)	14 (1)	9 (0.8)	4 (0.3)	5 (0.4)	81 (6.2)	47 (3.6)	75 (5.7)	17 (1.3)
L1	78 (6.2)	127 (9.8)	18 (6)	5 (0.4)	5 (0.4)	23 (1.8)	93 (7.1)	169 (13)	192 (14.8)	31 (2.4)
L2	133 (10.3)	119 (9.1)	16 (1.2)	7 (0.5)	7 (0.6)	19 (1.5)	181 (14)	94 (7.2)	269 (20.7)	43 (3.3)
L1A	37 (2.8)	4 (0.3)	13 (1)	1 (0.1)	0 (0)	12 (0.9)	163 (12.3)	7 (0.6)	53 (4.1)	7 (0.5)
L1B	23 (1.8)	8 (0.7)	4 (0.3)	2 (0.1)	0 (0)	4 (0.3)	42 (2.1)	4 (0.3)	37 (2.8)	1 (0.1)
L1C	12 (0.9)	2 (0.1)	5 (0.4)	1 (0.1)	0 (0)	2 (0.1)	27 (2)	4 (0.3)	23 (1.8)	4 (0.3)
L2A	43 (3.3)	9 (0.8)	2 (0.1)	0 (0)	2 (0.1)	5 (0.4)	61 (4.7)	6 (0.5)	58 (4.5)	9 (0.8)
L2B	22 (1.7)	4 (0.3)	1 (0.1)	0 (0)	1 (0.1)	1 (0.1)	30 (2.3)	6 (0.5)	32 (2.5)	0 (0)
L2C	8 (0.6)	3 (0.2)	1 (0.1)	1 (0.1)	0 (0)	0 (0)	14 (11.)	2 (0.1)	15 (1.1)	1 (0.1)
χ^2	1631.09						102.32		19.51	
P-value	0.001						0.001		0.001	

TVD: Tree-vessel disease; RCA: Right coronary artery; R1: Proximal RCA; R2: Mid RCA; R3: Distal RCA; R4: Posterior descending artery; L: Left coronary artery; L1: Circumflex artery; L2: Left anterior descending artery; L1A: Marginal artery; L1B: Posterior descending artery; L1C: Posterior lateral branch; L2A: 1st diagonal artery; L2B: 2nd diagonal artery; L2C: Distal left anterior descending artery.

Table 5 Univariate and multivariate Cox regression analyses for predicting the development of R to S ratio

	Univariate			Multivariate			Correlation	
	HR	95%CI	*P*-value	HR	95%CI	*P*-value	r	*P*-value
LVEF	0.964	0.919-0.933	0.001	0.945	0.896-0.410	0.001	-0.431	0.001
Mortality	3.924	2.748-5.708	0.001	1.463	0.989-2.219	0.004	0.242	0.001
TVD	2.197	1.592-2.911	0.001	3.471	1.419-7.133	0.001	0.189	0.001
cTn I	1.992	1.592-2.911	0.001	1.983	1.398-2.789	0.001	0.372	0.001
cTn II	1.145	1.117-1.189	0.001	1.278	1.233-1.491	0.001	0.326	0.001
cTn III	1.161	1.146-1.275	0.001	0.898	0.889-0.979	0.001	0.258	0.001
RDW	0.991	0.925-1.146	0.002	0.892	0.797-0.919	0.001	0.267	0.001
GS	1.109	1.102-1.114	0.001	0.996	0.986-1.005	0.386	0.208	0.001
Complication	1.368	1.322-1.469	0.001	0.993	0.989-1.178	0.752	0.267	0.001
Age	1.015	0.996-1.019	0.432	1.031	0.984-1.029	0.842	0.062	0.022
Gender	1.078	0.799-1.382	0.761	0.847	0.552-1.284	0.419	0.019	0.017

Multiple Cox proportional hazards model includes all the variables in univariate analysis with forward stepwise method. CI: Confidence interval; HR: Hazard ratio; LVEF: Left ventricular ejection fraction; TVD: Tree-vessel disease; RDW: Red cell distribution width; GS: Gensini score.

ARTICLE HIGHLIGHTS

Research background

New cardiac biomarkers and techniques that will help to provide rapid diagnosis are needed in order to evaluate risk in coronary artery patients.

Research motivation

The aim of this study was to evaluate the significance of R/S ratio (RSR) in the lead II derivation of electrocardiography in acute coronary syndromes (ACS) patients, in regard to the complications associated with myocardial infarction, three-vessel coronary artery disease, and mortality.

Research objectives
If the research is supported by prospective studies, it may be a guide for patients with ACS in the future.

Research methods
Between January 2014 and December 2018, 1,296 patients with ACS were included in the study. The patients were referred to group I for an RSR value less than I, and to group II for an RSR value greater than I.

Research results
In our study, 466 (35.9%) of the 1,296 patients (357 (38.3%) in group I and 109 (29.9%) in group II) were female, with a mean age of 61.56 ± 9.42. ST-elevation MI 573 (44.2%), unstable angina (UA) 502 (38.7%) and non-ST-elevation MI 220 (17%) were more prevalent in group I. In group 1, acute anterior MI 263 (20.3), and in group II acute inferior MI 184 (14.2) was higher. Ischemic heart failure was the most common complication. In group II, the red cell distribution width (RDW) was 15.42 ± 1.82, the gensini score was 48.39 ± 36.44, the left ventricular ejection fraction was 41.17 ± 10.41, the three-vessel disease (TVD) was 111 (8.5), and the mortality rate was 72 (5.6), which was significantly higher than group I RDW; in MI with ST and non ST-elevation, in TVD, mortality and complications was high and was low in UA. In single and multivariate regression analyses, the variables were associated with ACS risk.

Research conclusions
RSR levels may be an auxiliary predictive value in ACS in terms of complications developing after myocardial infarction, TVD, and mortality.

Research perspectives
R/S ratio, red cell distribution width.

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ORIGINAL ARTICLE

Clinical Trials Study

Comparative analysis of APACHE-II and P-POSSUM scoring systems in predicting postoperative mortality in patients undergoing emergency laparotomy

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Author contributions: Nag DS, Dembla A, Mahanty PR, and Chatterjee A planned and conducted of the study, scored the patients, and prepared and edited the manuscript; Kant S and Samaddar DP prepared manuscript, reviewed the literature, and analyzed the findings; Chugh P was involved in the study as a biomedical statistician and guided the plan of the study, determined appropriate sample size, and performed statistical analysis and interpretation.

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Abstract

BACKGROUND

Laparotomy remains one of the commonest emergency surgical procedures. Early prognostic evaluation would aid in selecting the high-risk patients for an aggressive treatment. Awareness about risks could potentially contribute to the quality of perioperative care and optimum utilization of resources. Portsmouth modification of Physiological and operative severity for the enumeration of mortality and morbidity (P-POSSUM) and the acute physiology and chronic health evaluation II (APACHE-II) have been the most widely used scoring systems for emergency laparotomies. It is always better to have a single scoring system to predict outcomes and audit healthcare organizations.

AIM

To compare the ability of APACHE-II and P-POSSUM to predict postoperative morbidity and mortality in patients undergoing emergency laparotomy.

METHODS

All patients undergoing emergency laparotomy at the Tata Main Hospital, Jamshedpur between December 2013 and November 2014 were included in the study. In this observational study, P-POSSUM and APACHE-II scoring were done, and the outcome analysis evaluated with mortality being the primary

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outcome.

RESULTS

For P-POSSUM, at a cut off value of 63 to predict mortality using receiver operating characteristics curve analysis, the area under the curve was 0.989; and for APACHE-II, at the cut off value of 24, the area under the curve was 0.965.

CONCLUSION

Because the ability of APACHE-II to predict mortality was similar to P-POSSUM and APACHE-II does not need scoring for intra-operative findings and histopathology reports, APACHE-II can be used pre-operatively to assess the risk in patients undergoing emergency laparotomy. However, for audit purposes, either of the two scoring systems can be used.

Key words: Laparotomy; Emergencies; Acute physiology and chronic health evaluation II; Morbidity; Mortality

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Core tip: Portsmouth modification of Physiological and operative severity for the enumeration of mortality and morbidity (P-POSSUM) and the acute physiology and chronic health evaluation II (APACHE-II) have been the most widely used scoring systems for emergency laparotomies. To date, no study with statistically significant sample size has compared them in predicting mortality in emergency laparotomies. P-POSSUM cannot be done for patients who are managed conservatively and can be scored only when histopathology reports are available. In this study, both P-POSSUM and APACHE-II were found to be equally accurate. Therefore, APACHE-II scoring system can be used as effectively as P-POSSUM with the added advantage that it can be used in the acute stratification of the patients into risk groups even before surgery.

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INTRODUCTION

Laparotomy remains one of the commonest emergency surgical procedures. Even after advances in surgical skills, antimicrobial agents and postoperative care, the mortality has remained high (14.9%-19.4%)[1,2]. Only over the last few years, various perioperative quality improvement initiatives involving early interventions, intensive postoperative care, and consultant led approaches have ensured a decrease in the average mortality rate to 11.1% in some studies[3].

Early prognostic evaluation would aid in selecting the high-risk patients for an aggressive treatment[3]. Awareness about risks could potentially contribute to the quality of perioperative care and optimum utilization of resources[4]. Regular audit and continuous improvement of clinical practice is essential to providing quality medical care[5] . The doctor is legally bound to discuss the prognosis and the possible outcomes of the available treatment modalities[6] . Estimating the risk preoperatively will help predict which patients would need aggressive treatment, which patients would need damage control surgery *versus* definitive procedure, and who would benefit from postoperative intensive care and organ support[7].

An ideal scoring system should accurately predict outcomes, help determine who deserves more aggressive care, guide in deciding the extensiveness of surgery, and can be used broadly across emergency laparotomies for various disease pathologies[8] . The scoring system should also be capable of analyzing risk-adjusted morbidity and mortality amongst various healthcare providers[9] .

Portsmouth modification of Physiological and operative severity for the enumeration of mortality and morbidity (P-POSSUM) and the acute physiology and

chronic health evaluation II (APACHE-II) have been the most widely used scoring systems for emergency laparotomies. While P-POSSUM remains the tool of choice in the United Kingdom[6] , disparities have been observed between APACHE II and P-POSSUM in their discriminatory ability to predict mortality[10].

It has been suggested that preoperative assessment of individual risk would help the treating team and the patient make shared decisions[10]. Although P-POSSUM is the commonest scoring system used for audit purposes in the United Kingdom[10] for National Emergency Laparotomy Audit, Enhanced Peri-Operative Care for High-risk patients or Emergency Laparotomy Pathway Quality improvement Care (ELPQuiC), it needs 18 data points as compared to 12 data points for APACHE-II. In addition, it needs intraoperative details such as blood loss, peritoneal contamination, and histopathology reports to suggest malignancy.

It is always better to have a single scoring system to predict outcomes and audit of healthcare organizations. Therefore, it has been suggested that studies should "update the performance (primarily the calibration) of APACHE-II and P-POSSUM" and compare its ability to predict postoperative morbidity and mortality[6,10].

MATERIALS AND METHODS

After approval from the institutional ethics committee, this single center prospective observational study was conducted from December 2013 to November 2014. All patients undergoing emergency laparotomy at the Tata Main Hospital, Jamshedpur, India, during this period were included in this study. All patients below 18 years, those with acute trauma, undergoing re-exploratory laparotomy, or any laparotomy for vascular surgery were excluded from the study.

All patients were scored with APACHE-II on being posted for emergency surgery. Twelve components of the Physiologic Scores of and one component of the Operative Score for P-POSSUM were scored at the time of being posted for surgery. Four of the six components of the Operative Score for P-POSSUM were done intraoperatively (category, number of procedures, blood loss, and peritoneal soiling), while one was done postoperatively on availability of histopathology reports (malignancy).

The patients were followed up for at least 30 d after discharge or death (during admission or within 30 d after discharge) by telephonic interview. While postoperative mortality was the primary outcome that was analyzed, the following secondary outcomes were also compared: (1) Length of stay (LOS); (2) Need for postoperative ventilator support (any time during the postoperative period, either immediate based on the assessment of the anesthesiologist or later due to respiratory failure); (3) Need for postoperative ionotropic support (inotropic support would be initiated if the patient remained hypotensive despite fluid resuscitation to maintain a mean arterial pressure ≥ 65 mmHg); (4) Acute kidney injury (AKI) (diagnosed based on the Kidney Disease: Improving Global Outcomes Acute Kidney Injury Work Group (2012) guidelines[11]); (5) Patients needing re-exploration; and (6) Cardiac morbidity (acute myocardial infarction or arrhythmias needing treatment).

Statistical analysis

Receiver operating characteristics curve (ROC) was used as a statistical method to measure the diagnostic accuracy. Area under the curve (AUC) was used to measure the "size" of the prediction, and it consisted of graphically plotting "sensitivity" and the "1–specificity" relationship[12]. AUC can range from 0.5 to 1.0, and a result of 1.0 indicates a perfect discriminatory ability. An AUC value > 0.8 is considered good, a range between 0.60-0.80 is considered as moderate, and an AUC value < 0.60 is regarded as poor. The ROC curve was used to display the optimal cut-off point when sensitivity and specificity reached an optimum for both values, by which the point on the ROC curved line was closest to the upper left corner on the curve. Statistical analysis was performed using the SPSS program for Windows, version 17.0 (Chicago, IL, United States). Continuous variables are presented as mean ± standard deviation (SD) or median (min-max), and categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired t-test, whereas the Mann-Whitney U-test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the chi square test or Fisher's exact test.

In previous studies of perforative peritonitis[13], it was found that the sensitivity of APACHE-II was 87.5% at cut off value 16–20. For the sample size calculation, using a two tailed alpha value (0.05) and a beta value (0.2), 150 patients would have been sufficient to detect a significant difference of 10% between APACHE-II and P-

POSSUM scoring systems in predicting postoperative mortality in patients undergoing emergency laparotomy. Thus, our sample size of 157 appears to be adequate to assess if there is any difference between the two scoring systems to predict mortality.

RESULTS

A total of 159 patients met the inclusion criteria. Two patients sought referral to a higher center and were lost on follow up and were excluded from the study.

Of the total 157 studied patients, 89 had perforative peritonitis, 57 had intestinal obstruction, and 11 were operated because of other reasons that included pancreatitis (4), cholecystitis (2), ruptured liver abscess (1), liver hematoma (1), rectal prolapsed (1), empyema gall bladder (1), and spontaneous hemoperitoneum because of thrombocytopenia (1).

The age of the patients ranged from 18 to 82 years. Of the 157 analyzed patients, 99 (63.1%) were male and 58 (36.9%) were females. Twenty-three (14.6%) of the total patients analyzed died, and 134 (85.4%) survived. The mean ± SD of LOS was 10.18 ± 8.24 and ranged from 1 to 70 d. Sixty-three patients (40.1%) required postoperative ventilatory support, 48 (30.6%) required perioperative ionotropic support, and 32 (20.4%) developed AKI in the postoperative period. Four out of the 157 analyzed patients required re-exploration. A total of eight patients developed postoperative cardiac morbidity.

The median age [interquartile range (IQR)] amongst the survivors was 46 (30-60) years and 60 (44-69) years for those who died in the postoperative period. The statistically significant *P* value (0.029) indicated that increasing age is associated with a higher risk of mortality. While 43.5% of the patients who died were males, 56.5% of the patients who died were females, indicating a statistically significant ($P = 0.035$) increased risk of mortality amongst female patients.

While the median APACHE-II score amongst the patients who died in the postoperative period was 31 (min-max 25-35), the median P-POSSUM Physiologic Score and Operative Score amongst them was 52 and 22, respectively (min-max 46-58 and 20-24, respectively). $P < 0.001$ signifies that higher scores are associated with statistically significant increased mortality.

For APACHE-II, the cut off value was found to be 24 to predict Mortality ROC analysis. In our studied patients, APACHE-II score of < 24 was associated with a significantly lower mortality of 17.4% as compared to an APACHE-II score of ≥ 24, which was associated with a mortality of 82.6% ($P < 0.001$) (Table 1). Using ROC, at cut off value 24, the AUC [95% confidence interval (CI)] was 0.965 (0.928–1.000). Sensitivity, specificity, positive predictive value, and negative predictive value of APACHE-II was found to be 82.6%, 98.5%, 90.5%, and 97.1%, respectively.

In comparison, for P-POSSUM the cut off value found to be 63 to predict Mortality using ROC analysis. P-POSSUM score of < 63 was associated with a significantly lower mortality of 8.7% as compared to a score of ≥ 63 which was associated with a mortality of 91.3% ($P < 0.001$) (Table 2). Using ROC, at cut off value 63, AUC (95%CI) was 0.989 (0.974–1.000). Sensitivity, specificity, positive predictive value, and negative predictive value of P-POSSUM was found to be 91.3%, 99.3%, 95.5%, and 98.5% respectively.

Using Pearson's Linear Correlation Coefficient, APACHE-II showed an overall predictive value of 95.5% with an odds ratio (OR) of 1.315, 95%CI of 1.193-1.448, and a $P < 0.001$. Similarly, P-POSSUM showed an overall predictive value of 98.1% with an OR of 1.364, 95%CI of 1.193-1.559, and a $P < 0.001$. Box-plots in R (Pearson Correlation Coefficient) using APACHE-II and P-POSSUM are depicted in Figures 1 and 2 respectively.

Multivariate logistic regression model has been used to identify independent risk factors (APACHE-II and P-POSSUM) for mortality. A ROC, the graphic display between the "sensitivity" and the "1–specificity" relationship to measure diagnostic accuracy of the true positives *versus* the false positives for APACHE-II and P-POSSUM, is depicted in Figure 3. AUC was 0.965 (using a cut-off value 0f 24) for APACHE-II and 0.989 (using a cut-off value 63) for P-POSSUM. AUC can range from 0.5 to 1.0, and a result of 1.0 indicates a perfect discriminatory ability.

Although both the scores were significantly good in predicting postoperative mortality in patients undergoing emergency laparotomy, the AUC of P-POSSUM (0.989) appeared better than APACHE-II (0.965). However, on comparing the sensitivity and specificity of APACHE-II and P-POSSUM (Table 3), there appears to be no statistically significant difference between their ability to predict postoperative mortality. Except for APACHE-II's inability to predict re-exploration, both were able

Table 1 Discriminating ability of APACHE-II

APACHE-II	Survived		Mortality		*P* value
	Frequency	%	Frequency	%	
< 24	132	98.5	4	17.4	< 0.001
≥ 24	2	1.5	19	82.6	
Total	134	100	23	100	

APACHE-II: Acute physiology and chronic health evaluation II.

to predict all the secondary outcomes in a statistically significant manner ($P < 0.001$) (Table 4).

DISCUSSION

Emergency laparotomy "describes an exploratory procedure for which the clinical presentation, underlying pathology, anatomical site of surgery, and perioperative management vary considerably"[1]. The mere fact that over 400 different surgical procedures have been described as a part of emergency laparotomy reflect the diversity in pathology[1]. Often there is little time to optimize these patients, resulting in significant adverse outcomes. The unadjusted 30-d postoperative mortality rate was 14.6% at our hospital. A study published in 2011 from a 650-bed general hospital (Royal United Hospitals, Bath) serving a population of over half a million reported a 30-d mortality of 16.9% amongst 124 patients undergoing emergency laparotomy[2]. Like their study, we also excluded emergency vascular surgery, re-exploration, and simple appendectomy[2]. Similarly, the Emergency Laparotomy Network[1] covering 35 NHS hospitals reported a 30-d mortality of 14.9% amongst 1853 patients who underwent emergency laparotomy. Similar incidence of mortality after emergency laparotomy of 20.2%[14] and 17%[15] were reported in 2017. Without adjusting for age, patient comorbidity, surgical presentation, and complexity of the involved pathology, we cannot be certain whether our 30-d postoperative mortality (14.6%) represents equivalent or better quality of care in comparison to that provided in European countries (14.9%-20.2%)[1,2]. However there is increased understanding that standardization of care and quality improvement bundles can improve morbidity and mortality after emergency surgery[7]. The male preponderance in our study group (63.1%) and statistically significant increased mortality amongst females (56.5% as compared to 43.5% in males) was in stark contrast to the UK Emergency Laparotomy Network observations[1]. However, there is some evidence supporting our observation. Similar studies in India have shown a male preponderance for patients undergoing emergency laparotomy (69.5%)[16]. Certain scoring systems, like the Mannheim Peritonitis Index, assign a higher risk for the female patients[17], a risk validated by our study also.

While no mortality was observed in any of our patients who were less than 20 years of age, it increased from 11.11% in the 21-40 year age group to 13.33% in the 41-60 year age group, 23.68% in the 61-80 year age group, and 33.33% amongst those above 80 years of age. Amongst the patients analyzed by Emergency Laparotomy Network, the risk of mortality increased by approximately 4% for each additional 10 years of age[1]. Increasing age has been identified as an independent risk factor, and increase in mortality with age has been observed in most studies, thus validating the inclusion of age as risk factor[2,18,19].

In our study, the LOS (± SD) was 10.18 (± 8.24) d. This was similar to the observations by the Emergency Laparotomy Network in whom the median [IQR (range)] postoperative length of stay for all patients was 11 d [6–21 (0–216)][1]. Although 30-d mortality after implementation of the ELPQuiC bundle indicated a reduction in the risk of death (14% to 10.5%), it had no bearing on the LOS, which remained at its median value of 11 d both before and after ELPQuiC[7]. A number of factors, including the survival of patients who would not previously have survived surgery and the availability of suitable discharge facilities, may explain the lack of reduction of LOS even with improved quality of care. Similar LOS of a median [IQR (range)] of 13 [8–24 (1–176)] d following emergency laparotomy has been reported by other studies as well[20]. While higher scores of APACHE-II a P-POSSUM do indicate some correlation with the LOS, the degree of correlation expressed by the Spearman Rank Correlation Coefficient is relatively small, 0.322 for APACHE-II and 0.374 for P-

Table 2 Discriminating ability of P-POSSUM

P-POSSUM	Survived		Mortality		*P* value
	Frequency	%	Frequency	%	
< 63	133	99.3	2	8.7	< 0.001
> 63	1	0.7	21	91.3	
Total	134	100	23	100	

P-POSSUM: Physiological and operative severity for the enumeration of mortality and morbidity.

POSSUM.

In our studied patients, APACHE-II score of < 24 was associated with a significantly lower mortality of 17.4%, as compared to a score of ≥ 24 which was associated with a mortality of 82.6%. At cut off value 24, the AUC (95%CI) was 0.965 (0.928-1.000). While all studies have so far shown the ability of APACHE-II scores to predict mortality and similar AUC has been reported in other studies as well for patients undergoing emergency laparotomy either for varied causes[21] or for perforative peritonitis[22], our study has demonstrated the strongest correlation to date with AUC of 0.965 (as compared to 0.74-0.86 in other studies)[21,22].

In our study, P-POSSUM at cut off value of 63 to predict mortality using ROC analysis, a score of < 63 was associated with a significantly lower mortality of 8.7% as compared to a P-POSSUM score of ≥ 63 which was associated with a mortality of 91.3% (P < 0.001). Using ROC, at cut off value 63, AUC (95%CI) was 0.989 (0.974–1.000). While our observations are similar to other studies[9,16,23,24], which demonstrates the ability of the P-POSSUM to predict mortality, our AUC of 0.989 at the cut off value of 63 shows a fairly high degree of accuracy of P-POSSUM. Studies have shown that P-POSSUM is a poor predictor in trauma[9], possibly resulting in our study showing a higher predictive ability of mortality, as we had excluded such cases.

While some studies have tried comparing APACHE-II and P-POSSUM across all surgeries[25], others have used it for specific pathologies[26,27]. To date, no study with statistically significant sample size has compared APACHE-II and P-POSSUM in its ability to predict mortality in patients undergoing emergency laparotomy. Our study can potentially fill in the present void in published literature comparing APACHE-II and P-POSSUM in predicting mortality in patients undergoing emergency laparotomy.

ELPQuiC[7] has used P-POSSUM as a scoring system to assess the impact of introduction of quality improvement bundles, but our study shows that either of the scoring systems (APACHE-II or P-POSSUM) can be used as a tool for surgical audit and the impact of quality improvement initiatives on hospital mortality.

In the present study, both scoring systems were found to be accurate in predicting the mortality of patients, with patients having higher scores having a higher mortality. APACHE-II scores correlate well with mortality and are effective in the prediction of outcome. It considers the acute physiology of the patient and can be completed before surgery. Therefore, it is very useful in the acute stratification of the patients into risk groups and in predicting which patients can be considered for more extensive procedures. However, the APACHE-II score does not consider the etiology of peritonitis or the nature of peritoneal contamination, which has an important bearing on the outcome. In comparison, the P-POSSUM system appears to be of value as the physiologic status is assessed just before the operation or more accurately after full resuscitation and also takes the operative findings into consideration.

However, the P-POSSUM model also has its limitations. First of all, it does not include the patients who are managed conservatively and those who have refused or been denied surgery due to the significant associated risk of mortality. Secondly, while recording the operative variables such as estimated blood loss or peritoneal contamination the surgeon's eye may be biased. And finally, the scores are not complete until the histopathology reports are available and may significantly delay the scoring and assessing of the risk. Possibly, that is the reason why in our study APACHE-II, being a physiologic score, was a poor indicator of the need for a re-exploration surgery (Spearman Rank Correlation Coefficient of 0.112) (P = 0.112). P-POSSUM is possibly a better predictor of the need for re-exploration (Spearman Rank Correlation Coefficient of 0.178) as it includes the intra-operative finding with a P = 0.026. This indicated that although P-POSSUM has some correlation with possible need re-exploration as compared to APACHE-II (which had no correlation), the correlation was quite low.

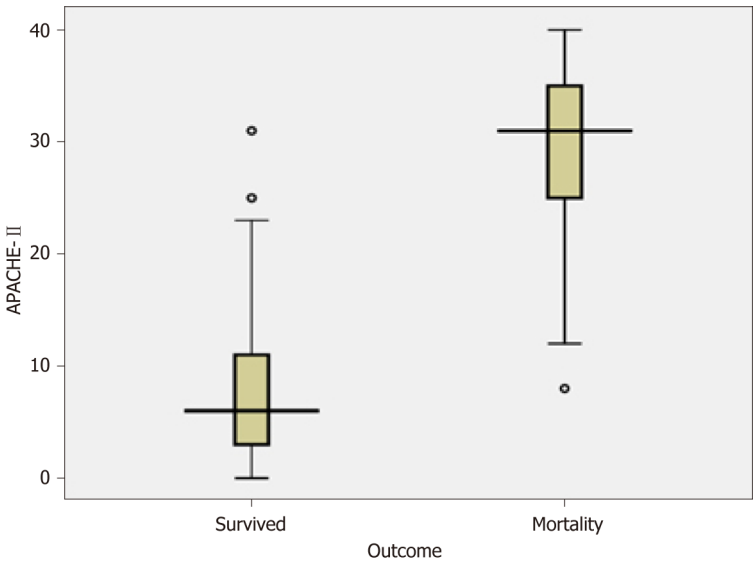

Figure 1 Box-plots in Pearson correlation coefficient using APACHE-II. APACHE-II: Acute physiology and chronic health evaluation II.

Higher APACHE-II and P-POSSUM correlated well with our secondary outcomes like the postoperative need for inotropic support or ventilatory support or AKI. Such patients who need postoperative organ support are best managed in a critical care setup. Ability of APACHE-II to predict these sicker patients (without relying on the intraoperative or histology findings as for P-POSSUM) could allow us to plan better, optimize and utilize such scarce resources.

Because the ability of APACHE-II to predict mortality is similar to P-POSSUM, and the fact that APACHE-II does not need scoring for intra-operative findings and histopathology reports, APACHE-II can be used pre-operatively to assess the risk in patients undergoing emergency laparotomy. However, for audit purposes, either of the two scoring systems can be used.

Table 3 Sensitivity and specificity of APACHE-II and P-POSSUM

	APACHE-II	P-POSSUM	*P* value
Sensitivity	82.6%	91.3%	0.665
Specificity	98.5%	99.3%	1.000

APACHE-II: Acute physiology and chronic health evaluation II; P-POSSUM: Physiological and operative severity for the enumeration of mortality and morbidity.

Table 4 Discriminating ability of APACHE-II and P-POSSUM in predicting the secondary outcomes

		APACHE-II	P-POSSUM
LOS	*R*	0.322	0.374
	P value	< 0.001	< 0.001
Ventilatory support	*R*	0.554	0.572
	P value	< 0.001	< 0.001
Ionotropic support	*R*	0.573	0.544
	P value	< 0.001	< 0.001
Re-exploration	*R*	0.112	0.178
	P value	0.161	0.026
AMI or arrhythmia	*R*	0.507	0.518
	P value	< 0.001	< 0.001
AKI	*R*	0.507	0.518
	P value	< 0.001	< 0.001

APACHE-II: Acute physiology and chronic health evaluation II; P-POSSUM: Physiological and operative severity for the enumeration of mortality and morbidity; LOS: Length of stay; AKI: Acute kidney injury; AMI: Acute myocardial infarction.

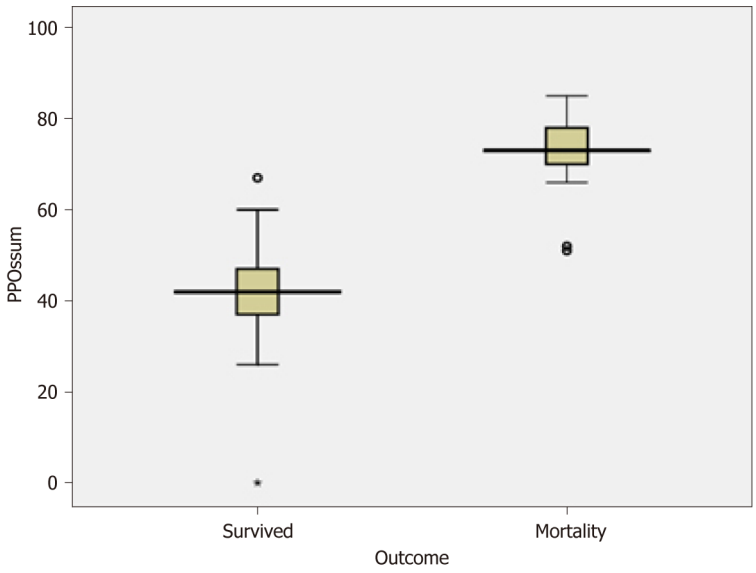

Figure 2 Box-plots in Pearson correlation coefficient using P-POSSUM. P-POSSUM: Physiological and operative severity for the enumeration of mortality and morbidity.

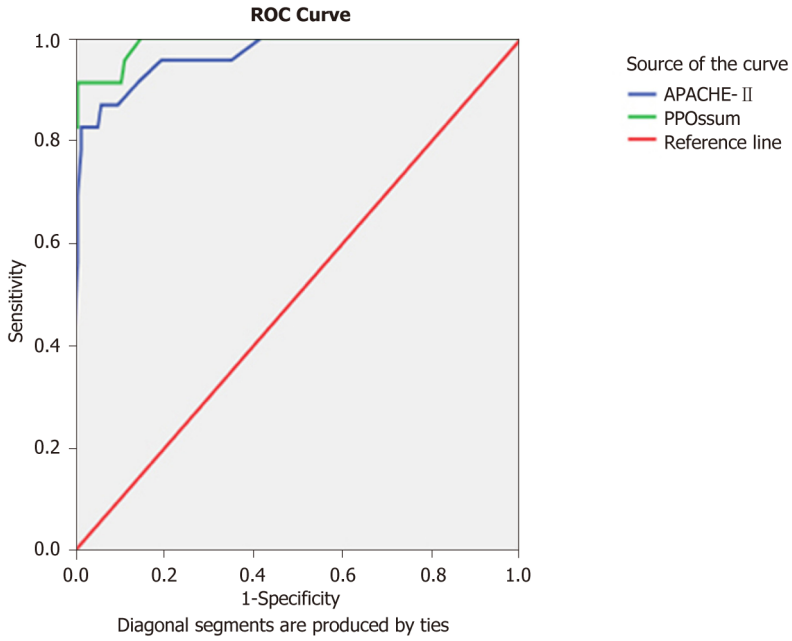

Figure 3 Receiver operating characteristics curve for APACHE-II and P-POSSUM using the Multivariate logistic regression model. APACHE-II: Acute physiology and chronic health evaluation II; P-POSSUM: Physiological and operative severity for the enumeration of mortality and morbidity; ROC: Receiver operating characteristics curve.

ARTICLE HIGHLIGHTS

Research background

Various scoring systems have been used historically to predict outcomes in patients who are at increased risk of morbidity and mortality during their hospital stay. Emergency laparotomy, despite being one of the commonest surgical procedures, continued to have reasonably high postoperative mortality. Doctors are legally bound to discuss with their patients and relatives the potential risk of complications and adverse outcomes. A robust scoring system enables us to quantify the risk and serves as a tool to measure risk-based outcomes and enable audit of clinical results and impact of improvement initiatives.

Research motivation

Portsmouth modification of Physiological and operative severity for the enumeration of mortality and morbidity (P-POSSUM) and the acute physiology and chronic health evaluation II (APACHE-II) have been the most widely used scoring systems for emergency laparotomies. P-POSSUM remains the tool of choice in the United Kingdom. However, it is subject to observational bias while quantifying intraoperative blood loss and peritoneal contamination. It is always better that we have a single scoring system to predict outcomes and audit healthcare organizations. Besides, delay in histopathology reports would delay the P-POSSUM score of the patient, and patients managed conservatively or refused surgery could not be scored. In these circumstances, the APACHE-II score had the advantage of being available in the pre-operative period itself. However, to date no study with statistically significant sample size has compared P-POSSUM and APACHE-II in their ability to predict mortality in emergency laparotomies. This study aims to bridge this gap and assess if APACHE-II can be used as a single scoring system to predict outcomes and for audit of outcomes across healthcare organizations.

Research objectives

The study was conducted to compare the predictability of APACHE-II and P-POSSUM scoring systems on postoperative mortality and to see any correlation between these scoring systems and length of stay, requirement of postoperative ventilatory support, inotropic support, development of acute kidney injury (AKI), cardiac morbidity, and need for re-exploration. While the study showed that both APACHE-II and P-POSSUM can equally predict mortality, it also demonstrated comparability in predicting increased length of stay and need for postoperative ventilatory support, higher incidence of AKI, and increased risk of cardiac morbidity. However, P-POSSUM was a better predictor of the need for re-exploration as compared to APACHE-II. The study was successful in demonstrating that both APACHE-II and P-POSSUM can be interchangeably used not only for postoperative mortality but also for effectively predicting morbidity. With the advantage that the APACHE-II scoring can be done preoperatively, the study justifies the fact that APACHE-II can be the single scoring system to predict outcomes and audit healthcare organizations for emergency laparotomies.

Research methods

All patients undergoing emergency laparotomy at Tata Main Hospital (Jamshedpur, India) form December 2013 to November 2014 were included in the study. All patients were scored with APACHE-II and P-POSSUM scoring systems. Receiver operating characteristics curve (ROC) was used as a statistical method to measure the diagnostic accuracy. Area under the curve (AUC) was used to measure the "size" of the prediction, and it consisted of graphically plotting "sensitivity" and the "1–specificity" relationship. The ROC curve was used to display the optimal cut-off point when sensitivity and specificity reached an optimum for both values, by which the point on the ROC curved line was closest to the upper left corner on the curve.

Research results

Out of a total of 159 patients who met the inclusion criteria, only 157 could be included in the study. For APACHE-II, the cut off value was found to be 24 for predicting mortality by ROC analysis. In comparison, for P-POSSUM, the cut off value was found to be 63 to predict mortality using ROC analysis. Multivariate logistic regression model was used to identify independent risk factors for mortality. A ROC, the graphic display between the "sensitivity" and the "1–specificity" relationship to measure diagnostic accuracy of the true positives *versus* the false positives for APACHE-II and P-POSSUM, depicted that AUC was 0.965 (using a cut-off value of 24) for APACHE-II and 0.989 (using a cut-off value 63) for P-POSSUM. Both the scores were significantly good in predicting postoperative mortality in patients undergoing emergency laparotomy and on comparing the sensitivity and specificity of APACHE-II and P-POSSUM, there appears to be no statistically significant difference between their ability to predict postoperative mortality. Except for APACHE-II's inability to predict re-exploration, both can predict all the secondary outcomes in a statistically significant manner.

Research conclusions

This is possibly the first adequately powered study with alpha value (0.05) and a beta value (0.2) and statistically significant sample size that has compared P-POSSUM and APACHE-II in predicting mortality in emergency laparotomies. P-POSSUM above 63 and APACHE-II above 24 not only indicates higher risk, it also increases the risk of postoperative morbidity. However, APACHE-II, being a physiologic score, was a poor indicator of the need for a re-exploration after laparotomy. P-POSSUM is a significantly better predictor of the possibility of re-exploration. While P-POSSUM continues to be the most commonly used scoring system for audit purposes, risk-based outcome comparisons across hospitals and impact of quality improvement initiatives using APACHE-II would ensure that a single scoring system can be used not only for individual patient's risk assessment and prognostication but also used interchangeably with P-POSSUM for audit purposes as well.

Research perspectives

This study demonstrates that compared to the more widely used P-POSSUM, which needs 18 data points, APACHE-II needs only 12 data points, is easily available for risk assessment in the preoperative period, and does not need subjective assessments (intraoperative blood loss or peritoneal contamination) or wait for histopathology reports. While this study was an adequately powered single center study, future research should focus on multi-center trials to strengthen the findings of our study.

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ORIGINAL ARTICLE

Observational Study

TAZ and myostatin involved in muscle atrophy of congenital neurogenic clubfoot

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Author contributions: Sun JX, Yang ZY, and Cai AL designed research; Sun JX, Xie LM, and Wang B performed clinical ultrasound examination; Sun JX and Bai N performed the biological experiments; Sun JX and Yang ZY analyzed data; and Sun JX, Yang ZY, and Cai AL wrote the paper.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Shengjing Hospital of China Medical University.

Informed consent statement: The clinical data used in this study were anonymous.

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

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Abstract

BACKGROUND

Muscular atrophy is the basic defect of neurogenic clubfoot. Muscle atrophy of clubfoot needs more scientific and reasonable imaging measurement parameters to evaluate. The Hippo pathway and myostatin pathway may be directly correlated in myogenesis. In this study, we will use congenital neurogenic clubfoot muscle atrophy model to verify *in vivo*. Further, the antagonistic mechanism of TAZ on myostatin was studied in the C2C12 cell differentiation model.

AIM

To identify muscle atrophy in fetal neurogenic clubfoot by ultrasound imaging and detect the expression of TAZ and myostatin in gastrocnemius muscle. To elucidate the possible mechanisms by which TAZ antagonizes myostatin-induced atrophy in an *in vitro* cell model.

METHODS

Muscle atrophy in eight cases of fetal unilateral clubfoot with nervous system abnormalities was identified by 2D and 3D ultrasound. Western blotting and immunostaining were performed to detect expression of myostatin and TAZ. TAZ overexpression in C2C12 myotubes and the expression of associated proteins were analyzed by western blotting.

RESULTS

The maximum cross-sectional area of the fetal clubfoot on the varus side was reduced compared to the contralateral side. Myostatin was elevated in the atrophied gastrocnemius muscle, while TAZ expression was decreased. They

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were negatively correlated. TAZ overexpression reversed the diameter reduction of the myotube, downregulated phosphorylated Akt, and increased the expression of forkhead box O4 induced by myostatin.

CONCLUSION
Ultrasound can detect muscle atrophy of fetal clubfoot. TAZ and myostatin are involved in the pathological process of neurogenic clubfoot muscle atrophy. TAZ antagonizes myostatin-induced myotube atrophy, potentially through regulation of the Akt/forkhead box O4 signaling pathway.

Key words: Congenital clubfoot; Neurogenic; Muscle atrophy; Myostatin; TAZ

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Core tip: This study aimed to identify muscle atrophy in fetal neurogenic clubfoot, detect expression of TAZ and myostatin in gastrocnemius muscle, and establish the mechanisms through which TAZ antagonizes myostatin-induced atrophy in an *in vitro* cell model. Muscle atrophy in fetal unilateral clubfoot with nervous system abnormalities was identified by ultrasound. TAZ overexpression in C2C12 myotubes induced atrophy by myostatin. Both TAZ and myostatin are involved in the process of neurogenic clubfoot muscle atrophy, and they are negatively correlated. TAZ antagonizes myostatin-induced myotube atrophy, potentially through regulation of the Akt/forkhead box O4 pathway.

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INTRODUCTION

Muscle atrophy is the basic defect of clubfoot and is important for functional outcomes[1]. Treatment that targets muscle atrophy is insufficient due to the lack of research on the mechanism of the disease. Muscular atrophy affects a patient's physical activities and even impairs their cognitive function. Therefore, it is important to explore the pathogenic mechanism and treatment of muscular atrophy.

Although it is still controversial, the occurrence of congenital clubfoot muscle atrophy is thought to be a neuromuscular abnormality[2], and studies have clearly confirmed that the changes in clubfoot muscle atrophy with neuropathy are more dramatic[3-5]. Therefore, the present study investigated patients with clubfoot atrophy with neurological abnormalities by ultrasound examination.

The Hippo signaling pathway plays a crucial role in the process of myogenesis and skeletal muscle regeneration[6,7]. Previous studies have confirmed that TAZ has a positive role in muscle function by upregulating myoD and activating gene transcription of myogenin and MCK[8,9]. Our previous studies showed that upregulation of TAZ in C2C12 cells could enhance the combination of myoD and myogenin promoter, promote myoD-dependent gene transcription, and antagonize the inhibition of muscle differentiation induced by myostatin. These effects were diminished by endogenous knockdown of TAZ[10].

Myostatin, a member of the transforming growth factor-β super family, is specifically expressed in embryonic and adult skeletal muscle and acts as an inhibitor of skeletal muscle protein production and hypertrophy[11,12]. Myostatin has been shown to play an important role in the process of denervation of gastrocnemius atrophy[13]. Myostatin signal transduction is mediated by two different types of serine/threonine kinase receptors. It can transduce signals into the nucleus through SMAD, MAPK, and Akt pathways[14,15].

Our previous studies confirmed the role of TAZ in muscle atrophy in a variety of *in vivo* models[10,16]. In this study, we confirmed our theory *in vivo*, using a new model of neurogenic muscular atrophy of congenital clubfoot with nervous system abnormalities and further explored how TAZ acts on myostatin-induced C2C12

myotube atrophy.

MATERIALS AND METHODS

Tissue specimens

Muscle tissue specimens were obtained from eight fetuses that underwent induced abortion due to the prenatal diagnosis of congenital clubfoot combined with nervous system abnormalities in Shengjing Hospital of China Medical University. For better comparison, fetuses with bilateral varus were excluded. The study protocol was approved by the Ethics Committee of China Medical University (protocol number 2016PS055K). Ultrasound equipment was Voluson E10 color Doppler ultrasound diagnostic instrument (GE, Boston, MA, United States). The frequency of the conventional abdominal convex probe was 1.0–5.0 MHz.

Cell culture and treatment

C2C12 cell line (mouse myoblasts) was obtained from the American Type Culture Collection (Manassas, VA, United States). Cells were cultured in DMEM (Gibco-BRL, Grand Island, NY, United States). Culture medium was supplemented with 10% fetal calf serum (Gibco-BRL), 10 mmol/L HEPES–NaOH (Gibco-BRL), 100 IU/mL penicillin (Sigma, St. Louis, MO, United States) and 100 μg/mL streptomycin (Sigma). Cells were grown on sterile tissue culture dishes at 37 °C in a humid atmosphere with 5% CO_2 and were passaged every 3–4 d using 0.25% trypsin (Gibco-BRL) when the density of cells was around 70%–80%. To induce muscle differentiation, the medium was replaced with DMEM containing 2% horse serum for 3 consecutive days when cells grew up to 100% confluence. DMSO (Sigma), 100 ng/mL myostatin (R and D) and 10 mmol/L IBS008738 were added to the medium alone or in combination, and the medium was changed every day. We observed the formation of myotubes under inverted microscope.

TAZ overexpression and myostatin treatment in C2C12 cells

TAZ overexpression plasmid and control constructions were synthesized by Bioengineering (Shanghai) Co. Ltd. (Shanghai, China). Cells were transfected using the Lipofectamine 3000 transfection reagent. Following transfection, the protein levels were assessed 48 h later. The cells were cultured to confluence and myotube formation was induced by differentiation medium with DMSO or 100 ng/mL myostatin. Three days later, myotubes were observed and measured.

Western blotting

Total proteins from primary tissues and cell lines were extracted in lysis buffer (Thermo Fisher Scientific, Rockford, IL, United States) and quantified using the Bradford method. Fifty micrograms of protein were separated by 12% SDS–PAGE. After transferring, the polyvinylidene fluoride membranes (Millipore, Billerica, MA, United States) were incubated overnight at 4 °C with the following antibodies TAZ (560235, 1:1000; Becton Dickinson, Franklin Lakes, NJ, United States), myostatin (98337, 1:1000; Abcam, Cambridge, MA, United States), Akt (#9272, 1:500; GST), p-Akt (4060S, 1:500; GST), forkhead box (FOX)O4 (#9472, 1:500; GST), and GAPDH (TA-08, 1:500; ZSGB-BIO, Beijing, China). After incubation with peroxidase-coupled anti-mouse IgG (Beyotime, Jiangsu, China) at 37 °C for 2 h, bound proteins were visualized using Bio-Rad GS-800 and analyzed using Image J software. The relative protein levels were calculated based on GAPDH as the loading control.

Immunohistochemistry

Muscle tissue specimens were fixed with 10% neutral formalin, embedded in paraffin, and 5-μm-thick sections were prepared. Immunostaining was performed using the avidin–biotin–peroxidase complex method (Ultra Sensitive TM; Maixin, Fuzhou, China). The sections were deparaffinized in xylene, rehydrated in graded alcohol series, and boiled in 0.01 mol/L citrate buffer (pH 6.0) for 2 min in an autoclave. Endogenous peroxidase activity was blocked using hydrogen peroxide (0.3%), which was followed by incubation with normal goat serum to reduce nonspecific binding. Tissue sections were incubated with antibody to TAZ (560235, 1:100; BD) or myostatin (98337, 1:100; Abcam). Staining for all primary antibodies was performed at 4 °C overnight. Biotinylated goat anti-mouse serum IgG (ready-to-use) (Maixin) was used as the secondary antibody at room temperature for 25 min. After washing three times in phosphate-buffered saline, the sections were incubated with horseradish-peroxidase-conjugated streptavidin–biotin for 20 min, followed by 3, 3'-diaminobenzidine tetrahydrochloride to develop the peroxidase reaction. Counterstaining of the sections was done with hematoxylin and then dehydrated in

ethanol before mounting.

Statistical analysis

Data are expressed as mean ± SD. Statistical analyses were performed using paired-samples *t* test for comparison between two samples or one-way analysis of variance followed by Bonferroni's test for multiple comparisons using GraphPad Prism 5.0. (GraphPad Software, La Jolla, CA, United States). $P < 0.05$ means the difference was statistically significant.

RESULTS

Identification of muscle atrophy in fetuses with clubfoot using 2D or 3D ultrasound

Two-dimensional ultrasound showed the fetal calf and foot, and the inversion side and healthy side images were obtained (Figure 1A and 1B). Ultrasound clearly showed bilateral calf muscles and bones and confirmed clubfoot. Three-dimensional tomographic ultrasound imaging was used to position the largest cross-section perpendicular to the tibia, and the cross-sectional area of bilateral calves was measured (Figure 1C and 1D). Quantitative results showed that the area of the varus side was significantly reduced (Figure 1E), and muscle atrophy was confirmed.

Expression of TAZ and myostatin in muscle tissue specimens from congenital neurogenic clubfoot

Gastrocnemius muscle tissue specimens were obtained from eight fetuses. We defined the varus limb as a positive experimental group and the normal limb of the same fetus as a negative control group. Western blotting was used to detect the protein levels of endogenous TAZ and myostatin. Western blotting (Figure 2A) and immunostaining (Figure 2B) showed that myostatin was increased in the atrophied gastrocnemius muscle, while TAZ expression was decreased. There was a negative correlation between expression of TAZ and myostatin.

TAZ rescues myostatin-induced C2C12 myotube atrophy via Akt/FOXO4 pathway

To investigate the interaction between TAZ and myostatin in muscle atrophy, we created TAZ-overexpressing and control C2C12 cells by transfection. We induced myotube atrophy by adding exogenous myostatin. Three days later, photomicrographs and quantitative data showed that myotube diameter in myostatin-treated control cells was smaller than that in DMSO-treated cells (Figure 3A and 3B left two columns), and TAZ overexpression reversed this condition (Figure 3A and 3B right two columns). As a key element that cross talks with myostatin in skeletal muscle atrophy, phosphorylated Akt was expectedly decreased by myostatin treatment, while TAZ recovered it to nearly its original level. FOXO4, a downstream factor of Akt, is also reported to be closely related with clubfoot, but it showed the opposite trend to phosphorylated Akt (Figure 3C).

DISCUSSION

Previous studies have focused on the use of magnetic resonance after birth to determine the presence of muscle atrophy in clubfoot[17-19]. We first attempted to use ultrasound to prenatally diagnose muscle atrophy. Our results show that combination of 2D and 3D ultrasound can achieve accurate localization, measurement, and quantitative comparison. As it is convenient to operate and there is no radiation, it is the preferred method for prenatal diagnosis of muscle atrophy of fetal clubfoot.

The Hippo pathway is important for regulating differentiation of mesenchymal stem cells, and its downstream cotranscription factor TAZ has been shown to promote muscle differentiation[6,20,21]. However, there are few reports about the relationship between TAZ and myostatin in muscle differentiation. In our previous screening assay, we found that the low molecular weight compound that could activate TAZ had an antagonistic effect on myostatin in a TAZ-dependent manner[10] . This compound has been commercialized as a TAZ activator and muscle differentiation promoter (TAZ activator, IBS008738: Calbiochem; 530961: Merck Millipore). The results encouraged us to further investigate the interaction between the Hippo and myostatin pathways and explore the possibility of TAZ activators as myogenic drugs. The myostatin pathway is a classical inhibitory pathway of muscle differentiation[22-25]. It plays an important role in neurogenic gastrocnemius muscle atrophy, so we chose fetal specimens with abnormal neurological malformation in clubfoot. The myostatin pathway plays its role by interacting with the IGF-1/PI3K, mTOR, and MAPK

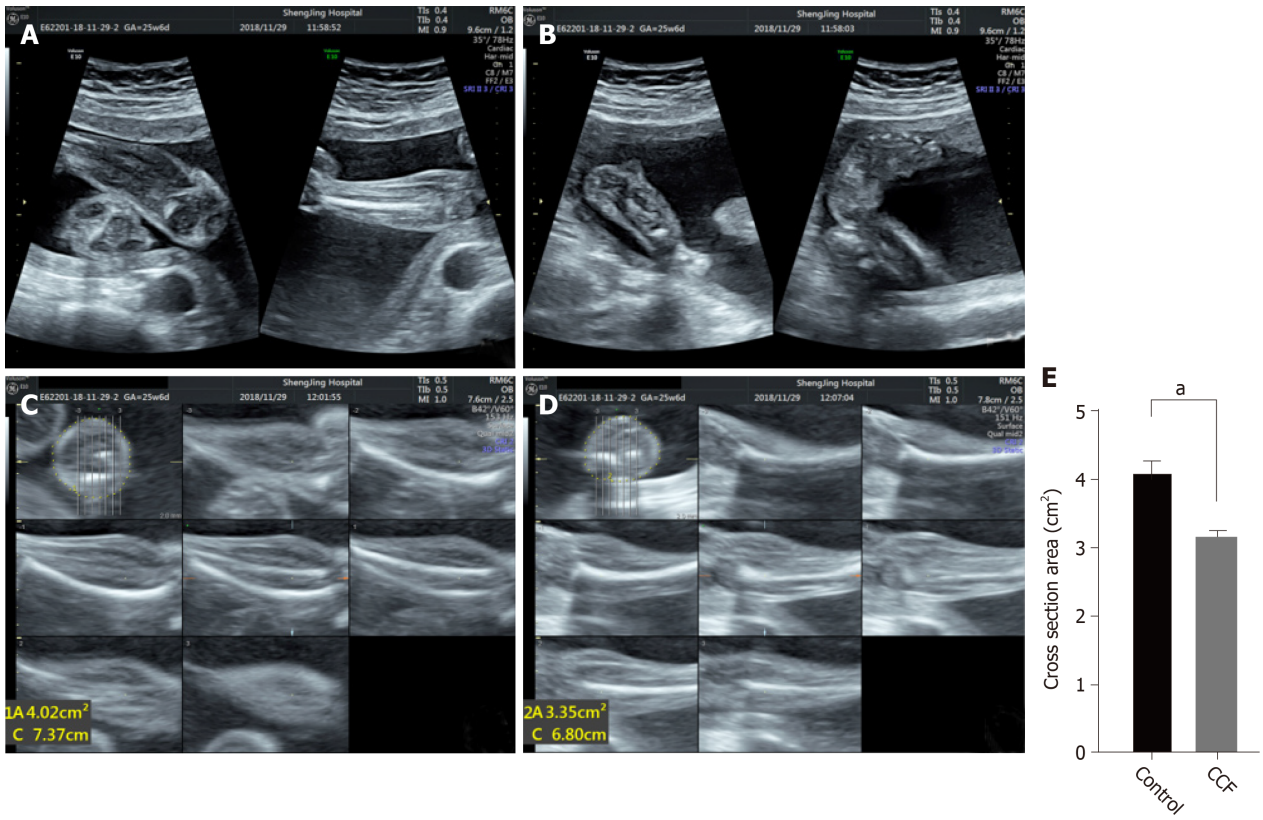

Figure 1 Identification with 2D or 3D ultrasound of muscle atrophy in fetus with clubfoot. The 2D ultrasound image of calves (A) and feet (B). The left side shows the normal condition and the right side the clubfoot condition. The 3D tomographic ultrasound imaging [normal (C); clubfoot (D)] fixed the positioning line at the largest cross-section perpendicular to the tibia (center of the nine-square image), and measurement of the area was done at the cross-section image (upper left of the nine-square image); E: Quantitative data and statistical analysis of cross-section area in paired fetal calves. $^{a}P < 0.05$ *vs* control.

pathways[26,27]. IGF-1/PI3K/Akt signaling reduces during muscle atrophy induced by denervation, unloading, and joint immobilization[28].

Myostatin inhibits Akt and thereby downregulates mTOR, S6K, and Akt inhibition and protein degradation associated with the FOXO family[27], while FOXO4 is reported to be closely associated with clubfoot[29]. Previous studies have shown that TAZ activators upregulate TAZ and p-Akt in a TAZ-dependent manner (data not shown). Therefore, we speculated that TAZ antagonizes myostatin partially because it reverses the inhibition of Akt by myostatin and the resulting increase of FOXO4.

Our previous studies confirmed that TAZ and its activator promoted muscle differentiation and resisted muscular atrophy, based on *in vivo* animal models of muscular atrophy induced by toxins, glucocorticoids[10], traumatic brain injury[16], disuse, starvation, and cancer-related cachexia[30]. These results suggest the potential of TAZ activators for treatment of muscle atrophy. In this study we selected gastrocnemius muscle tissue specimens from fetuses with congenital clubfoot. We confirmed that both TAZ and myostatin are involved in the pathological process of neurogenic clubfoot muscle atrophy, and they are negatively correlated. TAZ antagonizes myostatin-induced myotube atrophy, potentially through regulation of the Akt/FOXO4 signaling pathway. We also provide a theoretical basis for further application of TAZ activators in the treatment of neurogenic muscle atrophy.

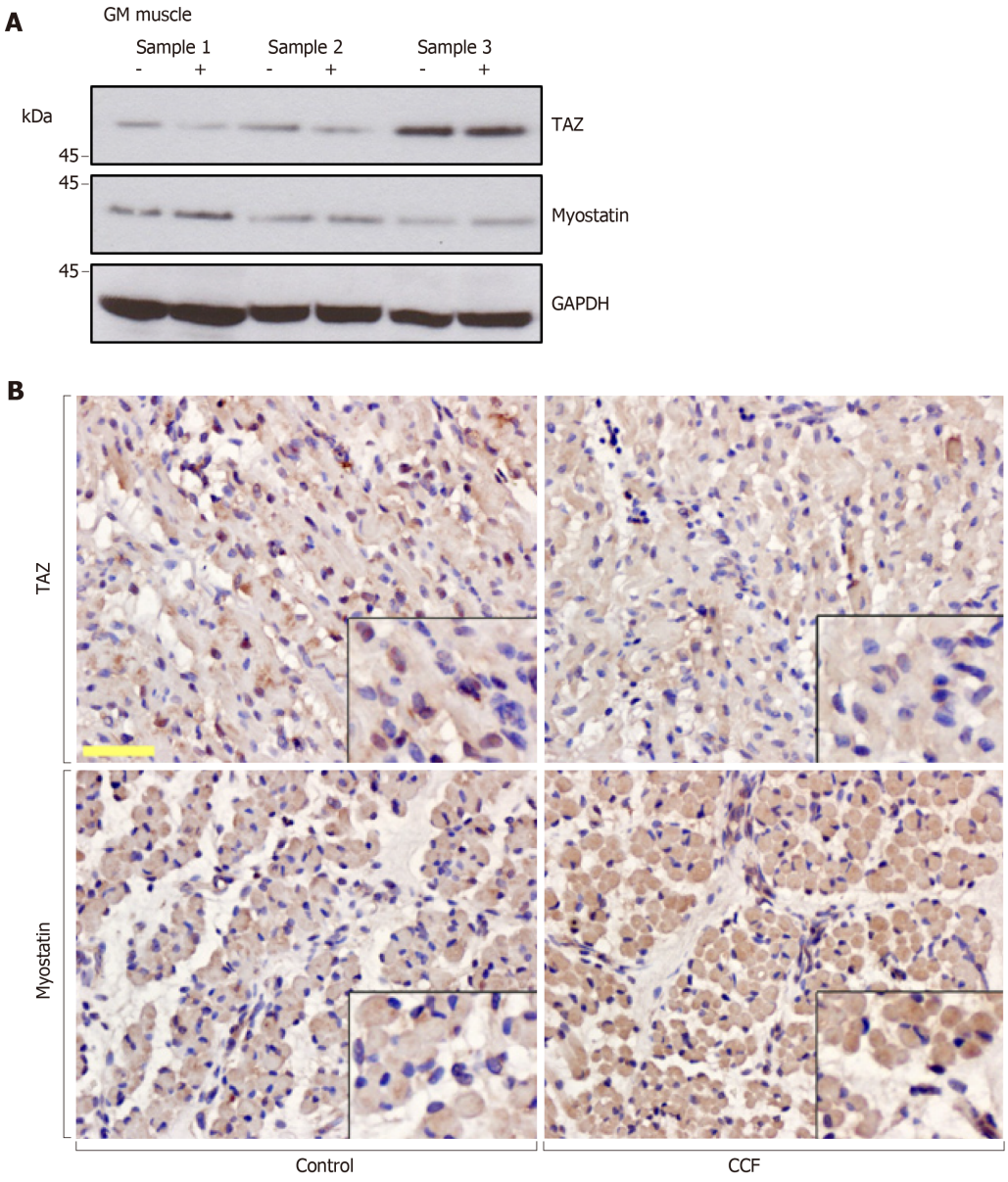

Figure 2 Expression of TAZ and myostatin in gastrocnemius muscles of fetus with congenital neurogenic clubfoot. A: Paired muscle specimens from three different fetuses are shown: normal limb (−); varus limb (+). Western blotting shows protein expression; GAPDH was used as the loading control; B: Immunohistochemical staining showed muscle fiber morphology and expression and location of TAZ and myostatin. Bar: 100 μm.

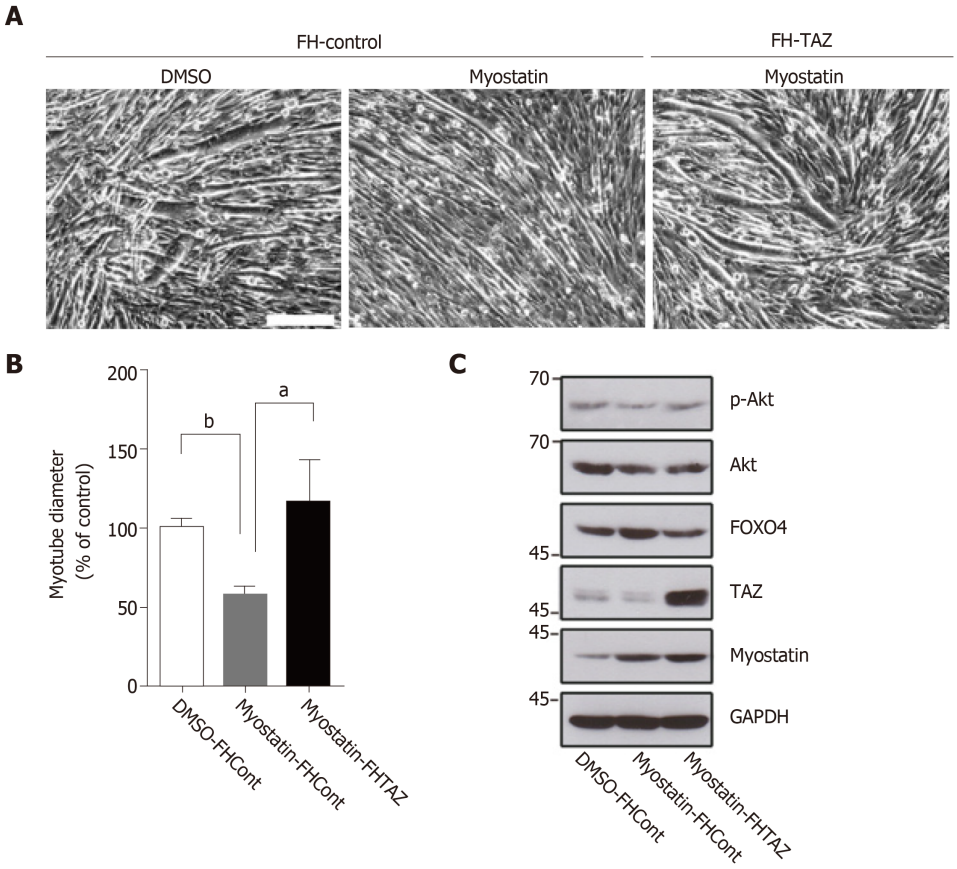

Figure 3 Myotube formation in each group of C2C12 cells and protein expression. A: Images of the myotube formation. Bar: 100 μm; B: Quantitative data and statistical analysis of myotube diameter in each group. $^{a}P < 0.05$, $^{b}P < 0.01$; C: Western blotting showing protein expression; GAPDH was used as the loading control.

ARTICLE HIGHLIGHTS

Research background

Muscle atrophy and volume reduction in neurogenic congenital clubfoot are the main factors causing limb mobility disorder, which seriously affects the quality of life of children. The rehabilitation treatment of muscle atrophy has great significance. At present, there are few reports on the study of clubfoot muscle atrophy. Therefore, it is of great clinical significance to use effective methods to diagnose and study in-depth mechanism research of clubfoot muscle atrophy.

Research motivation

Until now, the diagnose of clubfoot muscle atrophy is mainly depended on magnetic resonance imaging. Ultrasound is simple and real-time, and it is more suitable for follow-up prenatal observation and postnatal treatment. Therefore, this study clarifies the diagnostic value of 3D tomographic ultrasound imaging (TUI) for neurogenic congenital clubfoot muscle atrophy. There is no targeted drug treatment for clubfoot muscle atrophy currently, so this study attempts to reveal the mechanism of TAZ involvement in clubfoot muscle atrophy and to find drug therapeutic targets.

Research objectives

The objective of this study was to establish an ultrasound evaluation system for clubfoot muscle atrophy and to reveal the possible mechanisms of TAZ and myostatin involvement in clubfoot muscle atrophy and provide a theoretical basis for the development of potential drug therapy.

Research methods

Prenatal 2D and 3D ultrasound imaging was used to diagnose fetuses with neurological clubfoot. Quantitative evaluation of muscle atrophy was performed by 3D TUI. Muscle specimens were obtained, and protein expression was determined by western blotting and immunostaining. TAZ overexpressed C2C12 cells were differentiated and treated with myostatin, muscle differentiation of each group was compared quantitatively, and the Akt/FOXO4 signaling pathway expression was detected by western blotting.

Research results

The 3D TUI can detect the muscle atrophy on the varus side, which is significantly smaller than

the contralateral side. In the gastrocnemius specimens, TAZ and myostatin had opposite expression trends and were negatively correlated. Myostatin inhibited C2C12 muscle differentiation, manifested as thinner myotubes, and was rescued by TAZ overexpression. Overexpressed TAZ reduces the increased p-Akt and FOXO4 expression caused by myostatin.

Research conclusions

The 3D TUI technology can be used to evaluate neurogenic clubfoot muscle atrophy. TAZ and myostatin expression are negatively correlated in muscle samples. TAZ antagonizes muscle differentiation inhibition induced by myostatin, and the mechanism may potentially work through repression of the Akt/FOXO4 signaling pathway.

Research perspectives

Using 3D ultrasound to evaluate the clubfoot muscle atrophy prenatally and postpartum is effective. Appling previously developed TAZ activators for future research in order to develop new drug therapy of clubfoot muscle atrophy is necessary.

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ORIGINAL ARTICLE

Prospective Study

Effects of dual sofosbuvir/daclatasvir therapy on, chronic hepatitis C infected, survivors of childhood malignancy

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Author contributions: Yakoot M, Sherief LM, El-Shabrawi MH, Kamal NM and Helmy S designed the concept of study; Sherief LM, Yakoot M, El-Shabrawi MH, Almalky MA, AbdElgawad MM, Mahfouz AA, Kamal EM, Attia D and El-Khayat HR recruited the patients, conducted the study procedures and collected the data; Yakoot M interpreted the data and wrote the first draft of the manuscript; all authors reviewed the manuscript and approved the final version to be published; all authors have contributed to the manuscript in significant ways, have reviewed and agreed upon the manuscript content.

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Clinical trial registration statement:

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Abstract

BACKGROUND

Childhood cancer survivors are potentially at a higher risk of infection with hepatitis C virus (HCV). The effects of all-oral direct-acting antiviral therapy (DAA) on both the HCV infection as well as the state of cancer remission have not been well investigated in this population.

AIM

To test the effects of dual sofosbuvir/daclatasvir (SOF/DCV) therapy in the treatment of chronic HCV in survivors of hematologic malignancy in pediatric age group.

METHODS

The protocol was registered with a WHO Clinical Trial Registration ID: ACTRN12617000263392.

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

Conflict-of-interest statement: Mostafa Yakoot had conducted clinical trials on Pharco products. Sherine Helmy works and has stocks in Pharco Corporate. Other authors have nothing to disclose.

Data sharing statement: There is no additional data available.

CONSORT 2010 statement: The authors have read the CONSORT 2010 Statement, and the manuscript was prepared and revised according to the CONSORT 2010 Statement.

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We conducted a prospective, uncontrolled, open-label multicenter study. A total of 20 eligible, chronic HCV, genotype-4, infected children who had been in continuous complete remission from hematologic cancer (leukemia/lymphoma) for at least one year were included in the study. All patients were treated with combined SOF/DCV for 12 wk. Patients were monitored throughout the study till 12 wk after end of treatment for safety and efficacy outcomes including the sustained virologic response 12 (SVR12) rate, hematological indices, liver and kidney functions.

RESULTS

The intent-to-treat SVR12 rate was 20 of 20 (100%; 95%CI: 84%-100%). All patients showed normalized liver enzymes from week-4. All hematological indices, liver and kidney functions were kept normal throughout the study. No fatalities or treatment-emergent serious or severe adverse events were reported throughout the study.

CONCLUSION

SOF/DCV combined therapy could be used safely and effectively in the treatment of chronic HCV genotype-4 infection in leukemia/lymphoma treated children. No relapses were detected during treatment and throughout the follow up period for either the original malignant disease or the HCV infection.

Key words: Chronic hepatitis C; Survivors of childhood cancer; Sofosbuvir; Daclatasvir; Efficacy

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Core tip: Dual Sofosbuvir/Daclatasvir (SOF/DCV) treatment was found to be as effective in the treatment of chronic hepatitis C virus (HCV) in survivors of childhood hematologic malignancy as in others with no history of malignancy. No relapses detected during study for the original malignant disease or the HCV infection. The results of this study could be assuring that the treatment of chronic HCV infection with direct-acting antivirals (DAAs) might not adversely affect the state of remission of survivors of hematologic malignancy in pediatric age groups; though larger studies with different DAAs and longer follow up are needed to confirm.

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INTRODUCTION

Although the risk of transfusion-related-infection with hepatitis C virus (HCV) has become markedly low after the implementation of routine screening of the blood supply by the year 1992[1]; risk factors for HCV acquisition reported by many studies in middle and low income countries still include among others exposures to healthcare procedures, parenteral administration of drugs, chemotherapy and blood products transfusions[2,3].

Childhood cancer survivors are potentially at a higher risk of infection with HCV as they are more exposed to receive multiple transfusions of blood products and/or intravenous drug therapy[2,3].

HCV infection was shown to be associated with significantly increased morbidity and mortality from several cancers[3-5]. while, immunosuppression from cancer and/or the cancer-related treatment could increase the risk of chronicity, activation and progression to cirrhosis in patients infected with HCV[3,6].

Apart from hepatocellular carcinoma (HCC), the prevalence of HCV antibodies was reported to be much higher in patients harboring malignant diseases than others; in particular hematological malignancies which in some reports were associated with

HCV antibodies in as high as 30% of cases[7,8]. This has led The European Conference on Infection in Leukemia (ECIL-5) group of experts to recommend screening for all patients with hematological malignancies for hepatotropic viruses before hematological treatment and that patients with markers of past or current viral hepatitis should be assessed by an expert[9].

Nowadays, the direct-acting antivirals (DAAs) have changed the treatment paradigm for chronic HCV infection and improved virologic outcomes in all studied populations including HCV-infected adult patients with cancer[10]. As a result of the great advances in our understanding of cancer biology and the better survival of cancer patients with modern therapy; the populations of survivors of childhood cancer are becoming now of increased prevalence and importance worldwide. We have previously screened a group of 100 consecutive patients attending the follow up outpatient pediatric-hematology-oncology clinics in a university hospital in Egypt after achieving cure from hematologic cancers and we found that the prevalence of HCV infection is very high approaching 50% of the screened cases (unpublished data). The effects of DAAs treatment on both the HCV infection as well as the state of cancer remission have not been well studied in survivors of cancer in pediatric age groups.

There have been conflicting reports that survivors of hepatocellular carcinoma may be at increased risk of early recurrence with more aggressive course of malignancy after treatment with DAAs[11,12]. Although many other recent reports demonstrated that DAAs are not causative for de-novo cancer[13]; many hepatologists are still afraid to expose survivors of cancer (of any type) to be treated with DAAs.

We aimed to study the efficacy, safety and tolerability of dual sofosbuvir/daclatasvir (SOF/DCV) therapy in survivors of childhood hematologic malignancy infected with chronic HCV.

MATERIALS AND METHODS

This study is a part of our clinical evaluation program conducted by our group of investigators to test safety and efficacy of the new DAA therapy on different pediatric populations.

Study design and setting

The study had been designed as a prospective, single cohort, open-label, multi-center study and conducted in an outpatient setting.

Study cohort

Consecutive male or female patients, from eight to seventeen years of age, who had been detected from our HCV screening program for survivors of childhood hematologic malignancies to be infected with chronic HCV (by a positive HCV antibody test and/or positive serum HCV RNA), were subjected to screening for inclusion in the study according to our common protocol eligibility criteria described in our previous study[14]. Key exclusion criteria were: Pregnancy or lactation; concurrent other causes of hepatitis or HIV virus infection; active schistosomiasis; Child-Pugh score > six; alanine or aspartate aminotransferase (AST) > seven times the upper limit of normal, albumin < 2.8 g/dL; international normalized ratio > 2.3; transient elastography (by FibroScan®) result of more than 12.5 kPa at screening and/or an AST to platelet ratio index (APRI) of more than two; platelet count less than 50 × 10^9/L; severe anemia [hemoglobin grade-3 or higher (< 8 g/dL)]; any malignancy; Alfa-fetoprotein (AFP) level above 200 ng/mL; critically ill or more than slight limitation of activity; unwilling to participate or to sign the informed consent.

The study common protocol was reviewed and approved by the Research Ethics Committee of Faculty of Medicine, Alexandria University (IRB00007555) according to the Declaration of Helsinki. All subjects and their parents/guardians signed the informed consents before the start of the study interventions.

Procedures

Starting from September 2017, we contacted a convenient sample of 80 survivors of pediatric hematologic cancer (who had been in continuous complete remission for at least one year after completion of their treatment protocol for their malignant disease) from 4 pediatric centers in Egypt. All were subjected to blood analysis for HCV antibodies by the third generation enzyme-linked immunosorbent assay (ELISA-III) test and those found to be HCV antibody positive were further tested for serum HCV-RNA positivity and virus load using the polymerase chain reaction quantitative measurements by COBAS Amplicor 2.0, Roche Molecular Diagnostics, Pleasanton, CA, United States (lower limit of detection of 10 IU/mL). Those with a virus load

10000 IU/mL or above were subjected to full screening for eligibility criteria. The first 20 consecutive patients who fulfilled all eligibility criteria were included in this study as a single treatment group and received the study medications and regular assessments at study visits.

Study medications: All patients were subjected to a dual therapy in the form of a weight based daily doses of Gratisovir (Sofosbuvir) plus Daclatasvir (generic products produced by Pharco Pharmaceuticals, Alexandria, Egypt) for 12 wk duration. The daily dose of Sofosbuvir tablets was based on the body weight: One 400 mg tablet once daily for body weight 35 kg or above; or a dose of 200 mg once daily for those who weighed less than 35 kg. Daclatasvir was given in a daily dose: 60 mg once daily for body weight 35 kg or above and 30 mg once daily for those who weighed less than 35 kg.

Study visits: All other procedures done during all the study visits were the same as described in our previously published report[11]. All patients were subjected to full physical examination and investigations including the complete blood count, serum bilirubin, serum albumin, alanine aminotransferase (ALT), AST, prothrombin time, serum creatinine, serum HCV-RNA level (virus load) and ultrasonographic abdominal scan during the baseline visit (week 0) and all other study visits [week 2, 4, 12 (EOT)] and 24 (12 wk after EOT). Patients were then followed up for further 12 wk (till week 36) for detection of any relapse for either the HCV infection or the original malignant disease. Tests to exclude ineligible cases, tests for virus genotyping [using GEN-C 2.0 Reverse Hybridization Strip Assay (Nuclear Laser Medicine, Settala, Italy)] as well as liver biopsy /or transient elastography by FibroScan® (Echosens, Paris, France) were performed only at the screening visit (week 0). All patients were interrogated throughout the study for any adverse events and were requested to call by phone or visit at any unscheduled time for reporting any adverse event or query.

Outcome measures

Efficacy outcome measures: The primary efficacy outcome of this study was the proportion of patients achieving sustained virologic response 12 (SVR12) defined as serum HCV RNA below lowest level of quantification (LLOQ) at the end of 12 wk after end of treatment on intent-to-treat basis. Failure to sustain a virus load less than LLOQ either by the end of treatment or by the end of 12 wk after end of treatment is considered a virologic failure [on-treatment or post-treatment (relapse) failure] respectively.

Safety outcome measures: The primary safety outcome of this study was the incidence of any relapse or reactivation of the original treated malignant disease or any treatment-emergent adverse event (TEAE) as defined in previous study report[14]. All TEAEs were reported using the Medical Dictionary for Regulatory Activities version 20 (MedDRA v.20) lowest level and preferred terms. TEAEs deemed to have certain, probable or possible causality category according to Uppsala Monitoring Center causality categorization were considered in analysis for incidence rate and graded according to seriousness (serious/non-serious) and severity using the Common Terminology Criteria for Adverse Events v3.0.

Statistical methods

We presented our qualitative data as counts, proportions or percentage with the confidence interval using Wilson Score Interval Method. For quantitative data, descriptive statistics are the arithmetic mean, the standard deviation, the median and the 95% confidence interval whenever found appropriate. A repeated measure ANOVA design with one "within-factor" (liver enzymes) and one group with a total of 20 subjects; each subject is measured 3 times. This design achieves 81% power to test the "within-factor" if a Geisser-Greenhouse Corrected F Test is used with a 5% significance level and the actual effect standard deviation is 0.3.

RESULTS

Out of the 80 cancer survivors that were screened for anti-HCV antibodies, 34 (42.5%) were found positive. The confirmed positive patients for HCV RNA were 28 (35%), all were genotype-4. Twenty patients who fulfilled all the eligibility criteria and signed the informed consent were included in this study. All received treatment with dual SOF/DAC therapy and were compliant to the protocol throughout all the study visits (Figure 1).

The baseline demographics and clinical characteristics are presented in Table 1.

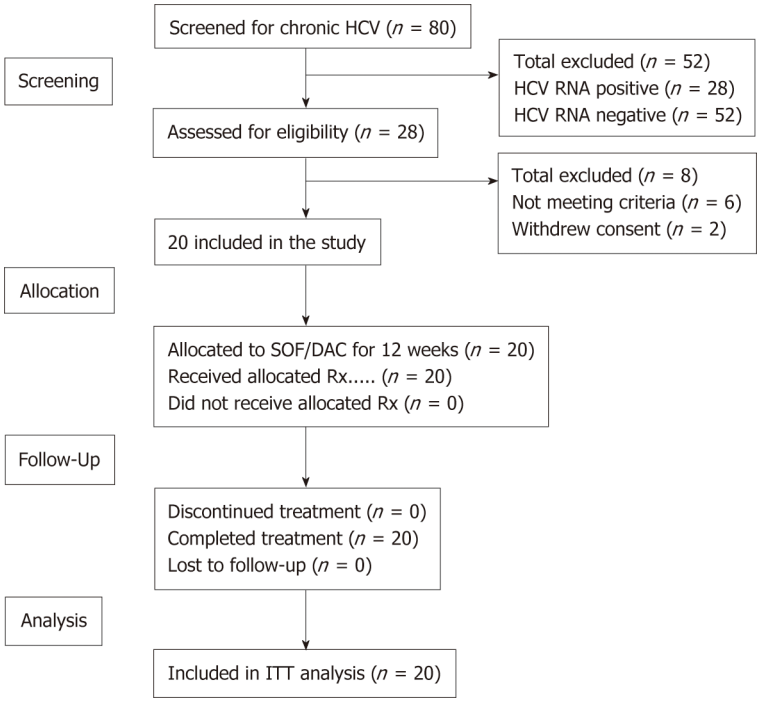

Figure 1 Patient flowchart.

The age ranged from eight to sixteen years with a mean (SD) of 11.6 (2.48) and 50% were males.

Seventeen were survivors of acute lymphoblastic leukemia (ALL); two were survivors of Hodgkin lymphoma and one of non-Hodgkin lymphoma. All had been in continuous complete remission for at least one year after the completion of their chemotherapy protocol.

All the 20 patients (100%) achieved viral negativity (serum HCV RNA below level of quantification) by the end of week 4 and remained so at the end of week 12 (end of treatment (EOT), week 24 (12 wk after EOT) and week 36 (24 wk after EOT). The intent-to-treat (ITT) sustained virologic response at week 12 after end of treatment (SVR12) rate was 20/20 (100%; 95%CI: 84%-100%).

Both mean serum ALT and AST enzymes were above normal levels at baseline and showed significant reduction towards full normalization by week 4 through the end of the study (Table 2 and Figure 2).

While all other tested biochemical and hematological parameters were within the normal ranges at baseline and remained so with no significant changes throughout the study visits (Table 2 and Figures 3, 4).

Safety outcome

No fatalities or serious adverse events were reported during the period of the study. Only seven (35%) patients reported 14 non-serious TEAEs throughout the study with causality assessment reports as possible or above. Nausea was reported by four (20%) patients, abdominal pain reported by three (15%) patients, fatigue reported by three (15%) patients, headache by 2 (10%) patients and pruritus or skin rash by 2 (10%) patients. All were mild to moderate (≤ grade-2) in severity.

No relapses were detected during treatment and throughout the follow up period for either the original malignant disease or the HCV.

DISCUSSION

To our knowledge this is the first study conducted to test the effects of dual SOF/DCV therapy on survivors of childhood malignancy, infected with chronic HCV. The SVR12 rate was 100% (95%CI: 84%-100%) which is concordant with the results of other published studies that tested efficacy of DAA agents on chronic HCV in other populations in pediatric age groups with no history of malignancy[14-17]. Also, the effects of treatment on other biochemical and hematological tests as well as other safety data matched the results of other studies conducted on other patients without history of malignancy.

Table 1 Baseline demographics and clinical characteristics, *n* (%)

Characteristic	Patients total (*n* = 20)
Age (y): Median (range)	11.5 (8-16)
Sex, male:	10 (50)
Duration (yr) after end of cancer treatment	4 (1- 9)
Type of cancer history	
ALL	17 (85)
Hodgkin Lymphoma	2 (10)
Non-Hodgkin Lymphoma	1 (5)
Weight (kg): Median (range)	37 (24-64.5)
Height (cm): Median (range)	137.5 (114-77)
BMI (kg/m^2): Median (range)	17.7 (14.56-24.89)
HCV RNA ($\log_{10}$ IU/mL): Mean (SD)	6.09 (0.72)
HCV RNA ≥ 800000 IU/mL	10 (50)
Interferon or ribavirin treatment experience	
Naïve:	19 (95)
Non-respondent to IFN Rx	1 (5)
METAVIR Score by Fibroscan	
F1	17 (85)
F2	3 (15)
WBCs (× 10/L): Median (range)	7.5 (4.1-13.8)
Hemoglobin (g/dL): Median (range)	12.05 (10-13.8)
Platelet Count (× 10/L): Median (range)	236.5 (66-444)
AST (IU/L): Mean (SD)	50.7 (29.4)
ALT (IU/L): Mean (SD)	54.3 (40.2)
S. Total Bilirubin (mg/dL): Median (range)	0.7 (0.2-1.7)
S. Creatinine (mg/dL): Median (range)	0.65 (0.2-0.8)

ALL: Acute lymphoblastic leukemia; BMI: Body mass index; WBCs: White blood cells; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; S: Serum; SD: Standard deviation; mg: Milligrams; DL: Deciliter; L: Liter; IU: International units.

Of particular importance in our findings that, there were no relapses detected for either the original malignant disease or the HCV during the study and all throughout the follow up period.

This finding is rather important and assuring to pediatricians treating survivors or patients with history of hematologic malignancy that this new class of DAA drugs (or at least SOF/DCV) might not adversely affect the state of remission of the original malignant disease, though further larger studies with longer follow up are needed.

This study also highlights the importance of screening for both survivors and patients with hematologic malignancy and goes well with the recommendations of ECIL-5[9]. Particularly that the rate of HCV infection in our screened "cancer survivors" sample (though small and non-random) was found unacceptably high 35%.

We acknowledge the small sample size and the short follow up duration of this pilot study which addresses a critical research question in a critical study population. But we hope that the results of this study might give other investigators and clinicians more confidence to include such population in their studies and real practice.

We hope that this study will prompt many other larger and longer studies to address the problem of HCV infection in both patients and survivors of cancer.

In conclusion, SOF/DCV combined therapy could be used safely and effectively in the treatment of chronic HCV genotype-4 infection in leukemia/lymphoma treated children. We detected no relapses/recurrence during treatment and throughout the follow up period for either the original malignant disease or the HCV infection.

Table 2 Mean (95% confidence interval) of main biochemical and hematological tests at 3 time points

Variable [Mean (95%CI)]	At baseline	At wk 4	At wk 12	Sig.
Serum. ALT (IU/L)	54.3 (35.49– 73.11)	19.55 (11.02-28.08)	17.45 (15.11-19.80)	< 0.05
Serum AST (IU/L)	50.7 (36.95-64.45)	24.05 (16.40-31.70)	19.95 (17.37-22.53)	< 0.05
T. Bilirubin (mg/dl)	0.66 (0.49-0.82)	0.53 (0.42-0.64)	0.55 (0.45-0.65)	No
S. Creatinine (mg/dl)	0.6 (0.52-0.68)	0.59 (0.51-0.66)	0.61 (0.54-0.68)	No
WBCs (× 10/L)	7.27 (6.3-8.2)	7.62 (6.8-8.5)	7.3 (6.5-8.1)	No
Hemoglobin (g/dL)	12.16 (11.7-12.6)	11.9 (11.38-12.35)	11.70 (11.13-12.27)	No
Platelets (× 10/L)	243 (203-283)	235.5 (200-271)	247.3 (213-281)	No

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; WBCs: White blood cells; g: Grams; mg: Milligrams; DL: Deciliter; L: Liter; IU: International units.

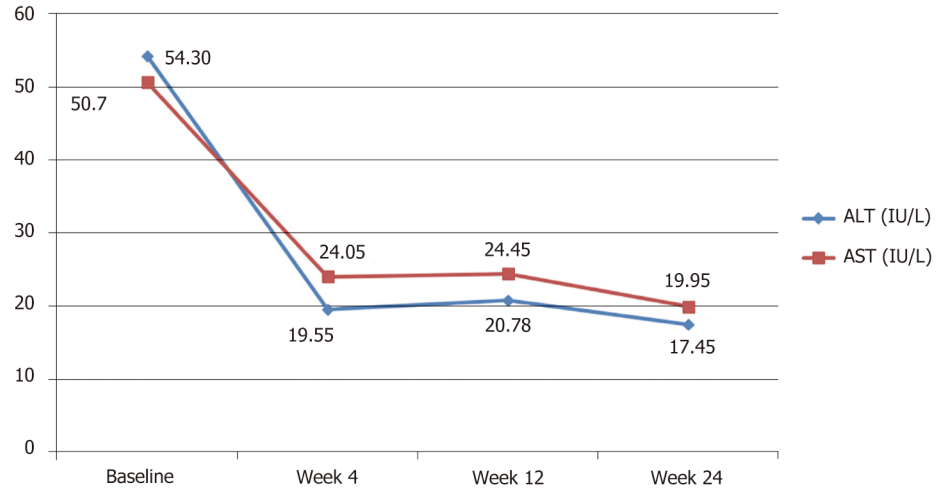

Figure 2 The mean serum alanine aminotransferase and aspartate aminotransferase (IU/L) at baseline and during follow up. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

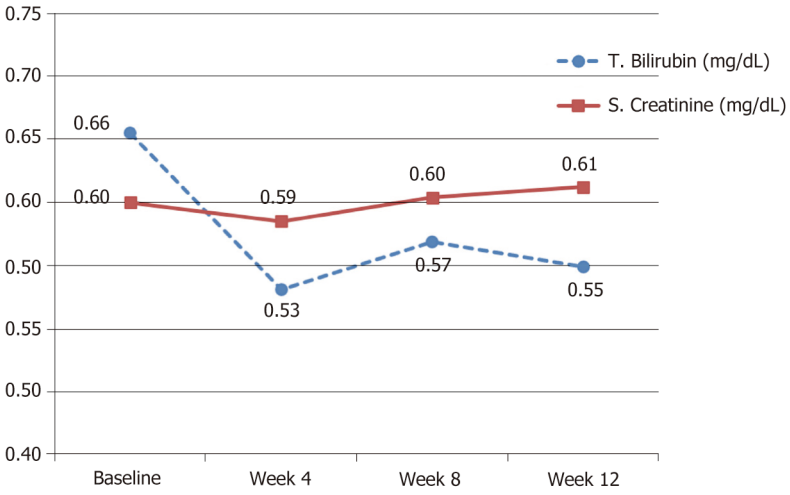

Figure 3 The mean serum bilirubin and creatinine at baseline and during follow up.

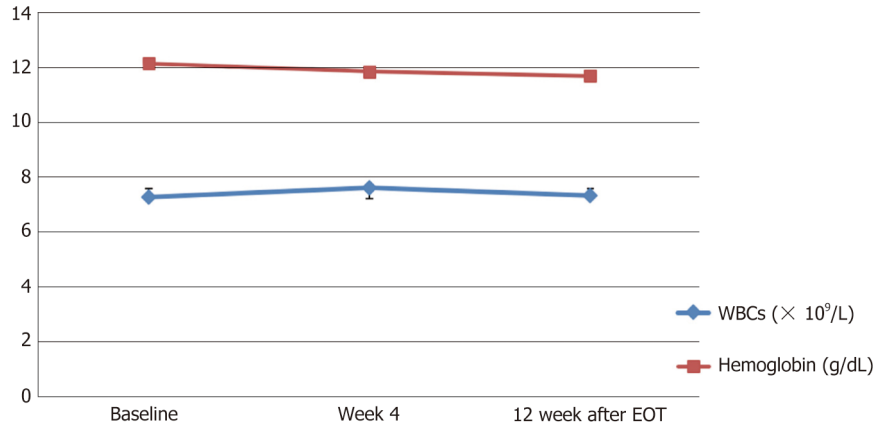

Figure 4 Mean hemoglobin and white blood cells count at baseline and during follow up. WBCs: White blood cells.

ARTICLE HIGHLIGHTS

Research background

Childhood cancer survivors are at a higher risk for infection with chronic hepatitis C (HCV). Sofosbuvir/Daclatasvir (SOF/DCV) dual therapy is currently a successful pan-genotypic option recommended by the world health organization for the treatment of adults infected with chronic HCV.

Research motivation

The effect of directly acting antiviral drugs (DAAs) on childhood cancer survivors infected with chronic hepatitis C (HCV) has not been well investigated. There is still hesitancy among pediatricians to start treatment with DAAs in this population for fear of negative impact on the state of complete remission.

Research objectives

To test the effects of SOF/DCV on both the HCV infection and the state of cancer remission in survivors of childhood hematologic malignancy.

Research methods

We conducted a prospective, uncontrolled multicenter study. A total of 20 eligible, chronic HCV, genotype-4, infected children who had been in continuous complete remission from hematologic cancer (leukemia/lymphoma) for at least one year were included in the study. All patients were treated with combined SOF/DCV for 12 wk. Patients were monitored throughout the study till 12 weeks after end of treatment for safety and efficacy outcomes including the sustained virologic response 12 (SVR12) rate, hematological indices, liver and kidney functions.

Research results

The sustained virologic response rate at 12 weeks after end of treatment (SVR12) was 20 of 20 (100%; 95%CI: 84%-100%). All patients showed normalized liver enzymes from week-4. All hematological indices, liver and kidney functions were kept normal throughout the study. No fatalities or treatment-emergent serious or severe adverse events were reported throughout the study.

Research conclusions

SOF/DCV combined therapy could be used safely and effectively in the treatment of chronic HCV genotype-4 infection in leukemia/lymphoma treated children. No relapses were detected during treatment and throughout the follow up period for either the original malignant disease or the HCV infection.

Research perspectives

The results of this study could indicate that dual SOF/DCV therapy is effective and safe in the treatment of chronic HCV in survivors of childhood hematologic malignancy. These results could also be assuring to pediatricians that the treatment of chronic HCV infection with DAAs might not adversely affect the state of complete remission of hematologic malignancy in pediatric age groups. This is hoped to encourage treatment and more studies on this population.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Hypoallergenicity of a thickened hydrolyzed formula in children with cow's milk allergy

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Author contributions: All authors conceptualized and designed the study; The authors all participated in the recruitment and follow-up of patients and were involved in data collection; the authors critically reviewed and approved the final manuscript.

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Abstract

BACKGROUND

Allergy to cow's milk is the most frequent allergy occurring in infants and young children. The dietary management of these patients consists of the elimination of any cow's milk proteins from the diet, and for formula-fed infants, the substitution of the usual infant formula with an adapted formula that is generally based on extensively hydrolyzed cow's milk proteins. The American Academy of Pediatrics has established specific criteria to confirm the hypoallergenicity of a formula intended for these children.

AIM

To assess the hypoallergenicity of a new thickened extensively hydrolyzed casein-based formula (TeHCF) in children with cow's milk allergy (CMA).

METHODS

Children diagnosed with CMA through a double-blind placebo-controlled food challenge (DBPCFC) were randomly administered increased doses of a placebo formula or the TeHCF [Allernova, new thickener including fibres (Novalac)] under double-blind conditions and medical surveillance on two separate days. Otherwise, both of these formulas and a cow's milk-based formula were randomly introduced to children who were highly suspected of having CMA on three separate days. Immediate and late reactions occurring after the introduction of any of these formulas were thoroughly recorded by the physician at the hospital and reported by parents to the physician after hospital discharge, respectively. If the children tolerated the TeHCF during the DBPCFC, they were exclusively fed this formula during a 3-mo period where potential allergic symptoms, anthropometric parameters, as secondary outcomes, and adverse events were registered. The Cow's Milk-related Symptoms Score (CoMiSS™) was assessed and anthropometric parameters were compared to World Health Organization (WHO) reference data.

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RESULTS

Of the 30 children included in the study, the CMA diagnosis of 29 (mean age: 8.03 ± 7.43 mo) patients was confirmed by a DBPCFC. The children all tolerated the TeHCF during both the challenge and the subsequent 3-mo feeding period, which they all completed. During the latter period, the CoMiSS™ remained at a very low level, never exceeding its baseline value (1.4 ± 2.0), growth parameters were within WHO reference standards and no adverse event related to the TeHCF was reported. Over the first week of this period, the proportion of patients with digestive discomfort significantly decreased from 20.7% (6/29) to 3.4% (1/29), $P = 0.025$. The proportion of satisfaction with the overall effect of the formula reported by the parents and investigator was high, as was the formula acceptability by the child.

CONCLUSION

The new TeHCF meets the hypoallergenicity criteria according to the American Academy of Pediatrics standards, confirming that the tested TeHCF is adapted to the dietary management of children with CMA. Moreover, growth was adequate in the included population.

Key words: Cow's milk allergy; Hypoallergenicity; Tolerance; Thickened extensively hydrolyzed formula; Digestive comfort; Dietary management

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Core tip: The hypoallergenicity of the new tested formula as the primary criterion was rigorously confirmed through a properly designed double-blind placebo-controlled food challenge (DBPCFC) in children with a diagnosis of cow's milk allergy (CMA) that was confirmed by a DBPCFC, the gold standard diagnostic procedure for food allergies. The subsequent 3-mo open exclusive feeding with the tested formula showed that the formula was still well tolerated by the 29 children with CMA included, their growth was adequate, and parents and the investigator were very satisfied with the effect of the formula.

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INTRODUCTION

In the EuroPrevall birth cohort, the incidence of cow's milk allergy (CMA) was reported to range from 1% to less than 0.3% in European children up to age 2, depending on the country considered[1]. The most rigorous food allergy diagnosis procedure, the double-blind placebo-controlled food challenge (DBPCFC), was used, and only allergic reactions occurring within 2 h of the DBPCFC and/or an increase in eczema within 48 h were considered for the CMA diagnosis, which might explain the very low incidences reported in some countries. In addition, the screening procedure might have lacked sensitivity for non-immunoglobulin (Ig) E-mediated gastrointestinal (GI) manifestations[2]. The CMA prognosis was quite good, as all children with non IgE-mediated CMA tolerated cow's milk as soon as one year after diagnosis and approximately 60% of children with IgE-mediated CMA displayed tolerance[1]. According to paediatric guidelines, the cornerstone of CMA dietary management is the implementation of a cow's milk protein (CMP) elimination diet. For non-breastfed infants and young children, it mainly consists of feeding them special infant formulas whose protein fraction comprises extensively hydrolyzed CMP (eHF), soy or rice proteins[3,4,5,6,7]. These formulas are well tolerated by most children with CMA. However, up to 10% of these children may react to eHF. In these cases, or when CMA is severe, the use of an amino-acid based formula (AAF) is recommended[6,7]. The American Academy of Pediatrics established criteria to determine the hypoallergenicity of any formula intended for children with CMA[8].

The primary objective of this study was therefore to evaluate the hypoallergenicity of a new thickened extensively hydrolyzed casein-based formula (TeHCF) in children with CMA proven by a DBPCFC.

MATERIALS AND METHODS

The primary outcome was the tolerance/hypoallergenicity of the tested formula, which was defined as the absence of intolerance during the DBPCFC with the TeHCF in infants with a proven CMA. In addition, CMA symptoms, growth, tolerance of the study formula and investigator's and parents' satisfaction with the study formula were analysed as secondary outcomes. This clinical trial comprised two phases, the first consisting of the DBPCFC with the TeHCF, and the second consisting of exclusive TeHCF feeding for 3 mo (Figure 1) by all patients. Once reconstituted, the tested study formula [Allernova, new thickener (Novalac, United Pharmaceuticals, Paris, France)] had an energy density of 67 kcal/100 ml and contained 1.6, 3.5, 6.9 and 0.5 g of proteins, lipids (including arachidonic acid and docosahexaenoic acid), carbohydrates and fibre per 100 mL, respectively. The new thickener was a patented mix of fibres, including pectin and locust bean gum. The formula was lactose free and complied with the European regulation in force at the start of the study[9].

Infants (1) Aged between 1 and 36 mo old; (2) Who were strongly suspected of having CMA or were diagnosed with a CMA that was confirmed by a DBPCFC performed within the last 2 mo; (3) Successfully fed an elimination diet for at least 2 wk as recommended by guidelines on food challenge procedures[10,11] and (4) Whose parents signed the informed consent form were included. The main exclusion criteria were the exclusive or major consumption of mother's milk at study enrolment, a past anaphylactic reaction, a history of a lack of improvement of allergic symptoms when previously fed an eHF since for these children an AAF is recommended[4,6,7,8], or any situation that, according to the investigator, might interfere with study participation.

If a CMA was already proven by a DBPCFC before study inclusion, the child underwent a 2-d DBPCFC: the placebo formula, namely, an AAF (Neocate, Nutricia), or the TeHCF were introduced in a random order on two different days [food challenge (FC) 1 and FC2] separated by at least 7 d. Otherwise, a "combined food challenge" was conducted: the placebo formula, the TeHCF and a cow's milk-based formula were introduced in a random order on three different days (FC1, FC2 and FC3), each separated by at least 7 d. This schedule was chosen since participation in two DBPCFCs, one to assess the hypoallergenicity of the TeHCF and one to prove CMA, would have been too cumbersome for both parents and the child.

Before each FC day at the hospital, the investigator ensured that the child did not present any clinical abnormalities and had stopped all medications including antihistamines that could have interfered with the administration of the challenge. Increased volumes of TeHCF, placebo or cow's milk-based formula were fed to the child in a blinded manner every 20 min under medical supervision. The placebo formula and the TeHCF were reconstituted according to the manufacturer's instructions; for blinding, the latter was mixed with the placebo formula at a 2:1 ratio. The cow's milk-based formula was standard infant formula, full fat or semi-skimmed cow's milk, that was mixed with or without the placebo formula, according to the usual practice of the service. The prescribed schedule was 0.5, 1, 3, 10, 30, 50 and 100 mL. The administered formulas were prepared by a staff member who was not involved in the patient's care. The investigator, the nursing staff, and the family were therefore not informed of what formula the child was being fed.

All objective and subjective symptoms were registered using a standardized symptom score[10,11] (Supplementary Table 1). If subjective clinical symptoms occurred, the last dose administered was repeated without an increase. The challenge was normally pursued in the absence of objective symptoms; otherwise, it was stopped, and the child was treated as deemed necessary by the investigator. The child was monitored for 2 h after the administration of the last dose, or longer if required according to his condition.

Once at home and until the next FC day, the child continued his usual CMP elimination diet by being fed the formula that was successfully consumed before study inclusion; moreover, his parents were instructed not to introduce any new foods. The child's parents noted any change in their usual child's regurgitations, stools, or mood in a diary to detect any late-occurring reactions on each FC day and the two subsequent days. The parents also recorded the appearance of any allergic symptoms within the week after each FC day. At the onset of any delayed reaction, the parents were advised to immediately contact the investigator to discuss further actions. At the end of each follow-up period, the child was clinically examined and

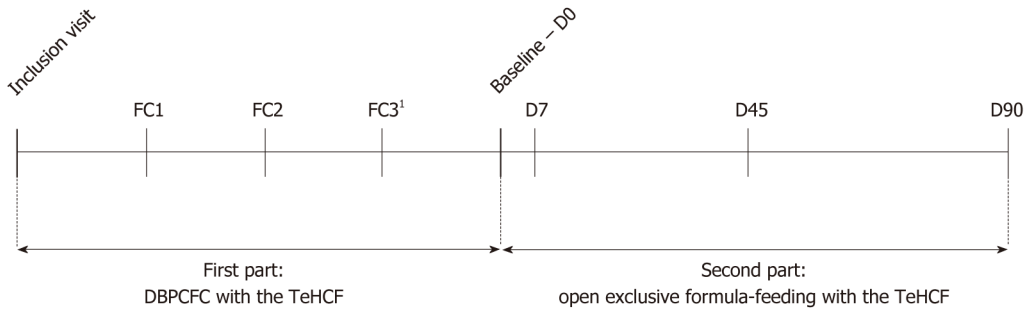

Figure 1 Study design.[1]FC3 was only performed as a combined challenge for patients with a cow's milk allergy that was not proven by a double-blind placebo-controlled food challenge before study inclusion. FC: Food challenge; D: Day; DBPCFC: Double-blind placebo-controlled food challenge; TeHCF: Thickened extensively hydrolyzed casein-based formula.

the potential symptoms reported by his parents were assessed by the investigator with regard to allergy.

If the TeHCF was tolerated during the DBPCFC and the CMA was confirmed, the child continued the study and started the open exclusive formula-feeding with the TeHCF at D0 visit (also named Baseline visit, Figure 1) that consisted of a total replacement of the substitution formula used before D0 visit by the TeHCF. In addition, the child continued his usual CMP elimination diet. Then, the child was monitored at a consultation on days 7, 45 and 90 (D7, D45 and D90 visits). On each of these visits, the investigator asked parents about the presence of potential allergic symptoms in their child. Based on this information, the investigator administered the Cow's Milk-related Symptoms Score (CoMiSS™), a tool aiming to evaluate and quantify the evolution of symptoms during therapeutic interventions[12]. CoMiSS™ consists of 6 sub-scores and ranges from 0 to 33 points (Supplementary Table 2). If eczema was present, the SCORing Atopic Dermatitis (SCORAD) index was also measured[13]. As the SCORAD index has previously been reported during dietary interventions in infants with CMA and eczema, it was chosen for comparison purposes. The frequency of vomiting episodes and the intensity of digestive discomfort were assessed using 4-item scales (Supplementary Table 2). The presence of angioedema and blood in stools were noted; the mean number of stools passed on the last three days, the child's sleep quality (quiet, *i.e.*, the absence of or few awakenings, or agitated, *i.e.*, excessive waking with no clear cause) and the parental satisfaction with the child's sleeping time were registered. The compliance was evaluated by the investigator at each visit by asking parents if the child accepted the formula's taste, if he/she had stopped the exclusive formula-feeding with the TeHCF or if he/she had taken another formula and what was the average volume of TeHCF taken by the child over the last 3 d. In case of poor compliance to feeding recommendation, or definitive interruption of the TeHCF feeding, the investigator could decide to end the child's study participation. The investigator also ranked his overall satisfaction with the effect of the study formula on the child using a 4-item scale ranging from very unsatisfied to very satisfied. Body weight, length and head circumference were recorded at each study visit. Adverse events experienced by all patients included in the study who received at least one of the two or three products administered on FC days were monitored.

During the first 7 d of the open feeding with TeHCF, and 3 d before the D45 and D90 visits, parents noted the volumes of formula consumed by the child, the types of foods eaten, and the same parameters recorded in the diaries after FC days. They also rated their satisfaction and the formula acceptability by their child, from very unsatisfied to very satisfied.

The results of atopy patch test (APT), Skin Prick Test (SPT) and the determination of the dosage of specific IgE (sIgE) to any type of allergen performed before or during the study period, and if deemed necessary by the investigator according to his usual practice, were registered. The APT was conducted as recommended[14] with a specific patch test system on which fresh cow's milk and a negative control were placed. The SPT was performed using the appropriate specific allergen extracts and positive and negative controls; the tests were interpreted 15 – 20 minutes after application. The dosage of serum sIgE was determined with a standardized ELISA. IgE-mediated CMA was defined as the presence of one sIgE to CMP (α-lactalbumin, β- lactoglobulin or casein) at a concentration greater than 0.1 kU/L in plasma or, for the SPT, a difference in the diameter between CMP wheal and negative control greater than 3 mm.

Table 1 Clinical and allergy characteristics of patients in the hypoallergenic population at inclusion (*n* = 29 patients), *n* (%)

Characteristics	*n* (%)
Age, mo	8.03 (7.43)
Male	16 (55.2)
Anthropometric characteristics at birth	
WFA z-score, mean ± SD	0.1 (1.0)
LFA z-score, mean ± SD	0.2 (1.3)
HCA z-score, mean ± SD	1.0 (1.0)
Gestational age, mean ± SD, wk	38.5 (2.3)
Preterm	6 (20.7)
Anthropometric characteristics at inclusion	
WFA z-score, mean ± SD	-0.3 (1.2)
LFA z-score, mean ± SD	0.0 (1.4)
WFL z-score, mean ± SD	-0.4 (1.0)
BMI-for-age z-score, mean ± SD	-0.4 (1.0)
HCA z-score, mean ± SD	0.7 (1.2)
Feeding history	
Breastfeeding in the past	22 (75.9)
Duration of (exclusive and/or partial) breastfeeding, mean ± SD, wk	17.6 (16.2)
Duration of feeding with the formula taken before inclusion, mean ± SD, wk	10.8 (13.1)
Average volume of the formula taken before inclusion, mean ± SD, mL/d	655.2 (199.3)
Solid foods diversification	18 (62.1)
Allergy history	
At least one parent or sibling with a medically confirmed allergy	14 (48.3)
Parents' smoking habits	
Past only	5 (17.2)
Current	11 (37.9)
Mother only	4 (13.8)
Father only	6 (20.7)
Both parents	1 (3.4)
Age at onset of first CMA symptoms, mean ± SD, mo	3.6 (4.4)
Time since beginning of the elimination diet, median (min–max; IQR), wk	8.3 (2.0–125.3; 9.7)
Time elapsed between the onset of allergy symptoms and the initiation of the elimination diet, median (min – max; IQR), wk	1.9 (0.1-46.0; 5.7)
Type of first CMA symptoms	
Exclusively digestive	10 (34.5)
Exclusively cutaneous	2 (6.9)
Digestive and cutaneous	3 (10.3)
Digestive symptoms and other symptoms such as crying, irritability, abdominal pain, and agitated sleep	5 (17.2)
Digestive symptoms and failure to thrive	6 (20.7)
Digestive, cutaneous and other symptoms such as crying, irritability, and agitated sleep	3 (10.3)
Delay of first CMA symptoms	
Immediate	1 (3.4)
Delayed	28 (96.6)
Type of food triggering the first CMA symptoms	
Mother's milk	4 (13.8)
Infant formula	25 (86.2)

SD: Standard deviation; WFA: Weight-for-age; LFA: Length-for-age; WFL: Weight-for-length; BMI: Body mass index; HCA: Head circumference-for-age; CMA: Cow's milk allergy; IQR: Interquartile range.

The study was conducted in the Pediatric Gastroenterology, Liver and Digestive Endoscopy Unit of University Hospital Umberto I, Roma, Italy. The study protocol was approved by the independent Ethics Committee of the Sapienza University, Roma, Italy. This study was conducted in accordance with ethical standards established in the Declaration of Helsinki. Parents/legal guardians provided written

Table 2 Characteristics of reactions (immediate and delayed) observed after cow's milk introduction during the double-blind placebo-controlled food challenge performed before (*n* = 19) or after study inclusion (*n* = 10), *n* (%)

Characteristics of patients with immediate reactions (*n* = 5)	
Types of reactions	
Objective gastrointestinal complaints	5 (100.0)
Severe reaction	1 (20.0)
Moderate reaction	1 (20.0)
Mild reaction[1]	3 (60.0)
Time of reaction after the ingestion of first CMP dose, mean ± SD, min	98.0 (4.5)
Eliciting dose of CMP, mean ± SD, g	2.2 (1.3)
Cumulative dose of CMP, mean ± SD, g	4.7 (2.9)
Characteristics of patients with delayed reactions only (*n* = 24)	
Types of reactions	
Digestive and cutaneous symptoms[2]	2 (8.3)
Upper digestive symptoms (regurgitations and vomiting)[2]	8 (33.3)
Lower digestive symptoms (changes in stools frequency/consistency and bloody stools)[2]	9 (37.5)
Lower and upper digestive symptoms[2]	3 (12.5)
Eczema	1 (4.2)
Irritability/crying[3]	1 (4.2)
Cumulative dose of CMP, mean ± SD, g	3.9 (1.8)
Time of reaction after hospital discharge	
Within 6 h	11 (45.8)
Between 6 and 12 h	5 (20.8)
> 24 h	8 (33.4)

[1]These patients also presented delayed digestive reactions, such as diarrhoea or regurgitations, at an average of 3.7 ± 2.1 h after hospital discharge;
[2]associated with or without general symptoms such as crying, irritability, and changes in behaviour;
[3]this patient had a positive Skin Prick Test to cow's milk. CMP: Cow's milk proteins.

consent regarding their willingness to participate and the study procedures.

Statistical analysis

A formula must show at 95% confidence that it does not provoke allergic reactions in 90% of subjects with a confirmed CMA to be considered hypoallergenic[8]. In a study with a binomial outcome (reaction versus no reaction), the sample size is determined by calculating a binomial confidence interval (CI) for p, the probability of having a reaction, as reported in a previous study[15] . In the case of 0 observed reactions, the upper 95%CI for P is < 0.10 when the sample size is 29 participants. Thus, a study including at least 29 subjects in which none were classified as positive in the DBPBFC enables the investigator to conclude that the study provided 95% confidence that at least 90% of children with a confirmed CMA who ingest the tested formula would not experience a reaction. The primary criterion was assessed using the hypoallergenic population, namely, patients with a proven CMA and complete DBPCFC. A DBPCFC was considered as complete when the child was administered the FC on all days (two or three days) and was monitored at a consultation up to 7 d after each FC day. The secondary outcomes were evaluated using patients from the hypoallergenic population who completed the second part of the study.

All statistical analyses were performed at the 0.05 global significance level using two-sided tests. Data obtained at each visit were compared to baseline data (D0 visit corresponding to the start of exclusive formula-feeding with the TeHCF) using a Wilcoxon test or Student's *t*-test for quantitative parameters, depending on the normality of the distribution. For qualitative parameters, McNemar's test or a symmetry test (if more than 2 classes) was used. Statistical analyses were conducted using SAS version 9.2.

The body mass index (BMI) was calculated and z-scores of weight-for-age (WFA), length-for-age (LFA), weight-for-length (WFL), BMI-for-age and head circumference-for-age (HCA) were computed based on World Health Organization (WHO) growth standards for healthy breastfed infants[16]. Specific charts were used for preterm infants less than 64 wk of postmenstrual age[17]. For older preterm infants, z-scores were computed using WHO growth charts and the corrected age.

RESULTS

Thirty patients were included in this clinical trial from April 2016 to July 2017. One patient did not have a confirmed CMA and was therefore excluded from the hypoallergenic population. The remaining 29 patients were aged from 1 to 31 mo at inclusion (median age: 6 mo). At that time, 4 patients were partially breastfed, but their mother's diet was devoid of CMP. Twenty-two subjects were fed an eHF and 7 a vegetable-based infant formula: One was fed a rice-based drink not adapted to infant feeding and the other 6 were fed a rice-based infant formula. Other characteristics of the patients' feeding history are detailed in Table 1.

At inclusion, subjects had received a CMP elimination diet for an average of 15.6 ± 24.8 wk. One patient also eliminated egg albumen and soy. Other details on the allergic history are described in Table 1. Of the 3 patients on whom an APT to cow's milk was performed, only one showed a moderate reaction. Three of 9 patients had a positive SPT to cow's milk. Another patient underwent a blood test for sIgE to CMP and the titre of the antibodies against β-lactoglobulin exceeded 0.35 kU/L; therefore, 4 patients were considered as having an IgE-mediated CMA. One patient had a positive SPT to cat, another to egg albumen and house dust mites, and another to tomatoes.

The CMA of 19 patients was confirmed by a DBPCFC that was performed at an average of 2.7 ± 1.8 wk before study inclusion. In the hypoallergenic population, 5 patients experienced an immediate reaction after cow's milk introduction, *i.e.*, within 2 h after the last administered dose, and 24 only experienced delayed reactions (Table 2).

All patients tolerated the TeHCF; none showed an immediate or delayed reaction after having ingested the entire planned volume of formula. Likewise, all patients tolerated the placebo formula. During the food challenge follow-up, parents of one patient reported an increase in regurgitations and irritability after the administration of the TeHCF; parents of another child noticed a higher frequency of stools and a change in their consistency, although these changes were also reported after the administration of the placebo formula. Finally, the parents of 2 other children reported changes after the administration of the placebo formula: For one, a higher stool frequency and lower stool consistency and for the other, irritability and an increase in crying duration. The investigator did not consider any of these changes as allergic reactions related to the TeHCF or placebo formula.

All patients were fed the TeHCF for the open 3-mo period and none dropped out of the study due to intolerance to the formula, poor compliance to feeding recommendation or definitive interruption of the TeHCF feeding. No significant differences in CoMiSS™ or any of its sub-scores were noted between the baseline and D7. The TeHCF was therefore well tolerated when consumed as the exclusive formula. At baseline, the CoMiSS™ score was very low, on average 1.4 ± 2.0. Notably, 51.7% (15/29) of patients had a null CoMiSS™ score and the maximum CoMiSS™ score was 6 for only 3 patients (10.3%). After 7 d of treatment, the mean CoMiSS™ score decreased slightly to 0.7 ± 1.2, and remained very low, never exceeding the mean baseline CoMiSS™ score, during the entire course of the study.

At baseline, the most severe intensity of regurgitations on the CoMiSS™ scale was observed for only 3 patients who experienced more than 5 episodes of regurgitations/day with a volume of more than one coffee spoon; at D7, regurgitations improved for all these patients. The stools sub-score was also very low at baseline, an average of 0.62 ± 1.21, and did not change significantly throughout the study. Additionally, 20.7% (6/29) of patients cried for more than 1.5 h daily at baseline; after 7 d of TeHCF feeding, only 3.4% (1/29) of patients cried for more than 1.5 h per day, this change tended to be statistically significant (P = 0.06). One patient presented eczema lesions: The SCORAD index decreased from 20.5 at D0 to 5.1 at D45. None of the patients presented urticaria or respiratory symptoms from baseline to the end of the study. Vomiting was reported for only one patient on one visit (D45), and another patient presented bloody stools also only once during the study (D90). Notably, 20.7% (6/29) patients presented digestive discomfort at baseline; this percentage significantly decreased after 7 d of TeHCF feeding to 3.4% (1/29, P = 0.025).

The majority of patients (51.7%, 15/29) did not show changes in their daily stool frequency, which remained in the normal range throughout the study, *i.e.*, from 1 to 4 stools passed/day[18]. During the entire study course, the great majority of parents (86.2% (25/29)) were satisfied with the sleeping time of their child. At baseline, approximately 90% of patients (26/29) experienced quiet sleep; this proportion reached 96.6% at D90.

The mean WFA z-score significantly increased from -0.3 ± 1.2 at baseline to -0.2 ± 1.3 at D45 (P = 0.025) and to 0.1 ± 1.2 at D90 (P < 0.001) (Figure 2). The same significant evolution was observed for the mean WFL and BMI z-scores, which were also slightly negative (Table 3) at baseline and increased by 0.3 ± 0.6 and 0.6 ± 0.9 after

45 and 90 d of TeHCF feeding, respectively.

At each visit and for all patients, the investigator was satisfied or very satisfied with the overall effect of the formula. Regardless of whether they were interviewed after seven or 45 d of TeHCF feeding, more than 80% of parents were satisfied to very satisfied with the global effect of the formula (Supplementary Figure 1). Moreover, throughout the study, more than 85% of parents were satisfied to very satisfied with the formula acceptability by their child.

All children in the hypoallergenic population complied to feeding recommendations, with the exception of 5 who were introduced to a new food allergen within days following the challenge with CMP, but without consequence, as these foods were subsequently consumed with no reaction reported. On average, the patients consumed between 500 to 600 ml of TeHCF throughout the study and their diet was strictly devoid of CMP.

Children participated in the study for an average of 112.5 ± 20.7 d and were fed the study formula for 90.4 ± 17.2 d. Except for reactions observed after cow's milk introduction in patients exposed to a combined challenge, only one adverse event was reported (viral gastroenteritis) during the study and was not considered related to the TeHCF.

DISCUSSION

Based on the findings of the present study, the new TeHCF tested in children with a proven CMA and which correspond to the patient population the most likely to benefit from this type of formula, *i.e*. children aged 0-36 mo, meets the criteria of hypoallergenicity according to the AAP[8]. No allergic reactions were noted after the ingestion of this TeHCF, whether it was progressively administered at increasing doses under medical surveillance at the hospital or when consumed as the exclusive formula at home under the parents' observation. In addition, children who were fed the tested TeHCF for 3 mo showed increased growth *z*-scores within WHO reference standards. In conclusion, the new TeHCF is hypoallergenic and appropriate for consumption by children who are allergic to CMP because of its good tolerance and safety.

The methodology adopted in this study was rigorous. First, the CMA was proven through a DBPCFC, the strictest diagnostic tool, and the same procedure was used to introduce the TeHCF. In addition, immediate reactions occurring during food challenge were graded using an allergy symptoms scale derived from the PRACTALL consensus report[10] to ensure that each subject's challenge was conducted, monitored, and interpreted in a uniform manner. Finally, children who were highly suspected of having a CMA were included in that study, but underwent a combined food challenge. This approach was used to avoid the excessive burden imposed by 2 DBPCFCs on children and their parents. Therefore, the introduction of the placebo formula was not repeated and was used for both the CMA diagnosis and the evaluation of the TeHCF hypoallergenicity. Conversely, no control group was investigated during the second study phase, which is the main methodological limitation of this clinical trial. The TeHCF could have been compared to a control formula, especially for the evaluation of the satisfaction of the formula effect by clinician and parents. However, the design was adequate for confirming the hypoallergenicity of the new TeHCF in allergic children, the primary objective of the present study. Another limitation is that information on late reactions after the introduction of cow's milk, TeHCF or placebo formula was initially obtained from the parents. However, the hospitalization of children for more than a day to perform a DBPCFC is hardly feasible. Moreover, children were clinically re-examined by the investigator and parents were required to accurately register the occurrence of any delayed reactions in diaries, as already performed before[19,20] to enable their objective evaluation by the investigator at follow-up visit.

When tested for a CMA diagnosis, most children reacted within the day following the introduction of intact CMP, highlighting the importance of the open feeding period to ensure that no delayed symptoms will appear after the first introduction of a new formula. The AAP requests a 7-d open feeding period and a proper monitoring of the onset of potential symptoms to confirm the hypoallergenicity of a formula[8]. Some studies employing a similar design to the present clinical trial also included an open feeding period where parents were requested to note any occurrence of allergic symptoms. Among the most recent studies, in 30 infants with CMA who underwent a DBPCFC with an AAF, no serious adverse events were reported during the ensuing 7-d feeding period that was completed by 24 patients[20]. Another AAF was tested in 30 of 33 included children with CMA during a similar period[19]. Vomiting (4 subjects)

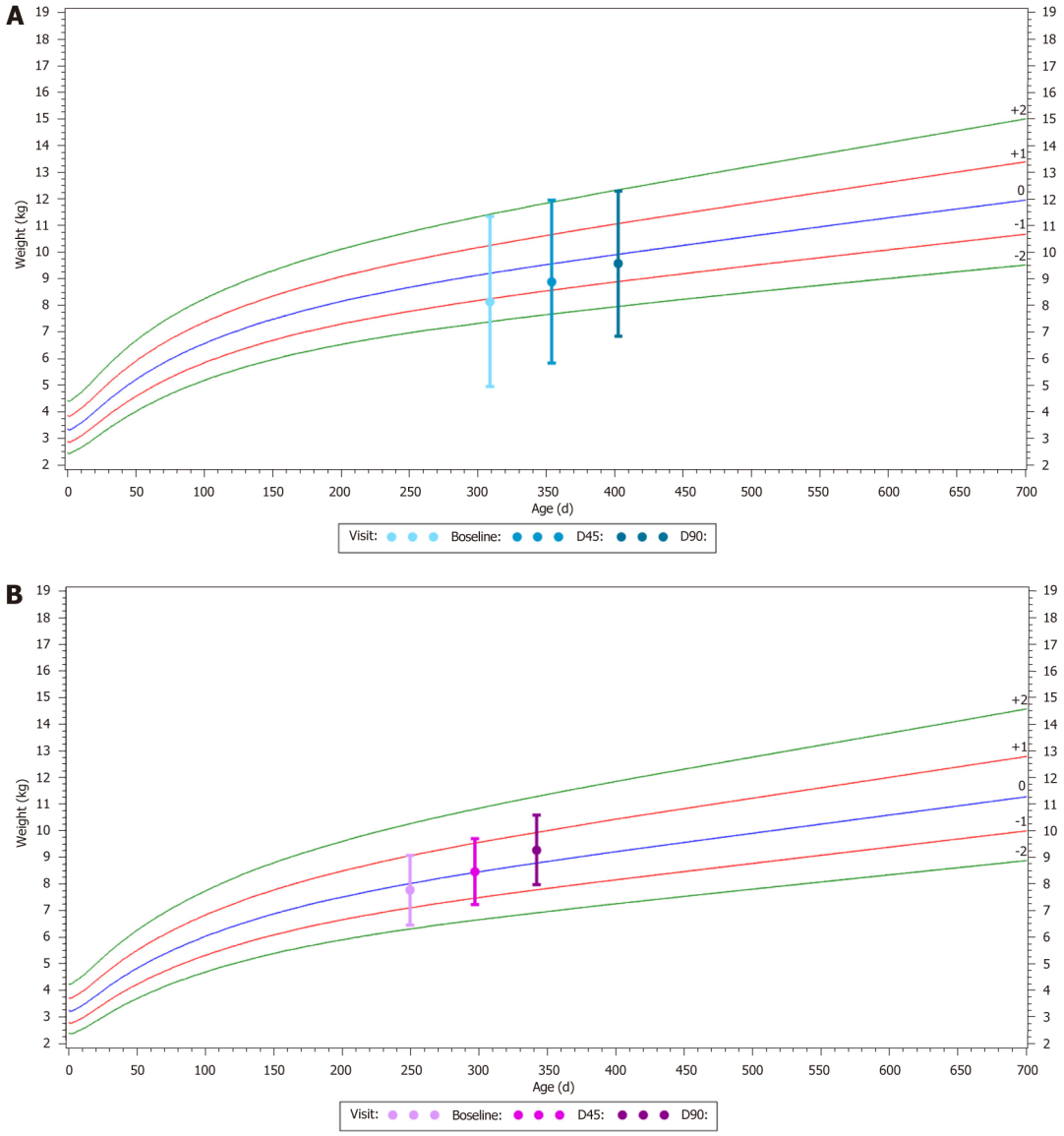

Figure 2 Mean weight ± SD at each visit shown compared with the World Health Organization growth standards for boys (A) and girls (B) fed the thickened extensively hydrolysed casein-based formula.

due to the formula palatability, erythema for one subject, itchy skin on the back for one subject and a mild stomach ache in another subject appeared but then subsided, and the consumption of the AAF was not discontinued. The tested TeHCF showed a good tolerance as well: none of the patients ceased its consumption during the open exclusive formula feeding period, and the CoMiSS™ decreased slightly after the first 7-day feeding period.

Globally, during the second study phase, the CoMiSS™ remained at a low level, in contrast to findings reported in previous studies, *i.e.*, significant decreases in CoMiSS™ after interventions with therapeutic formulas such as eHCF, eHF based on whey proteins or rice-based infant formula[21,22,23,24]. In the present study, children were asymptomatic for at least two weeks upon inclusion and then underwent the DBPCFC with an at least one-week interval between each FC day to allow potential symptoms to resolve. Between each FC day and until the start of the exclusive formula-feeding with the TeHCF (*i.e.*, at Baseline – D0 visit), the child was fed the formula that was successfully consumed before study inclusion; thus, the CoMiSS™ was very low at baseline. The tolerance of the new TeHCF was evidenced by the maintenance of the CoMiSS™ at a low level when children were exclusively fed this formula. Recently, the mean CoMiSS™ score of 413 healthy infants (median age: 7.0 wk) was reported to be 3.7 ± 2.9[25], which was higher than the mean CoMiSS™ reported at baseline in the present study 1.4 ± 2.0; the difference might be explained by the lower median age of the healthy infants (7 wk) than the included children (7 mo at baseline).

An increasing amount of data on the growth of children with food allergies,

Table 3 mean ± SD anthropometric Z-scores at baseline, Day 45 and Day 90

		LFA z-score	WFL z-score	BMI z-score	HCA z-score
Baseline	*n*	29	27	27	28
	mean ± SD	0.0 (1.5)	-0.4 (1.0)	-0.4 (1.0)	0.5 (1.2)
D45	*n*	29	27	27	28
	mean ± SD	0.0 (1.7)	0.0 (1.1)	-0.1 (1.1)	0.5 (1.2)
	P-values[1]	NS	0.011[2]	0.023[2]	NS
D90	*n*	29	27	27	28
	mean ± SD	0.1 (1.7)	0.3 (1.2)	0.2 (1.2)	0.6 (1.1)
	P-values[1]	NS	< 0.001[3]	0.002[2]	NS

[1]*P*-values *vs* baseline;
[2]Student's *t*-test;
[3]Wilcoxon test. D: Day; LFA: Length-for-age; WFL: Weight-for-length; BMI: Body mass index; HCA: Head circumference-for-age; NS: Not significant.

particularly CMA, is accumulating[26,27]. Children with food allergies might indeed be at risk of growth failure for several reasons, such as the mismanagement of an elimination diet or a delay in CMA diagnosis. Paediatrician recommendations stress careful nutritional guidance to manage CMA[3,6,28,29] , including the use of an adapted formula that meets the nutritional needs of non-breastfed infants and young children[30,31]. Therefore, evidence on the safety and suitability of these formulas should be obtained from children with CMA in particular, as recently reported for some eHFs[23,24] or AAFs[32,33]. Growth parameters of the children fed the TeHCF were within normal range throughout the 3-mo period, indicating that this hypoallergenic formula is safe.

Henceforth, the TeHCF constitutes a new option among the various formulas already available for the dietary management of non-breastfed children with CMA[34]. The aim of the eHCF thickening is the management of the concomitant presence of CMA and regurgitations occurring when gastro-oesophageal reflux (GER) is present[34] in some infants. Previously, a formula with non-hydrolyzed CMP supplemented with the same thickeners complex as the one in the TeHCF induced a significant decrease in the daily number of regurgitations, from 7.3 ± 3.4 to 1.1 ± 1.3, in 90 infants within 14 d[35]. Therefore, the new TeHCF deserves further investigation in a patient population whose allergic symptoms are still present because the CMP have not yet been eliminated from their diet, on the contrary to children included in the present study which had to be successfully fed an elimination diet before inclusion so that their allergic symptoms were well improved. The effect of the TeHCF on gastro-intestinal symptoms in children presenting at enrolment symptoms suggesting a CMA, including severe regurgitations, should be compared to a control formula. CMA is often difficult to separate from functional gastro-intestinal disorder (FGID) in these patients when they present adverse GI reactions to cow's milk for several reasons: Regurgitation is the most common FGID observed in the first year of life[36,37] and occur often concurrently with other FGIDs, such as colic[38], and no pathognomonic symptom or sign exists for the diagnosis of CMA or GER disease[4,5,6,7,34]. A DBPCFC should be conducted, but because this procedure is time-consuming, expensive and requires specialized facilities, some experts suggest that it may even be more clinically relevant in daily clinical practice to propose a thickened eHF that addresses both of these conditions[39]. Moreover, the new TeHCF might be an interesting option within the context of a widely use of acid suppressant medicines in infants[40,41], despite paediatric guidelines urging physicians to exercise caution before prescribing them[34], and particularly when multiple FGIDs are present[38].

ARTICLE HIGHLIGHTS

Research background

Cow' milk protein allergy is the most frequent allergy in infant and young children. Its dietary management consists of the elimination of any cow's milk protein from the diet. In infant and young children, infant formula has to be replaced by an adapted formula which protein do not provoke reaction. This has to be demonstrated by a double-blind placebo controlled food challenge according to the American Academy of Pediatrics.

Research motivation

A new thickened extensively hydrolyzed casein-based formula (TeHCF) has been developed to manage concomitant presence of cow's milk allergy (CMA) and regurgitation. However, as a first step, its hypoallergenicity had to be assessed.

Research objectives
The objective of this study was to assess the hypoallergenicity of a new TeHCF in children with CMA.

Research methods
In children diagnosed with CMA through a double-blind placebo-controlled food challenge (DBPCFC), the hypoallergenicity of the new thickened formula was assessed through a DBPCFC: children were randomly administered increased doses of a placebo formula or the TeHCF under double-blind conditions and medical surveillance on two separate days. In children highly suspected of CMA, the hypoallergenicity of the formula and the CMA diagnosis were assessed simultaneously during a 3-days food challenge. Immediate and late reactions occurring after the introduction of any of these formulas were thoroughly recorded by the physician at the hospital and reported by parents to the physician after hospital discharge, respectively. If the children tolerated the TeHCF during the DBPCFC, they were exclusively fed this formula during a 3-mo period

Research results
30 children have been included in the study between April 2016 to July 2017. CMA diagnosis was confirmed by a DBPCFC in 29 (mean age: 8.03 ± 7.43 mo) patients. The children all tolerated the TeHCF during both the challenge and the subsequent 3-mo feeding period, which they all completed. During the latter period, the Cow's Milk-related Symptoms Score remained at a very low level, never exceeding its baseline value (1.4 ± 2.0), growth parameters were within World Health Organization reference standards and no adverse event related to the TeHCF was reported. Over the first week of this period, the proportion of patients with digestive discomfort significantly decreased from 20.7% (6/29) to 3.4% (1/29), P = 0.025. The proportion of satisfaction with the overall effect of the formula reported by the parents and investigator was high, as was the formula acceptability by the child. The efficacy on regurgitations in a specific population of infants having CMA and regurgitation should be assessed.

Research conclusions
This study demonstrates that the new thickened extensively hydrolysed formula is hypoallergenic. The design of our study, allowing to combine DBPCFC for CMA diagnosis and evaluation of the hypoallergenicity reduced burden for the family while allowing a sure diagnosis of CMA. Moreover, the tolerance was not assessed only during 7 d feeding period as per the American Academy of Pediatrics but through a 3-mo feeding period, in conditions similar to daily practices.

Research perspectives
Further studies should investigate the effect of this new thickened extensively hydrolysed formula in a patient population whose allergic symptoms are still present.

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SYSTEMATIC REVIEWS

Surveillance and diagnosis of hepatocellular carcinoma: A systematic review

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Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) appears in most of cases in patients with advanced liver disease and is currently the primary cause of death in this population. Surveillance of HCC has been proposed and recommended in clinical guidelines to obtain earlier diagnosis, but it is still controversial and is not accepted worldwide.

AIM

To review the actual evidence to support the surveillance programs in patients with cirrhosis as well as the diagnosis procedure.

METHODS

Systematic review of recent literature of surveillance (tools, interval, cost-benefit, target population) and the role of imaging diagnosis (radiological non-invasive diagnosis, optimal modality and agents) of HCC.

RESULTS

The benefits of surveillance of HCC, mainly with ultrasonography, have been assessed in several prospective and retrospective analysis, although the percentage of patients diagnosed in surveillance programs is still low. Surveillance of HCC permits diagnosis in early stages allows better access to curative treatment and increases life expectancy in patients with cirrhosis. HCC is a tumor with special radiological characteristics in computed tomography and magnetic resonance imaging, which allows highly accurate diagnosis without routine biopsy confirmation. The actual recommendation is to perform biopsy

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only in indeterminate nodules.

CONCLUSION
The evidence supports the recommendation of performing surveillance of HCC in patients with cirrhosis susceptible of treatment, using ultrasonography every 6 mo. The diagnosis evaluation of HCC can be established based on noninvasive imaging criteria in patients with cirrhosis.

Key words: Surveillance; Hepatocellular carcinoma; Ultrasonography; Cirrhosis; Imaging diagnosis

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Core tip: Hepatocellular carcinoma is one of the tumors with the worst prognosis and the 5-year survival is discouraging. The advantages of surveillance of hepatocellular carcinoma in patients with cirrhosis remains controversial, but the best strategy considered is to diagnose the tumor in early stage, which gives the opportunity to access better curative treatment. The current review will focus on the more recent available evidence about surveillance and diagnosis of hepatocellular carcinoma.

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INTRODUCTION

Primary liver cancer is the 6th most commonly diagnosed cancer and was the 4h cause of cancer death worldwide in 2018, including hepatocellular carcinoma (75%-85%) and intrahepatic cholangiocarcinoma (10%-15%)[1]. In 2018, 841,080 new cases of hepatocellular carcinoma (HCC) were diagnosed (4.7% of all new cases of cancer) and 781,631 patients died of this disease. It is more common in men and is currently the 2nd leading cause of cancer death worldwide in men and the 6th in women[2]. According to data from the surveillance, epidemiology and end results program, the 5-year survival for liver cancer is only 18%[3].

Incidence, mean age at diagnosis, and risk factors for HCC vary regionally according to the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV)[4,5]. However, the increasing incidence of non-alcoholic fatty liver disease (NAFLD) in high socioeconomic countries, the viral load suppression with chronic antiviral treatment of HBV, the high rates of HCV curation with the new direct-acting antiviral (DAA) therapy as well as HBV vaccination programs could change this paradigm in the next decades[6].

The improvement in cirrhotic patient care and better management of clinical complications associated with chronic liver disease in the last years has led to a sustained decrease in mortality, and currently HCC development is the most severe and life threatening complication in these patients[7-9]. Consequently, any action carried out to improve the prognosis of patients with end stage liver disease, must take into account early diagnosis of this cancer. In fact, HCC accomplishes the recommendation for surveillance programs established by the World Health Organization: it is an important health problem with high morbidity and mortality. The target population is clearly defined, the diagnosis test is easy to apply and there is a well-designed and accepted diagnosis process. Also, the diagnosis in early stages of the tumor allows access to curative treatment and a better prognosis.

MATERIALS AND METHODS

In this first part of the chapter, we review the evidence available on surveillance of HCC in patients with chronic liver disease according to the etiology and fibrosis status, the cost-effectiveness of such programs, and the impact in survival of

surveillance. In the second part of the article, we review the diagnosis tools in these patients.

The review was conducted using the Preferred Reported Items for Systematic Reviews guidelines. A computer-aided systematic literature search of PubMed and Scopus databases was performed. This review has been divided into two different parts: screening and diagnosis. The development of this article was conducted by members of the multidisciplinary team for diagnosis and evaluation of HCC at our hospital (two hepatologists and three radiologists). The literature search was carried out by each author according to the part of the paper assigned to each physician. The combination of keywords in the first part were as follows: "Screening AND/OR Surveillance AND Hepatocellular Carcinoma" and for the second part: "Diagnosis AND Hepatocellular Carcinoma AND LIRADS". In addition, the references of the more relevant studies (excluding case reports and articles in non-English languages) and specially the review and meta-analysis articles were manually searched to identify additional studies not detected in the previous selection.

In the first step, the title and abstract of each identified record was screened in order to explore the accuracy of the search and select only those really related to the topics. After this, the final list of selected articles was retrieved as full text for detailed assessment.

According to the topic assigned to each author, the most appropriate studies were selected to carry out the specific part of the review.

RESULTS

Survival advantages of surveillance diagnosis

The efficacy of any medical procedure should be based on objective data extracted from randomized and controlled studies. In the case of the hypothetical efficacy of surveillance programs in HCC, only two studies have assessed this aspect, both performed in Asia, and both in carriers of hepatitis B surface antigen (HBsAg). In the first, the screening test used was the determination of alpha-fetoprotein (AFP) every 6 mo in the study group (n = 3712) *versus* a control group without follow-up (n = 1869). Despite earlier diagnosis in the screening group, there were no differences in 5-year survival between the groups[10].

In the latter study, in the screening group (n = 9373), an ultrasonography (US) performed as well as an AFP have been performed every 6 mo comparing with control group without intervention (n = 9373). Despite a low adherence of 60%, it showed an improvement in survival in the screening group, achieving a reduction in mortality to approximately 37%[11].

To date, no other study carried out in this context (Asian HBsAg-carrying patients) has evaluated the profitability of screening and therefore, these data have not been able to be extrapolated to other populations (*e.g.*, western countries), as in other causes of chronic liver disease. This could be explained because the approach of a study of these characteristics (surveillance *vs* no surveillance) faces the refusal of the patients to sign an informed consent that includes the possibility of being part of the control group, as has been described in an article published a few years ago[12].

Given the absence of evidence with prospective series, several studies have tried to demonstrate the effectiveness of screening indirectly. American series reported that surveillance is associated with improved early stage detection, curative treatments and survival, despite adherence rates as low as less than 20%[13,14]. A recent meta-analysis of studies published between 1990 and 2014, including abstracts presented in congresses from 2009 to 2012, identified a total of 45 original articles (most of them retrospective, n = 38) that included a total of 15,158 patients with HCC, of which 41% had been diagnosed in screening programs. In most of the studies, the surveillance test employed was a combination of US and AFP (n = 39) and was conducted in Europe (n = 13), America (n = 15), and Asia (n = 15). This meta-analysis confirmed that these patients had tumors diagnosed in earlier stages of the disease, with greater possibility of curative treatment and better survival[15].

It must be taken into account that observational studies, especially in the setting of screening tools, have important bias that can confound assessment of screening test efficacy, that include lead-time bias (apparent improving survival because of an anticipated diagnosis), that can be minimized using correction formulas and length time bias (overrepresentation of slower growing tumors), that is inherent to this kind of study.

Based on the available evidence, the last published clinical practice guidelines from AASLD and EASL recommend the conduct of surveillance in patients with liver cirrhosis, with a moderate evidence level and a strong recommendation[16,17].

A more recent meta-analysis published in 2018, which included 19511 patients from 22 studies, showed an overall real world adherence rate to HCC surveillance imaging every 6-12 mo in 52%, better than previously reported[18]. The authors observed that the prospective studies had an adherence rate of 71%, when compared with 39% of retrospective studies, suggesting that being aware of surveillance may have a positive effect on adherence rates. On the other hand, they did not identify any other factor related to HCC adherence (geographical area, etiology of liver disease, surveillance test or interval). Nevertheless, another study comparing HCC survival in Japan (with intensive national surveillance program, n = 1174) *versus* Hong Kong (none program, n = 1675) over similar time periods (Japan 2000–2013, Hong Kong, China 2003–2014) showed that in Japan over 75% of cases are currently detected by surveillance, whereas in Hong Kong less than 20%. Median survival was 52 mo in Japan and 17.8 mo in Hong Kong; this survival advantage persisted after allowance for lead-time bias. A total of 63% of Japanese patients had early disease at diagnosis and 63% received curative treatment *versus* 31.7% and 44.1%, respectively in Hong-Kong. This suggests a clear benefit of the surveillance program[19].

In United States population, (a country where surveillance remains controversial), in real world, a matched case-control study carried out in the Veterans Affairs health care system, surveillance with ultrasound (US) and AFP was not associated with decreased HCC-related mortality[20].

Table 1 summarizes the more recent, retrospective studies, published in the last years about surveillance of HCC in real life, and not included in previous metanalyses. As shown in the table, surveillance adherence remains low, both in Europe and the United States.

Cost-effectiveness of surveillance programs

As we have explained, HCC is a potential target for cancer surveillance as it occurs in well-defined at-risk populations, and curative therapy is possible when small tumors are diagnosed. Surveillance has been recommended by regional and national liver societies all over the world and is practiced widely. However, there is a lack of randomized controlled trials in real settings that could help to address the incidence from which the surveillance should be applied because it is the key parameter which determines the cost-effectiveness of HCC screening[26].

Most studies use decision models (Markov chain or decision tree), which usually include the full economic evaluation of HCC screening programs, a comparison between HCC techniques, and the outcome measures expressed in terms of quality adjusted life years[27-32]. In general, a screening strategy is likely to be cost-effective in every setting considered, and a semiannual surveillance has been shown to be the most cost effective timing strategy.

Discrepancies in the results exist when determining the type of technology to be used. US alone or in association with AFP technology is likely to be the most cost-effective, and the use of computed tomography (CT) shows controversial results. Screening should be implemented to detect HCC at an early stage of cirrhosis and is likely not cost-effective in advanced HCC or after liver transplantation[33].

Optimal surveillance interval

The interval between screening examinations for HCC has been established based on both the tumor growth rate and the tumor incidence in the target population and its cost-effectiveness[17]. In studies carried out on the growth rate of untreated HCC, the time of duplication of tumor size is variable depending on factors such as their degree of differentiation[34]. Currently, the recommended interval between scans for screening is 6 mo. This strategy increases the detection of small size lesions compared with the annual screening, in which curative treatments can be applied more frequently with greater patient survival, and has proved to be cost-effective[27,35]. It does not seem that shortening to 3-mo screening interval improves the detection rate of small HCC (candidates for more radical treatments) or that it has an impact on survival over screening every 6 mo[36].

Surveillance tools

HCC screening includes imaging techniques and biomarkers.

Radiological: US: It is the most used test for the screening of HCC due to its wide availability, non-invasiveness, acceptable diagnostic accuracy, and cost. In addition, US provides additional information useful for the monitoring and assessment of the cirrhotic patient such as the appearance of ascites and portal thrombosis, but has the limitation of being an operator-dependent technique. Although it is difficult to establish its sensitivity and specificity due to the heterogeneity of the studies and their limitations, a meta-analysis that included 13 studies and 3571 patients found a

Table 1 Studies about the advantages and results of surveillance in HCC

Ref.	Location UNI/MULTI	Inclusion period	*n*	Screening (%)	Results surveillance group
Edenvik *et al*[21]	Sweden UNI	2005-2012	616	22%	Better survival
van Meer *et al*[22]	Netherlands MULTI	2005-2012	1074	27%	Smaller tumor size, earlier tumor stage, more often curative treatment and improving 1, 3, 5 years survival rates
Singal *et al*[23]	United States MULTI	2012-2013	374	42%	Early tumor detection and improved survival
Mittal *et al*[24]	United States MULTI	2005-2010	887	46.5%	Reduction in mortality
Atiq *et al*[25]	United States UNI	2010-2013	680	11.5%	Early HCC

UNI: Uni-center; MULTI: Multicenter; HCC: Hepatocellular carcinoma.

sensitivity and specificity of 94% for the detection of HCC[37]. However, this sensitivity is lower (63%) when it comes to lesions in early stages. Sensitivity of US can be affected by certain conditions such as obesity, the presence of ascites, or very advanced liver disease, so in some cases it may be necessary to use alternative techniques[38].

CT and magnetic resonance imaging: They are useful for the diagnosis of liver lesions, but in terms of screening tests, they are not cost-effective. Although these are more sensitive tests for the detection of lesions, especially in early stages, this greater sensitivity does not justify in most cases the increase in cost[27]. It does not seem that annual CT or magnetic resonance imaging (MRI) is preferable to US every 6 mo given the estimated doubling time of HCC. Apart from the cost, there are other disadvantages in these techniques that limit their usefulness as screening tests such as radiation, the risk of nephrotoxicity, allergic reactions by CT contrast, the availability of MRI equipment in some centers, the duration of the MR as well as the discomfort and the use of contrasts with gadolinium. However, in patients in whom US assessment is difficult and may have a low sensitivity, the benefit of using alternative techniques such as CT or MRI and as well as its periodicity should be individualized.

Biomarkers: Serum biomarkers cannot be used alone for HCC surveillance because of their relatively low sensitivity and specificity. However, combined with imaging techniques, they can increase the sensitivity although this increases false-positive results.

AFP: It is the most studied biomarker of HCC. A positive result is considered to be higher than 20 ng/mL, although with these values it has a relatively low specificity and with levels above 200 ng/mL the technique has a high specificity, but a low sensitivity. According to the results of some studies, adding the determination of AFP to the imaging controls could increase the sensitivity to an additional 6%-8% of in the detection of HCC in in early stages, at the expense of slightly decreasing the specificity[39,40]. This low yield of AFP is due to the fact that in certain chronic liver diseases, altered levels of this molecule can be observed without relation to HCC and only 10%-20% of HCC in initial stages has high values[17]. In addition, adding AFP to US significantly increases the cost of screening[27]. For all these reasons, a categorical recommendation of adding AFP to the imaging test cannot be established.

Although studies that demonstrate an increase in survival with the addition of AFP are lacking, since it could improve the detection of early lesions (and therefore susceptible to a more radical treatment) and the fact that a progressive increase in this determination in semi-annual controls may increase the suspicion of HCC, the decision to add AFP to imaging tests should be individualized[39].

Currently, the main guidelines do not establish a clear recommendation on its use. While the EASL guide does not recommend adding AFP to image screening, the AASLD guideline recommends screening with or without AFP along with the semester imaging test, so its use should be individualized[16,17].

Other biomarkers: There are other markers, des-gamma-carboxy prothrombin (DCP), the ratio of glycosylated AFP (L3 fraction) to total AFP and others, but for now they cannot be recommended as a screening technique. DCP and AFP-L3 have been

associated with advanced HCC and portal vein invasion, but currently cannot be recommended as a screening technique, because none of them have been adequately studied as surveillance tests[17].

Populations

Screening should be performed in populations considered to be high risk. It is established that screening is cost-efficient in cirrhotic patients with a risk of developing HCC of 1.5% per year or more and in non-cirrhotic HBV patients with a risk of 0.2% per year or more[17]. There are populations whose risk is not clearly established, such as non-cirrhotic NASH patients or patients with HCV who have reached a sustained viral response.

Cirrhosis/advanced fibrosis: Almost 80% of HCC develop on cirrhotic livers by any etiology. The studies carried out suggest a cost-effective screening for cirrhotic patients with a risk of developing HCC of 1.5% per year or more. This risk is equal or greater in cirrhotic patients by any etiology, in which this strategy would be beneficial. There are diseases such as cirrhosis of autoimmune origin in which although in several studies the incidence of HCC is less than 1.5% per year, a meta-analysis that included more than 6,000 patients obtained an annual incidence of 1.007%, but the 95% confidence interval (CI) was up to 1.47% per year[41,42]. Table 2 shows the observed incidences of HCC according to their etiology.

In patients with advanced liver disease (Child C or Child B patients with massive ascites or deep jaundice), who are not candidates for a liver transplant, screening is not cost-effective since due to their clinical situation, they would not benefit from HCC treatment[17,46].

In the case of patients included in the waiting list for liver transplantation, screening for HCC should still be carried out even if they have a decompensated disease since the HCC can alter their position on list or exclude them in the case of exceeding the accepted criteria for transplant.

Regarding the age of the patient, there are no data to support interrupting the screening at a certain age, but this decision would be given by the patient's clinical situation, their life expectancy and comorbidities that may prevent a treatment of HCC.

In patients with fibrosis grade 3, for any etiology, screening is also recommended, although in some of these groups the benefit and its cost-effectiveness are still unclear and need further studies[17].

NAFLD: NAFLD is a cause of liver disease that is gaining prominence given the growing number of cases diagnosed, especially in industrialized countries[47]. The natural history of HCC in patients with NAFLD and its pathogenesis is not known, although some theories involving proinflammatory cytokines, lipotoxicity, certain genetic polymorphisms in genes such as *PNPLA3* and *MBOAT7*, alterations in the microbiota, and possible increased absorption of iron have been discussed[48-50]. Although the risk of developing HCC is greater in those with a cirrhotic liver, this disease poses an increased risk of developing HCC even in non-cirrhotic patients[51]. In fact, a recent meta-analysis that included 168571 patients (13345 of them NASH) concluded that the risk of HCC in non-cirrhotic patients with NASH liver disease is 2.5 times higher than that in other etiologies[52]. These results should be interpreted with caution given the heterogeneity of the studies included in the meta-analysis and the lack of data on the degree of fibrosis. However, given the large number of patients included, it is a relevant result.

Some studies have suggested that the diagnosis is later than in other etiologies due to possible underdiagnosis of conditions that increase the risk of HCC such as cirrhosis and also due to the greater difficulty in interpreting US, because of the attenuation of the US by subcutaneous fat, and the difficulty of obtaining images of the entire liver[38,51]. Therefore, US has lower sensitivity for the detection of smaller tumors and in some series the prognosis could be worse. Although HCC may occur in patients with NAFLD in the absence of cirrhosis with a higher risk than in other etiologies, there is a lack of evidence on the cost-effectiveness of screening, which is why it is currently recommended in patients with cirrhosis or fibrosis 3 (advanced fibrosis can be diagnosed by elastography or by scoring systems like FIB-4), although it is based on expert opinions[53].

HCV: HCV is a risk factor for the development of cirrhosis and HCC. Achieving sustained viral response with interferon-based regimens has shown to be beneficial and reduce the risk of development of HCC for all degrees of fibrosis[54]. With the recent regimens based on direct-acting antivirals, sustained viral response rates are higher than with the interferon-based regimens and achieve a reduction in the risk of suffering HCC of more than 70%[55-57]. However, in some groups of patients, there is

Table 2 Annual incidence of HCC in cirrhotic patients by etiology

Ref.	Location UNI/MULTI	*n*	Follow-up period	Study design	Incidence
Tansel *et al*[42]	North America, Europe, Asia, Australia. MULTI	6528	Median 8 yr	Meta-analysis	1.007% (95%CI: 0.69–1.47)
Fattovich *et al*[43]	Europe MULTI	297	Median 66 yr	Retrospective	2.2% for hepatitis B virus and 2.5% for hepatitis C virus
Mancebo *et al*[44]	Spain UNI	450	Median 42 mo	Prospective	2.6%
Shibuya *et al*[45]	Japan MULTI	396 (134 stage III or IV)	Median 43 mo	Prospective	1.5% for PBC stage III/IV

UNI: Uni-center; MULTI: Multicenter.

evidence to suggest that there may be a relationship between the use of DAA and early development of HCC after treatment. In these studies, risk factors have been identified, including the presence of non-characterizable nodules in cirrhotic patients prior to treatment initiation in which the response rate is 2.83 (95%CI: 1.55, 5.16) compared to those without nodules or with benign nodules[58]. This possibility makes adequate compliance in screening especially important in these patients. Other studies have also shown a higher incidence of HCC de novo in patients treated with DAA *versus* IFN. This may be due to the fact that treatments based on DAA are used in older patients with more advanced liver disease, so after adjusting the incidence for these risk factors, patients treated with DAA that reach sustained viral response (SVR) present no greater risk of HCC than those treated with IFN[59].

Hepatitis treatment aims to reduce the risk of developing HCC, although it does not diminish completely[60]. The risk is present especially in cirrhotic patients, although patients with fibrosis grade 3 also continue to present an increased risk, so the screening should be performed every 6 mo[57]. There are several reasons that could justify this, such as an underestimation of the degree of fibrosis due to causes such as "sampling error" in the case of biopsies due to the size of the sample. In addition, it is important to note that after reaching the sustained viral response, the diagnostic accuracy of the elastographic techniques changes and can also underestimate fibrosis, so with the current evidence these techniques cannot be recommended in patients on SVR to decide on the need for screening[61,62]. For this reason and although after the SVR it seems that there may be a reduction in fibrosis, these results should be interpreted with caution since this reduction may be overestimated by elastographic techniques and there are also a lack of data that correlates the reduction of fibrosis after SVR with a risk reduction that would allow interrupting the screening; so the current recommendation is to continue performing lifelong screening for fibrosis 3 and cirrhotic patients according to the pre-treatment fibrosis assessment despite a reduction in elastographic measures after achieving SVR[16,17]. Table 3 shows the most recent studies and meta-analysis with the incidence of de novo HCC in patients treated with DAA.

HBV: HBV is associated with HCC even in non-cirrhotic patients (30% of the HCCs associated with HBV occur in these patients). This is due to the ability of viral DNA to integrate into host cells and act as a mutagenic agent. Therefore, although the presence of liver cirrhosis is the major risk factor in these patients, presenting a chronic B virus infection already constitutes an increased risk to develop HCC even in the absence of cirrhosis. The incidence of HCC in non-cirrhotic HBV patients ranges from 0.1% to 0.8% per year and in cirrhotic patients from 2.2% to 4.3% per year[16,17].

In non-cirrhotic patients with HBV, the recommendation is established when the risk of developing HCC is 0.2% per year. This is because non-cirrhotic patients diagnosed with early-stage HCC are better candidates for radical treatments (such as surgery), so the cost-benefit of screening is different to cirrhotic patients in whom the liver function can limit the applicability of some treatments and therefore the annual incidence threshold to initiate screening in this group is lower[17].

The AASLD guide establishes populations at risk of HCC that require screening of cirrhotic and non-cirrhotic HBV patients with the following characteristics[16,64]: (1) High-risk HBsAg patients including African or Asian men (these ethnic groups have an increased risk of HCC older than 40 years and Asian women older than 50 years[65,66]. (2) Patients with first-degree relatives diagnosed with HCC. (3) Patients with delta virus. (4) It is difficult to establish the risk in children and adolescents, but

Table 3 Annual HCC incidence in cirrhotic patients with HCV

Ref.	Location UNI/MULTI	*n*	Follow-up period	Study design	Incidence
Li *et al*[55]	US MULTI	17836	Median 2719.2 d in patients treated with IFN, and 396.4 d for the ones treated with DAAs	Retrospective	Annual incidence in cirrhotic patients 2.28% treated with DAA and 2.12% in patients treated with IFN. Annual incidence in patients with no treatment of 4.531%
Piñero *et al*[57]	Latin America MULTI	1400	Median 16 mo	Prospective	Accumulated incidence in cirrhotic patients of 3% at 1 year and 6% at 2 yr
Waziry *et al*[59]	Europe, Asia, North America, South America MULTI	11523	Median 5.5 yr in patients treated with IFN and 1 yr in patients treated with DAA	Meta-analysis	Annual incidence 1.14% in patients with SVR treated with IFN and 2.96% in patients SVR treated with DAA. After adjusting for age and follow-up period, no greater risk is observed in those treated with DAA
Nahon *et al*[63]	France Multi	1270	Median 67.5 mo	Prospective	2.6% in cirrhotic patients in SVR with DAA. In patients with SVR the annual incidence is 12%

UNI: Uni-center; MULTI: Multicenter; IFN: Interferon; DAA: Direct-acting antiviral; SVR: Sustained viral response.

it seems reasonable to recommend screening patients with fibrosis grade 3 or cirrhosis and patients with first-degree relatives diagnosed with HCC.

In addition, predictive models have been proposed to assess the risk of HCC such as REACH-B and PAGE-B (in Caucasian patients on antiviral treatment), based on risk factors (viral load, male sex, age, *etc*). These models stratify HCC risk in at least three groups: low, intermediate, and high risk groups. Patients in the PAGE-B risk class have less than the 0.2%/year risk for HCC[67,68]. Although the use of these scores has some limitations and cannot be universally applied, they can be useful to assess the need for antiviral treatment in certain cases or to assist in the decision to initiate HCC screening in patients who do not belong to the groups indicated.

Alcohol: Alcohol, due to its genotoxicity is directly related to the development of HCC, although it seems that this role is entirely due to the development of cirrhosis in patients without other etiological factors[17,69,70]. Therefore, there is currently no recommendation on screening in patients with alcohol abuse who do not have advanced fibrosis/cirrhosis. However, it is relatively frequent that alcohol is not presented as the only cause of liver disease, but as an added factor to other etiologies such as viral hepatitis. In these patients, the sum of risk factors such as the consumption of large amounts of alcohol and others such as diabetes could increase the risk of HCC[71-73]. It will be necessary in the future to identify the role that these etiological factors play in order to decide to initiate a screening program in non-cirrhotic patients.

Other etiologies: In other pathologies such as primary biliary cholangitis and autoimmune hepatitis, although the evidence is limited, screening does not seem beneficial if they do not present cirrhosis[17].

DISCUSSION

Imaging diagnosis

All current guidelines on the management of HCC accept that this tumor can only be diagnosed by means of imaging techniques if the lesion meets specific criteria and if it is a patient at risk for developing this neoplasm. If both conditions are not met, the

biopsy will be necessary for the diagnosis[16,17,74].

The typical scenario is usually a lesion detected by surveillance US in a patient with liver disease, or a casual finding in imaging techniques that initially had another objective (being the liver disease previously known, or discovered at that time).

Characterization by means of imaging tests is based on the fact that HCC has specific vascular characteristics that reflect the results of the process of hepatocarcinogenesis: there is an increase in arterial supply and a decrease in portal vein branches. In a multiphasic study (CT or MRI), this means that a nodule will show greater vascularization in the arterial phase than the rest of the parenchyma, whereas in venous phases the opposite will occur, presenting lower contrast uptake than the surrounding liver[75-78]. Demonstrating this behavior in a lesion of at least 1 cm, identified in a patient at risk, is diagnostic of HCC with values of specificity and positive predictive value that approach 100%[79-82]. Sensitivity values are variable, depending on several factors, largely on the size of the lesion (for lesions between 1 and 2 cm, they are about 60%, increasing these values with lesion size)[78]. For nodule(s) < 1 cm the specificity is lower, because even benign entities as arteriovenous fistula can have the same appearance. Therefore a close follow-up with US at 4-mo intervals is recommended. If the lesion remains stable for 12 mo, can return to regular surveillance (US every 6 mo)[17].

When the previous conditions are met for the diagnosis of HCC, biopsy is not considered necessary, since it will not improve the accuracy of the imaging tests. In addition, the biopsy can have diagnostic limitations (false negatives due to error in the sample or complicated differentiation between dysplasia *vs* carcinoma), technical difficulties for the procedure (obesity, ascites, location of the tumor that makes access difficult) and, above all potential complications because it is an invasive technique like bleeding or dissemination of the disease[79].

Currently, the imaging techniques validated for the diagnosis of HCC are multiphasic studies using CT and MRI. This conditions a maximum demand on the image, since not only is demanded to detect a lesion, but the objective is to characterize it. Therefore, in these cases it is essential to define quality criteria related to the technique itself, so that in concluding that a lesion "does not meet HCC criteria," we can be sure that the study by image cannot reach the diagnosis. This avoids, for example, indicating a biopsy without being sure of having exhausted the non-invasive diagnostic path or ceasing to diagnose an injury that, depending on the context, can change the patient's management in a crucial way.

As for purely technical requirements, both CT and MRI are described in the LIRADS guidelines and the OPTN/UNOS has also published similar standards[83,84]. These recommendations are in line with the current availability of techniques in the vast majority of centers (for example, it is suggested as a minimum, a multidetector CT of at least eight rows of detectors, and a MRI with 1.5 Tesla).

The intravenous contrast should be administered in a suitable dose, adapted to each patient and in CT the injection rate should be high whenever possible (recommended at least 4 mL/s). Regarding the phases to be performed, three are essential in CT (late arterial, portal and delayed phase) and in MRI the precontrast phase must be added (which is optional in CT, unless previous treatments have been performed that have used radiopaque contrast agents like Lipiodol®). Lesions in MRI are intrinsically T1 hyperintense lesions, due to the presence of elements like proteins, hemoglobin degradation products, copper or melanine, in which visual determination of hyperintensity attributable to contrast enhancement can be difficult; so precontrast imaging provides a baseline against which this enhancement can be identified and allows for subtraction imaging if necessary.

MRI provides the availability of numerous additional sequences unlike CT, which can provide more information.

The review of the quality of the images in the dynamic study is essential, which are similar in CT and MRI, whose compliance ensures that the images have been obtained at the optimum moment of hepatic vascularization.

Late arterial phase: The hepatic arterial branches have to show an intense and homogeneous enhancement (which is especially guaranteed with a high flow rate of intravenous contrast injection on CT); in the portal vein the enhancement must be starting, but with non-opacified suprahepatic veins (Figure 1A,B). These three conditions indicate that the phase is adequate, allowing time for the hypervascular lesion to become opaque, but without there being a hepatic venous return. For an optimal moment of image acquisition, it is recommended to use automatic contrast detection techniques -bolus tracking or bolus test-, which adapt the phases to the cardiac output of each patient.

If the portal vein still does not show contrast, we are in an early arterial phase, much less sensitive to detect HCC (Figure 2). On the other hand, if the suprahepatic

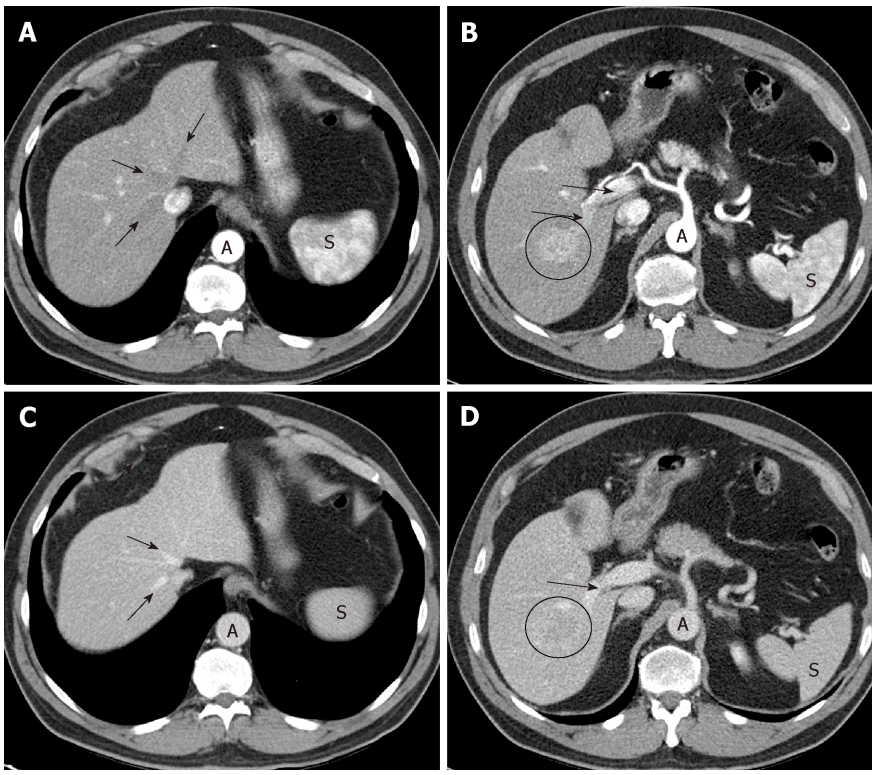

Figure 1 Optimal late arterial phase and portal phase. A, B: Hepatic artery and branches are fully enhanced. Portal vein is enhanced (arrows) but hepatic veins not yet enhanced by antegrade flow (arrows). Heterogeneous spleen. Aorta of very high density; C, D: Portal phase: portal veins are fully enhanced (D: arrows). Hepatic veins are enhanced by antegrade flow (C: arrows). Liver parenchyma is at peak enhancement. Homogeneous spleen. Portal vein even denser than aorta.

veins are opacified by hepatic anterograde flow (not by retrograde contrast flow from the right atrium), it will be too late of a phase. In both cases, the exploration will have lost the ability to detect a hypervascular lesion.

Portal phase: Maximum hepatic parenchymal and portal vein enhancement (mainly depend on an adequate dose of contrast) is given; the suprahepatic veins are opacified by anterograde flow (Figure 1C,D). In this case, what is of interest is the maximum density in the hepatic parenchyma, which will make more evident the differences between a focal lesion and the surrounding non-tumor tissue.

Late venous phase (also known as delayed or equilibrium phase): Obtained between 2 and 5 minutes after injection of the contrast, with which both the liver and the vessels will have a lower density than in the portal phase.

If the multiphasic study does not meet these quality criteria, the next step is to consider whether the study is repeated or an alternative imaging technique is needed.

Once the imaging technique is considered valid, the behavior of the lesion in the different phases is assessed to determine if it meets diagnostic criteria for HCC, which is applicable when it reaches at least 1 cm in maximum diameter. As previously stated, the behavior of HCC correlates with its vascular characteristics, and the diagnosis is based on the findings depicted in Table 4.

Demonstrating an arterial phase hyperenhancement (APHE) with washout in venous phases, allows the diagnosis of HCC (Figure 3). The LIRADS criteria give the presence of the "enhancing capsule" in nodules ≥ 20 mm the same value as the washout. The diagnosis of HCC is also considered as the growth of a hypervascular nodule by ≥ 50% of its diameter in ≤ 6 mo (Table 5).

These are the only criteria that allow the diagnosis by image of HCC. Several "ancillary findings" are described, which can guide or increase the suspicion of HCC, but in no case establish a diagnosis, if the previously defined criteria are not met. Examples of these findings are outlined in Table 6.

Treated lesions have special considerations at LIRADS classification, establishing 3 categories known as nonviable, equivocal or viable. Viable tissue after treatment is considered when APHE, washout or similar pretreatment enhancement is seen. Multidisciplinary discussion for consensus management is mandatory in these patients.

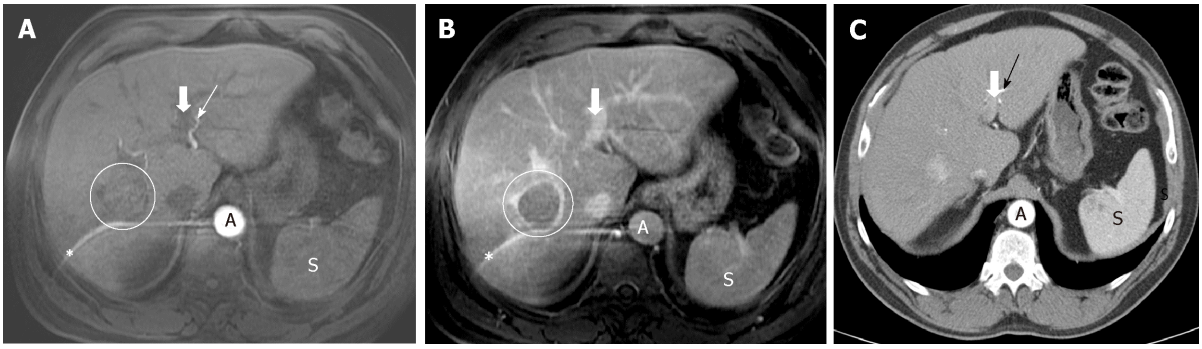

Figure 2 Importance of precise late arterial phase. A: Too early arterial phase. Aorta and left hepatic artery (thin arrow) with high signal intensity, but no contrast is seen in portal vein (thick arrow). No contrast in the suspicious lesion; B: Late venous phase: washout and capsule in the lesion (but no diagnoses due to lack of hyperintensity in arterial phase due to bad technique). Note the artifact in MRI images (*); C: CT was performed in the same patient with a good late arterial phase depicting hyperattenuation of the lesion that it is now diagnostic. Contrast in left portal vein can be seen (thick arrow).

Hepatospecific contrasts

Hepatobiliary agents in MRI are a type of intravenous contrast with dual properties: on one hand it has an initial extracellular distribution, so that, like the rest of contrasts, it allows to assess the vascularization of the lesion; on the other, it is captured and excreted by the hepatocyte, in a different proportion: Gadoxetate disodium has 50% uptake and hepatobiliary elimination, while in Gadobenate dimeglumine it has a 5% hepatobiliary elimination (the rest in both has a renal elimination). Its application in patients with suspected HCC is also based on the process of hepatocarcinogenesis: the evolution from regeneration nodule to HCC, there is a decrease in hepatocyte capacity for uptake and elimination of the bile duct of hepatospecific contrast due to alterations in membrane transporters[75,76,85]. Thus, when images of the liver are obtained at the moment when the peak of contrast uptake by the hepatocyte exists (after 20 min for the Gadoxetic and around 60 min for the Gadobenate), the non-tumor parenchyma should show enhancement, while most HCCs will show low signal intensity, as will any other lesion that does not have functioning hepatocytes (for example, benign lesions such as a cyst or a hemangioma, or malignant lesions, such as a metastasis).

The use of this type of contrast may increase the sensitivity and the negative predictive value, but it does not improve the specificity and is still considered an "ancillary finding" without constituting a major criteria for the diagnosis of HCC[17].

CEUS

The US contrast is based on microbubbles, and has a purely intravascular distribution, without passage to the interstitium, which results in a lesion behavior somewhat different to that seen in iodinated contrast media for CT and in extracellular gadolinium-based media for MRI[86]. This is especially important in cholangiocarcinoma, which in CEUS may show homogeneous enhancement in the arterial phase, followed by rapid washing, so that this behavior would no longer be specific for HCC in this technique[87,88]. It has been described as a differentiating factor between both entities that the HCC has an earlier enhancement and a less intense and later washing (> 60 s) than the cholangiocarcinoma[86,89].

The use is recommended only in centers with experience, being a highly operator-dependent technique, less reproducible than CT or MRI, and serves for a targeted assessment of a lesion, without allowing a study of the entire liver.

Thus, CEUS is not suitable for screening or surveillance. Rather, it is used to characterize lesion(s) identified on a screening and surveillance US or on CT/MRI. It is not recommended as a first-line imaging technique, because CT or MRI will be needed for staging, but it can be utilized when both CT and MRI are contraindicated or are inconclusive for the HCC diagnosis[17]. In this way, a CEUS LIRADS has also been developed[83].

CEUS is not only a valuable contributor to multimodality imaging for characterizing nodules in a cirrhotic liver, but may also be used, for example, to guide biopsy or treatment of lesions that are difficult to visualize with pre-contrast US, or to detect enhancement in a portal thrombus, in order to differentiate bland thrombus from neoplastic thrombosis[86]. This differentiation takes on importance due to the increased incidence of non-neoplastic portal vein thrombosis[90].

TC versus RM

Table 4 Findings for HCC diagnosis

Vascular phase (CT/MRI)	Feature	Comments
Late arterial phase	Arterial phase hyperenhancement also known as "wash-in"	The lesion must be hypervascular with an enhancing part higher in attenuation or intensity than the liver, depicting a nonrim-like enhancement unequivocally greater in whole or in part of the lesion than the surrounding liver parenchyma
Portal phase or late venous phase	Washout	The lesion will present lower contrast uptake than the surrounding parenchyma
	"Capsule appearance"	A ring of peripheral uptake in the lesion

CT: Computed tomography; MRI: Magnetic resonance imaging.

There are no conclusive studies demonstrating the superiority of one technique over another for the diagnosis of HCC, although the tendency is for a slight advantage of MRI, due to greater sensitivity, especially for small lesions (< 20 mm), and the MRI provides more information for ancillary findings, in addition to the added value of being able to use an hepatospecific contrast[16,17].

The choice between CT or MRI will depend to a great extent on other variables, beyond the diagnosis such as the availability of the technique, the radiologist's experience or certain characteristics of the patient like obesity, ascites or difficulty in performing apneas that can significantly limit the quality of the MR image, with CT being a more reliable technique in these circumstances. Claustrophobia can also prevent a patient from having an MRI. As for CT, it is necessary to take into account ionizing radiation (especially in young patients and multiphasic studies, which need several series) and allergy to iodinated contrast. Renal insufficiency limits the use of contrasts, both in CT and MRI, although patients in hemodialysis can have performed a CT scan, while MRI is contraindicated in this case because of the risk of nephrogenic systemic fibrosis[16].

LI-RADS®

Standardized report and assessment by reference center and multidisciplinary committee. The introduction of LIRADS aims to achieve a standardized process that is part of the study by image of the patient at risk of developing HCC, from the technique of completion to the written report of the examination[83].

In each liver lesion detected, the size (larger diameter), the liver segment where it is located and the degree of suspicion of HCC should be described according to their behavior (Table 7). To determine the likelihood of HCC, the LI-RADS categories are suggested (from LI-RADS 1, corresponding to lesion with benign features, to LI-RADS 5, which is a lesion with diagnostic criteria for HCC). The intermediate categories (LI-RADS 2, 3 and 4) refer to an increase in the probability of HCC, but without making it possible to achieve diagnostic imaging, so that management in these cases will always depend on a multidisciplinary and individualized assessment of each patient, being able to decide on options as different as a biopsy, to perform an alternative imaging technique as well as having a more or less narrow follow-up, or even a treatment[16].

The evaluation of imaging studies in a multidisciplinary committee and reference centers is recommended, which results in better results in diagnosis, management and even patient survival[16].

The last studies about regular surveillance of HCC in advanced liver diseases, suggest that it could be cost-beneficial in this context, although the evidence in clinical practice is still limited. US at 6-mo interval appears the most extending tool for surveillance and a CT and/or MRI are the most accepted imaging techniques for HCC diagnosis, relegating the biopsy procedure only for selected cases. Advances in HBV control viremia and HCV definitive curation is decreasing the HCC incidence. In the next decades, the high risk subgroups that will benefit from surveillance remains an important research goal in this new stage.

Table 5 Computed tomography/magnetic resonance imaging diagnostic table

	Nonrim-like APHE	
Observation size	10-19 mm	≥ 20 mm
Enhancing "capsule"	LR-4	LR-5
Non-peripheral washout or threshold grown	LR-5	LR-5

APHE: Arterial phase hyperenhancement; LR-5: LI-RADS lesions.

Table 6 Ancillary findings (LIRADS 2018)

Favoring HCC in particular	Favoring malignancy in general	Favoring benignity
Non-enhancing "capsule"	US visibility as discrete nodule	Size stability > 2 yr
Nodule-in-nodule	Subthreshold growth	Size reduction
Mosaic architecture	Restricted diffusion	Parallels blood pool
Blood products in mass	Mild-moderate T2 hyperintensity	Marked T2 hyperintensity
Fat in mass, more than adjacent liver	Fat sparing in solid mass	Undistorted vessels
	Iron sparing in solid mass	Iron in mass, more than liver
	Transitional phase hypointensity	Hepatobiliary phase isointensity
	Hepatobiliary phase hypointensity	
	Corona enhancement	

HCC: Hepatocellular carcinoma; US: Ultrasonography.

Table 7 LI-RADS 2018 recommendations for untreated ≥ 1 cm lesions without pathologic proof in patients at high risk for HCC

LR-NC	Cannot be categorized (image degradation, lack of key phases)
LR-1	Definitely benign (*e.g.*, cyst, hemangioma, perfusion alteration)
LR-2	Probably benign (probable but no definitive LR-1 findings)
LR-3	Intermediate probability of malignancy (nonmalignant and malignant entities each have moderate probability)
LR-4	High probability but no certainty of HCC
LR-5	Definitively HCC
LR-M	Probably or definitely malignant, not HCC specific (*e.g.*, HCC not meeting LR-5 criteria, intrahepatic cholangiocarcinoma, metastases to liver)
LR-TIV	Tumor in vein

HCC: Hepatocellular carcinoma.

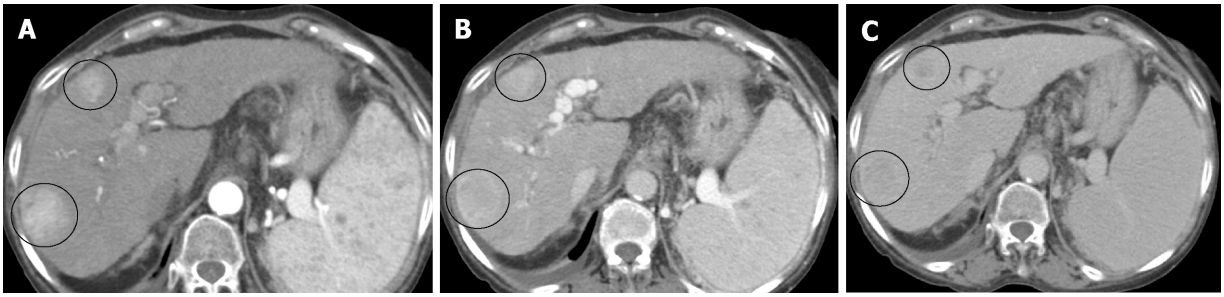

Figure 3 Importance of delayed phase. A: Late arterial phase, 2 hypervascular lesions (circles); B: Portal phase, no washout is seen. Non-diagnostic imaging findings; C: Delayed phase: Washout in both lesions (circles). Diagnosis by imaging.

ARTICLE HIGHLIGHTS

Research background

Surveillance of hepatocellular carcinoma (HCC) has been proposed and recommended in clinical

guidelines, in order to obtain earlier diagnosis but it is still controversial and it is not accepted worldwide.

Research motivation
Emerging populations like non-alcoholic fatty liver disease patients or hepatitis C virus (HCV) after achieving sustained viral response (SVR) are at risk of developing HCC. Should they be screened? What is the ideal screening tool attending cost-effectiveness?

Research objectives
Support the surveillance programs in patients at risk of developing HCC because of the cost-effectiveness of early diagnosis.

Research methods
Systematic review of recent literature of surveillance (tools, interval, cost-benefit, target population) and the role of imaging diagnosis (radiological non-invasive diagnosis, optimal modality and agents) of HCC.

Research results
The benefits of surveillance of HCC, mainly with ultrasonography, have been assessed in several prospective and retrospective analysis. Surveillance of HCC permits diagnosis in early stages allowing better access to curative treatment and increased life expectancy in patients at risk.

Research conclusions
The actual evidence supports the recommendation of performing surveillance of HCC in patients with cirrhosis or advanced fibrosis of any etiology susceptible of treatment, using ultrasonography every 6 mo. In some populations of non-cirrhotic hepatitis B virus patients the screening can be cost-effective. The diagnosis evaluation of HCC can be established based on noninvasive imaging criteria in patients with cirrhosis.

Research perspectives
Further studies need for evaluating the cost-effectiveness of screening in emerging populations like non-cirrhotic non-alcoholic fatty liver disease patients or HCV who achieved SVR. Utility of hepatospecific contrasts needs further evaluation.

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META-ANALYSIS

Neuraxial adjuvants for prevention of perioperative shivering during cesarean section: A network meta-analysis following the PRISMA guidelines

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Abstract

BACKGROUND

Perioperative shivering is clinically common during cesarean sections under neuraxial anesthesia, and several neuraxial adjuvants are reported to have preventive effects on it. However, the results of current studies are controversial and the effects of these neuraxial adjuvants remain unclear.

AIM

To evaluate the effects of neuraxial adjuvants on perioperative shivering during cesarean sections, thus providing an optimal choice for clinical application.

METHODS

A systematic review and network meta-analysis were conducted following the PRISMA (Preferred Reported Items for Systematic Review and Meta-analysis) guidelines. Analyses were performed using Review Manager 5.3 and Stata 14.0. We searched PubMed, EMBASE, Web of Science, and Cochrane Central databases for eligible clinical trials assessing the effects of neuraxial adjuvants on

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perioperative shivering and other adverse events during cesarean sections. Perioperative shivering was defined as the primary endpoint, and nausea, vomiting, pruritus, hypotension, and bradycardia were the secondary outcomes.

RESULTS

Twenty-six studies using 9 neuraxial adjuvants for obstetric anesthesia during caesarean sections were included. The results showed that, compared with placebo, pethidine, fentanyl, dexmedetomidine, and sufentanil significantly reduced the incidence of perioperative shivering. Among the four neuraxial adjuvants, pethidine was the most effective one for shivering prevention (OR = 0.15, 95%CI: 0.07-0.35, surface under the cumulative ranking curve 83.9), but with a high incidence of nausea (OR = 3.15, 95%CI: 1.04-9.57) and vomiting (OR = 3.71, 95%CI: 1.81-7.58). The efficacy of fentanyl for shivering prevention was slightly inferior to pethidine (OR = 0.20, 95%CI: 0.09-0.43), however, it significantly decreased the incidence of nausea (OR = 0.34, 95%CI: 0.15-0.79) and vomiting (OR = 0.25, 95%CI: 0.11-0.56). In addition, compared with sufentanil, fentanyl showed no impact on haemodynamic stability and the incidence of pruritus.

CONCLUSION

Pethidine, fentanyl, dexmedetomidine, and sufentanil appear to be effective for preventing perioperative shivering in puerperae undergoing cesarean sections. Considering the risk-benefit profiles of the included neuraxial adjuvants, fentanyl is probably the optimal choice.

Key words: Neuraxial adjuvants; Shivering; Cesarean section; Prevention; Network meta-analysis

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Core tip: Shivering is a common complication of obstetric anaesthesia, especially during caesarean section. Recently, several neuraxial adjuvants have been used for the prevention of shivering. However, the results of current studies are controversial and the role of these adjuvants in obstetric anesthesia remains unclear. The aim of our network meta-analysis is to evaluate the effects of neuraxial adjuvants on shivering and other side effects, thus providing an optimal choice for clinical application.

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INTRODUCTION

There are about 18.5 million cesarean deliveries performed each year worldwide[1]. Neuraxial anaesthesia is the commonest technique for obstetric anesthesia because it is easy to handle, underspent, and of less adverse effects or complications[2]. Perioperative shivering is very common during caesarean section under neuraxial anaesthesia, with an incidence of 29%-54%[3], which increases catecholamine excretion, maternal metabolic rate, carbon dioxide production, and oxygen consumption, thereby interfering with the operation and anesthesia monitoring[4,5]. In clinical practice, several neuraxial drugs have been used as adjuvants to local anaesthetics for obstetric anesthesia[6,7], and some of them have been found to alleviate the side effects, including shivering[8,9], but the results of these studies are still controversial, because there are also a few studies showing that some neuraxial adjuvants did not reduce the incidence of the side effects during cesarean section[6,10]. Furthermore, so far, there are no relevant guidelines or standards available for clinical practice, and it remains to identify the most preferable neuraxial adjuvant for shivering prevention in the puerperae undergoing cesarean sections.

In the present analysis, we performed a network meta-analysis (NMA) to comprehensively compare the effects of several neuraxial adjuvants on shivering and

other adverse reactions during caesarean section, with an aim to help guide clinicians in making optimal preventive regimen for puerperae undergoing cesarean sections.

MATERIALS AND METHODS

This systematic review and meta-analysis were conducted following the Preferred Reported Items for Systematic Review and Meta-analysis (PRISMA) guidelines.

Search strategy and criteria

We searched for available clinical trials in PubMed, EMBASE, Web of Science, and Cochrane Central databases that published by August 7, 2018. The retrieval was not restricted by age, data, or language. The combinations of our search terms were ("shivering" or "chill" or "chillness") AND ("lumbar anaesthesia" or "subarachnoid anaesthesia" or "regional anaesthesia" or "intrathecal anaesthesia" or "neuraxial anaesthesia" or "spinal anesthesia" or "peridural anesthesia" or "extradural anesthesia" or "epidural anesthesia") AND ("caesarean" or "caesarean" or "c-section").

Studies included in this study should meet the following criteria: (1) Surgery type: Caesarean section; (2) Anesthesia type: Spinal anesthesia (SA), epidural anesthesia (EA), or combined spinal-epidural anesthesia (CSEA); (3) Administration time: During the anesthesia; (4) Administration method: intrathecal or extradural; and (5) Original and prospective clinical trials.

Types of interventions were: Local anesthetics plus neuraxial adjuvant in the experimental group; local anesthetics plus placebo in the control group. All kinds of local anesthetics and neuraxial adjuvants were eligible.

We excluded descriptive literature reviews or systemic reviews, case reports, and studies that were unable to extract any data by reviewing titles, abstracts, and full papers. All the included studies met the inclusion criteria.

Outcomes

Our primary outcome was the incidence of shivering during and after caesarean section. Most studies graded shivering with a scale described by Crossley and Mahajan: 0: No shivering; 1: Piloerection or peripheral vasoconstriction but no visible shivering; 2: Muscular activity in only one muscle group; 3: Muscular activity in more than one muscle group but not generalized shivering; and 4: Shivering involving the whole body[11]. So we incorporated data only when the grade of shivering was greater than or equal to the grade 2.

The secondary outcomes were the incidence of other adverse reactions including: (1) Nausea; (2) Vomiting; (3) Pruritus; (4) Hypotension; and (5) Bradycardia. Postoperative nausea and vomiting were reported in a few eligible studies, and these data were extracted and pooled to evaluate the corresponding outcomes. Additionally, when the data came with time point, we took the data at the longest time point.

Data extraction

According to our protocol, all of our data were independently extracted and assessed by two investigators using a standard data table, and any disagreements were solved through consultation with the third party. The following contents were extracted from the included studies: first author and publication year, sample size, intraoperative ambient temperature, type of anesthesia, administration method, local anesthetic intervention, and clinical outcomes. We used the Cochrane Collaboration's Risk of Bias Tool to assess the risk of bias of eligible studies[12].

Statistical analysis

We performed NMA to synthesize evidence using STATA software (version 14.0). Odds ratio (OR) was used to estimate all outcomes. Surface under the cumulative ranking curve (SUCRA) represented the corresponding ranking of each outcome; the higher the value, the more effective the intervention. After that, the degree of inconsistency was analyzed by the node-splitting method, and the risk of publication bias is shown on funnel plots[13,14].

RESULTS

Study selection and characteristics

As showed in the flow diagram (Figure 1), 476 records were screened after initial

searches in PubMed, EMBASE, and the Cochrane Library. And 51 citations remained after exclusion of duplicate articles by screening title and abstracts. Finally, 26 studies with 2054 puerperae were selected for full text reviews (Figure 1), which were performed in 10 countries from 1990 to 2017[3,6-10,15-34]. The included studies compared the following 9 neuraxial adjuvants with placebo: Fentanyl, sufentanil, pethidine, morphine, dexmedetomidine, magnesium sulfate, clonidine, tramadol, and midazolam. Three types of anesthesia were used in the included studies: SA in 17 studies (65.4%), EA in 3 (11.5%), and CSEA in 6 (23.1%). Three kinds of local anesthetics were used in the studies: bupivacaine (76.9%), ropivacaine (15.4%), and lidocaine (7.7%). The specific concentrations of local anesthetics are presented in Table 1. The network of eligible comparisons of all adjuvants for shivering is showed in Figure 2; 21 studies were two-arm trials and 5 were three-arm trials.

Primary outcome (shivering)

Twenty-six studies with a total of 2054 patients reported data of the incidence of shivering. Compared with placebo, eight adjuvants decreased the incidence of shivering, and four of them demonstrated statistically significant effects (Figure 3) (pethidine *vs* placebo: OR = 0.15, 95%CI: 0.07-0.35; fentanyl *vs* placebo: OR = 0.20, 95%CI: 0.09-0.43; dexmedetomidine *vs* placebo: OR = 0.25, 95%CI: 0.11-0.54; sufentanil *vs* placebo: OR = 0.35, 95%CI: 0.16-0.78). Besides, as showed in SUCRA curve graph (Figure 4), the four largest SUCRA values were as follows: Pethidine (83.9), fentanyl (75.1), dexmedetomidine (66.9), and sufentanil (53.3).

Secondary outcomes (other adverse events)

Overall, fentanyl reduced the incidence of nausea (fentanyl *vs* placebo: OR = 0.34, 95%CI: 0.15-0.79) and vomiting (fentanyl *vs* placebo: OR = 0.25, 95%CI: 0.11-0.56) compared with the control groups. On the contrary, patients treated with pethidine showed a higher incidence of nausea (pethidine *vs* placebo: OR = 3.15, 95%CI: 1.04-9.57) and vomiting (pethidine *vs* placebo: OR = 3.71, 95%CI: 1.81-7.58). Besides, sufentanil reduced the incidence of vomiting (sufentanil *vs* placebo: OR = 0.34, 95%CI: 0.14-0.80) and hypotension [sufentanil *vs* placebo: OR = 0.47, 95%CI: 0.23-0.96). However, the incidence of pruritus increased when sufentanil was used (sufentanil *vs* placebo: OR = 20.37, 95%CI: 2.44-169.96)]. Differences in the incidence of bradycardia between the intervention and control groups were not statistically significant. A complete summary table with SUCRA values and effect size is displayed in Table 2.

Risk of bias assessment

The risk of bias assessment is presented in Figure 5. Three of the included studies[6,16,18] were rated as high risk of bias due to inappropriate allocation concealment, selective data reporting, and unclear reporting of statistical methods. The asymmetry in the funnel plots indicated publication bias (Figure 6).

Inconsistency

We used the node-splitting method to assess inconsistency. As shown in Figure 7, the majority of loops had no inconsistent results and the inconsistency of pruritus was relatively high compared with other outcomes.

DISCUSSION

Neuraxial anesthesia is widely used in lower abdominal surgery including cesarean section. Traditional neuraxial anesthesia only uses local anesthetic, which is often accompanied with the emergence of perioperative complications[35]. Shivering is one of the common complications of obstetric anaesthesia. Patients with shivering often suffer from uncontrolled muscular activity. The etiology of shivering is multiple and complicated. The risk factors responsible for shivering in puerperae undergoing caesarean sections may be intraoperative body heat and fluid loss, response to pain, or excitement of the sympathetic nervous system[36]. Because of the high incidence of shivering during caesarean section, the prevention of shivering has become an indispensable part of obstetric anaesthesia.

In current obstetric anaesthesia, combination of local anaesthetics and adjuvants has been a new choice for anesthetists to reduce side effects[37]. Several medications have been applied to obstetric anaesthesia as adjuvants and some of them have been reported to reduce shivering. We conducted the present NMA and comprehensively assessed the preventive effects of common adjuvants: Fentanyl, sufentanil, pethidine, morphine, dexmedetomidine, magnesium sulfate, clonidine, tramadol, and midazolam.

The results showed that pethidine, fentanyl, dexmedetomidine, and sufentanil had

Table 1 Characteristics of included trials and patients

First author, year	Size	Intraoperative ambient temperature	Type of anesthesia	Administration method	Local anesthetic	Intervention	Outcomes
Palmer *et al*[15], 1995	28	Unclear	SA	Intrathecal	5% lidocaine	Fentanyl *vs* Placebo	
Shehabi *et al*[16], 1990	62	21 °C	EA	Extradural	0.5% bupivacaine	Fentanyl *vs* Placebo	
Han *et al*[17], 2014	60	Unclear	EA	Extradural	0.75% ropivacaine	Fentanyl *vs* Dexmedetomidine *vs* Placebo	
Shami *et al*[34], 2016	150	24-26 °C	CSEA	Intrathecal	0.5% hyperbaric bupivacaine	Pethidine *vs* Placebo	
Techanivate *et al*[33], 2005	60	23 °C	SA	Intrathecal	0.5% hyperbaric bupivacaine plus 0.2 mg morphine	Fentanyl *vs* Placebo	
Roy *et al*[18], 2004	40	21–23 °C	SA	Intrathecal	0.75% hyperbaric bupivacaine plus 0.15 mg morphine	Pethidine *vs* Placebo	
Qi *et al*[19], 2016	118	Unclear	SA	Intrathecal	0.5% bupivacaine	Dexmedetomidine *vs* Morphine *vs* Placebo	
Chen *et al*[31], 2010	64	Unclear	SA	Intrathecal	0.75% ropivacaine	Sufentanil *vs* Placebo	
Bachmann-Mennenga *et al*[21], 2005	60	Unclear	EA	Extradural	1% ropivacaine	Sufentanil *vs* Placebo	
Abdollahpour *et al*[10], 2015	75	Unclear	SA	Intrathecal	0.5% bupivacaine	Midazolam *vs* Sufentani *vs* Placebo	
He *et al*[7], 2017	90	22–28 °C	SA	Intrathecal	0.5% hyperbaric bupivacaine	Dexmedetomidine *vs* Placebo	
Nasseri *et al*[22], 2017	50	22–26 °C	SA	Intrathecal	0.5% hyperbaric bupivacaine	Dexmedetomidine *vs* Placebo	
de Figueiredo Locks *et al*[3], 2012	80	Unclear	SA	Intrathecal	0.5% hyperbaric bupivacaine	Sufentanil *vs* Placebo	
Rastegarian *et al*[9], 2013	100	Unclear	SA	Intrathecal	5% hyperbaric lidocaine	Pethidine *vs* Placebo	
Khan *et al*[6], 2011	72	21–23 °C	SA	Intrathecal	0.5% hyperbaric bupivacaine	Pethidine *vs* Placebo	
Hong *et al*[23], 2005	120	23–25 °C	CSEA	Intrathecal	0.5% bupivacaine	Morphine *vs* Pethidine *vs* Placebo	
Agrawal *et al*[24], 2016	60	Unclear	SA	Intrathecal	0.3% bupivacaine	Morphine *vs* Fentanyl *vs* Placebo	
Hanoura *et al*[25], 2013	50	Unclear	CSEA	Extradural	0.5% hyperbaric bupivacaine (intrathecal) 0.25% bupivacaine plus 100ug fentanyl (extradural)	Dexmedetomidine *vs* Placebo	
Anaraki *et al*[26], 2012	156	21–23°C	SA	Intrathecal	0.5% hyperbaric bupivacaine	Pethidine *vs* Placebo	
Bajwa *et al*[27], 2012	100	Unclear	SA	Intrathecal	0.5% hyperbaric bupivacaine	Clonidine *vs* Placebo	
Bi *et al*[28], 2017	60	Unclear	CSEA	Intrathecal	0.5% bupivacaine	Dexmedetomidine *vs* Placebo	
Yousef *et al*[29], 2010	90	Unclear	CSEA	Extradural	0.5% hyperbaric bupivacaine (intrathecal) 0.25% bupivacaine plus 100ug fentanyl (extradural)	Magnesium sulfate *vs* Placebo	

Subedi *et al*[30], 2013	77	Unclear	SA	Intrathecal	0.5% hyperbaric bupivacaine	Tramadol *vs* Fentanyl
Qian *et al*[31], 2008	80	Unclear	CSEA	Intrathecal	0.6% ropivacaine	Sufentanil *vs* Placebo
Faiz *et al*[8], 2013	72	23–25 °C	SA	Intrathecal	0.5% bupivacaine	Magnesium sulfate *vs* Placebo
Sadegh *et al*[32], 2011	80	24 °C	SA	Intrathecal	0.5% hyperbaric bupivacaine	Fentanyl *vs* Placebo

SA: Spinal anesthesia; EA: Epidural anesthesia; CSEA: Combined spinal-epidural anesthesia; Outcomes: Shiver; Nausea; Vomiting; Pruritus; Hypotension; Bradycardia.

preventive effects on perioperative shivering during caesarean sections. Of note, pethidine, fentanyl, and sufentanil are opioids, and according to the research, opioids have a hyperthermic effect through the activation of μ-receptor, which might be the anti-shivering mechanism of opioids[38]. Moreover, compared with the other two opioids, pethidine has a better preventive effect on shivering. Several studies have indicated that the anti-shivering mechanisms of pethidine are different from those of other opioids. Besides activating the μ-receptors, it has a modulatory effect on shivering threshold and thermoregulation[39,40], which may help explain why pethidine has the highest rank of anti-shivering effect.

Dexmedetomidine is approved for procedural sedation use, but it is mainly for non-intravenous administration or peadiatric use. Recent studies have shown that dexmedetomidine may be a safe intrathecal supplement in Cesarean delivery[7,22].

Several studies demonstrated that α_2 adrenoreceptor (α_2-AR) agonists (including clonidine and dexmedetomidine) have a potential prophylactic effect on shivering in patients[41,42]. Another study showed that α_2-AR agonists markedly inhibited shivering in rats[43]. Dexmedetomidine is one of the emerging α_2-AR agonists, possessing almost eight times higher α_2-AR affinity compared to clonidine[44]. As our results indicated, clonidine had a weak preventive effect on shivering. Dexmedetomidine can quickly be absorbed and subsequently agitate α_2-ARs in the spinal cord, leading to the inhibition of sympathetic activity and central thermoregulation[45]. The attenuation of hyperadrenergic response to perioperative stress could be another mechanism of action of dexmedetomedine for shivering control.

In terms of other adverse events, the present study indicated that pethidine significantly increased the risk of nausea and vomiting, while fentanyl significantly reduced the risk to the contrary. Both drugs are opioid receptor agonists and it is well known that opioids often increase the risk of nausea and vomiting in the clinical situation. The mechanism of nausea and/or vomiting after opioid use is complex[46]. However, interacting with μ-opioid receptors in the vomiting center may be the main mechanism of the anti-nausea and anti-vomiting effects of higher dose opioids[47]. Barnes *et al*[48] reported that the appropriate dose of fentanyl has a great inhibitory effect on drug-induced emesis. The different dose of opioids used as adjuvants may lead to the opposite results, which can help to explain the above findings of our study.

In addition, our study revealed that sufentanil significantly increased the incidence of pruritus than other drugs, including morphine, although pruritus was mostly mild, and no puerperae required treatment. As shown in Table 2 and Figure 7, the relatively high heterogeneity variance of pruritus, wide confidence interval, and potential inconsistency exist; further research is needed to confirm these findings.

The present analysis is the first NMA of the preventive effects of neuraxial adjuvants on perioperative shivering during caesarean section, revealing that pethidine, fentanyl, dexmedetomidine, and sufentanil could decrease the incidence of perioperative shivering in puerperae. In addition, our study comprehensively analyzed the effects of neuraxial adjuvants on the other adverse reactions, indicating the optimal adjuvant, which can not only prevent shivering, but also reduce other adverse events.

There are several limitations of this NMA. First, some outcomes, such as the Apgar score, could not be analyzed due to the lack of sufficient studies. Second, heterogeneity and potential risk of bias weakened the reliability of the results. Third, the incidence of adverse events may be confounded by different kinds of local anesthetic, the type of anesthesia, the dose of adjuvant, individual characteristic, or different type of intraoperative warming. Because of the limited number of included studies and inadequate information, the relevant subgroup analyses and stratified analyses were not possible.

In conclusion, the results of our study clearly suggest that, based on the available evidence, neuraxial pethidine, fentanyl, dexmedetomidine, and sufentanil are more

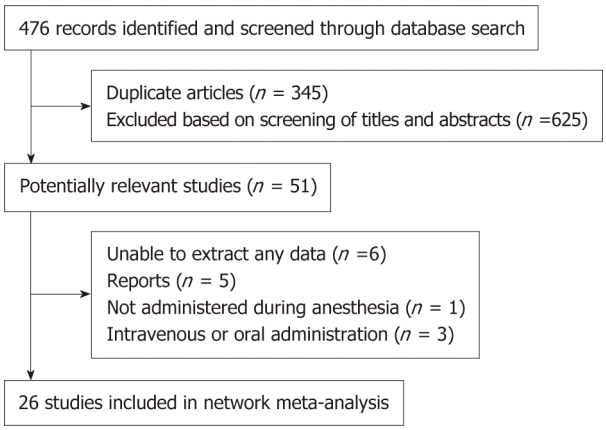

Figure 1 Flow chart of literature search and selection process, inclusion, and exclusion.

efficacious than other medications in the prevention of shivering during caesarean sections. Although pethidine is the most effective adjuvant for shivering prevention, it significantly increases the incidence of nausea and vomiting. Considering the risk-benefit profiles of the included neuraxial adjuvants, fentanyl is probably the optimal choice.

Future clinical trials are still needed to further assess the efficacy and safety of neuraxial adjuvants for the puerperae and neonates, the optimal doses of the medications, and the timing of administration, *etc*., thus contributing to the establishment of a guideline of neuraxial adjuvant administration for obstetric anesthesia during caesarean sections.

Table 2 SUCRA values and effect size in comparison to placebo

Nausea (Heterogeneity variance = 0.17)					Vomiting (Heterogeneity variance = 0.00)				
Rank	Treatment	SUCRA	OR	95%CI	Rank	Treatment	SUCRA	OR	95%CI
1	Midazolam	86.10	0.20	(0.04, 0.96)	1	Fentanyl	79.90	0.25	(0.11, 0.56)
2	Fentanyl	77.10	0.34	(0.15, 0.79)	2	Sufentanil	70.70	0.34	(0.14, 0.80)
3	Pethidine	4.30	3.15	(1.04, 9.57)	3	Pethidine	1.10	3.71	(1.81, 7.58)
1	Clonidine	79.30	0.26	(0.06, 1.10)	1	Midazolam	89.90	0.11	(0.01, 1.00)
2	Tramadol	58.50	0.51	(0.06, 4.58)	2	Clonidine	69.30	0.35	(0.10, 1.26)
3	Magnesium sulfate	53.00	0.65	(0.09, 4.88)	3	Magnesium sulfate	48.70	0.65	(0.10, 4.10)
4	Dexmedetomidine	44.50	0.82	(0.38, 1.76)	4	Dexmedetomidine	38.10	0.87	(0.43, 1.74)
5	Sufentanil	37.20	0.97	(0.45, 2.06)	5	Placebo	32.20		
6	Placebo	34.80			6	Morphine	20.10	1.42	(0.62, 3.26)
7	Morphine	25.10	1.30	(0.47, 3.56)					
Pruritus (heterogeneity variance = 1.18)					Hypotension (heterogeneity variance = 0.15)				
Rank	Treatment	SUCRA	OR	95%CI	Rank	Treatment	SUCRA	OR	95%CI
1	Morphine	22.60	6.54	(1.02, 41.88)	1	Midazolam	99.70	0.04	(0.01, 0.19)
2	Sufentanil	7.40	20.37	(2.44, 169.96)	2	Sufentanil	74.50	0.47	(0.23, 0.96)
1	Tramadol	76.90	0.40	(0.01, 12.33)	1	Magnesium sulfate	56.50	0.68	(0.16, 2.87)
2	Clonidine	75.90	0.34	(0.00, 29.96)	2	Morphine	55.20	0.68	(0.11,4.42)
3	Dexmedetomidine	71.90	0.74	(0.13, 4.17)	3	Placebo	41.20		
4	Placebo	68.00			4	Dexmedetomidine	32.50	1.22	(0.31, 4.85)
5	Magnesium sulfate	48.60	2.05	(0.08, 52.19)	5	Pethidine	31.70	1.18	(0.59, 2.36)
6	Pethidine	39.60	2.98	(0.50, 17.73)	6	Fentanyl	31.50	1.18	(0.52, 2.67)
7	Fentanyl	39.10	2.84	(0.58, 13.91)	7	Tramadol	27.30	1.39	(0.33, 5.77)
Bradycardia (heterogeneity variance = 0.00)									
Rank	Treatment	SUCRA	OR	95%CI					
1	Pethidine	82.30	0.32	(0.07, 1.42)					
2	Dexmedetomidine	52.70	0.84	(0.14, 4.87)					
3	Fentanyl	49.50	1.00	(0.06, 16.44)					
4	Morphine	47.70	1.00	(0.02, 40.86)					
5	Placebo	46.70							
6	Sufentanil	39.10	1.21	(0.44, 3.37)					
7	Tramadol	32.00	1.72	(0.07, 41.24)					

SUCRA: Surface under the cumulative ranking curve.

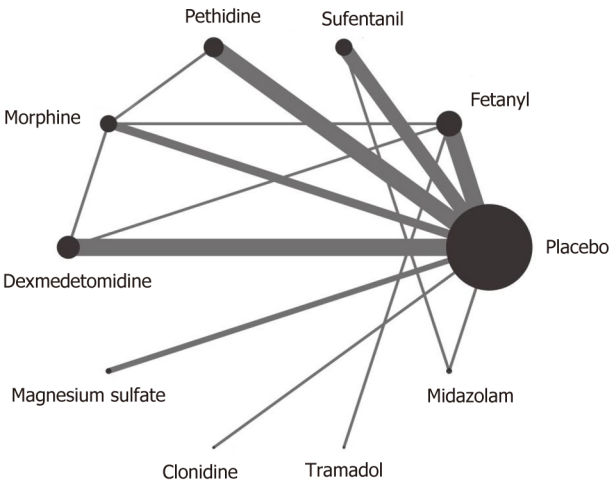

Figure 2 Network plot of eligible comparisons of all neuraxial adjuvants for the prevention of shivering. The width of the lines is proportional to the number of each pair of direct comparisons, and the size of the point is proportional to sample size.

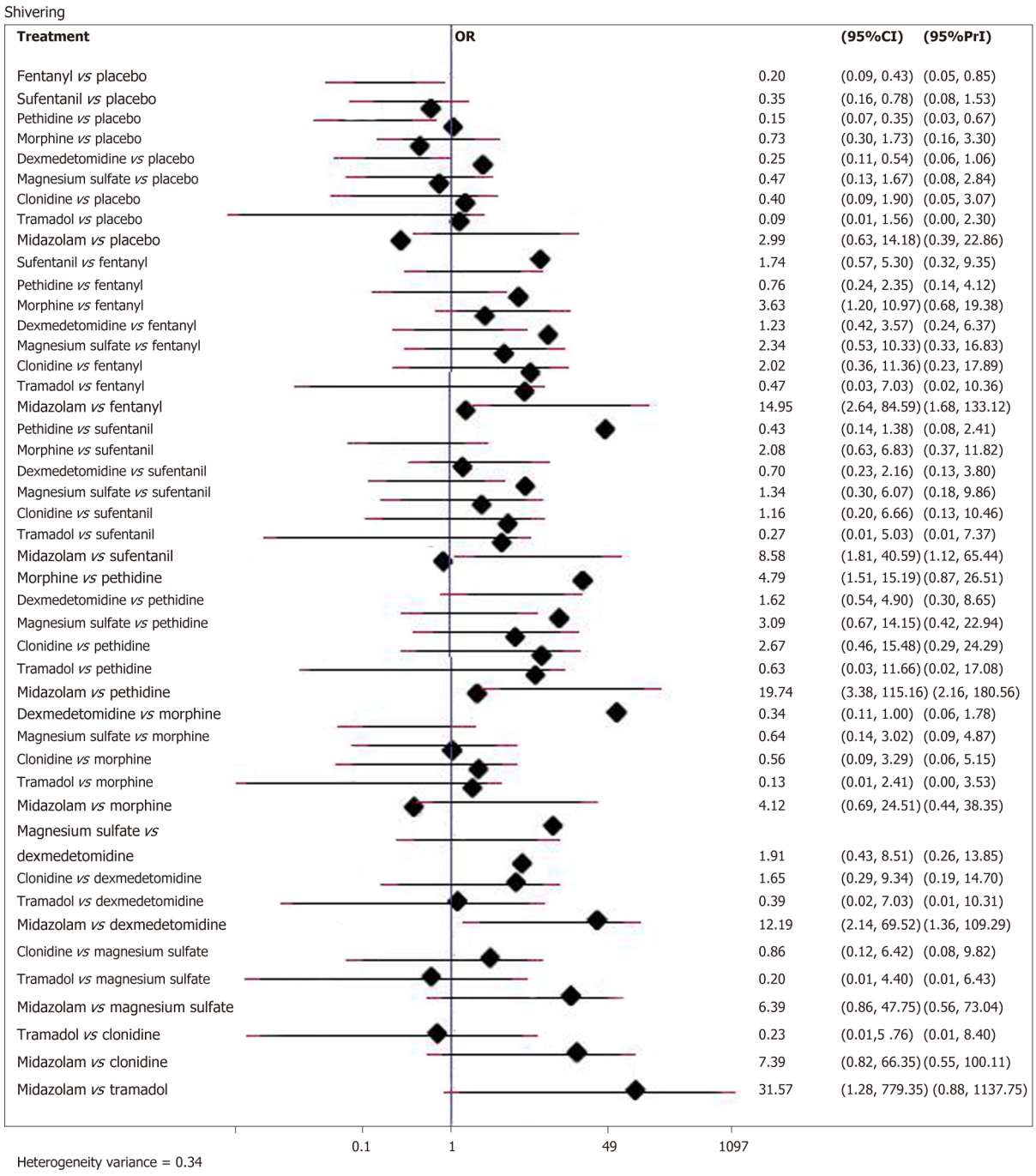

Figure 3 Forest plots of the effect of all neuraxial adjuvants in the prevention of shivering.

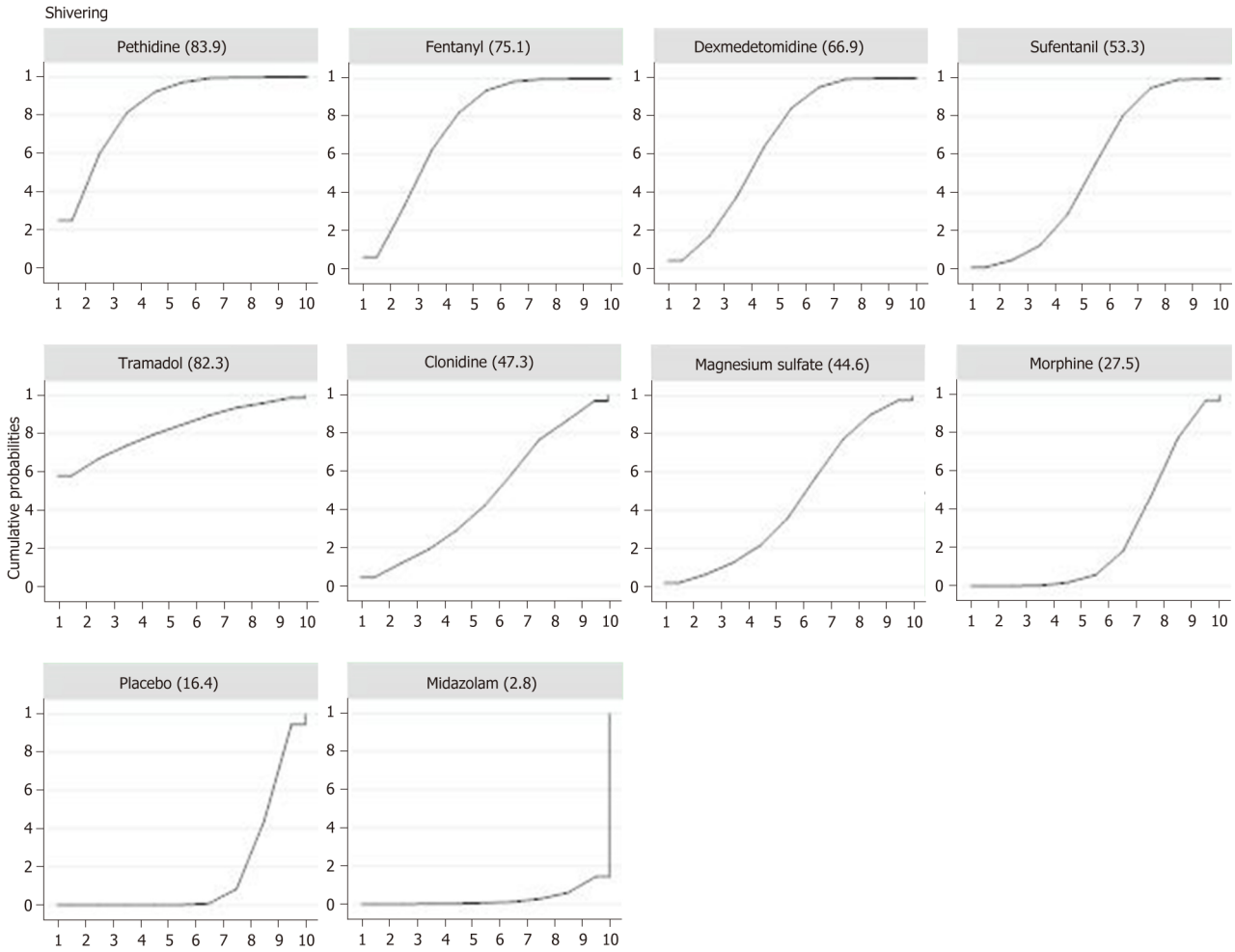

Figure 4 Ranking of SUCRA values of the incidence of shivering.

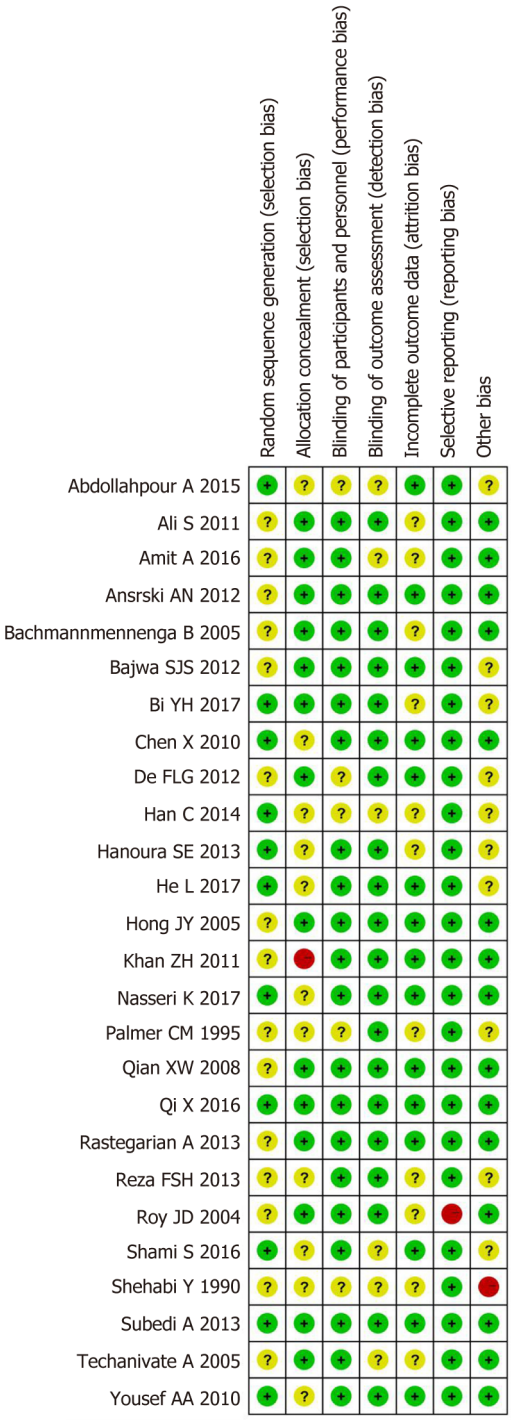

Figure 5 Risk of bias assessment. Green circle: Low risk of bias; red circle: high risk of bias; yellow circle: Unclear risk of bias.

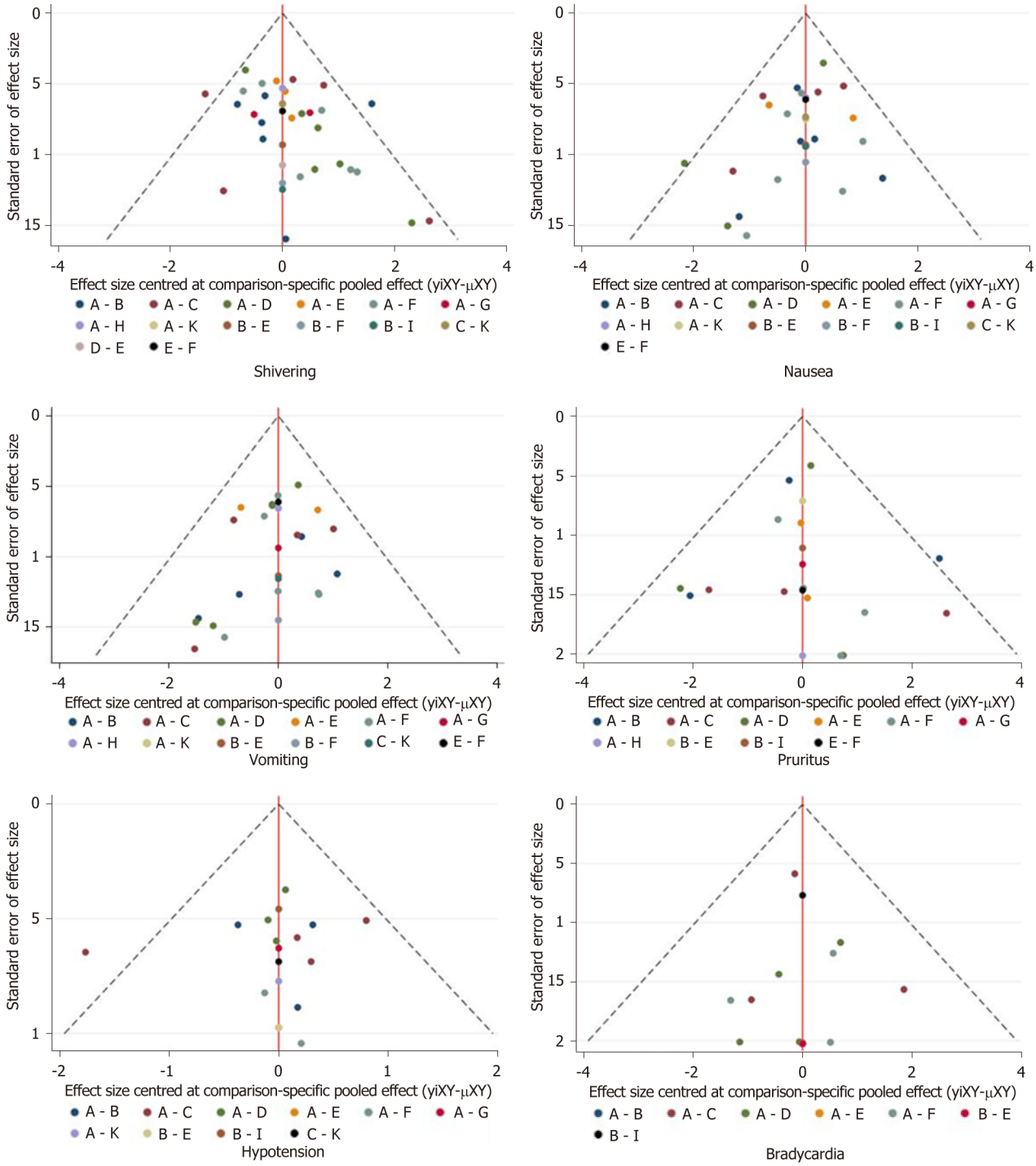

Figure 6 Funnel plots of publication bias.

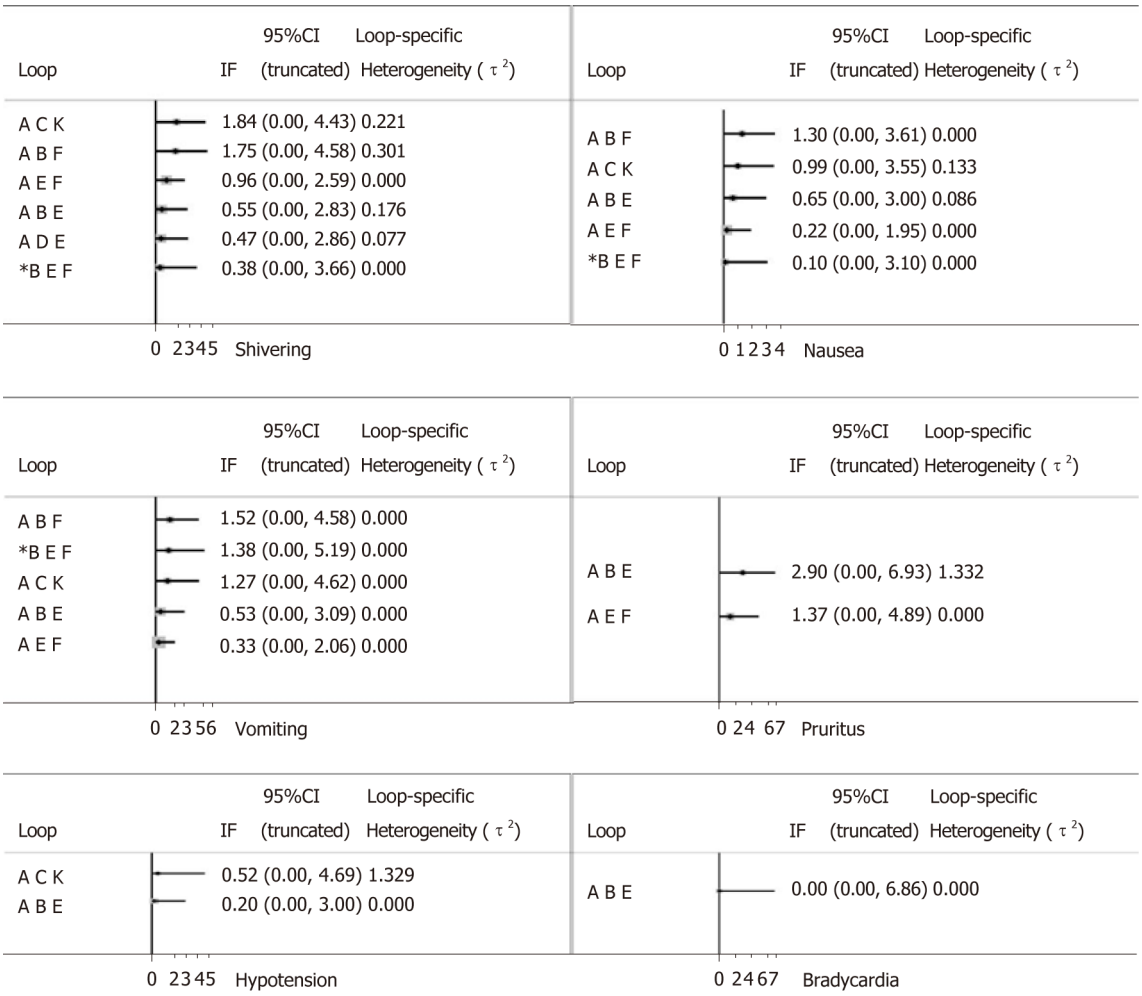

Figure 7 Inconsistency plots, node-splitting assessment.

ARTICLE HIGHLIGHTS

Research background

Perioperative shivering is clinically common during caesarean section, and several neuraxial adjuvants have been used to prevent perioperative shivering. However, the effects of these neuraxial adjuvants and which one is preferred remain elusive.

Research motivation

To provide evidence for clinicians to choose the optimal neuraxial adjuvant to reduce perioperative shivering during cesarean section.

Research objectives

To evaluate the effects of different neuraxial adjuvants on perioperative shivering during cesarean section.

Research methods

A systematic review and network meta-analysis (NMA) were conducted following the PRISMA guidelines. We performed a comprehensive search of PubMed, EMBASE, Web of Science, and Cochrane Central databases for eligible clinical trials assessing the effects of neuraxial adjuvants on perioperative shivering. Analyses were performed using Review Manager 5.3 and Stata 14.0.

Research results

Pethidine, fentanyl, dexmedetomidine, and sufentanil are more efficacious than other medications in the prevention of shivering during caesarean sections. Among the above four adjuvants, pethidine was most effective for shivering prevention (OR = 0.15, 95%CI: 0.07-0.35, SUCRA 83.9), but with a high incidence of nausea (OR = 3.15, 95%CI: 1.04-9.57) and vomiting (OR = 3.71, 95%CI: 1.81-7.58). The efficacy of fentanyl for shivering prevention was slightly inferior to pethidine (OR = 0.20, 95%CI: 0.09-0.43), with a significantly decreased incidence of nausea (OR = 0.34, 95%CI: 0.15-0.79) and vomiting (OR = 0.25, 95%CI: 0.11-0.56). Furthermore, compared with sufentanil, fentanyl showed no impact on haemodynamic stability and the

incidence of pruritus.

Research conclusions

The results of this NMA indicated that neuraxial pethidine, fentanyl, dexmedetomidine, sufentanil appear to be more efficacious than other medications in the prevention of shivering during caesarean section. Considering the risk-benefit profiles of the included neuraxial adjuvants, fentanyl is probably the optimal choice for the prevention of perioperative shivering during cesarean section. Although several neuraxial adjuvants have been reported to prevent shivering during caesarean section, very few clinical trials directly compared the neuraxial adjuvants during caesarean section. Thus, it is currently impossible to perform a pairwise meta-analysis to directly compare the difference between two neuraxial adjuvants. More clinical trials that directly compare these neuraxial adjuvants (*e.g.*, neuraxial pethidine *vs* fentanyl) are needed to fully explore the possible differences between the effects of these adjuvants.

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CASE REPORT

Primary malignant melanoma of the biliary tract: A case report and literature review

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Abstract

BACKGROUND

Primary malignant melanoma of the biliary tract (MBT) is a rare condition whose diagnosis requires excluding a primary origin in another location. This paper reviews the most important characteristics of MBT cases published in the literature and reports a new case. The patient reported here is the first case of primary malignant melanoma of the biliary tract with pulmonary metastasis treated with immunotherapy. This patient remains disease-free 36 mo after the treatment of metastatic lung lesions.

CASE SUMMARY

A 51-year-old man was admitted to the gastrointestinal department to study obstructive jaundice of a 1 wk clinical course. Magnetic resonance cholangiopancreatography revealed dilatation of the intrahepatic biliary tract and stenosis of the common hepatic duct. Given the suspicion of biliary tract neoplasia, cholecystectomy and resection of the common hepatic duct were performed with hepatic jejunostomy free of complications. Anatomo-pathological diagnosis was melanoma. After intervention, the patient was referred to the Department of Medical Oncology, where a primary origin was excluded in the skin, mucosa, and eyes. This confirmed diagnosis of primary biliary tract melanoma. Computed tomography was performed 12 mo after the procedure revealed several subcentimetric lung nodules. Wedge resection was performed. After confirming the diagnosis of pulmonary metastasis of primary melanoma of

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the biliary tract, the patient was started on immunotherapy with nivolumab. Tolerance to treatment was excellent. The patient remains disease-free 36 mo after the treatment of metastatic lung lesions.

CONCLUSION
The patient reported here is the first case of primary malignant melanoma of the biliary tract with lung metastases successfully treated with immunotherapy.

Key words: Biliary tract; Case report; Immunotherapy; Malignant melanoma

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Core tip: Primary malignant melanoma of the biliary tract is a very rare entity that mainly affects men (men/women ratio 12:2) and has an average age of presentation of 47 years (range: 26-67). Cases usually present as abdominal pain and jaundice. Lesions are usually black, polypoid, and have endoluminal growth. In cases of localised disease, the treatment of choice is surgery. Mortality because of this disease was 50% after average follow-up of 37 mo (range: 4-204 mo). Here, for the first time, we describe a case of primary malignant melanoma of the biliary tract with pulmonary metastases, successfully treated with immunotherapy.

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INTRODUCTION

Melanoma is a malignant tumour arising from melanocytes whose origin is the neural crest[1]. This is a very aggressive neoplasia with a high capacity for remote dissemination[2]. In the last few years there has been a major increase in the incidence of this neoplasia[3,4] due to early diagnosis and overdiagnosis of benign lesions[5,6], whereby mortality remains virtually stable[4,7,8]. Its location in the mucosa is less common and associated with a delay in diagnosis[2]. Location in the biliary tract in the form of primary tumour is exceptional with only 13 cases reported in the literature[9-20]. Increased knowledge of molecular biology has enabled more development of treatments for this disease[2,4]. This paper reviews the most important features of primary malignant melanoma of the biliary tract (MBT) cases using the PubMed database (up to January 2019) and reports the first patient treated with immunotherapy.

CASE PRESENTATION

Chief complaints

A 51-year-old man with a personal history of smoking (one pack/d) and high blood pressure. The patient was admitted to the gastrointestinal department to study obstructive jaundice of a 1 wk clinical course.

Laboratory examinations

Initial analysis revealed: total bilirubin 13.9 mg/dL, GGT 900 U/L, and AF 324 U/L.

Imaging examinations and treatment

Magnetic resonance cholangiopancreatography (MRC) revealed dilatation of the intrahepatic biliary tract and stenosis of the common hepatic duct. Cytological samples obtained by endoscopic retrograde cholangiopancreatography were negative for malignancy (non-representative samples). Abdominal computed tomography (CT) confirmed the findings of the MRC and was negative for distant metastasis. Given the suspicion of biliary tract neoplasia, cholecystectomy and resection of the common hepatic conduct were performed with hepatic jejunostomy free of complications.

Anatomopathological examination, including the immunohistochemical study, revealed a malignant melanoma measuring 1 cm in diameter (Figure 1). A total of two adenopathies were analysed on a perichole-cystic and peri-portal level; these did not reveal metastasis.

After intervention, the patient was referred to the Department of Medical Oncology, where primary origin was excluded in the skin, mucosa, and eyes. This confirmed diagnosis of primary biliary tract melanoma.

CT performed 12 mo after the procedure revealed several subcentimetric lung nodules in the posterior and apical segments of the left upper lobe (LUL), in the anterior and superior segments of the left lower lobe (LLL), and in the posterior-basal region of the right lower lobe (RLL). These nodules revealed pathological increased uptake on positron emission tomography-CT (Figure 2). Wedge resection was performed on the LUL and LLL free of complications. Second, atypical segmentectomy was performed in the RLL. Anatomopathological study confirmed the diagnosis of lung metastasis from melanoma (positive for HMB45, vimentin, and S100 protein but negative for cytokeratin in the immunohistochemical studies). *BRAF* gene mutation in tumour tissue was studied by real-time polymerase chain reaction (Cobas 4800 BRAF V600 Mutation Test; Roche Diagnostics, Indianapolis, IN, United States); this proved to be negative.

FINAL DIAGNOSIS

Pulmonary metastasis from primary melanoma of the biliary tract.

TREATMENT

After confirming the diagnosis of pulmonary metastasis from primary malignant melanoma of the biliary tract, the patient started treatment with intravenous nivolumab at a dose of 3 mg/kg every 2 wk.

OUTCOME AND FOLLOW-UP

Positron emission tomography-CT studies performed 4 mo from starting nivolumab, subsequently every 6 mo and recently at 36 mo from pulmonary surgery, revealed a prolonged complete response (Figure 3). Tolerance to treatment was excellent; no immunomediated toxicity presented.

DISCUSSION

Melanoma is considered a multifactorial disease that arises from interactions between genetic susceptibility and environmental exposure[2,4]. Ultraviolet radiation (UVR) is the most significant modifiable risk factor. Both UVA-A and UVR-B have genotoxic effects either because of generation of oxygen radicals or abnormality in the nucleotide sequence, among others[21]. Melanomas are mainly classified according to their location and relationship to solar exposure[2]. Types of melanoma that arise on the skin exposed to sun are: Melanoma with superficial extension/melanoma with low cumulative solar damage, lentigo-malignant melanoma/melanoma with significant solar damage, and desmoplastic melanoma[2]. Melanomas that arise on skin protected from the sun or free of aetiological association UVR are: spitz melanoma, acral melanoma, melanoma of mucosa, melanoma that arises in congenital naevi, melanoma that arises in blue naevus, and uveal melanoma[2]. The four most common histological subtypes of melanoma are superficial spreading melanoma, lentigo maligna melanoma, acrallentiginous melanoma, and nodular melanoma[2].

Although these are very useful classifications in clinical practice, new classifications are being developed based on genetic abnormalities, which have therapeutic indications. In general, melanoma is a tumour with a high mutational load[2,22]. Mutations are common in *driver* genes such as *BRAF* and *RAS*[2,23]. Those melanomas that appear in exposed areas have more abnormalities in the genes *BRAF*, *N-RAS*, and *PTEN*. However, it appears that those not exposed to UVR (melanoma of mucosa and acral melanoma) have a higher number of chromosomal abnormalities (amplifications and deletions) and gene amplification, essentially of CDK4 and CCND1[2,23]. Therefore, it appears that melanomas that arise in areas not exposed to radiation would have a higher mutational load, and therefore, predict a greater response to immuno-

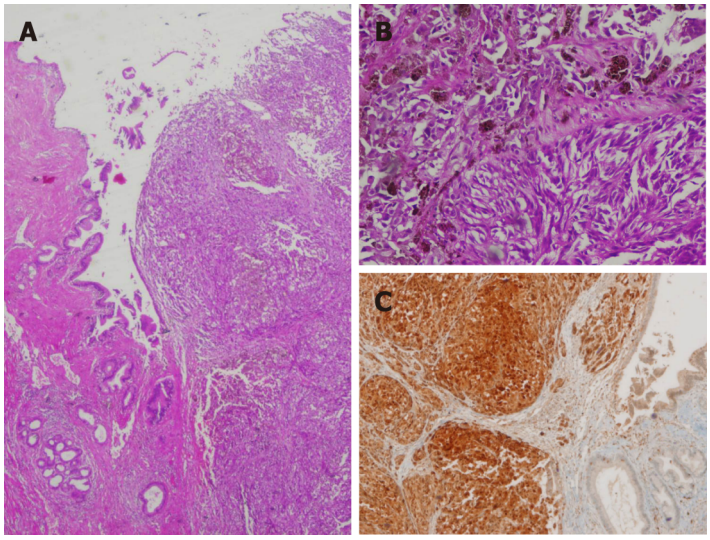

Figure 1 Primary melanoma of the biliary tract. A: The melanoma formed a polypoid mass that infiltrates the wall of the extrahepatic bile duct; B: At high magnification, some malignant melanocytes revealed finely granular cytoplasmic melanin; C: Tumour cells revealed strong immunopositivity for the HMB45 antigen.

therapy[22].

The most common melanoma location is on the skin, mainly the trunk (43.5%) and the limbs (33.9%)[2,7]. Melanoma is a highly aggressive tumour that can disseminate to any part of the body, although metastases are more common in the lymph nodes, liver, lungs, and brain. Only 2% to 4% of patients present metastasis in the gastrointestinal system, mainly in the bowel, colon, and stomach[24]. There are unusual cases of solitary metastasis of melanoma to the gallbladder[25] and asymptomatic metastasis has been reported in the gallbladder and biliary tract in up to 4% to 20% of autopsies performed on patients with metastatic melanoma[26,27]. Therefore, another primary origin in the event of existence of melanoma in the biliary tract should be ruled out. Along these lines, Ricci *et al*[28] proposed five criteria that could provide guidance around diagnosis of primary melanoma in the biliary tract: (1) Absence of previous melanoma; (2) Absence of melanoma in other locations; (3) Single solitary lesion; (4) Polyploid or papillary form; and (5) Existence of biliary obstructive clinical symptoms.

After reviewing the literature and including this case, there are only 14 cases of MBT reported to date (Table 1). MBT is a very rare entity that mainly affects men (men/women ratio 12:2) and has an average age of presentation of 47 years (range: 26-67). The most common form of presentation is abdominal pain together with jaundice and at times a general syndrome. In cases with macroscopic data lesions were black, polyploid, and with endoluminal growth, which conditioned obstructive clinical symptoms associated with clinical presentation. Although only three patients were revealed to have metastatic disease at diagnosis, up to seven revealed remote course after the procedure. The most common site of metastatic dissemination documented was the liver, although other locations were the brain, lung, mesentery, and pelvis. In all cases of localised disease, the treatment of choice was surgery. Radical surgery was performed in those with good "performance status"; duodenopancreatectomy was the most common intervention. Only two patients were not candidates for surgery due to their general condition and/or disease extension at the time of diagnosis.

In the last few years, immunotherapy has changed the paradigms for treatment of metastatic melanoma. Currently, first-line treatment of metastatic melanoma or non-resectable wild-type *BRAF* is considered, which demonstrates benefits on overall survival, progression-free survival, response rates, and duration of response[29]. While most immunotherapy clinical trials only include malignant melanomas of the skin, this case exemplifies that the excellent response of malignant melanoma to this therapy is not limited exclusively to primary malignant melanomas of the skin.

CONCLUSION

Until a few years ago, malignant melanoma was considered one of the most aggressive tumours and there were hardly any effective systemic treatment options.

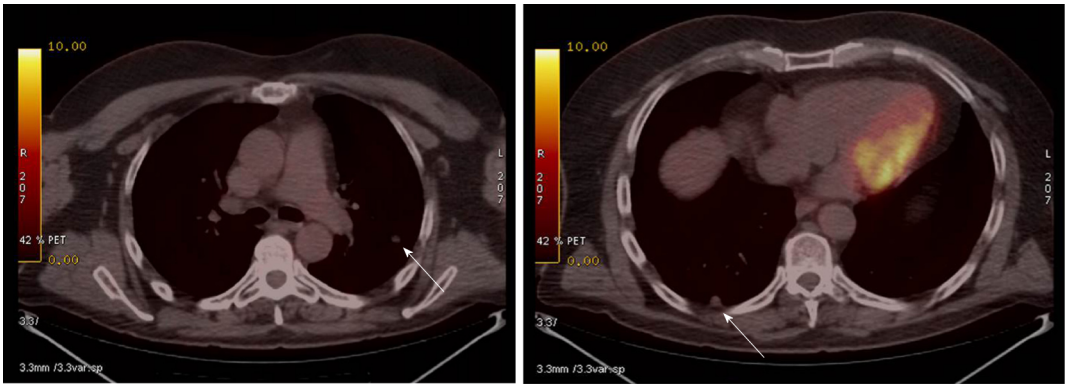

Figure 2 Thoracic positron emission tomography/computed tomography scan showing metastatic nodules (arrows).

The overall survival of a patient with metastatic melanoma was less than 1 year. However, knowledge of melanoma's molecular biology has meant a spectacular change in regard to treatment and survival of melanoma patients. Contrary to what was believed, a greater mutational load has been revealed in those melanomas that appear in sites not exposed to UV[2]. This has meant a greater efficacy of immunotherapy[30]. Here, for the first time, we describe a case of primary biliary malignant melanoma with pulmonary metastases, successfully treated with immunotherapy.

Table 1 Cases of patients with primary melanoma of common bile duct

Ref.	Age/sex	Clinical presentation	Tumour location	Metastasis at presentation	Treatment	Outcome
Zaide[9]	47/M	Jaundice	Proximal CHD	No	Cholecystectomy and resection of CHD	DFS (6 mo)
Carstens *et al*[10]	30/M	Jaundice; Abdominal pain	Distal CBD	No	Whipple resection	PD (liver); Expired (6 mo)
Deugnier *et al*[11]	34/F	Jaundice; Abdominal pain	LHD	No	Left hepatectomy with partial right hepatic and CBD resection	PD (skin and lymph nodes); Expired (18 mo)
Zhang *et al*[12]	58/M	Jaundice	CBD and gallbladder	No	Whipple resection	PD; Expired (48 mo)[13]
Washburn *et al*[13]	43/M	Jaundice; Abdominal pain; Loss of weight	CHD and RHD	No	Right hepatic lobectomy and cholecystectomy	DFS (11 mo)
Washburn *et al*[13]	45/M	Jaundice; Loss of weight	Distal CBD and ampulla	No	Whipple resection	DFS (72 mo)
Wagner *et al*[14]	48/M	Jaundice and acute cholecystitis	CBD	No	Whipple resection	PD (widespread metastases); Expired (9 mo)
González *et al*[15]	67/F	Jaundice; Abdominal pain	Proximal CHD	No	Extrahepatic BD resection	DFS (17 yr)
Bejarano *et al*[16]	47/M	Jaundice; Abdominal pain; Loss of weight	Distal CBD	Yes (gallbladder)	Chemotherapy; (dacarbacin + IFN2B, Carboplatin	PD (stomach, spleen, lymph nodes, subcutaneous kidney); Expired (23 mo)
Hoshi *et al*[17]	55/M	Jaundice	CHD	Unknown	Bile duct resection	PD; Expired (4 mo)
Agrawal *et al*[18]	26/M	Abdominal pain	Intrahepatic biliary tract	No	Hepatic segmentectomy (II-III)	DFS (72 mo)
Smith *et al*[19]	55/M	Jaundice	Distal CBD	No	Whipple resection	DFS (12 mo)
Addepally *et al*[20]	52/M	Jaundice; Abdominal pain; Loss of weight	CHD	No	BCS	PD (liver, lymph nodes, mesentery and brain) Expired (3 mo)
Present case	51/M	Jaundice	CHD	No	Cholecystectomy and CHD resection PD (lung): Lung segmentectomy + nivolumab	PD (lung, 12 mo); OS (31 mo)

BCS: Best care support; CBD: Common bile duct; CHD: Common hepatic duct; DFS: Disease-free survival; F: Female; LHD: Left hepatic duct; M: Male; OS: Overall survival; PD: Progressive disease; RHD: Right hepatic duct.

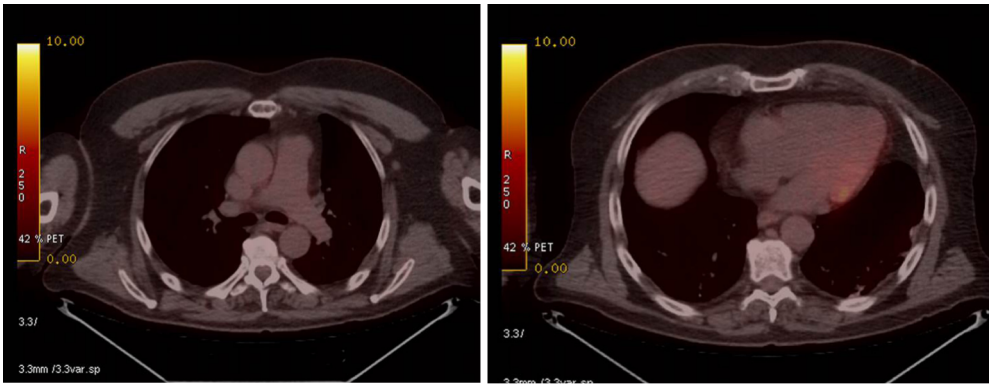

Figure 3 Thoracic positron emission tomography/computed tomography scan with no evidence of metastases.

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CASE REPORT

Successful treatment of tubulointerstitial nephritis in immunoglobulin G4-related disease with rituximab: A case report

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Abstract

BACKGROUND

Immunoglobulin G4-related disease (IgG4-RD) is an immune-mediated condition that consisted of disorders that share particular clinical, serologic and pathologic properties. The common presentation of disease includes tumor-like swelling of involved organs and the histopathological findings are a lymphoplasmacytic infiltrate enriched with IgG4-positive plasma cells, and a variable degree of fibrosis that has a characteristic "storiform" pattern in biopsy specimens of tumor-like masses. Major presentations of this disease, which often affects more than one organ, include autoimmune pancreatitis, salivary gland disease (sialadenitis), orbital disease and retroperitoneal fibrosis. The steroid treatment is essential for the treatment of the disease however, other immunosuppressive drugs including cyclophosphamide or rituximab could be an option in resistant cases.

CASE SUMMARY

Herein, we reported a 34-year-old woman whom previously had diagnosed with asthma, rheumatoid arthritis and Sjögren's syndrome (SS) referred our nephrology department due to acute kidney failure development at the last rheumatology visit. After kidney biopsy she has been diagnosed with IgG4-RD and tubuluointerstitial nephritis. She had been accepted resistant to steroid, mycophenolate mofetil, methotrexate and azathioprine therapies due to receiving

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in last two years. She refused to receive cyclophosphamide due to potential gonadotoxicity of the drug. Thus, rituximab therapy was considered. She received 1000 mg infusion, 15 d apart and 6 mo later it has been administered same protocol. After one year from the last rituximab dose serum creatinine decreased from 4.4 mg/dL to 1.6 mg/dL, erythrocyte sedimentation rate decreased from 109 mm/h to 13 mm/h [reference range (RR) 0-20], and C-reactive protein decreased from 55.6 mg/L to 5 mg/L (RR 0–6). All pathologic lymph nodes and masses were also disappeared.

CONCLUSION
Patients with IgG4-RD usually misdiagnosed with rheumatologic diseases including systemic lupus erythematous or SS and also they were screened for the presence of malignancy. Rituximab could be an important treatment option in cases with steroid resistant tubulointerstitial nephritis in IgG4-RD.

Key words: Immunoglobulin G4-related disease; Tubulointerstitial nephritis; Rituximab; Case report

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Core tip: Immunoglobulin G4-related disease is an immune-mediated condition that mimics several rheumatologic disorders and malignancies. The diagnosis can be provided by detecting lymphoplasmacytic infiltration with storiform fibrosis in the histopathological examination of the affected organ. However, steroid is the mainstay treatment agent, rituximab should be addressed in resistant cases.

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INTRODUCTION

Immunoglobulin G4-related disease (IgG4-RD) is a recently recognized systemic fibro-inflammatory condition that mimics several autoimmune, malignant, and rheumatological diseases[1]. The hallmarks of the disease are swelling of affected tissue(s)/organ(s) caused by a lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells, usually accompanied by fibrosis and an increased number of eosinophils[2]. Serum IgG4 levels are elevated in about two-thirds of these patients. In addition, a minority of patients have normal serum IgG4 levels despite the presence of the typical IgG4-RD-related histopathological changes[3,4].

IgG4-RD can involve one or multiple organs. Patients often present with subacute development of mass/masses or diffuse enlargement of the affected organ(s). Lymphadenopathy is an important finding, and asthma or allergy is present in approximately 40% of IgG4-RD patients. One of the most common conditions in these patients is autoimmune pancreatitis. This condition often presents as a pancreatic mass or painless obstructive jaundice and, often which can mimics pancreatic cancer. Moreover, patients often present with lacrimal and salivary gland involvement (IgG4-related Mikulicz's disease), which was once thought to be a subset of Sjögren's syndrome (SS). Orbital diseases, including orbital masses and retroperitoneal fibrosis, are other clinical presentations observed in IgG4-RD patients. Depending upon the multiorgan nature of the condition, several clinical phenotypes have recently been identified. These phenotypes include four main groups: (1) Pancreatic hepatobiliary disease; (2) Retroperitoneal fibrosis and/or aortitis; (3) Head-and neck-limited disease; and (4) Classic Mikulicz syndrome with systemic involvement. Renal involvement in patients with IgG4-RD is rarely seen. Only individual case series have been reported in the literature and the most frequent finding in these patients is tubulointerstitial nephritis (TIN)[5-7].

In this case report, we present a case previously misdiagnosed as SS because of the presence of sialadenitis. The patient was referred to our nephrology department

because of development of acute kidney dysfunction that had been detected during her last rheumatology visit. Histopathological examination of the kidney biopsy revealed TIN with fibrosis. Serum IgG4 levels were predominantly increased, and the patient was diagnosed with IgG4-RD. Steroid therapy failed to lead to remission; however, complete remission achieved as a result of rituximab administration.

CASE PRESENTATION

Chief complaints

A 34-year-old female patient was admitted to the rheumatology department in July 2017 with complaints of nausea, joint pain, and swelling in the neck.

History of present illness

Her medical history was notable for asthma, rheumatoid arthritis (RA), SS, and sialadenitis.

History of past illness

She had been undergoing follow-up for RA and SS in the rheumatology department since 2015. Her last follow-up was in November 2016, and laboratory results revealed a rheumatoid factor of 273 IU/mL [reference range (RR) 0-20], anti-cyclic citrullinated peptide (anti-CCP) of < 1.5 U/mL (RR 1.5-1.93), creatinine of 0.74 mg/dL (RR 0.5-0.9 mg/dL), erythrocyte sedimentation rate (ESR) of 109 mm/h (RR 0-20), and C-reactive protein (CRP) of 55.6 mg/L (RR 0–6).

Physical examination upon admission

Upon initial physical examination, a 2-3 cm palpable cervical lymphadenomegaly was detected. She had clear lungs and normal heart sounds with no murmurs or gallops upon auscultation. Her current medication upon admission included methotrexate (15 mg once weekly injection), methylprednisolone (16 mg once daily), calcium carbonate (2500 mg) plus cholecalciferol/vitamin D3 (880 IU once daily).

Laboratory examinations

Laboratory results revealed several abnormal findings: Hemoglobin: 10.8 g/dL (RR 14-18 g/dL); blood urea nitrogen 33 mg/dL (RR 8-23 mg/dL); creatinine 4.4 mg/dL (RR 0.5-0.9 mg/dl); K: 5.6 mmol/L (3.5-5.1 mmol/L); phosphorus 4.6 mmol/L (RR 2.5-4.5 mmol/L); uric acid: 8.4 mg/dL (RR 2.4-5.7 mg/dL); ferritin: 221.8 ng/mL (RR 15-150 ng/mL); ESR: 84 mm/h (RR 0-20); and CRP: 23 mg/L (RR 0-6 mg/L). All laboratory results are depicted in Table 1. The patient was referred to the nephrology department. A urine stick test revealed one positive protein. Urine microscopy was negative for casts. The 24-h urine protein level was 0.8 g. Anti-nuclear antibody, anti-double-stranded DNA (dsDNA), anti-glomerular basement membrane, and antineutrophil cytoplasmic antibodies profiles were all negative. The C3 level was 60 mg/dL (RR 90-180 mg/dL), and the C4 level was 15.3 (RR 10-40).

Hypereosinophilia was detected on a peripheral blood smear (eosinophil count was 3.99×10^3/L; RR is 0-0.2 $\times 10^3$/L), eosinophil percentage was 37.3% (RR 0.9%-2.9%), and the IgG4 level was 2602 mg/dL (RR 3-201 mg/dL).

Imaging examinations

Renal ultrasonography indicated enlarged bilateral kidneys. The right and left kidneys had long axes of 159 and 181 mm, respectively. Bilateral renal echoes were significantly decreased, and hypoechoic areas were observed in both kidneys. Among the enlarged area, the largest one was 54 mm × 42 mm in the lower middle section of the left kidney.

Computed tomography (CT) revealed nodular lesions in the liver, a pancreatic mass, cervical and mediastinal lymph nodes of 2 to 3 cm in diameter.

Positron emission tomography/CT (PET-CT) screening was used based on suspicion of metastatic malignancy or lymphoma. It has been revealed that cervical and mediastinal lymph nodes had high metabolic activity. Infiltrative soft tissue masses were observed ranging from 2 to 4 cm in size in both kidneys. These masses have showed intense hypermetabolic activity in the cortical areas of the kidney parenchyma (standardized uptake value max: 8.01) (Figure 1).

Pathological examinations

A kidney biopsy was performed. The biopsy showed intense, connective tissue infiltration between the glomerular and tubular structures. Lymphoplasmacytic and eosinophilic cell infiltrations were observed. IgG4-stained cells were detected on the biopsy specimen (Figure 2).

Table 1 Laboratory parameters of the patient on the admission in July 2017

Parameter	Value	Normal Range
WBC (10^3/μL)	6.63	4.8-10.7
Hemoglobin (g/dL)	10.8	12-16
Platelet (10^3/μL)	286	130-400
MCV (fl)	92	80-100
Glucose (mg/dL)	98	82-115
BUN (mg/dL)	33.5	8-23
Creatinine (mg/dL)	4.4	0.5-0.9
Calcium (mg/dL)	9.6	8.8-10.2
Phosphorus (mg/dL)	4.6	2.5-4.5
Sodium (mmol/L)	139	136-145
Potassium (mmol/L)	5.6	3.5-5.1
Uric acid(mg/dL)	8.4	2.4-5.7
Total bilirubin (mg/dL)	0.51	0.2-1.2
Direct bilirubin (mg/dL)	0.16	0-0.3
GGT (U/L)	11	6-42
LDH (U/L)	141	135-214
AST (U/L)	9	0-32
ALT (U/L)	12	0-33
ALP (U/L)	46	35-105
Total protein (g/dL)	8.3	6.4-8.3
Albumin (g/dL)	3.82	3.5-5
Iron (μg/dL)	80	70-180
Iron binding (μg/dL)	320	225-480
Ferritin (ng/mL)	221.8	15-150
Folic acid (ng/mL)	4.5	3.89-26.8
Vitamin B12 (pg/mL)	386.6	197-771
ESR (mm/h)	84	0-20
CRP (mg/L)	23	0-6
iPTH (pg/mL)	35	15-65

WBC: White blood cell; MCV: Mean corpuscular volume; BUN: Blood urea nitrogen; GGT: Gamma-glutamyl transferas; LDH: Lactate dehydrogenase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ESR: Erythrocyte sedimentation rate; CRP: C-Reactive Protein; iPTH: Intact parathyroid hormone.

FINAL DIAGNOSIS

The patient was diagnosed with TIN and IgG4-RD.

TREATMENT

A two-hundred fifty milligram intravenous (iv) pulse of methylprednisolone treatment was administered for three days. Afterwards 1 mg/kg (64 mg) oral methylprednisolone initiated and continued. After ten weeks with steroid treatment, her creatinine level was 3.5 mg/dL in October 2017. Steroid resistance was considered and steroid dose was tapered gradually. She had been treated with mycophenolate mofetil, methotrexate and azathioprine previously in last two years and she had been accepted resistant to these therapies. She refused to receive cyclophosphamide due to her child-bearing potential. Thus, rituximab therapy was considered. The 2 infusions of 1000 mg, 15 d apart. After six months dose was repeated due to partial response in May 2018. Total dose of rituximab reached to 4000 mg.

OUTCOME AND FOLLOW-UP

Her last visit was in May 2019. After one year of rituximab therapy serum creatinine

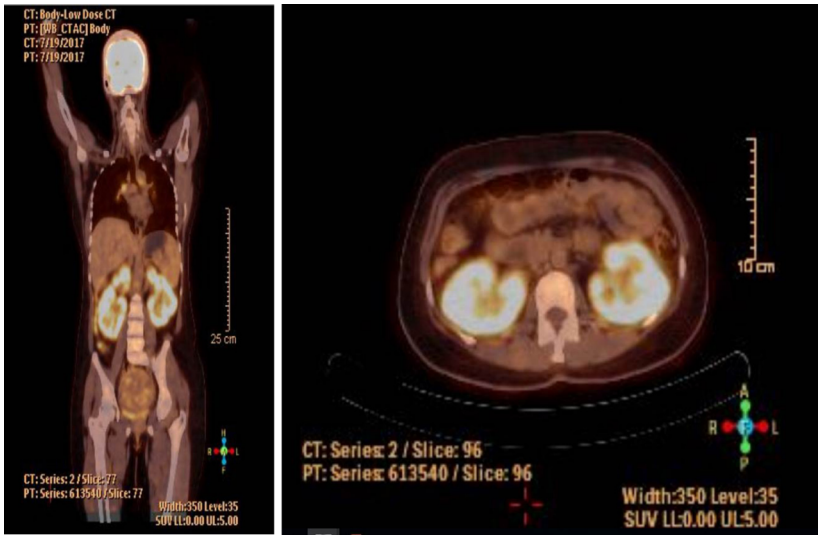

Figure 1 Positron-emission tomography-computed tomography scan reported as increased fluorodeoxyglucose uptake in infiltrative soft tissue areas in both kidneys (standardized uptake value max: 8.01).

level decreased from 4.4 mg/dL to 1.6 mg/dL, ESR decreased from 109 mm/h to 13 mm/h (RR 0-20), and CRP decreased from 55.6 mg/L to 5 mg/L (RR 0–6). She has no complaint. All pathologic lymph nodes and masses were also disappeared. Clinical and laboratory remission was achieved. The steroid therapy withdrawal was achieved six months ago.

DISCUSSION

The diagnosis of IgG4-RD is often challenging due to a complex presentation as this condition mimics malignancies and autoimmune rheumatic diseases. Herein, we reported a case of IgG4-RD and TIN that was diagnosed by kidney biopsy. IgG4-RD rarely involves the kidneys, but the finding of kidney involvement has been described in some case series and in individual case reports. The most common renal-related finding is TIN. However, it has been reported that many of the affected IgG4-RD patients with kidney disease are middle-aged and older men, and our particular case involved a middle-aged woman[1,8]. Lymphoplasmacytic infiltration of the renal interstitium and presence of fibrosis were distinct features to distinguish IgG4RD-TIN from other TIN cases as has been shown in our case. Immunohistochemistry staining of renal biopsy specimen have revealed increased numbers of IgG4-positive plasma cells supported the diagnosis of IgG4RD-TIN. On the other hand, nodular lesions in kidneys mimicking metastatic malignancy is a very rare condition as in this case[2,9]. Kawano *et al*[10] recently reported a series of 41 Japanese IgG4-RD patients identified between 2004 and 2011 who presented with histopathological findings consistent with kidney involvement. Twenty-nine patients underwent CT scans, and the most common radiological findings were multiple low-density lesions with subsequent diffuse bilateral renal swelling as the second most common involvement. A solitary hypovascular parenchymal nodule was detected in just one patient in that study. More recently, Bianchi *et al*. reported an IgG4-RD case that presented with a solid mass in kidney, which mimicked a malignancy[9]. Our case also showed both bilateral enlarged kidneys with multiple lesions in both kidneys. This is important because establishing a differential diagnosis between IgG4-RD and malignancy is challenging. It has been well-established that conventional imaging techniques (ultrasound, CT, magnetic resonance imaging, and PET/CT) have limited efficiency in diagnosing IgG4-RD[11]. A biopsy from the affected organ remains the cornerstone for the IgG4-RD diagnosis[6].

However, the optimal treatment for IgG4-RD has not yet been well defined yet, an international consensus guideline has recently been published[12]. The treatment recommendations consist of several issues: (1) Glucocorticoids are the first-line drugs for remission induction in all patients with active, IgG4-RD, unless contraindications to steroids are present; (2) After successful achievement of induction therapy, certain patients will need maintenance therapy; and (3) Retreatment with glucocorticoids is indicated in some patients in whom relapse following successful remission induction

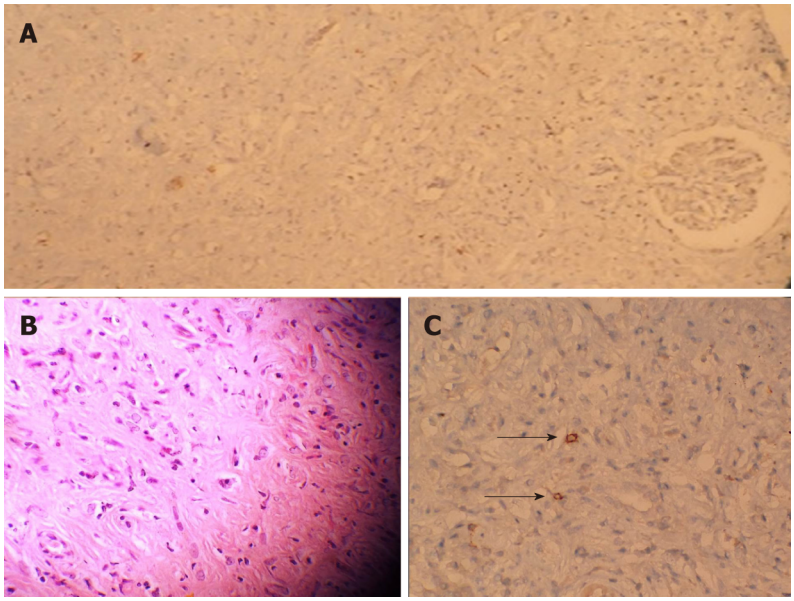

Figure 2 Histological section of kidney staining. A: In the examination of kidney biopsy specimen only one glomerulus and a few tubules were observed due to both intense inflammation and fibrosis (H and E: × 10); B: Histological section of kidney parenchyma showing interstitial nephritis characterized by the presence of inflammatory infiltration by lymphocytes and eosinophlis (H and E: × 40); C: Black arrows show the IgG4 staining positive cells in immunostaining with anti- IgG4 antibody (× 40).

was observed. Otherwise, some experts recommend the initiation of steroid-sparing immunosuppressive agents from treatment start due to the ultimate failure of steroid therapy and potential toxicities associated with long-term steroid treatment. Steroids and cyclophosphamide combinations have recently been reported to cause a low risk of relapse in these patients[13]. The risk of infertility could also be a barrier to this treatment combination, especially in women of child-bearing potential as in our case.

It has been shown that B-cell depletion leads to the targeted and quick reduction of serum IgG4 concentrations, with relative preservation of the concentrations of other immunoglobulins and immunoglobulin subclasses. Authors also speculate that rituximab achieves its effects in IgG4-RD by depleting the pool of B lymphocytes that replenish short-lived IgG4-secreting plasma cells[14-16]. Thus, rituximab was considered as a promising option for these cases. When we review the literature, the efficacy of B-cell depletion with rituximab in patients with that is resistant to steroids and other therapies has been recently demonstrated. Khosroshahi *et al*[14,15] have been treated successfully total 14 patients with IgG4-RD in a two different case series in the literature. They showed that almost all patients had clinical and laboratory response to rituximab. Only a few patients (4 of 14) have received second infusion six months after first infusion. More recently a retrospective study has been conducted in France with 156 patients with IgG4-RD and 33 patients have been treated with rituximab. Clinical response was noted in 29/31 (93.5%) symptomatic patients. Maintenance therapy (second dose six months after initial dose before occurrence of a relapse) with rituximab have been associated with longer relapse-free survival (41 *vs* 21 mo; P = 0.02) comparing to patients whom had been administered single rituximab dose[16]. They concluded that rituximab might be a novel treatment option for both induction and maintenance therapy in these patients. However, rituximab has not been evaluated in a randomized trial in patients with IgG4-RD, and its use for this disease might be evaluated for off-label use by drug control agencies.

In this unique case, we showed that rituximab therapy was successful in IgG4-RD and TIN treatment especially resistant to steroid and other therapies. Future randomized trials with larger patients are needed to establish the usage of rituximab in these patients.

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CASE REPORT

Effectiveness of vedolizumab treatment in two different anti-tumor necrosis factor alpha refractory pouchitis: A case report

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Abstract

BACKGROUND

Refractory pouchitis is a common cause of pouch failure, which may require surgical excision of the pouch or permanent diversion. We aimed to show the effect of vedolizumab on treatment of the patient with refractory pouchitis.

CASE SUMMARY

A 32-year-old male with pancolonic ulcerative colitis since the age of 25 with primary failure of infliximab and mesalamine and intolerance of azathioprine, underwent a total proctocolectomy with ileal pouch-anal anastomosis in 2012. He developed chronic diarrhea in 2014, which was watery, 30 per day and accompanied with blood and mucus affecting his quality of life.

CONCLUSION

Vedolizumab is safe and effective in the management of anti-tumor necrosis factor alpha refractory pouchitis.

Key words: Anti-tumor necrosis factor alpha; Refractory pouchitis; Vedolizumab; Ulcerative colitis

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Core tip: Vedolizumab, a humanized immunoglobulin G1 monoclonal antibody to α4β7 integrin, has been shown to moderate gut lymphocyte trafficking with an efficacy in treatment of both Crohn's disease and ulcerative colitis. In our patient who had two different anti-tumor necrosis factor refractory pouchitis, the gut-specific immune modulation mediated by vedolizumab treatment resulted in good responses. This case is important because vedolizumab is the novel therapy for refractory pouchitis. However, further large and prospective studies are needed for efficacy and the underlying mechanisms of efficacy of vedolizumab in treatment of refractory pouchitis.

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INTRODUCTION

Refractory pouchitis is a common cause of pouch failure, which may require surgical excision of the pouch or permanent diversion. Vedolizumab, a humanized immunoglobulin G1 monoclonal antibody to α4β7 integrin, has been shown to moderate gut lymphocyte trafficking with an efficacy in treatment of both Crohn's disease and ulcerative colitis (UC)[1,2]. Although tumor necrosis factor-alpha (TNF-α) inhibitors have been reported to be effective as treatment for pouchitis[3], there is little data regarding the use of vedolizumab in refractory pouchitis[4]. The effect of vedolizumab treatment on chronic antibiotic refractory pouchitis is very limited. Chronic antibiotic refractory pouchitis is a challenging complication in patients with UC who undergo proctocolectomy with ileal pouch-anal anastomosis. Chronic antibiotic refractory pouchitis occurs when patients do not respond to a 2-wk course of ciprofloxacin, metronidazole or rifaximin for pouchitis[5].

CASE PRESENTATION

Chief complaints

We report on a 32-year-old male with pancolonic UC.

History of present illness

A 32-year-old male with pancolonic UC since the age of 25 with primary failure of infliximab and mesalamine and intolerance of azathioprine, underwent a total proctocolectomy with ileal pouch-anal anastomosis in 2012.

History of past illness

He developed chronic diarrhea in 2014, which was watery, 30 per day and accompanied with blood and mucus affecting his quality of life. He could not work. He lost a lot of weight. He had fallen from 55 kg to 43 kg during pouchitis. His body mass index was 15.2 kg/m^2. He used meselamine 3 g orally, steroid intermittently, lavman and loperamide orally three times daily.

Personal and family history

His family history was unremarkable.

Physical examination upon admission

His abdominal physical examination was normal.

Laboratory examinations

Laboratory work-up revealed erythrocyte sedimentation rate of 56 mm/h and C-reactive protein of 3.6 mg/dL with no liver function abnormalities. Autoimmune markers including IgG4, anti-nuclear antibody and anti-mitochondrial antibody were negative. His blood tests for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus antibodies were negative. Stool studies for *Clostridium difficile*, viruses and bacteria were negative. Blood tests for Epstein-Barr virus and cytomegalovirus antibodies were negative.

Imaging examinations

An ileoscopy and pouchoscopy were performed that demonstrated normal proximal ileal mucosa, but there were diffuse edema, erythema and nodularity and multiple superficial and deep ulcers in the pouch. His pouchitis disease activity index score was 16. Biopsies obtained were negative for cytomegalovirus. An upper endoscopy was done at the same time to evaluate diarrhea, and it was normal. Duodenal biopsy was negative for the presence of celiac disease. Serum antibodies for celiac disease including anti-gliadin antibodies, endomysial antibodies and anti-transglutaminase antibodies were negative. Therefore, gluten restricted diet was not given to the patient. His chest X-ray was normal. Purified protein derivative skin test was 0 mm.

FINAL DIAGNOSIS

Chronic pouchitis.

TREATMENT

He was prescribed metronidazole 500 mg orally three times daily and ciprofloxacin 500 mg orally two times daily. But his symptoms did not improve. Then we added rifaximin 550 mg orally three times daily. We continued meselamine 3000 mg orally two times rectally and loperamide three times daily. He also used probiotics. He continued to have diarrhea with blood and mucus 20 to 30 times per day. Then adalimumab was started at 160 mg, 80 mg and 40 mg subcutaneously at 0, 2, and every 2 wk, respectively. He reported improvement of diarrhea without blood 10 to 15 per day the first week of adalimumab treatment. However, this response decreased within 4 wk, and the diarrhea and weight loss increased. His pouchoscopy was the same as before treatment at 6 mo after the beginning of treatment. Therefore, we stopped adalimumab. We tested the patient again for other etiologies like infections that were negative. Finally, we decided to start vedolizumab. The patient was given 300 mg parenterally at 0, 2, and 6 wk then every 8 wk.

OUTCOME AND FOLLOW-UP

He reported improvement in clinical symptoms at 4 wk for frequency of diarrhea (six to eight per day) without blood and mucus. He did not have any abdominal complaints. A pouchoscopy at 6 wk and 15 wk after beginning vedolizumab demonstrated that there were less ulcers after 6 wk, and there was only one small superficial ulcer after 15 wk (Figure 1). A pouchoscopy before beginning vedolizumab treatment is shown in Figure 2. His laboratory tests including C-reactive protein, erythrocyte sedimentation rate and liver test were normal. He gained almost 9 kg during vedolizumab treatment, and his quality of life improved (he started to work again).

DISCUSSION

There is only one retrospective study on the efficacy of vedolizumab for refractory pouchitis of the ileo-anal pouch in the literature[6]. This study suggested that vedolizumab is safe and effective for treatment of refractory pouchitis. The other studies found in the literature are case presentations and case series. These presentations showed us vedolizumab was a good choice for refractory pouchitis[7-11]. The effects of vedolizumab for treatment of pouchitis is summarized in Table 1[8-13]. We differentiated our case from other cases in the literature by taking an effective clinical and endoscopic response with vedolizumab treatment in two different anti-TNF alpha refractory pouchitis.

Vedolizumab, a monoclonal antibody, selectively blocks gut lymphocyte trafficking by interacting with α4β7 heterodimer[1]. There is severe infiltration of the mucosa by both innate and adaptive immune cells in active pouchitis. An increased proportion of mucosal dendritic cells expressing integrin β7 in patients with pouch inflammation has been shown[14]. The integrin signaling in the pathogenesis of this clinical condition of pouchitis may have a pathogenic role. Therefore, blockade of α4β7 integrin with vedolizumab treatment might represent a promising therapeutic strategy for this clinical condition[14].

Vedolizumab has been shown to be beneficial for the treatment of chronic antibiotic-refractory pouchitis[15,16]. After 3mo of therapy with vedolizumab in patients with refractory pouchitis, the small case series of four patients had symptomatic and endoscopic improvements[17].

Vedolizumab may be a new choice as a treatment option in patients with refractory pouchitis who showed no improvement with steroids and other biological therapies such as anti-TNFs. Future studies may show when to start vedolizumab and the advantages of vedolizumab therapy in patients with refractory pouchitis.

CONCLUSION

In our patient who had anti-TNF refractory pouchitis, the gut-specific immune

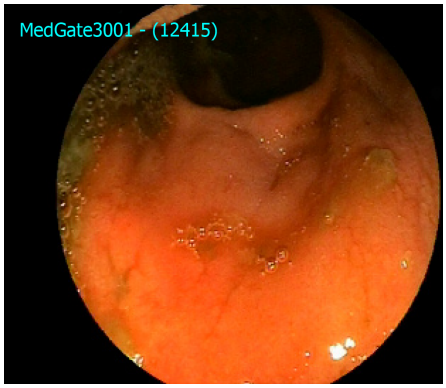

Figure 1 Fifteen weeks after vedolizumab treatment.

modulation mediated by vedolizumab treatment resulted in good responses. Further large and prospective studies are needed for the efficacy and the underlying mechanisms of efficacy of vedolizumab in treatment of refractory pouchitis.

Table 1 Effect of vedolizumab treatment of pouchitis in the literature

Country and reference	Number of patients	Age and gender	Features of inflammatory bowel disease	Outcomes
United States and reference 8	1	41-year-old female	She had pouchitis; 2 years later IPAA	Improvement in clinical symptoms and decreased frequency of bowel movements to four to six per day without blood or mucus were reported with 6 wk of vedolizumab treatment. There were no side effects
Italy and reference 9	1	33-year-old male	Anti-TNF-refractory chronic pouchitis and concomitant PSC	3 mo after ileostomy closure, chronic pouchitis occurred, refractory to antibiotics and anti-TNF. Thus, vedolizumab was started, leading to a marked improvement in clinical symptoms, which was maintained to the end of follow up (wk 34). There were no side effects
Germany and reference 10	20	12 male, 8 female; The median age was 22.5 years old	All of the patients were diagnosed with pouchitis	Improvement of clinical symptoms, the Oresland score and the PDAI score. There were no side effects
Greece and reference 11	1	22-year-old female	She was first diagnosed with pouchitis 1 year after surgery. Administered infliximab followed by adalimumab, both of which she discontinued after an early severe allergic reaction	Vedolizumab was subsequently initiated, together with a single course of antibiotics, and the patient experienced improvement in clinical symptoms and laboratory results with no documented relapse since then. A new pouchoscopy at wk 33 showed significant improvement
Greece and reference 11	1	22-year-old female	She was first diagnosed with pouchitis 1 year after surgery. Administered infliximab followed by adalimumab, both of which she discontinued after an early severe allergic reaction	Vedolizumab was subsequently initiated, together with a single course of antibiotics, and the patient experienced improvement in clinical symptoms and laboratory results with no documented relapse since then. A new pouchoscopy at wk 33 showed significant improvement
United States and reference 12	12	9 female, 3 male; The mean age was 41 years old	All of the patients had active pouchitis. Five patients (41.7%) used mesalamine, six (50.0%) took budesonide and four (33.3%) took prednisone prior to using vedolizumab. Eight (66.7%) had used anti-TNF agents prior to vedolizumab use	Eight (66.7%) patients demonstrated significant reduction in mPDAI symptom subscores before and after vedolizumab therapy
Portugal and reference 13	1	20-year-old female	She was diagnosed with pouchitis and a severe symptomatic autoimmune hemolytic anemia 1 year after IPAA	Patient reported symptom improvement at wk 12 and a pouchoscopy revealed only mucosal edema after 6 mo of therapy. Her inflammatory markers and hemoglobin normalized on repeat testing, allowing steroid withdrawal

IPAA: Ileal pouch-anal anastomosis; TNF: Tumor necrosis factor; PSC: Primary sclerosing cholangitis; PDAI: Pouchitis disease activity index; mPDAI: Modified pouchitis disease activity index.

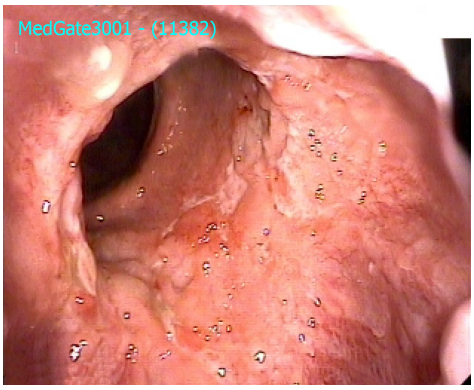

Figure 2 Before vedolizumab treatment.

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CASE REPORT

Clinical outcomes and safety of high-resolution manometry guided superficial partial circular muscle myotomy in per-oral endoscopic myotomy for Jackhammer esophagus: Two cases report

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Abstract

BACKGROUND

Jack hammer esophagus is a relatively rare disease and to date, there is no dramatic treatment option. Recently, conventional per-oral endoscopic myotomy (POEM) have expanded their area into Jackhammer esophagus. However, several complications such as post procedure motility disorders (*e.g.*, passage disturbance) are issues after POEM. To overcome these issues, we here introduced high-resolution manometry (HRM)-guided superficial partial circular muscle myotomy, which involves cutting only the superficial layer of the esophageal circular muscle.

CASE SUMMARY

We report two cases of patients with Jackhammer esophagus who were treated with HRM-guided extremely superficial partial circular muscle myotomy during POEM. Case 1 was a 53-year-old female with medication-refractory odynophagia and case 2 was a 47-year-old man who presented with chest pain. They were diagnosed with Jackhammer esophagus using HRM, and the hypercontractile segments of the esophagus were identified. HRM-guided extremely superficial partial circular muscle myotomy was performed while preserving the lower esophageal sphincter. Therefore, the circular and longitudinal muscle layers are preserved but hypercontractile movements are reduced, even after POEM. Patients' clinical symptoms dramatically improved right after POEM, and 6-mo follow-up HRM revealed completely resolved status. During a 1-year follow-up period, patients were still in good health and remained symptom free.

CONCLUSION

HRM-guided superficial partial circular muscle myotomy may be a promising

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alternative to conventional POEM for treating Jackhammer esophagus with improved efficacy.

Key words: Jackhammer esophagus; Hypercontractile; Partial circular muscle myotomy; Case report

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Core tip: Jack hammer esophagus is a relatively rare disease and to date, there is no dramatic treatment option. Recently, conventional per-oral endoscopic myotomy (POEM) have expanded their area into Jackhammer esophagus. However, several complications such as post procedure motility disorders (*e.g.*, passage disturbance) are issues after POEM. To overcome these issues, we here introduced high-resolution manometry-guided superficial partial circular muscle myotomy, which involves cutting only the superficial layer of the esophageal circular muscle for two patients.

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INTRODUCTION

Jackhammer esophagus, also referred to as hypercontractile peristalsis, is a rare esophageal motility disorder characterized by hypertensive but normally propagated peristaltic contractions[1-3]. The manometric criteria for Jackhammer esophagus are an initial average peristaltic amplitude > 180 mmHg in the distal esophagus using conventional manometry[1,3,4] > 20% of swallows having a distal contractile integral (DCI) value > 8000 mmHg.s.cm, and normal latency on high-resolution manometry (HRM)[2,5-8]. The therapeutic options for Jackhammer esophagus are pharmacologic agents such as nitrates, phosphodiesterase 5 inhibitors, low-dose antidepressants, proton pump inhibitors, and endoscopic botulinum toxin injection into the esophageal body. However, the efficacy of these methods is not satisfactory[1-3,9,10]. Per-oral endoscopic myotomy (POEM) has been used as an alternative treatment to overcome the limitations of the above therapies. However, there are still concerns regarding post-POEM complications, such as passage disturbance and sigmoid esophagus[2,11-14]. Jack hammer esophagus is a relatively rare disease and to date, there is no definitive and dramatic treatment options. including medication, endoscopic treatments or surgical treatments.

To reduce the risk of complications after conventional POEM[11,15], we introduced HRM-guided extremely superficial partial circular muscle myotomy during the POEM procedure for two Jackhammer esophagus cases. partial circular muscle myotomy involves cutting only the superficial layer of the esophageal circular muscle. Therefore, the circular and longitudinal muscle layers are preserved but hypercontractile movements are reduced, even after POEM. Moreover, hyper-contractile segments were specifically targeted and measured though HRM.

Between April 2016 and August 2018, a total of 350 patients underwent HRM and 8 were diagnosed with Jackhammer esophagus in our hospitals. Two patients with medication-refractory Jackhammer esophagus underwent partial circular muscle myotomy during POEM. Herein, we describe two patients who presented with Jackhammer esophagus and were successfully treated using HRM-guided partial circular muscle myotomy during POEM.

CASE PRESENTATION

Chief complaints

Case 1: A 53-year-old woman was referred to our hospital for odynophagia and regurgitation.

Case 2: A 47-year-old man was referred to the gastrointestinal department for atypical chest pain for 6 mo.

History of present illness

Case 1: She had previously presented to a local hospital and had been prescribed oral proton pump inhibitors and nitroglycerin for several months, but her symptoms did not improve.

Case 2: He was previously presented to the cardiovascular department for atypical chest pain. However, his symptom was not improved even after stent angiography and administration of anti-angina medications and oral proton pump inhibitors for several months. After then he was referred to the gastrointestinal department.

History of past illness/ Personal and family history

Case 1: She had no known medical or surgical history. Her family history was negative.

Case 2: He had two-vessel cardiovascular disease with angina and a stent was inserted 5 years ago. Despite use of a patent stent, cardiovascular medications including nitrates, and a 3-mo trial of proton pump inhibitors, atypical squeezing pain in the epigastric region remained. His family history was negative.

Physical examination upon admission

Case 1: Physical examination on admission revealed no abnormal palpable mass on head and neck area.

Case 2: Physical examination on admission revealed no abnormal findings.

Laboratory examinations

Case 1: There were no abnormal findings on electrocardiogram, and laboratory tests including total blood count, liver function, renal function, and other basic chemical tests were normal.

Case 2: Laboratory tests were normal, including total blood count, liver function, renal function, and other basic chemical tests.

Imaging examinations

Case 1: Esophagography (barium radiography) showed spasmodic contraction of the distal esophagus and a narrowing of the esophageal cavity (Figure 1). HRM showed high-amplitude distal esophageal contractions with a DCI value > 8000 mmHg.s.cm for 6 of a total of 10 swallows. The highest DCI value was 13553 mmHg.s.cm (Figure 1). HRM showed high-amplitude distal esophageal contractions located 25-38 cm from the incisors according to the distance gauge of the pressure measuring tubes.

Case 2: On upper endoscopy, no abnormal findings were reported. Barium radiography showed spasmodic contractions of the distal esophagus and a narrow esophageal cavity (Figure 2). HRM showed high-amplitude distal esophageal contractions with a DCI value > 8000 mmHg.s.cm for 8 of a total of 10 swallows (Figure 2B). The highest DCI value was 21024 mmHg.s.cm. POEM was performed. HRM showed high-amplitude distal esophageal contractions located 28-39 cm from the incisors according to the distance gauge of the pressure measuring tubes.

FINAL DIAGNOSIS

Case 1: The final diagnosis was medication refractory Jack hammer esophagus without involvement of low esophageal sphincter.

Case 2: The final diagnosis was medication refractory Jack hammer esophagus without involvement of low esophageal sphincter.

TREATMENT

Case 1: The patient underwent HRM-guided superficial partial circular muscle myotomy (Figure 3). Since HRM showed high-amplitude distal esophageal contractions located 25-38 cm from the incisors, we performed partial circular muscle myotomy of the esophageal muscle on the right side (Figure 3). We preserved the lower esophageal sphincter.

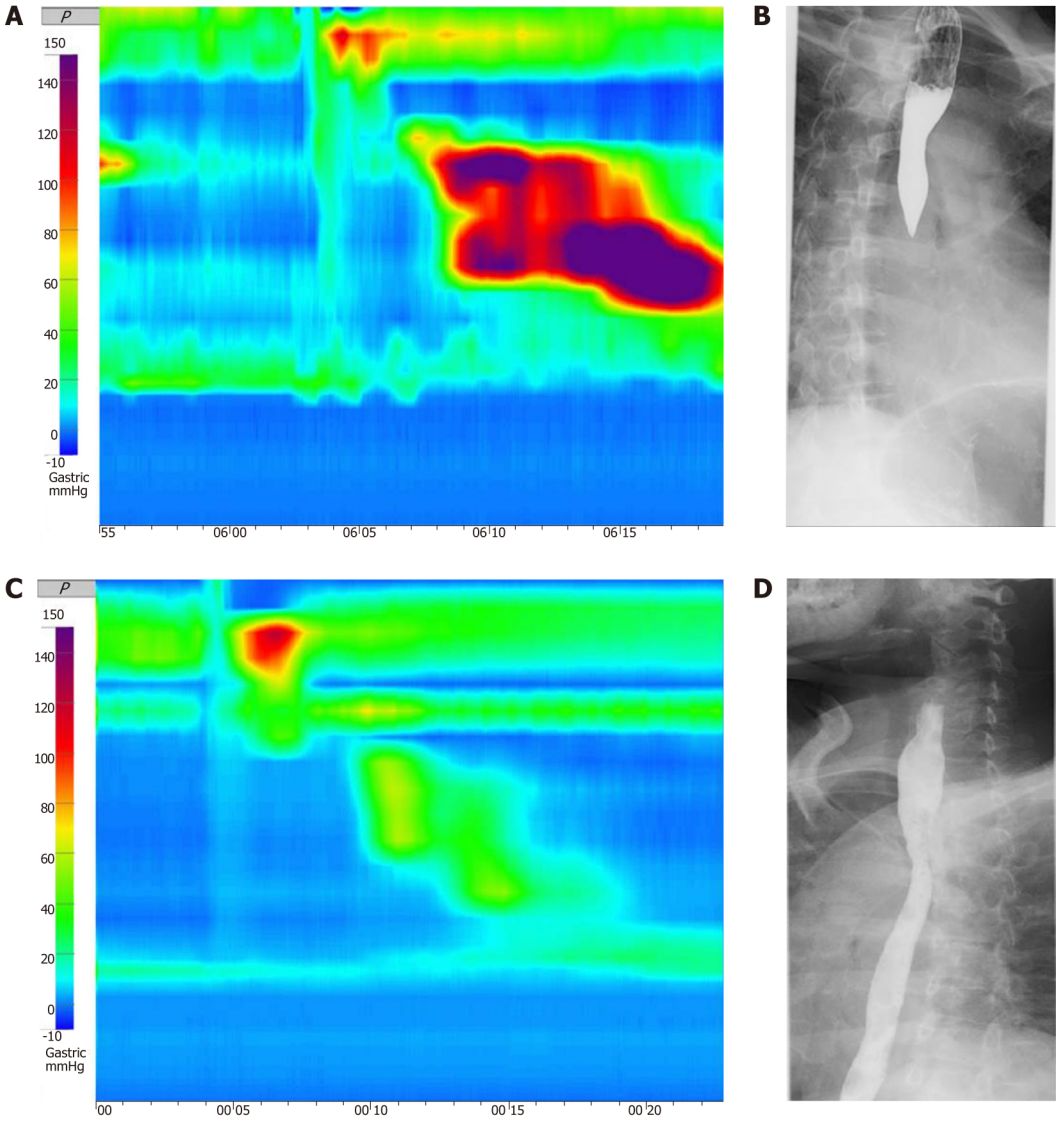

Figure 1 The examination of 53-year-old woman conducted before superficial circular muscle per-oral esophageal myotomy. A: Pre-treatment high-resolution esophageal manometry (HRM) image depicted a jackhammer esophagus patient with distal contractile index over 8000 mmHg.s.cm; B-D: Barium radiography showed spasmodic contraction of the distal esophagus and a narrowing of the esophageal cavity (C) Post-treatment HRM revealed distal contractile integral of 980 mmHg.s.cm (D) Post-treatment esophagogram showed improved.

Case 2: The patient underwent HRM-guided superficial partial circular muscle myotomy (Figure 3). Since HRM showed high-amplitude distal esophageal contractions located 25-38 cm from the incisors, we performed partial circular muscle myotomy of the esophageal muscle on the right side (Figure 3). We preserved the lower esophageal sphincter.

We performed HRM-guided superficial partial circular muscle myotomy of the esophageal muscle. Since HRM showed high-amplitude distal esophageal contractions located 28-39 cm from the incisors, we performed partial circular muscle myotomy of the esophageal muscle on the right side (Figure 3) and preserved the lower esophageal sphincter.

OUTCOME AND FOLLOW UP

Case 1: After the procedure, the patient's symptoms dramatically improved and post-POEM HRM was within the normal range. During a 1-year follow-up period, patients were in good health and remained symptom free.

Case 2: After the procedure, the patient's symptoms dramatically improved and post-POEM HRM was within the normal range (Figure 2C). During a 6-mo follow-up period, patients were in good health and remained symptom free.

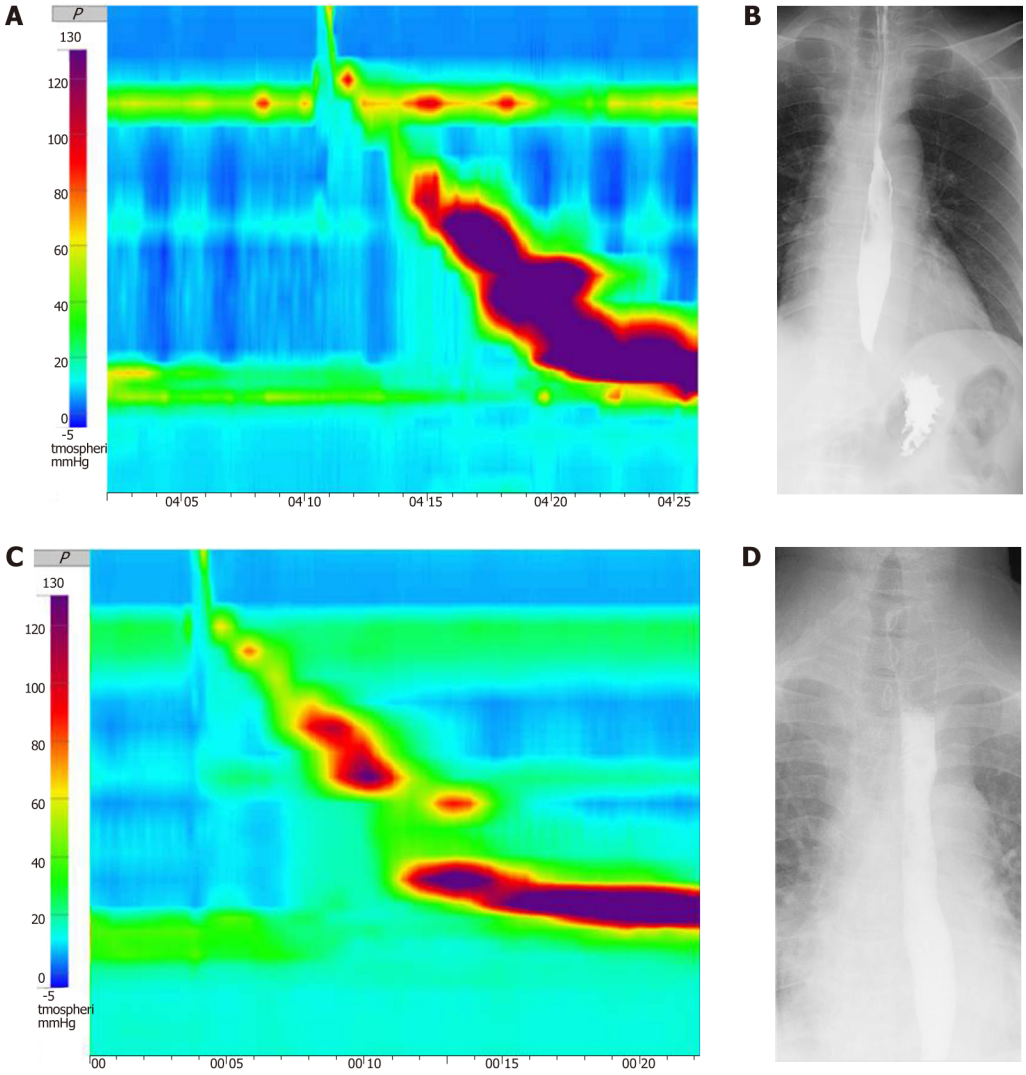

Figure 2 The examination of 47-year-old man conducted before superficial circular muscle per-oral esophageal myotomy. A: Pre-treatment high-resolution manometry (HRM) showed high-amplitude distal esophageal contractions with a distal contractile integral (DCI) value > 8000 mmHg.s.cm for 8 of a total of 10 swallows and the highest DCI value was 13553 mmHg.s.cm; B-D: Pre-treatment esophagography showed spasmodic contraction of the distal esophagus (C) Post-treatment HRM revealed within normal range of DCI (D) Post-treatment esophagogram showed improved function of passage of esoaphagus.

DISCUSSION

We here report two patients with Jackhammer esophagus who were successfully treated with HRM-guided superficial partial circular muscle myotomy during POEM. After the procedures, both patients reported improve symptoms with no side effects. The current cases suggest that HRM-guided superficial partial circular muscle myotomy may be a potential treatment option for Jackhammer esophagus with a relatively low rate of post-procedure complications as compared to conventional POEM.

Jackhammer esophagus is rare and severe disease[2,16,17]. Jackhammer esophagus is extremely high amplitudes contractions and within normal limit of peristatic contractions[4]. Treatment strategy for Jackhammer esophagus includes medication for smooth muscle relation (nifedipine), anti-reflex medication, and pneumatic dilatation of LES[4]. Because of the rare incidence of Jackhammer esophagus, proper evaluation of incidence is not easy, the medication refractory Jackhammer esophagus has been increasing. Recently for medical refractory Jackhammer esophagus, POEM was introduced[11].

POEM is the first clinically efficacious natural orifice transluminal endoscopic surgery (NOTES) with an endoscopic safety profile[1,15,18,19]. However, despite its safety profile, post-POEM complications are not rare[6,7,9,20]. Conventional POEM for Jackhammer esophagus is associated with several side effects including post-procedure sigmoid esophagus and ineffective esophageal motility[14,19]. It remains debated whether the lower esophageal sphincter should be cut to prevent symptom

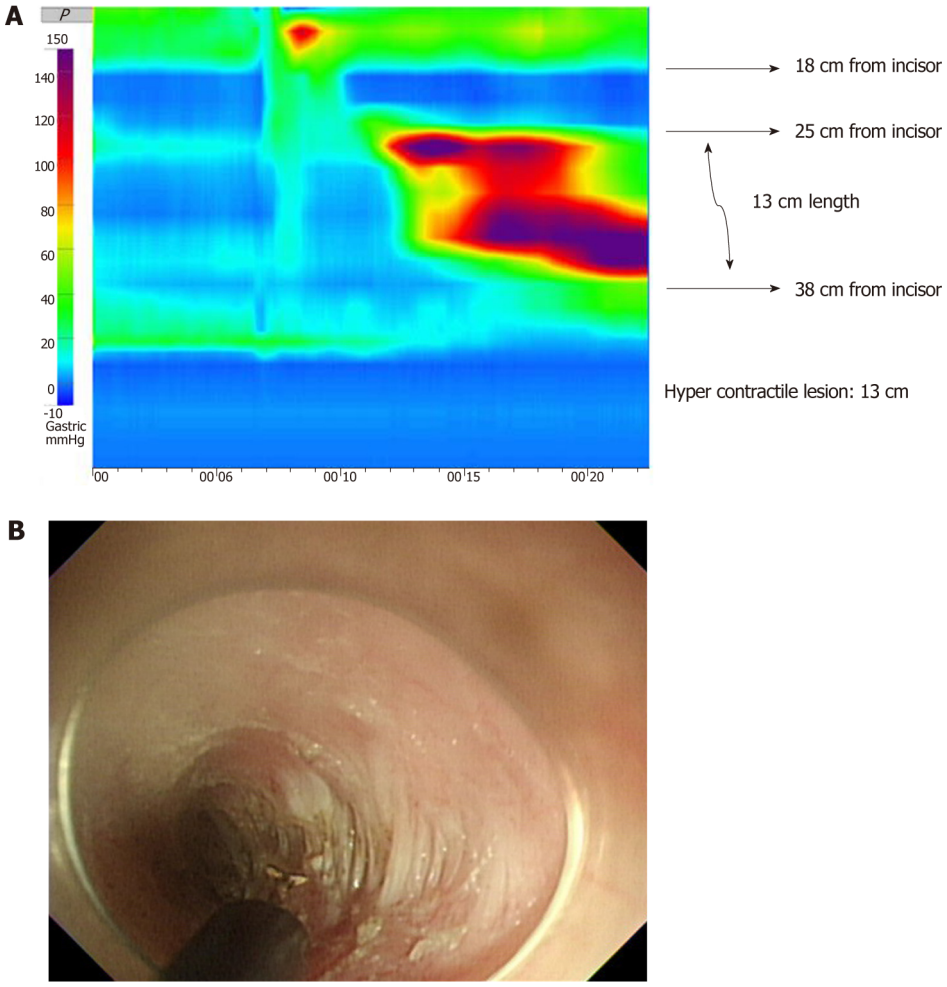

Figure 3 Procedures of high-resolution manometry guided superficial partial circular muscle myotomy. A: We first detect the hypercontractile lesion through high-resolution manometry (HRM), and in this patients' case, HRM showed high-amplitude distal esophageal contractions located 25-38 cm from the incisors according to the distance gauge of the pressure measuring tubes. B: Therefore, we performed superficial partial circular muscle myotomy of the esophageal muscle on the right side, (B) Superficial partial circular muscle myotomy during POEM. Remnant circular muscle.

development after the procedure[7,14,18]. Recent systemic review showed that the pooled rate of clinical success in patients of Jackhammer esophagus for POEM was 89.6%[21]. The success rates of both the length > 10 cm, and the length < 10 cm were 91.1% and 89.1%, respectively[21]. There are several researches on the symptom in patients with Jackhammer esophagus and the pre-peak and post-peak phase of contraction[3,22]. In these regards, to distinguish the contractile integral components of pre-peak and post peak phase contractile activity is important to treat Jack hammer disease[3,22]. However, there are still concerns regarding post-POEM complications for medication refractory Jackhammer esophagus after POEM, such as passage disturbance and sigmoid esophagus[2,11-14].

To improve treatment efficacies and reduce the complications in the treatment of Jack hammer esophagus after POEM, we focused two issues: (1) To measure the accurate segments which are hypercontratile in the esophagus; and (2) To conserve the esophageal motility after POEM procedures. HRM-guided superficial partial circular muscle myotomy which we introduced is a modified type of conventional POEM, and this method is expected to reduce side effects and increase treatment efficacy[5,11,14]. Because partial circular myotomy during POEM involves cutting only the superficial layer of the circular esophageal muscle and not the full thickness of the muscle nor the full circular muscle layer, even after POEM, the previously diagnosed Jackhammer esophagus consists circular and longitudinal layer of muscle with its nature but reduced hypercontractile movements[11,14,19]. Moreover, in addition to region-targeted therapy, partial circular muscle myotomy was performed under HRM guidance to selectively detect hypercontractile segments. Therefore, decreased esophageal motility after the procedure are prevented. Moreover, it is not necessary to cut the lower esophageal sphincter in a partial circular myotomy when low esophageal sphincter is not involved[23]. This method not only reduces the occurrence

of side effects associated with conventional POEM, including partial or full thickness POEM, but also improves treatment efficacy.

CONCLUSION

HRM-guided superficial partial circular muscle myotomy during POEM may be a promising alternative to conventional POEM for the treatment of patients with Jackhammer esophagus who are refractory to conventional medical therapy, which is associated with improved efficacy and safety profile.

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CASE REPORT

Cardiac arrhythmias and cardiac arrest related to mushroom poisoning: A case report

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Author contributions: Li S and Tian C carried out the initial diagnosis and resuscitation; Ma QB, Ge HX, Liang Y, Guo ZG, and Tian C also played essential roles in providing critical healthcare throughout the hospital stay and clinical follow-up of the patient; Geng JN performed genome analysis of the mushrooms; Ma QB and Li S conceived the idea of possible publication of the case; Li S and Riley F were major contributors in the literature research and in the process of writing the manuscript; all authors read and approved the final manuscript.

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Abstract

BACKGROUND

Mushroom exposure is a global health issue. The manifestations of mushroom poisoning (MP) may vary. Some species have been reported as rhabdomyolytic, hallucinogenic, or gastrointestinal poisons. Critical or even fatal MPs are mostly attributable to *Amanita phalloides*, with the development of severe liver or renal failure. Myocardial injury and even cases mimicking ST-segment elevation myocardial infarction (STEMI) have been previously reported, while cardiac arrhythmia or cardiac arrest is not commonly seen.

CASE SUMMARY

We report a 68-year-old woman with MP who suffered from delirium, seizure, long QT syndrome on electrocardiogram (ECG), severe cardiac arrhythmias of multiple origins, and cardiac arrest. She was intubated and put on blood perfusion. Her kidney and liver functions were intact; creatine kinase-MB was mildly elevated, and then fell within normal range during her hospital stay. We sent the mushrooms she left for translation elongation factor subunit 1α, ribosomal RNA gene sequence, and internal transcribed spacer sequence analyses. There were four kinds of mushrooms identified, two of which were

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found to be toxic.

CONCLUSION
This is the first time that we found cardiac toxicity caused by *Panaeolus subbalteatus* and *Conocybe lactea*, which were believed to be toxic to the liver, kidney, and brain. We suggest that intensive monitoring and ECG follow-up are essential to diagnose prolonged QT interval and different forms of tachycardia in MP patients, even without the development of severe liver or renal failure. The mechanisms need to be further investigated and clarified based on animal experiments and molecular signal pathways.

Key words: Mushroom poisoning; Arrhythmia; Cardiac arrest; Seizure; Case report

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Core tip: Critical or even fatal mushroom poisonings are mostly attributable to *Amanita phalloides*, with severe liver or renal failure developing. Myocardial injury was reported previously while cardiac arrhythmias of multiple origins or cardiac arrest are definitely rarely seen or reported in the literature. Also, this is the first time that we found cardiac toxicity caused by *Panaeolus subbalteatus* and *Conocybe lactea*, which were believed to be hepatic, renal, and brain toxic.

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INTRADUCTION

Mycetism, widely known as mushroom poisoning (MP), is an international health care issue. Some species of mushroom are edible or even medicinal and have been proven to have antioxidative effects. Nonetheless, some species initially classified as edible were later recognized as toxic. In general, the majority of MPs are the result of misidentification while attempting to collect wild mushrooms. More unusual intentional ingestion of hallucinogenic species can occur with recreational use. The clinical manifestations of MP may vary. Fatal poisonings are mostly attributable to *Amanita phalloides*, causing liver and renal failure, while cardiac arrhythmia is not commonly seen.

Here, we present a case of MP in which the patient suffered from severe cardiac arrhythmias and cardiac arrest but was then totally cured at our hospital.

CASE PRESENTATION

Chief complaints

A 68-year-old female patient presented to the emergency department (ED) complaining of dizziness, visual changes, and slight hearing loss.

History of present illness

She had picked mushrooms 2 d ago at a roadside lawn in the community in which she lived in central Beijing. She ate cooked mushrooms for lunch 2 h prior to her admission and reported nausea, dizziness, visual and hearing changes as well as thirst 30 min after ingestion without any other symptoms.

History of past illness

She had a 10-year history of hypertension and hyperlipidemia, taking candesartan 4 mg per day and atorvastatin 10 mg per day. She denied having taken any other prescribed or over-the-counter medications, alcohol, or illegal drugs.

Physical examination

Her vital signs upon arrival were as follows: Blood pressure 194/106 mmHg, heart rate 93 beats per minute, respiratory rate 12 times per minute, body temperature 37 °C, and oxygen saturation 100% on room air. On examination in the ED, she was awake, alert, and oriented. However, she claimed to be thirsty and kept asking "Am I dying?" with a euphoric smile. Her pupils were slightly dilated, with a diameter of 5 mm on both sides. Other than that, her physical examination was negative.

Laboratory examinations

Her ED laboratory tests showed normal complete blood count and chemistry panel with sodium 142.5 mmol/L; potassium 3.78 mmol/L; magnesium 0.9 mmol/L; and normal liver, renal, and coagulative functions as well as myocardial injury markers. Her electrocardiogram (ECG) revealed sinus rhythm with a normal QT interval.

Further progress, diagnosis, treatment, and outcome

The initial management included immediate fluid resuscitation and oral gastric lavage given the assumed diagnosis of MP, and an intravenous infusion of urapidil was started for blood pressure control. Her blood pressure was 157/96 mmHg after 5 h. A blood sample was sent to the laboratory for further poison screening. However, food residue could be easily seen in her vomitus, and she began to refuse treatment and entered a stage of confusion and then intensive agitation. Therefore, a nasogastric tube was placed for the prevention of aspiration.

Later, her heart rate gradually increased to approximately 120 beats per minute, and intermittent atrial arrhythmia began, which persisted for several minutes with every episode recovering spontaneously. It was quite unexpected that a seizure suddenly developed without arrhythmia onset. It lasted for approximately 1 min, and after termination of the seizure, she immediately had pulseless ventricular tachycardia and cardiac arrest. Fortunately, she had a return of spontaneous circulation after 2 min of CPR and 1 mg epinephrine administration. She was intubated because of vomiting, prior seizure, the need for sedation, and the probability of recurrent cardiac arrest based on her unstable status. No secretions were found during intubation, and her brain CT scan showed no intracranial bleeding or ischemia. The post-cardiac arrest ECG revealed a prolonged QT interval (QT interval 360 msec when heart rate was 135 beats per minute and corrected QT interval was 540 ms, Figure 1). We put her on blood perfusion for 2 days. She was extubated, totally conscious and had no recurrence of seizure. The toxicity screen, thyroid function, and brain MR were all negative. Her creatine kinase (CK) and kinase-MB (CKMB) were elevated mildly (37 U/L and 245 U/L, respectively) and trended to the normal range after fluid resuscitation. The QT interval also returned to normal. Troponin I (TNI) was negative on serial tests during the hospital stay. She underwent uneventful hospital stay and was discharged 7 days after admission.

Identification of the mushrooms

We sent the mushroom she left for translation elongation factor subunit 1α, ribosomal RNA gene sequence, and internal transcribed spacer sequence analyses. There were four kinds of mushrooms identified, including *Hebeloma sp*., *Psathyrella leucotephra*, *Panaeolus subbalteatus*, and *Conocybe lactea*. The latter two were found to be toxic (Figure 2A and B, respectively).

DISCUSSION

There are 100000 known species of fungi worldwide. Approximately 100 of the known species of mushrooms are poisonous to humans. The incidence of MP varies a great deal with respect to the global perspective. The mortality rate reported was 10–15%, or even as high as 21.2%. The prognosis of patients varies greatly and is affected by many factors, including the type of mushroom, intake amount, ED visit time, and treatment strategy. According to the incubation period, the stages of MP are separated into early (< 6 h), delayed (6–24 h), and late (> 24 h). There is a great diversity of clinical manifestations, which are generally divided into different subtypes such as gastrointestinal, hepatic, nephrological, neuropsychiatric, hemolytic, and photoallergic, depending on the type of mushroom ingested and the toxin contained.

Panaeolus subbalteatus, also having been named *Panaeolus cinctulus* in the past, is commonly known as "Weed Panaeolus". It is a very widely distributed psilocybin mushroom that causes hallucination[1]. The dramatic effects are caused by fungi holding neurotoxins, such as muscarine (*Clitocybe* and *Inocybe spp*.) and psilocybin (*Psilocybe* and *Panaeolus spp*.). Psilocybin and related substances are potent hallucinogens producing effects similar to those of lysergic acid diethylamide (LSD). Psilocybin and/or psilocin have also been detected in *Conocybe lactea*, named *Conocybe*

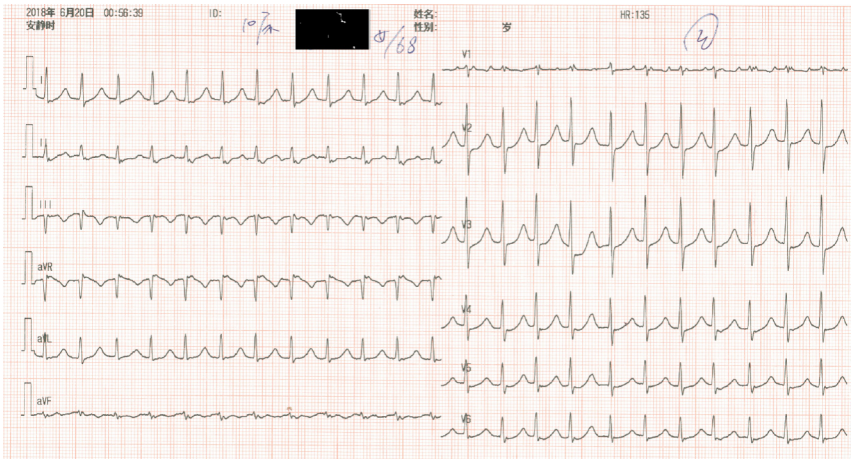

Figure 1 Electrocardiogram of the patient shortly after resuscitation.

apala or the White Dunce Cap, which was the other kind of mushroom involved in this case. They can both be found in areas with rich soil and short grass, such as pastures, playing fields, lawns, road sides, and meadows, as well as rotting manured straw or rotting vegetable remains. These "magic" mushrooms, also called "dancing mushrooms" or "laughing mushrooms" in China, are usually ingested intentionally for their hallucinogenic effects in developed countries or ingested accidentally due to being mistaken for other edible species. Symptoms occur within 20–60 min after ingestion and include a sense of altered time and space, euphoria, depersonalization, hallucinations, anxiety, agitation, mydriasis, vertigo, headache, nausea, enlarged pupils, tachycardia, flushing, fever, and seizures. Symptoms are usually maximal within 2 h and disappear within 4–6 h, although "flashback" may recur after weeks or months[2]. Our patient had an early onset (< 6 h) of symptoms and was conscious with hearing and sight alterations upon arrival. Then, she underwent a period of euphoria, hallucination, agitation, and seizure, which were all well explained by the toxicity of psilocybin.

As known to all, the consumption of wild mushrooms may cause serious toxicity to hepatic, renal, and neurological functions. *Conocybe lactea* can also be highly toxic to liver cells, containing phallotoxins which are famous as isolated from the death cap mushroom (*Amanita phalloides*)[3]. However, the toxic effects of MP on the cardiovascular system have been rarely investigated or reviewed systemically, and the conclusions have been controversial.

Elevated CKMB and TNI, ventricular dysfunction, and cardiogenic shock were reported in a few previous studies. All of the probable pathological mechanisms were previously based on speculation from the well-known species, *Amanita phalloides*, which has the toxicity of amatoxins, circulating antitroponin antibodies that impair myocardiocytes. Kalclk published a clinical case of a 25-year-old woman who presented with severe crushing chest pain after consumption of wild mushrooms. The ECG showed ST segment elevation in leads II, III, and aVF. Acute myocardial infarction was confirmed with profoundly elevated CKMB and TNI. Cardioangiography showed normal coronary arteries. It was thought that myocardial infarction was secondary to coronary spasm. A potential mechanism might be vasoconstrictive substances emerging as a result of endothelial damage by the toxins[4]. Furthermore, Krzysztof once reported that the patients' serum and urine indole alkaloids, which are agonists of the 5-HT receptors and may lead to coronary vasoconstriction, were strongly positive[5]. Later, on the other hand, Erenler reviewed 175 patients with MP over a 2-year period, and the cardiac markers of the patients were found to be normal[6].

Moreover, Erenler concluded that MP causes hypertension and ECG alterations in his article. Sinus tachycardia and sinus arrhythmia were the most common types of arrhythmia found in patients with MP. The prevalence of tachycardia was significantly higher in patients with late-onset symptoms (> 6 h). Furthermore, the blood pressure of the patients tended to increase as the interval between mushroom consumption and onset of symptoms prolonged. Other ECG manifestations included ST/T inversion, first degree AV block, and QT prolongation[6]. Another case was described in an 18-year-old man who developed Wolff-Parkinson-White syndrome, paroxysmal supraventricular tachycardia, myocardial infarction, and cardiac arrest[5]. Considering the life-threatening features of MP, Graeme reviewed the symptoms of

Figure 2 Two kinds of toxic mushrooms involved in the case. A: *Panaeolus subbalteatus*; B: *Conocybe lactea*.

palpitation, chest discomfort, dizziness, recurrent syncope, and seizure, followed by sudden death, in patients linked to mushroom ingestions. Ventricular tachycardia and ventricular fibrillation may precede death[7]. The mechanisms of cardiovascular damage were suggested to be related to peripheral sympathomimetic stimulation by psilocybin, which were contained in both of the toxic species of mushrooms we have identified here[5]. Unfortunately, almost none of the authors succeeded in the identification of the species of mushroom that causes unusual cardiovascular toxicity.

Our patient experienced several types of arrhythmia, including sinus tachycardia, atrial arrhythmia, ventricular tachycardia, prolonged QT interval, and cardiac arrest. Fortunately, she had a return of spontaneous circulation, and her CKMB was mildly elevated without cardiogenic shock or ventricular failure. She did not have severe liver or renal damage, except for periodic psychotic manifestations. All of her symptoms disappeared after hemoperfusion treatment. This is the first time that we found cardiac toxicity caused by *Panaeolus subbalteatus* and *Conocybe zeylanica*.

CONCLUSION

MP can be critical or fatal, even without the development of severe liver or renal failure. The patient may have sinus, atrial, or ventricular arrhythmias. Intensive monitoring and ECG follow-up are essential to catch prolonged QT interval and all kinds of tachycardia. The mechanisms need to be further investigated and clarified based on animal experiments and molecular signal pathways. Blood perfusion or hemodialysis is usually an effective treatment for severe MP.

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CASE REPORT

Role of abdominal drainage in bariatric surgery: Report of six cases

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Abstract

BACKGROUND

Abdominal drainage allows for timely detection of hemorrhage, but it cannot prevent hemorrhage. Whether routine abdominal drainage is needed during bariatric procedures remains controversial. Few reports describe the role of abdominal drainage in the diagnosis and treatment of abdominal hemorrhage in bariatric surgery.

CASE SUMMARY

Six cases of hemorrhage after bariatric surgery were described, including three cases with and three without abdominal drainage during the first surgery. The hemorrhage in five of the six cases was controlled by conservative treatment. Abdominal hemorrhage was found through the drainage tube on the day of the operation in the three patients with abdominal drainage during the first surgery. Emergency treatment was initiated, and their conditions gradually stabilized within 48 h. No patients required a reoperation. Abdominal hemorrhage was found later in the patients without abdominal drainage. Although the hemorrhage was controlled by conservative treatment in two cases (1 and 2), reoperation and percutaneous drainage were performed for abdominal infection and pelvic hemorrhage. An obsolete encapsulated effusion that may require treatment in the future was left in the abdominal cavity of a patient (Case 1).

CONCLUSION

The possibility of controlling abdominal hemorrhage after bariatric/metabolic surgery by conservative treatment is high. When hemorrhage occurs, abdominal drainage can reduce the probability of reoperation by reducing the formation of blood clots behind the stomach.

Key words: Abdominal drainage; Morbid obesity; Bariatric surgery; Hemorrhage; Case report

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Core tip: Abdominal drainage allows for timely detection of hemorrhage, but it cannot prevent it. Whether routine abdominal drainage is needed during bariatric procedures remains controversial. The possibility of controlling abdominal hemorrhage after bariatric/metabolic surgery by conservative treatment is high. When hemorrhage occurs, abdominal drainage can reduce the probability of reoperation by reducing the formation of blood clots around the stomach.

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INTRODUCTION

The prevalence of morbid obesity is increasing worldwide, and the number of bariatric surgery procedures is accordingly increasing each year[1]. As in other abdominal surgeries, hemorrhage is a challenging issue for bariatric surgeons. Abdominal drainage allows for timely detection of hemorrhage, but it cannot prevent hemorrhage. Whether routine abdominal drainage is needed during bariatric procedures remains controversial[2]. Few reports describe the role of abdominal drainage in the diagnosis and treatment of abdominal hemorrhage in bariatric surgery. We herein present six cases of abdominal hemorrhage in bariatric procedures performed in our center.

CASE PRESENTATION

Case 1

A 37-year-old man with a body mass index (BMI) of 33.73 kg/m^2 underwent laparoscopic sleeve gastrectomy (LSG) with cholecystectomy due to cholecystolithiasis. Abdominal hemorrhage was found on the first postoperative day, and the patient exhibited an accelerated heart rate of 100 to 120 beats per minute (bpm) compared with the preoperative baseline of 60 to 70 bpm. His blood pressure and urine volume remained steady, and conservative treatment was implemented (bed rest, hemostasis, and fluid infusion). The patient's condition stabilized on postoperative day 4. His hemoglobin level decreased from 15.7 g/dL to 8.0 g/dL, and transfusion of 8 U of red blood cells was therefore performed. On postoperative day 8, 800 mL of blood was percutaneously drained from a pelvic hematocele that had formed. The patient did not require a reoperation and was discharged on postoperative day 10. However, an encapsulated effusion was found in his abdominal cavity by abdominal magnetic resonance imaging at the 3-mo follow-up (Figure 1). Because he had no symptoms, conservative observation was still recommended.

Case 2

A 32-year-old man with a BMI of 33.46 kg/m^2 underwent LSG. Hemorrhage was found on the first postoperative day by routine blood tests and bedside ultrasound examinations. After 48 h of conservative treatment, his condition stabilized and his hemoglobin level decreased by 4.1 g/dL without a blood transfusion. However, he developed a fever of 39 ^{o}C on postoperative day 4. Physical examination revealed tenderness in the epigastrium without guarding or rebound. Antibiotics were administered and 200 mL of blood was percutaneously drained from a pelvic hematocele that had formed; however, the fever did not improve. Abdominal ultrasound findings were normal, but a computed tomography scan showed free air in the peritoneal cavity (Figure 2A). Considering the patient's increasing generalized abdominal pain and fever, a reoperation was performed on postoperative day 6 to place a drainage tube and confirm whether gastric leakage was present. A large number of blood clots were found in the greater curvature of the stomach during the operation, but neither the location of the abdominal hemorrhage nor the presence of gastric leakage was found. The blood clots were thoroughly cleared and an abdominal drainage tube was placed on the greater curvature side of the stomach. The patient's recovery was quite rapid after the second operation. On postoperative day 3, after the second surgery, no gastric leakage was detected by upper gastrointestinal

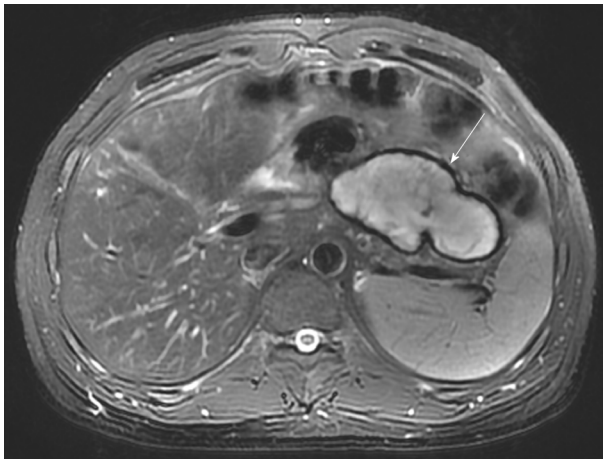

Figure 1 An encapsulated effusion was found in the abdominal cavity of the patient in Case 1 at the 3-mo follow-up.

radiography (Figure 2B), and oral liquid feeding was started. On day 10, the drainage tube was removed and the patient was discharged with no other complications.

Case 3

A 20-year-old woman with a BMI of 35.7 kg/m^2 underwent LSG. Hemorrhage was found on the first postoperative day by routine blood tests and bedside ultrasound examinations. After 12 h of conservative treatment, the patient's vital signs remained unstable and her hemoglobin level declined from 14.1 to 7.9 g/dL. A computed tomography scan showed a large number of blood clots behind the stomach (Figure 3), which was a difficult area to drain percutaneously. A reoperation was performed for hemostasis and placement of a drainage tube. The location of hemorrhage was not found by laparoscopy. The blood clots were thoroughly cleared, and an abdominal drainage tube was placed on the greater curvature side of the stomach. During the second postoperative period, the patient's recovery was quite rapid. On postoperative day 2, oral liquid feeding was started. On day 7, the drains were removed and the patient was discharged with no other complications.

Case 4

A 47-year-old man with a BMI of 32.8 kg/m^2 underwent laparoscopic Roux-en-Y gastric bypass (LRYGB).

Case 5

A 42-year-old man with a BMI of 30.2 kg/m^2 underwent LRYGB.

Case 6

A 32-year-old woman with a BMI of 30.9 kg/m^2 underwent laparoscopic single anastomosis gastric bypass.

In all of the last three cases, abdominal drainage was performed during the first surgery. Abdominal hemorrhage was found through the drainage tube on the day of the operation. The patients' heart rate, blood pressure, and urine volume remained steady, and conservative treatment was implemented (bed rest, hemostasis, and fluid infusion). The hemorrhage stopped after the hemoglobin level decreased to 4.4, 3.1, and 6.1 g/dL, respectively, within 48 h. The amount of hemorrhage from the drainage tube was 480, 910, and 750 mL, respectively. In Case 4, 370 mL of blood was percutaneously drained from a pelvic hematocele that had formed. No patients required a reoperation, and no abdominal infection developed.

DISCUSSION

According to previous reports, the probability of abdominal hemorrhage after bariatric surgery ranges from 1.5% to 2.0%[3]. In total, 343 metabolic procedures have been performed in our center this year, among which six cases of hemorrhage occurred (incidence of 1.7%). All six cases were treated by an experienced surgeon who had surgical experience with > 400 LSG procedures and > 100 gastric bypass procedures. The gastric staple line is reinforced by sutures in the LSG procedure, but

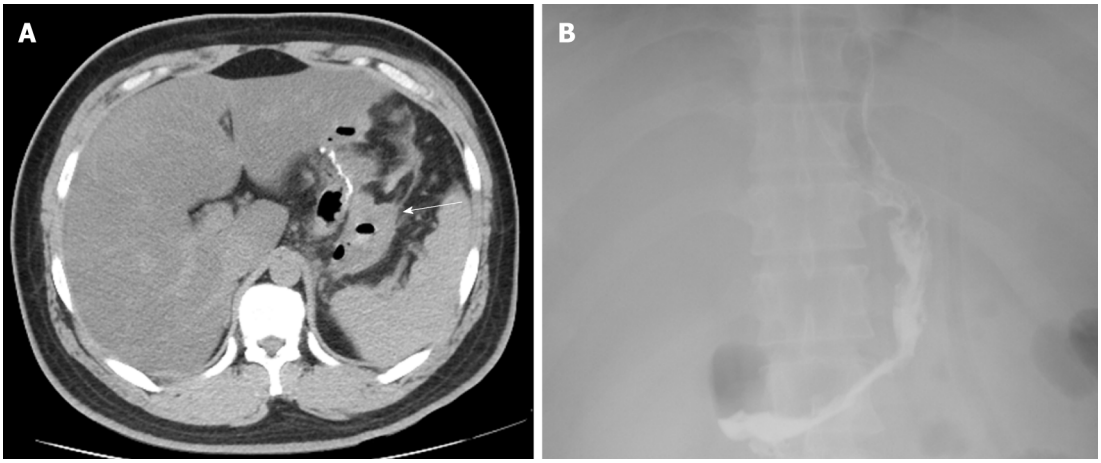

Figure 2 Imaging findings in Case 2. A: A blood clot and peritoneal cavity free air were found; B: No gastric leakage was detected by upper gastrointestinal radiography.

not in the gastric bypass procedure. A review of the operative videos showed that the surgical process was relatively smooth in all six cases. The origin of hemorrhage was most likely to be the omental margin in the LSG procedure and the gastric staple line in the gastric bypass procedure.

The concept of enhanced recovery after surgery has gradually gained popularity in recent years[4,5]. Day-case bariatric surgery has been adopted in some bariatric and metabolic centers[6]. Chang *et al*[7] suggested that omission of drainage may contribute to a shorter time to flatus passage. Doumouras *et al*[8] suggested that the use of routine abdominal drainage should be restricted to very select, high-risk cases. Drains can increase postoperative pain, lengthen the hospital stay, increase morbidity, and result in a marked peritoneal inflammatory response[9,10]. The concept of no routine abdominal drainage is being advocated by increasingly more surgeons.

The hemorrhage in five of the six cases was controlled by conservative treatment (success rate of 83.3%). Abdominal hemorrhage was found through the drainage tube on the day of the operation in the three patients with abdominal drainage during the first surgery. Emergency treatment was initiated, and their conditions gradually stabilized within 48 h. No patients required a reoperation. Abdominal hemorrhage was found later in the patients without abdominal drainage. Although the hemorrhage was controlled by conservative treatment in Cases 1 and 2, reoperation and percutaneous drainage were performed for abdominal infection and pelvic hemorrhage. An obsolete encapsulated effusion that may require treatment in the future was left in the abdominal cavity of a patient (Case 1). The clinical outcomes of the six cases varied greatly depending on whether abdominal drainage was implemented. Therefore, we believe that it should be in a very prudent way to popularize no routine drainage in bariatric surgery. Hemorrhage cannot be prevented by abdominal drainage, but it can be detected in a timely manner, allowing for immediate implementation of the necessary treatment. When hemorrhage occurs, the greater clinical significance of drainage is to drain the hematocele and reduce the formation of blood clots behind the stomach; such clots developed in the three cases without abdominal drainage in the present study. Blood clots are a likely reason for reoperation because they may lead to the development of infections and are difficult to drain out by other drainage methods.

However, controlled studies with a larger sample size are needed to evaluate the significance of drainage in bariatric/metabolic surgery more objectively.

CONCLUSION

The possibility of controlling abdominal hemorrhage after bariatric/metabolic surgery by conservative treatment is high. When hemorrhage occurs, abdominal drainage can reduce the probability of reoperation by reducing the formation of blood clots behind the stomach; such clots may lead to the development of infections or persistent residue in the abdominal cavity.

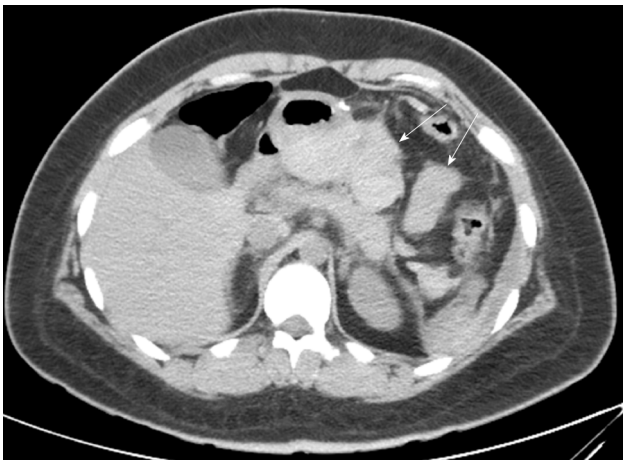

Figure 3 Blood clots behind the stomach were found in Case 3.

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CASE REPORT

A patient misdiagnosed with central serous chorioretinopathy: A case report

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Author contributions: Wan ZQ ordinated and provided the collection of the information of the patient; Peng Q designed the study; Wang TY wrote the manuscript.

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Abstract

BACKGROUND

Due to some similarities in the manifestations between central serous chorioretinopathy (CSC) and polypoidal choroidal vasculopathy (PCV), PCV may be misdiagnosed as CSC. More attention should be paid to distinguishing these two disorders.

CASE SUMMARY

A 52-year-old woman presented to our hospital with blurred vision in her left eye for approximately 1 wk. Anterior segment and intraocular pressure findings were normal in both eyes. Fundus photography of the left eye showed a seemingly normal adult oculus fundus without any obvious hard exudate or hemorrhage. Optical coherence tomography exhibited a hypo-reflective space beneath both the neurosensory retina and the pigment epithelium layer. The late phase of fluorescein angiography revealed increased leakage. The patient was initially diagnosed with CSC. At follow-up, however, the final diagnosis turned out to be PCV.

CONCLUSION

CSC and PCV are two different retinal entities. Lipid deposition and hemorrhage are the most important elements that lead to confusion between these two entities. Indocyanine green angiography should be performed to make a definitive diagnosis, especially in cases with suspected PCV.

Key words: Central serous chorioretinopathy; Polypoidal choroidal vasculopathy; Optical coherence tomography; Case report

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Core tip: Polypoidal choroidal vasculopathy and central serous chorioretinopathy are different diseases. While relatively mature research has been done on central serous chorioretinopathy, there is no unified understanding of polypoidal choroidal vasculopathy. These two diseases have some common symptoms, which have been plaguing clinicians. This case report provides a good example for the differential diagnosis between them. It also provides an alternative treatment for the clinical treatment of polypoidal choroidal vasculopathy.

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INTRODUCTION

The clinical manifestations of polypoidal choroidal vasculopathy (PCV) have been recognized for nearly two decades[1,2]. The clinical features of PCV include serosanguineous pigment epithelial detachment, recurrent sub-retinal hemorrhage, serous retinal detachment and sub-retinal exudation. Although PCV and central serous chorioretinopathy (CSC) are two different diseases, they have common characteristics[3]. PCV is often characterized by the presence of orange-red sub-retinal nodules and aneurysmal polypoidal lesions in the choroidal vasculature, with or without an associated branch vascular network. We report a patient with serous retinal detachments masquerading as CSC.

CASE PRESENTATION

Chief complaints

A 52-year-old female presented to our hospital with blurred vision in her left eye for approximately 1 wk.

History of present illness

The patient reported no headache or eye pain. She had no other diseases.

History of past illness

Unremarkable.

Physical examination

No abnormalities were found on slit lamp examination.

Laboratory and imaging examinations

The patient underwent a comprehensive ophthalmic examination, including decimal best corrected visual acuity, color fundus photography, spectral domain optical coherence tomography (OCT) and fluorescein fundus angiography. On first examination, her best corrected visual acuity was 20/40 in the left eye and 20/20 in the right eye. Anterior segment and intraocular pressure findings were normal in both eyes. Fundus photography of the left eye showed a seemingly normal adult oculus fundus without any obvious hard exudate or hemorrhage (Figure 1A). OCT demonstrated a hypo-reflective space beneath both the neurosensory retina and the pigment epithelium layer (Figure 1B). The late phase of fluorescein angiography revealed hyper fluorescence (Figure 1C). On the basis of these findings, a diagnosis of CSC was made. As the patient lived thousands of miles from Shanghai and did not perceive obvious changes in her eyes, she declined follow-up visits to the hospital.

One month later, her visual acuity deteriorated suddenly. On examination, her best corrected visual acuity in the left eye was 20/100. A sub-retinal hemorrhage, hard exudate and reddish-orange nodules were found on fundus photography (Figure 2A). OCT demonstrated a pigment epithelium detachment and sub-retinal fluid (Figure 2B). The late phase of fluorescein angiography revealed increased hyper-fluorescence compared to that observed one year previously (Figure 2C). These characteristics led

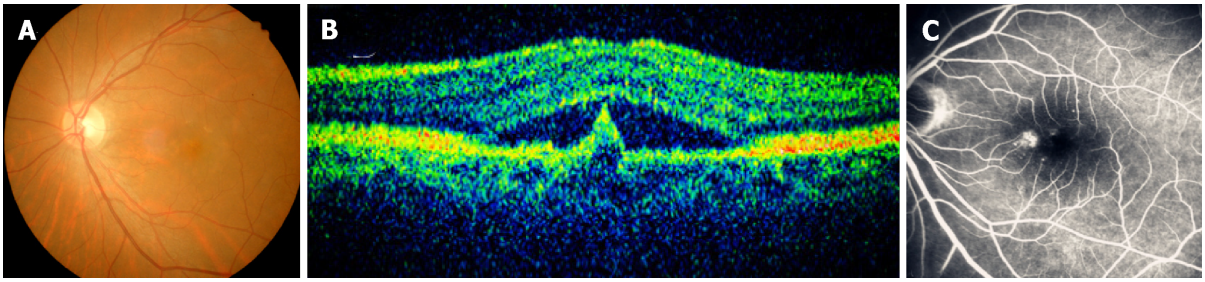

Figure 1 First examination. A: Fundus photography findings. Fundus photography showed a seemingly normal adult ocular fundus without any obvious hard exudate or hemorrhage; B: Optical coherence tomography findings. Optical coherence tomography demonstrated a hypo-reflective space beneath both the neurosensory retina and the pigment epithelium layer; C: Late phase of fluorescein angiography. The late phase of fluorescein angiography revealed increased leakage.

to the diagnosis of PCV.

FINAL DIAGNOSIS

Diagnosed as PCV.

TREATMENT

Most studies have shown that anti-vascular endothelial growth factor (VEGF) therapy may result in visual stabilization and a promising outcome in patients with PCV. Our patient received three monthly loading doses of intravitreal ranibizumab, which were injected 4 mm posterior to the corneal limbus into the vitreous cavity. During the follow-up period, her vision did not worsen. However, 6 mo later, the patient again complained of blurred vision, and fundus photography showed that there was an obvious sub-retinal hemorrhage at the posterior pole (Figure 3A). OCT demonstrated a pigment epithelium detachment (Figure 3B). Considering that this patient had received three intravitreal injections of ranibizumab at monthly intervals, three monthly intravitreal injections of conbercept were administered. Since then, the patient's vision has been stable. As a new antiangiogenic agent, conbercept is a novel VEGF receptor fusion protein that blocks all isoforms of VEGF-A, VEGF-B, VEGF-C and placenta growth factor (also known as PIGF). In addition, it has a higher binding affinity to VEGF. Six months after the first intravitreal injection of conbercept, fundus photography showed no recurrence of hemorrhage (Figure 3C). Her visual acuity was 40/300 in the left eye, which was not improved. The patient showed no change in her eye condition during the continuous follow-up.

DISCUSSION

PCV was once regarded as a variant of wet age-related macular degeneration. We now realize that it may also manifest with serous retinal detachments masquerading as CSC, and PCV patients often present with several unique clinical features that are obviously different from typical neovascular age-related macular degeneration[4-6]. PCV has been defined as "the presence of single or multiple focal areas of hyperfluorescence arising from the choroidal circulation within the first 6 min after the injection of indocyanine green (ICG), with or without an associated branch vascular network. The presence of orange-red sub-retinal nodules with corresponding ICG hyperfluorescence is pathognomonic of PCV." CSC is characterized by a serous retinal detachment in the posterior fundus accompanied by several leaks at the retinal pigment epithelium. It often has a favorable visual prognosis, with the resolution of sensory retinal detachment after several months[7,8]. The classic manifestations of PCV and CSC made it difficult to differentiate them from each other. It is important to make a distinction between these two entities, because they are different in their natural course, response to treatment and visual prognosis[9]. Fundus photography is crucial in the diagnosis and management of PCV and CSC, but ICG angiography (ICGA) is regarded as the gold standard for the diagnosis of PCV. OCT provides complementary information on the structure of the retina observed on ICGA[10].

ICGA is seldom used when PCV shows a focal macular detachment with one or

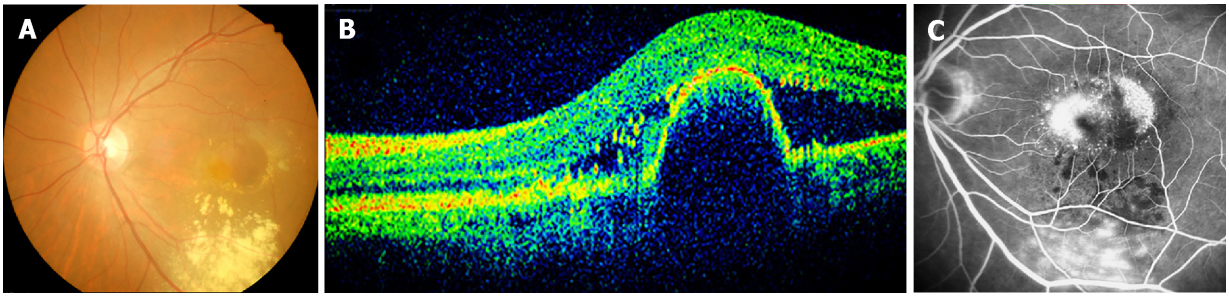

Figure 2 Examination 1 mo later. A: Fundus photography findings. Fundus photography showed a sub-retinal hemorrhage, hard exudate and reddish-orange nodules; B: Optical coherence tomography findings. Optical coherence tomography demonstrated a pigment epithelium detachment and sub-retinal fluid; C: Late phase of fluorescein angiography. The late phase of fluorescein angiography revealed more leakage than that seen one year previously.

more small pigment epithelial detachments. Because these clinical presentations are so similar, this type of disease is frequently diagnosed as CSC. However, as far as chronic CSC is concerned, it may also be associated with intra-retinal lipid deposition accumulation. In addition, sub-retinal hemorrhage and choroidal neovascularization are also possible complications of CSC, while PCV is characterized by lipid deposition and hemorrhage. In view of these findings, when neurosensory detachment in CSC is associated with lipid deposition or hemorrhage, both fluorescein fundus angiography and ICGA should be performed to make a definitive diagnosis. Certain demographic and clinical features can also help to distinguish PCV from CSC. PCV mainly occurs in middle-aged to elderly subjects, and most generally affects patients in their 50s or 60s, while the young are more prone to CSC. Most importantly, when the fundus photography of a patient with PCV shows dot hemorrhagic lesions, ICGA will show dot hyper-fluorescence corresponding to the hemorrhagic lesions in the early phase of angiography. In contrast, the CSC lesion is hyperfluorescent only 5 min after performing ICGA.

CONCLUSION

CSC and PCV are two different retinal entities. ICGA is essential in the diagnosis of PCV, and should be performed to make a definitive diagnosis, especially in patients with suspected PCV. Lipid deposition and hemorrhage are the most important elements that lead to confusion between these two entities. When CSC is associated with lipid deposition or sub-retinal hemorrhage, we should always bear in mind that ICGA may help to distinguish between CSC and PCV.

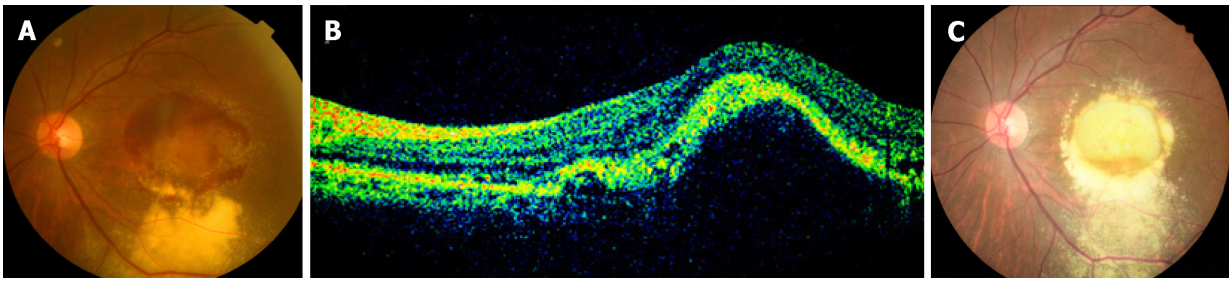

Figure 3 Examination 6 mo later. A: Fundus photography findings. Fundus photography showed that there was an obvious sub-retinal hemorrhage at the posterior pole; B: Optical coherence tomography findings. Optical coherence tomography demonstrated a pigment epithelium detachment; C: Fundus photography findings. Fundus photography showed no recurrence of hemorrhage.

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CASE REPORT

Large carotid body tumor successfully resected in hybrid operating theatre: A case report

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Author contributions: Li MQ and Zhao Y contributed equally to this article; Yang XY and Zhao Y were the patient's neurosurgeons; Li MQ reviewed the literature and contributed to manuscript drafting; Sun HY drew the diagram and reviewed the literature; Zhao Y interpreted the imaging findings; Yang XY was responsible for the revision of the manuscript for important intellectual; all authors issued final approval for the version to be submitted.

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Abstract

BACKGROUND

Surgical treatment for large carotid body tumor (CBT), particularly the Shamblin III type, is challenging and rarely reported.

CASE SUMMARY

In July 2014, a 63-year-old woman presented to our hospital with a large CBT (130 mm × 60 mm × 70 mm). The lesion was hypervascular, spanned from the first to the seventh cervical vertebra, and adhered to the right common carotid artery (CCA), internal carotid artery (ICA) and external carotid artery (ECA). The resection was carried out in a hybrid operating theatre. First, we used Onyx gel to embolize the feeding artery. An ICA balloon was used to prevent gel entry into the ICA. After shrinkage and hardening of the CBT, we quickly resected the CBT as well as a part of the ECA that adhered to the CBT. A vascular shunt was inserted between CCA and ICA, and the part where the ICA was cut off from the CCA was directly sutured. A follow-up at four years later showed no neurological damage.

CONCLUSION

For large hypervascular CBT, embolization of the feeding artery prior to resection is helpful. The hybrid operating theatre is the ideal platform to carry out such operations.

Key words: Carotid body tumor; Paraganglioma; Hybrid operating theatre; Interventional embolization; Case report

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Core tip: Carotid body tumor of giant Shamblin III type is very rare, and the treatment for which is a big challenge for a surgeon. Combination of interventional embolization and surgical resection in the hybrid operating room is an effective method to safely and completely remove carotid body tumor lesions.

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INTRODUCTION

Carotid body tumor (CBT) is the most common paraganglioma in the head and neck[1]. CBT typically originates from glomus caroticum in carotid bifurcation and consists of chemoreceptor cells of primitive neural crest origin[2-4]. CBT can be categorized into three types: Sporadic, familial and hyperplastic[1].

Surgical resection is the standard treatment for CBT but carried substantial risk for large CBT, including cranial nerve injury and stroke[5]. Intraoperative embolism has been used to successfully remove large CBT previously considered not resectable[6]. Hybrid operating rooms are equipped with digital subtraction angiography (DSA) and near-infrared indocyanine green video angiography, and thus could readily identify severe complications in vascular surgery[7-9].

Here, we report a case of large CBT successfully resected after Onyx gel embolization of the feeding arteries in a hybrid operating theatre.

CASE PRESENTATION

Chief complaints

In July 2014, a 63-year-old right-handed woman presented with a progressively enlarging mass over the past five years and emerging numbness of the right hand and heel.

History of present illness

Patient's symptoms started two months ago with recurrent numbness of the right hand and heel.

History of past illness

She had osteoarthritis of the knee for twenty years and hypertension for four years.

Physical examination

The mass was painless, non-moveable, and located between the right lower jaw and the right clavicle. No vascular murmur was heard over the mass upon auscultation. She had no dysphagia or voice hoarseness. No signs of hypoglossal neuropathy were detected.

Laboratory examinations

Laboratory blood tests were normal.

Imaging examinations

Magnetic resonance imaging (MRI) revealed a 130 mm × 60 mm × 70 mm mass with enhancement (Figure 1A and 1B). The mass spanned from the first to the seventh cervical vertebra, crossed over carotid bifurcation, and seemed to adhere to the right common carotid artery (CCA), right internal carotid artery (ICA), and right external carotid artery (ECA) (Figures 1C, D and Figure 2A).

FINAL DIAGNOSIS

The final diagnosis of the presented case is carotid body tumor; this is based on the clinical examination and postoperative pathology.

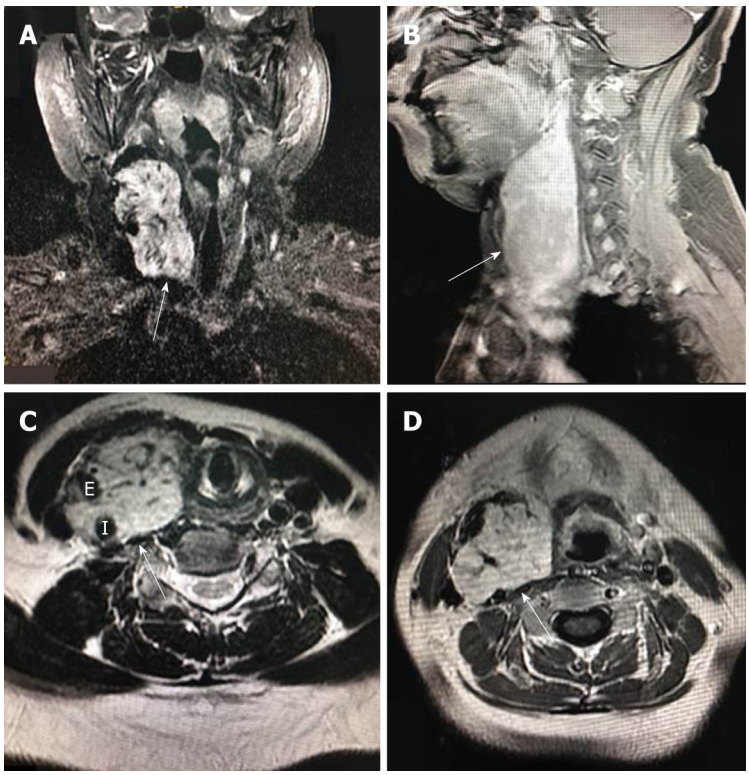

Figure 1 Enhanced magnetic resonance imaging. A: Coronal section (arrow indicates enhancement); B: Sagittal section (arrow); C, D: Axial section showing blood flow in the tumor (arrows). E: External carotid artery; I: Internal carotid artery.

TREATMENT

Surgical resection was conducted in a hybrid operating room, with a goal to completely resect the tumor (Figure 2B). DSA indicated that the right external carotid artery (ECA) was the major source of blood supply to the lesion (Figure 3A). A balloon test occlusion (BTO) was performed under general anesthesia. Upon angiography of the left ICA angiography, the right anterior cerebral artery and right middle cerebral artery were visualized. Twenty minutes after balloon occlusion, the patient's neurological reflex was normal. BTO revealed poor compensation of the posterior communicating artery (PCoA) (Figure 3B and C). Onyx gel was used to embolize the feeding artery superior thyroid artery (STA) (Figure 3D). The ICA balloon was inflated to prevent gel entry into the right ICA. The procedure resulted in significant decrease in blood supply (Figure 3D) and the tumor began to shrink and harden. The balloon was deflated and removed from ICA, and surgical resection started. After isolation of the CBT, the blood supply from the right CCA, ICA, and ECA was blocked. The distal end of the right ECA was ligated, and the (including the part that adhered to the right ECA) was removed. At this point, the blood flow had been blocked for over 30 min. We restored blood flow of the carotid artery and put a vascular shunt between ICA and CCA. The part where the ICA was cut from the CCA was sutured. Intraoperative angiography at this time revealed patency of the ICA and its branches (Figure 4A).

OUTCOME AND FOLLOW-UP

Figure 4B shows the removed tumor. Post-operative pathological examination confirmed paraganglioma (Figure 4C). Two months after the surgery, numbness of the right hand and heel disappeared. Ultrasound examination showed that CCA and RICA were unobstructed (Figure 4D). Upon the last follow-up visit four years later, the patient had no recurrence and no any neurological abnormalities (Figure 4E).

DISCUSSION

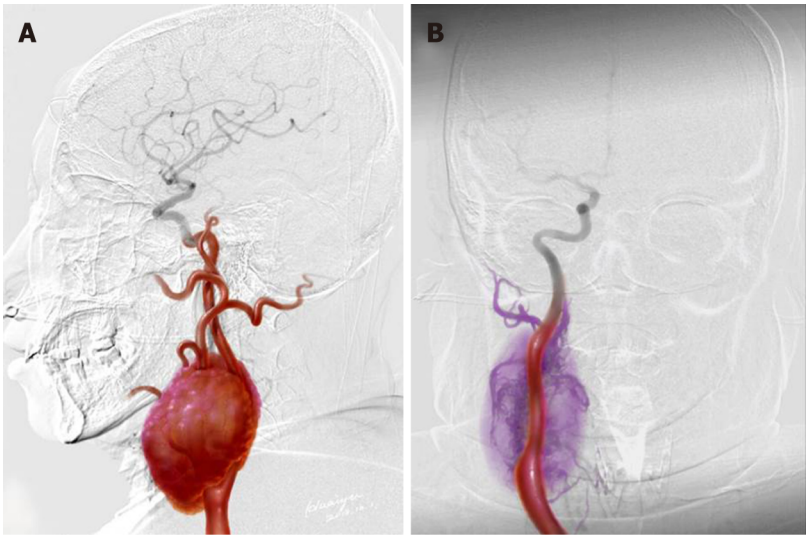

Figure 2 Hypervascular carotid body tumor spans and remove of tumor. A: The hypervascular carotid body tumor spans from C1 to C7, and adheres to the right common carotid artery, right internal carotid artery and right external carotid artery; B: Purple: Removed tumor and external carotid artery segment; Red: Right common carotid artery and right internal carotid artery.

To the best of our knowledge, the report represents the largest CBT that was successfully resected under interventional embolization in a hybrid operating room.

The CBT in the current case was very close to the base of the skull. As a result, the risk of significant blood loss and cranial nerve injuries during operation is high. A previous study showed that, for every 1-cm decrease in DTBOS, intraoperative blood loss (> 250 mL) increases by 1.8 times (95%CI: 1.25-2.55), and the cranial nerve injury increases by 1.5 times (95%CI: 1.19-1.92)[10]. To reduce the intraoperative bleeding, we used Onyx gel to embolize the feeding artery. The right CCA, ICA and ECA were also blocked. We believe that these procedures are important for complete resection of the tumor.

Based on Shamblin's classification[4], CBT is classified into three types. Type I: Tumor is small and confined to the bifurcation of carotid artery, and there is little adhesion to the carotid artery; Type II: Tumor is large and partly surrounds the carotid artery with certain degree of adhesion to the carotid artery; and Type III: The tumor is large and completely envelops the carotid artery. CBT with higher Shamblin grade tends to have more severe neurological complications upon the surgery[11], mostly cranial nerve injuries[12,13]. The tumor, in this case, was Shamblin type III, and unusually large, but neurological complications did not happen, possibly due to feeding artery embolization, blocking of the CCA, ICA and ECA, and experience of the surgeons.

It is noteworthy to point out that the management of large CBT should be individualized since the tumor could be located in varying positions and may invade different tissues[14,15]. Also, an interdisciplinary approach is needed.

CONCLUSION

For large hypervascular CBT, embolization of the feeding artery in a hybrid operating theatre could be helpful.

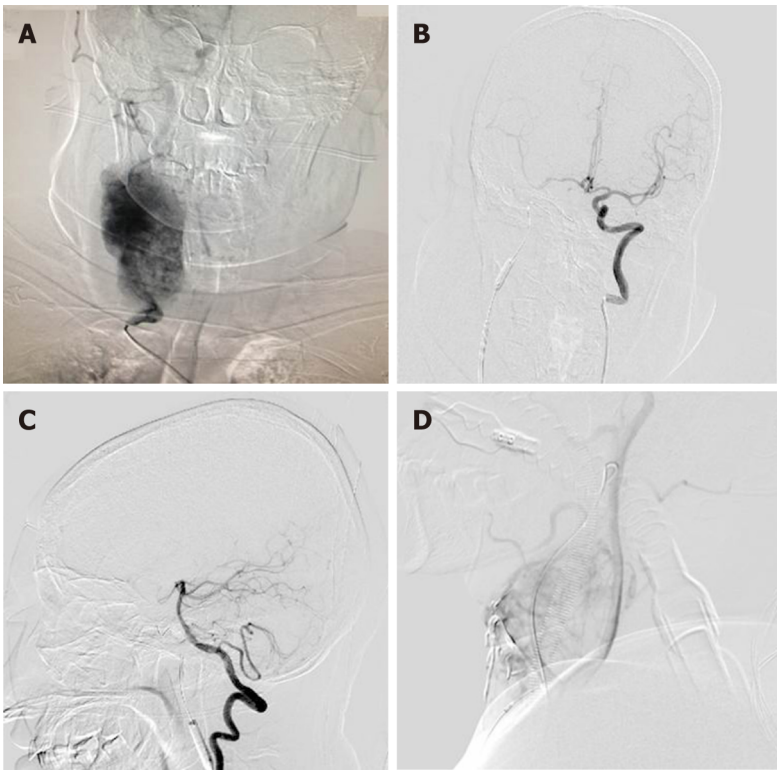

Figure 3 Preoperative angiography and balloon test occlusion. A: Right carotid artery angiography revealed a hypervascular tumor; B: Balloon test occlusion showed that the anterior communicating artery was open and the right internal carotid artery blood supply was compensated; C: The left vertebral artery angiography showed the posterior communicating artery was open; D: Embolization of the small feeding artery of carotid body tumor.

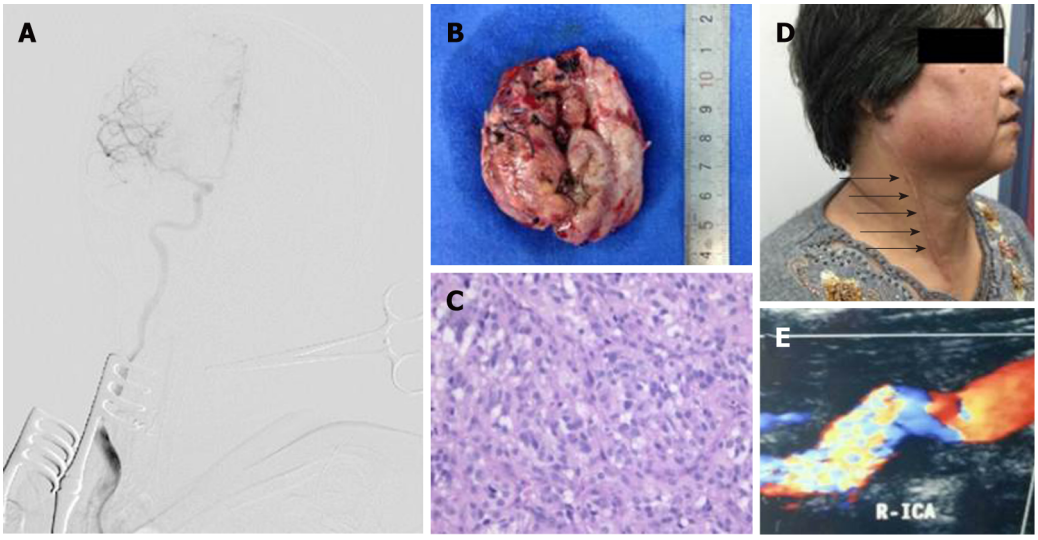

Figure 4 The tumor was completely removed and the right internal carotid artery flow was unobstructed. A: Intraoperative angiography showed that the internal carotid artery was unobstructed after the carotid body tumor was completely removed; B: The tumor volume decreased significantly due to blockade of blood supply; C: HE staining showed paraganglioma (× 400); D: Ultrasonography at 2 wk after the surgery (arrows: The site of surgical incision); EL 4-year follow-up.

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CASE REPORT

A huge pancreatic lipoma mimicking a well-differentiated liposarcoma: A case report and systematic literature review

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Author contributions: Xiao RY collected case data, prepared the photos and wrote the manuscript; Yao X and Wang WL proofread and revised the manuscript; all of the authors approved the final version to be published.

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Abstract

BACKGROUND

Pancreatic lipomas are thought to be very rare. Lipomas are usually easy to identify on imaging, particularly *via* computed tomography (CT). But sometimes it's quite difficult to distinguish a lipoma from a well-liposarcoma without histologic result.

CASE SUMMARY

Here, we present a case of pancreatic lipoma in a 59-year-old female. She was asymptomatic and had no medical history of note. CT and magnetic resonance imaging revealed a mass like well-differentiated liposarcoma in the pancreatic head, positron emission tomography/CT showed a low fluorodeoxyglucose uptake and laboratory tests revealed elevated transaminase and carbohydrate antigen-199 levels. Finally, the patient underwent a pancreaticoduodenectomy. Histologically, mature adipocytes were noted in the bulk of the tumor. Accordingly, the pathologic diagnosis of the pancreatic neoplasm was lipoma. To our knowledge, this case is the first example of a suspected well-differentiated liposarcoma that was actually a pancreatic lipoma. We also highlight the radiological features distinguishing a pancreatic lipoma from a pancreatic liposarcoma and briefly review the literature.

CONCLUSION

Pancreatic lipomas show no obvious gender bias and most commonly occur in the head of the pancreas, of which the maximum diameters are often less than 5 cm, and small, asymptomatic non-compressed lipomas require follow-up only. Surgical excision should be considered when the tumor has compressed important tissues or is difficult to distinguish from a liposarcoma, the choice of

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surgery depends on the intraoperative presentation.

Key words: Pancreatic Lipoma; Liposarcoma; Pancreas; Case report

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Core tip: Pancreatic lipomas are rare, especially the huge ones. Lipomas are usually easily identified on imaging, particularly *via* computed tomography. Here we present the first example of a suspected well-differentiated liposarcoma on imaging that was actually a pancreatic lipoma. We also highlight the radiological features distinguishing a pancreatic lipoma from a liposarcoma and briefly review the literature.

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INTRODUCTION

Mesenchymal tumors of the pancreas are rare, and are classified by their histological origin; they represent only 1%–2% of all pancreatic tumors[1]. Of these rare tumors, fat-originating tumors (lipomas and liposarcomas) are the rarest. Intrapancreatic lipomas were found in only 0.012% of all patients undergoing routine cross-sectional imaging[2]. A pancreatic lipoma must be distinguished from focal fat replacement, lipomatous pseudohypertrophy, and liposarcoma[3]. For the surgeon, the most important differential diagnosis is liposarcoma, which is generally easily identified on imaging [such as computed tomography (CT)]. Here, we report a huge asymptomatic pancreatic lipoma mimicking a well-differentiated liposarcoma pathologically confirmed after performing the Whipple procedure. Additionally, we found that no systematic retrospective review of pancreatic lipoma status has appeared since 2010[4]. Thus, we reviewed the literature in terms of clinical manifestations and treatments.

CASE PRESENTATION

Chief complaints

A 59-year-old female presented with a pancreatic mass that had been identified during a medical examination 10 d prior.

History of present illness

She was asymptomatic and didn't undergo any treatment at other hospitals.

History of past illness

The patient had a free previous medical history.

Personal and family history

Her medical history and family history were unremarkable.

Physical examination

she was 160 cm tall and weighed 64 kg. Her abdomen was soft and nontender with no palpable mass.

Laboratory examinations

The laboratory data were: Alanine transaminase 95.2 U/L (reference < 40 U/L); aspartate transaminase 67.2 U/L (reference < 35 U/L); conjugated bilirubin 7.3 μmol/L (reference < 6.8 μmol/L); γ-glutamyl transferase 91.4 U/L (reference < 45 U/L); carbohydrate antigen 19-9 46.0 U/mL (reference < 39 U/mL); and serum ferritin, 423 ng/mL (reference < 367.1 ng/mL).

Imaging examinations

Abdominal ultrasonography revealed a hypoechoic flaky lesion of maximum diameter 5.2 cm in the head of the pancreas. Subsequent contrast-enhanced CT revealed a 6.4 cm × 6.0 cm near-circular heterogeneous fat-containing lesion (−109 ± 19.2 HU on contrast-enhanced CT compared to 47.9 ± 14.9 HU for the liver) in the head of the pancreas (Figure 1). The borders were indistinct and a few fibroreticular septa were evident within the lesion. The surrounding parenchyma was slightly enhanced, and the lesion was not clearly distinguishable from the pancreas. The adjacent tissues were partially compressed, including the head of the pancreas, the duodenum, and certain blood vessels (the inferior vena cava, portal vein, and superior mesenteric artery/vein). The pancreatic duct and intrahepatic bile ducts were not obviously dilated. By reference to the CT data only, we first considered that the mass might be a liposarcoma derived from the retroperitoneum. On magnetic resonance imaging, the mass was of high signal intensity on T2-weighted axial imaging, being isointense to the subcutaneous and intra-abdominal fat. And the fat-suppressed T1- and T2-weighted images revealed signal intensity losses, indicating that the mass was composed principally of adipose tissue (Figure 2). A few fibroreticular septa were evident within the lesion. The boundary between the lesion and the pancreas was unclear. Thus, the mass was most likely a well-differentiated liposarcoma derived from retroperitoneal fat. Magnetic resonance cholangiopancreatography revealed no dilatation or stenosis of the intrahepatic bile duct or pancreatic duct, but the middle and lower parts of the common bile duct were partially compressed. An abnormal 6.2 cm × 6.0 cm circular mixed/fatty signal emanated from the head of the pancreas (Figure 3). On positron-emission tomography/CT, the lesion had the density of fat, exhibited low fluorodeoxyglucose uptake, excluded evident distant metastasis, and was thus thought to be a non-malignant fat-derived tumor first, but it still cannot be distinguished from a well-differentiated liposarcoma.

Treatment

Given the huge size and the compression of the middle and lower parts of the common bile duct and important blood vessels, we suggested surgery even if the lesion was benign. We planned total surgical excision, but found that the upper part of the mass was tightly connected to the pancreas and could not be completely excised. We feared that complete removal would increase the risk of injury to the pancreatic duct and superior mesenteric vein, which might trigger a major intraoperative hemorrhage and a postoperative pancreatic fistula that could erode the superior mesenteric vein and cause a massive hemorrhage or other complications. Thus, we switched to a pancreatoduodenectomy.

FINAL DIAGNOSIS

The final pathological examination confirmed a giant lipoma of the pancreas; the largest diameter was 13.0 cm (Figure 4). Two lymph nodes near the pancreas and three around the stomach evidenced chronic lymphadenitis. Pathology also revealed chronic cholecystitis with cholesterol polyps.

OUTCOME AND FOLLOW-UP

Postoperatively, we controlled an elevated blood glucose level, abnormal liver function, and hyperamylasemia, and the patient was discharged to home with a peritoneal drainage tube on postoperative day 25. She followed regularly to the department of general surgery.

LITERATURE REVIEW

In the time since the first report[5], 169 cases of pancreatic lipoma have been reported in 48 articles[1-3,5-49], including 10 in Chinese. Most cases were diagnosed by imaging (such as CT); only 22 were confirmed by pathology, 16 of which underwent surgery and 6 endoscopic ultrasound/fine needle aspiration (FNA). Only 2 patients underwent both FNA and surgery; these exhibited massive vascular compression by the tumor[34] and elevated serum bilirubin and alkaline phosphatase levels[15]. However, the FNA data were not described. Some have argued that pancreatic lipomas are not rare[2,22]. The sexes of 162 of the 169 cases were identified: 87 males and 75 females. Their ages ranged from 11 months to 88 years. Lipomas are most commonly found in the middle-aged and elderly, possibly because they undergo

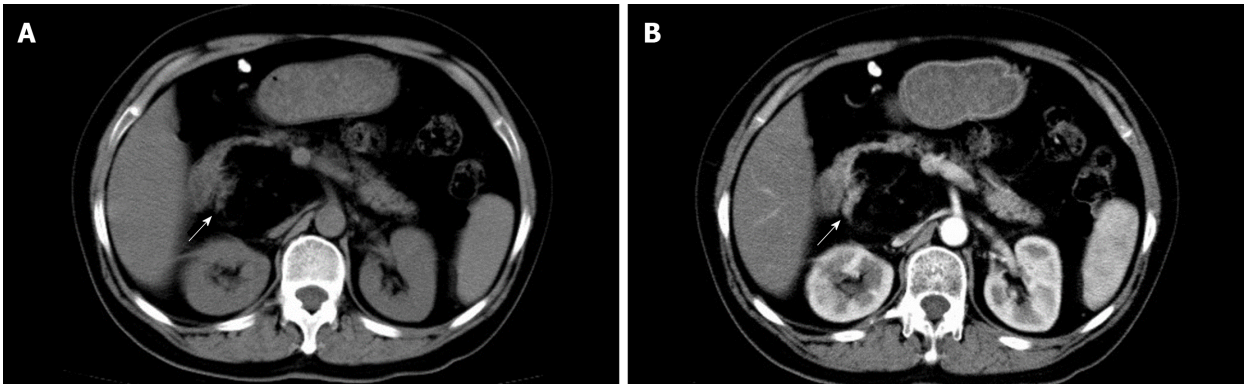

Figure 1 Computed tomography scans before treatment. A: Non-contrast abdominal computed tomography (CT) showed a 6.4 cm × 6.0 cm, nearly circular, heterogeneous lesion, owning indistinct borders, located in the head of the pancreas. B: Contrast-enhanced CT imaging indicated that the fat containing tumor (-109 ± 19.2 HU in the tumor and 47.9 ± 14.9 HU in the liver) had a few fibroreticular septa within it, and the surrounding parenchyma of the mass could be slightly enhanced (arrow).

physical examinations more often than do the young. The pancreatic lipoma locations were: The head (n = 70); the head and the uncinate process (n = 1); the uncinate process (n = 15); the head and neck (n = 2); the neck (n = 9); the neck and body (n = 1); the body (n = 30); the body-tail junction (n = 3); the tail (n = 34); and not mentioned (n = 4) . Only a few tumors were of diameter > 50 mm: < 50 (n = 150); 50–100 (n = 9); and > 100 mm (n = 3). Most patients were asymptomatic and required only follow-up or conservative treatment (n = 132); only 16 required operations, including pancreatoduodenectomy (7), tumor enucleation from the head (3), subtotal pancreatectomy and splenectomy (1), pancreatic tail resection (1), biliary bypass (1), and not mentioned (3). In patients who underwent surgery, postoperative complications were mentioned in only two cases; these were an elevated blood glucose level and a pancreatic fistula.

DISCUSSION

A pancreatic lipoma is a rare solid tumor, the etiopathogenesis of which remains unclear although lipomas located in the pancreatic head have been considered to be adipose tissue trapped during posterior rotation of the ventral pancreatic bud[22,48].

CT is the most useful radiological method to diagnose pancreatic lipoma[4]. The density of a liposarcoma in CT is higher than that of normal fat and benign fatty masses, and indistinct borders[50], thick septa[48,51] , a larger size[48,52] (> 5 cm, and in most cases > 10 cm)[1], calcification[48,52] and rapid growth[48] are significant indicators of malignancy. Features of well-differentiated liposarcoma include large lesion size, presence of thick septa, presence of nodular and/or globular or non-adipose mass-like areas, and decreased percentage of fat composition[52]. A lipoma is usually well circumscribed, of the density of normal fat, homogenous[4], noninvasive[50], stable and devoid of symptoms. However, it is not easy to distinguish a lipoma from a well-differentiated liposarcoma due to the radiographic similarities between these two lesions[4] (Table 1).

To our knowledge, our case is the first example of a suspected well-differentiated liposarcoma that was actually a pancreatic lipoma. The tumor of our present patient was around 6.2 cm × 6.0 cm in dimensions, indistinct from the pancreas, contained a few fibroreticular septa, and the surrounding parenchyma was slightly enhanced. We first thought that the mass was a well-differentiated liposarcoma derived from the retroperitoneum. Despite that positron emission tomography/CT showed low fluorodeoxyglucose uptake, the diagnosis was still uncertain. Thus, given the huge size and the compression of the middle and lower parts of the common bile duct and important blood vessels, we suggested surgery even if the lesion was benign. We planned total surgical excision, but found that the upper part of the mass was tightly connected to the pancreas and could not be completely excised. We feared that complete removal would increase the risk of injury to the pancreatic duct and superior mesenteric vein, which might trigger a major intraoperative hemorrhage and a postoperative pancreatic fistula that could erode the superior mesenteric vein and cause a massive hemorrhage or other complications. Thus, we switched to a pancreatoduodenectomy.

Pancreatic lipoma seems to exhibit no gender bias, is usually diagnosed *via* CT or

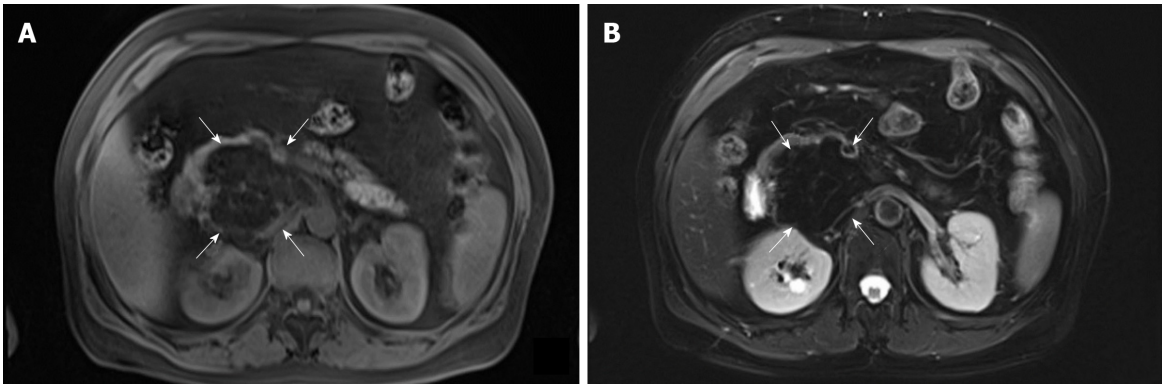

Figure 2 Magnetic resonance scans before treatment. A and B: Both fat-suppressed T1-weighted and fat-suppressed T2-weighted images showed a loss in signal intensity (arrow), which indicated the mass mainly composed of adipose tissue. And a few fibroreticular septa could be seen within the lesion. The boundary between the lesion and the pancreas was unclear.

other imaging methods, and most commonly occurs in the head of the pancreas. The maximum diameter is often less than 5 cm. Generally, small, asymptomatic non-compressed lipomas require follow-up only. Very few cases exhibit significant short-term changes, but the lipoma may grow in the long term[25]. Patients may elect to undergo trans-duodenal core needle biopsy if the tumor is difficult to identify on imaging. Surgery is recommended if a malignancy is in play. However, it is sometimes difficult to distinguish lipomas from well-differentiated liposarcomas[53]. A combination of FNA data and MDM2 genetic analysis improves the liposarcoma detection rate[54,55]. In addition, short-term close follow-up may identify patients with enlarging lesions that require surgery. Compressive lesions, such as that of our present case, require excision; the choice of surgery varies by the intraoperative presentation.

CONCLUSION

In summary, pancreatic lipomas are rare, especially the huge ones, no obvious gender bias, and most commonly occur in the head of the pancreas. Small, asymptomatic non-compressed lipomas require follow-up only. Surgical excision should be considered when the tumor has compressed important tissues or is difficult to distinguish from a liposarcoma, the choice of surgery depends on the intraoperative presentation.

Table 1 Clinical features of pancreatic lipoma

	Clinical Manifestation	Cases (*n*)	Percentage, %
Sex	Male	87	53.7
	Female	75	46.3
Locations of the tumor	Head	70	42.4
	Tail	34	20.6
	Body	30	18.2
	Uncinate process	15	9.1
	Neck	9	5.5
	Body-tail junction	3	1.8
	Head-neck junction	2	1.2
	Head - uncinate process junction	1	0.6
	Neck and body junction	1	0.6
Tumor size, mm	< 50	150	92.6
	50-100	9	5.6
	> 100	3	1.9
Treatment	Follow-up or conservative treatment	132	89.2
	Pancreatoduodenectomy	7	4.7
	Tumor enucleation in head	3	2.0
	Subtotal pancreatectomy with a splenectomy	1	0.7
	Pancreatic tail resection	1	0.7
	Biliary bypass	1	0.7
	Type of surgery not mentioned	3	2.0

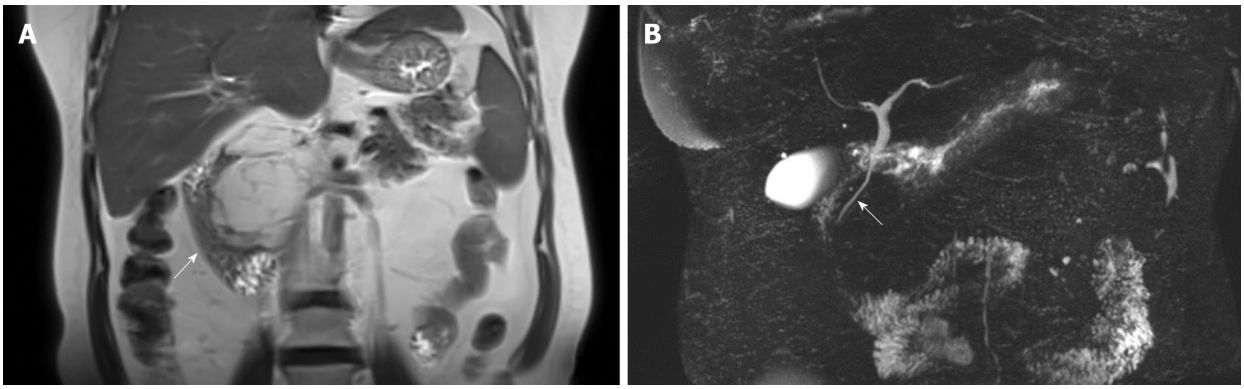

Figure 3 Coronary magnetic resonance scan and magnetic resonance cholangiopancreatography before treatment. A: Magnetic resonance imaging showed a fat-signal lobulated tumor compressing her duodenum (arrow); B: Magnetic resonance cholangiopancreatography demonstrated that there was no dilatation and stenosis in the intrahepatic bile duct and the pancreatic duct, but the middle and lower part of the common bile duct was partially compressed (arrow).

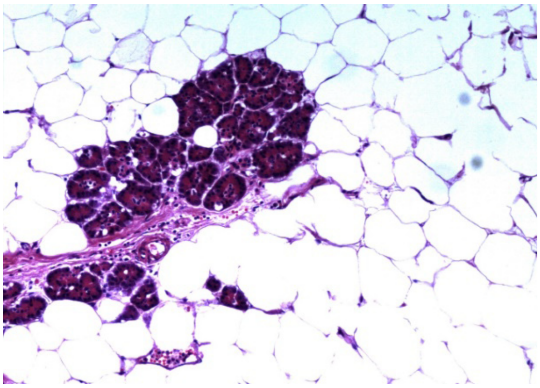

Figure 4 Final pathological examination. Mature adipocytes were noted adjacent to the pancreatic parenchyma (original magnification, × 100).

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CASE REPORT

Ulcerative colitis complicated with colonic necrosis, septic shock and venous thromboembolism: A case report

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Author contributions: Zhu MY drafted the manuscript; Sun LQ revised the article critically; All authors read and approved the final manuscript.

Informed consent statement: Written informed consent was obtained from the patient for publication of this case report.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

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Abstract

BACKGROUND

Severe total colonic necrosis, septic shock and venous thromboembolism secondary to ulcerative colitis (UC) are rare and life-threatening. No such severe complications have been reported in the literature.

CASE SUMMARY

We report a 36-year-old woman who developed total colonic necrosis and septic shock secondary to UC. The patient was treated with emergency surgery because computed tomography showed suspicious perforations. Persistent massive ascites occurred after operation and computed tomography angiography demonstrated portal vein, mesenteric vein and splenic vein thrombosis. The patient was discharged from hospital after active treatment.

CONCLUSION

Clinicians should pay attention to venous thrombosis, colonic necrosis and septic shock in UC patients. Close observation of surgical indications and timely surgical intervention are the key to reduce mortality and complications in UC.

Key words: Ulcerative colitis; Total colonic necrosis; Venous thromboembolism; Sepsis; Septic shock; Case report

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Core tip: Severe total colonic necrosis, septic shock and venous thromboembolism are rare but life-threatening complications of ulcerative colitis. Possibility of colonic necrosis in ulcerative colitis should be considered and close observation of surgical indications and timely surgical intervention are the key to reduce mortality and complications in ulcerative colitis.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease, is easy to relapse, and prognosis is poor once there are complications[1]. Patients with UC have mucosal inflammation, which may extend continuously to the entire colon[2]. Total colonic necrosis, a complication in UC, is rarely reported and can be life threating. We present a patient with UC who developed total colonic necrosis, septic shock and venous thromboembolism (VTE) accompanied by acute kidney injury (AKI). The patient was successfully treated with emergency surgery and support therapies. This case suggests that occurrence of colonic necrosis in UC should be noticed, and timely colectomy should be performed to prevent critical complications.

CASE PRESENTATION

Chief complaints

A 36-year-old female was admitted to our hospital with abdominal pain, diarrhea, mucus blood, pus and recurrent tenesmus attacks.

History of present illness

Her condition was improved with fecal microbiota transplantation. Nine days later, she complained of anuria, vomiting and diarrhea, which were more severe than before.

History of past illness

The patient was diagnosed with UC ten years previously. She was maintained on mesalazine and prednisone but without comprehensive treatment.

Physical examination

About 100 mL dark colored urine was drained, and bloody fluid was discharged from the urethral catheter. She appeared dyspneic with cold clammy limbs, increased pulse rate, abdominal cavity pressure of 25 mmHg and grade IV abdominal hypertension[3].

Laboratory and imaging examinations

The endoscopy demonstrated total colonic wall thickening, erosions in luminal surface of colon, hyperemia, friability, bleeding and ulcerations (Figure 1). Laboratory tests revealed white blood count of 40.77 × 10^9/L, red blood count of 2.41 × 10^{12}/L, hemoglobin of 60 G/L and sequential organ failure assessment scores of 10 (Table 1). Emergent computed tomography showed suspicious thin perforations at rectosigmoid colon and massive ascites (Figure 2).

FINAL DIAGNOSIS

Septic shock, colonic perforation, UC, acute renal failure, disseminated intravascular coagulation and severe anemia.

TREATMENT

She underwent urgent total proctocolectomy and ileostomy. During surgery, massive ascites, total colonic necrosis, edema and dilatation of small intestine were observed (Figure 3) but with no obvious perforation and arterial thrombosis. The ascites culture was negative. Histopathological examination of the resected colon showed mucosal inflammation, necrosis and hemorrhage (Figure 4). After etiological treatment, the patient was transferred to the intensive care unit. Her symptoms were alleviated, and urine excretion was normal with maintenance of renal perfusion. Ascites fluid of 1880-3880 mL was drained daily for 7 d in the intensive care unit. The possibility of

Table 1 Sepsis-related organ failure assessment score

System	Assessment	Index	Score
Respiratory	PaO2/FiO2 (mmHg)	382 (153/0.4)	1
Coagulation	Platelets×10^9/L	53	2
Liver	Bilirubin (mg/dL)	25.9	1
Cardiovascular	Mean arterial pressure	70 mmHg	0
Central nervous system	Glasgow coma scale score	15	0
Renal	Creatinine, mg/dL; Urine output, mL/d	215.2; 100	2 4
Total score			10

thrombosis was considered. Following anticoagulant therapy, the volume of ascites was reduced gradually. The condition of the patient gradually improved after active treatment. Computed tomography angiography displayed thrombosis in the trunk of portal vein and its intrahepatic branches, the superior mesenteric vein and the splenic vein (Figure 5). The patient recovered and was discharged from the hospital after antibacterial, anticoagulant and nutritional support treatment.

DISCUSSION

UC affects the colon and most commonly afflicting young adults aged 30-40 years[4]. UC complicated with total colonic necrosis has not been reported, and the mortality rate of septic shock secondary to colon necrosis is extremely high. Such severe UC is potentially a life-threatening condition that requires attention and early recognition[5]. The present patient had a long course of UC involving the whole colon. Fecal microbiota transplantation through the jejunum tube does not damage the colon. Related factors for colon necrosis in this patient included non-occlusive mesenteric ischemia, shock and long-term inflammation. Immediate surgical intervention for refractory UC could improve outcomes[6]. Restorative proctocolectomy with ileal pouch-anal anastomosis is the most commonly performed surgery for UC.

Sepsis is defined as life-threatening organ dysfunction caused by an overwhelming immune response to infection[7]. Septic shock is associated with a greater risk of mortality than sepsis alone[8]. Sepsis with a sequential organ failure assessment score of ≥ 2 in a patient with suspected infection indicates a risk of death[9]. The sequential organ failure assessment score of our patient was 10 and was highly associated with serious infection. Total colonic necrosis is the etiology of sepsis. Tissue hypoperfusion accompanied by septic shock, if persistent may lead to organ dysfunction and failure[10]. The cases of AKI account for more than 50% in sepsis[11]. Septic AKI is diagnosed with an increase in serum creatinine or a decrease in urinary output[12]. The patient developed anuria after abdominal pain, which is a sign of renal dysfunction. The compression of the renal artery by abdominal hypertension is also one of the causes of AKI. In this case, the renal function recovered, making computed tomography angiography imaging possible. Source control measures are important in the treatment of abdominal sepsis[13]. Removal of total colonic necrosis helps alleviate septic shock and lower the abdominal pressure. Volume resuscitation, vasopressor and antimicrobial therapy are the main treatments for septic shock[14]. Manifestations of sepsis depends on the infection, the organ dysfunction, the underlying health condition of the patient and the aggressive treatment used[15]. Septic shock in this patient was caused by abdominal infection and characterized by coagulation disorders and AKI rather than circulatory and respiratory dysfunctions.

The risk of VTE in patients with UC is 3-4 times higher than that in the normal population with an overall incidence up to 7%[16], especially in those with a long-term use of prednisone. VTE is associated with disease activity in UC[17]. Portal vein thrombosis and mesenteric venous thrombosis in our patient were severe complications associated with UC and surgery. The prevalence of portal vein thrombosis in the general population is about 1%[18]. A large amount of ascites before, during and after surgery was considered to be associated with portal vein thrombosis, but angiography could not be performed in these patients because of AKI. Patients with mesenteric venous thrombosis can present with acute abdominal pain, but most of them have vague clinical presentation, which makes it difficult to be diagnosed in time[19]. VTE is often a critical condition and anti-coagulate therapy should be given as soon as the diagnosis is made[20]. It is important to prevent VTE during hospitalization.

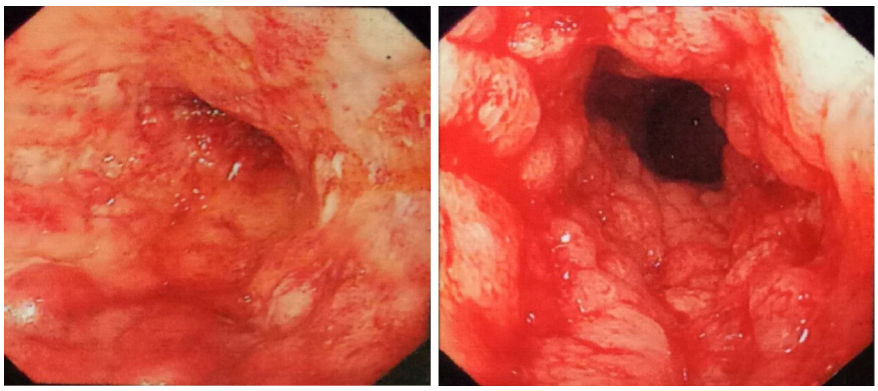

Figure 1 Endoscopy demonstrated total colonic wall thickening, erosions in luminal surface of colon, hyperemia, friability, bleeding and ulcerations.

CONCLUSION

Early computed tomography angiography is necessary in patients with severe UC. Total colonic necrosis is a rare complication in UC. If medical therapy fails, close observation of surgical indications and timely colectomy should be performed to prevent critical complications.

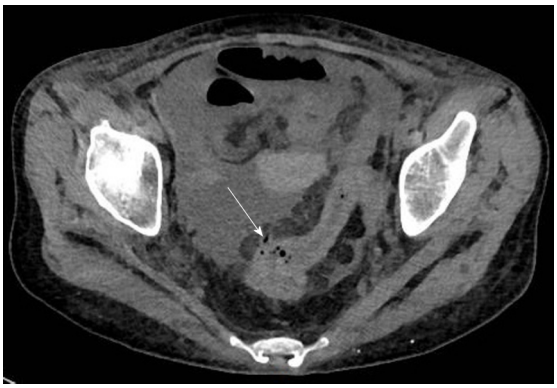

Figure 2 Thin flaws and perforations at rectosigmoid colon and a large amount of fluid in abdominal cavity and pelvis were found by emergency computed tomography.

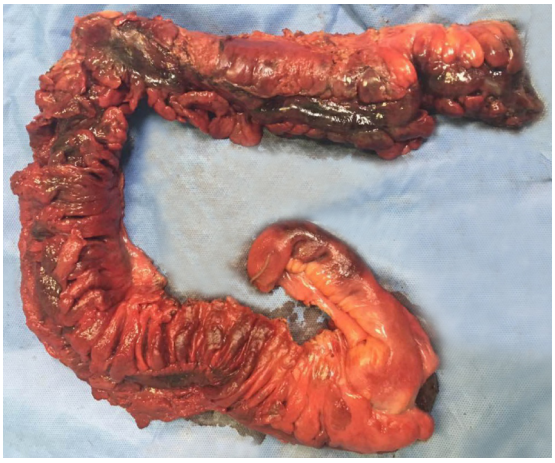

Figure 3 Total colonic necrosis was seen during operation.

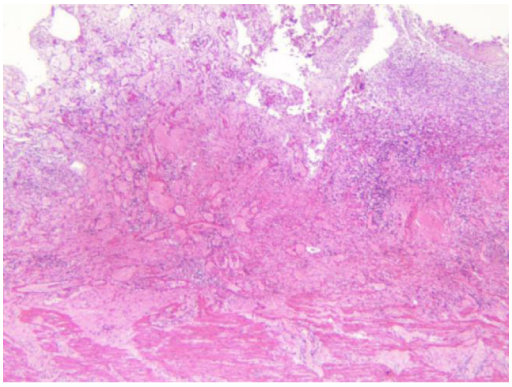

Figure 4 Histopathological examination of the resected colon in the patient showed extensive hemorrhage, necrosis and exudation involving the whole intestinal wall and the extraserous adipose tissue.

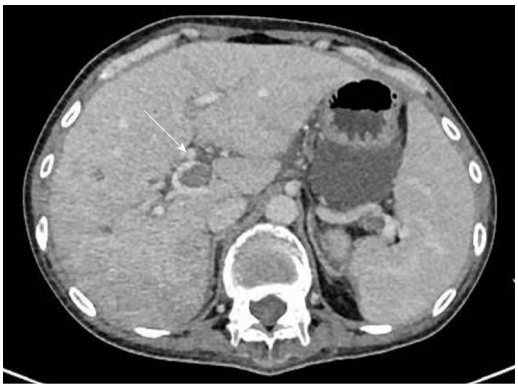

Figure 5 Plain computed tomography scanning and contrast enhancement of abdomen display thrombosis in the trunk of portal vein and its intrahepatic branches, the superior mesenteric vein and the splenic vein.

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CASE REPORT

Acute pancreatitis connected with hypercalcemia crisis in hyperparathyroidism: A case report

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Author contributions: Duan YF designed the research and diagnosed and treated the patient; Hu J and Ma YB collected the patient's clinical data and wrote the paper.

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Abstract

BACKGROUND

The association between primary hyperparathyroidism (PHPT) and acute pancreatitis is rarely reported. Here we describe the process of acute pancreatitis-mediated PHPT induced by hypercalcemia in a male patient. Hypercalcemia induced by undiagnosed PHPT may be the causative factor in recurrent acute pancreatitis.

CASE SUMMARY

We report a case of hypercalcemia-induced acute pancreatitis caused by a functioning parathyroid adenoma in a 57-year-old man. The patient initially experienced a series of continuous gastrointestinal symptoms including abdominal distension, abdominal pain, nausea, vomiting, electrolyte disturbance, renal dysfunction, and acute pancreatitis. Due to prolonged hypercalcemia, the patient subsequently underwent surgical resection of the parathyroid adenoma. Two weeks after surgery, his serum calcium, amylase, and lipase concentrations were normal. The patient had a good recovery after a series of other relevant therapies.

CONCLUSION

Acute pancreatitis as the first presentation is a rare clinical symptom caused by PHPT-induced hypercalcemia.

Key words: Acute pancreatitis; Hypercalcemia; Hyperparathyroidism; Case report

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Core tip: Acute pancreatitis as the first presentation is a rare clinical symptom caused by primary hyperparathyroidism-induced hypercalcemia. Surgical

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INTRODUCTION

Primary hyperparathyroidism (PHPT) is commonly characterized as an endocrine disorder with hypercalcemia attributable to overexpression of parathyroid hormone (PTH) from one or more parathyroid glands[1]. The occurrence of hypercalcemia crisis is usually associated with an elevation of PTH. Hypercalcemia in patients with PHPT can result in various comorbidities such as gastrointestinal symptoms, electrolyte disturbance, renal dysfunction, acute pancreatitis, or can be asymptomatic[2-5]. Hypercalcemia with acute pancreatitis as an initial symptom is an uncommon presentation of PHPT and its prevalence is estimated to be between 1.5% and 7%[6,7]. Upon routine laboratory testing, PHPT may be diagnosed incidentally as hypercalcemia can be asymptomatic in a large number of patients.

The regulation of serum calcium in humans mainly depends on the secretion of PTH. The normal serum levels of calcium range from 2.25 to 2.75 mmol/L. Multi-organ clinical manifestations can occur due to excessive serum calcium. However, acute pancreatitis as the first clinical presentation in PHPT is rare. Hypercalcemia caused by undiagnosed PHPT may be the only causative factor in acute pancreatitis[8]. Therefore, this disease is often misdiagnosed or overlooked completely during clinical consultation. We report a case of hypercalcemia-induced acute edematous pancreatitis as the first manifestation of a benign parathyroid adenoma in a male patient. This man was diagnosed with PHPT and subsequently underwent parathyroidectomy with complete resolution of all symptoms.

CASE PRESENTATION

Chief complaints

A 57-year-old man presented to the emergency room with sudden onset of severe epigastric pain with nausea and vomiting for less than one day.

History of present illness

The patient's initial symptoms consisted of sudden onset of severe epigastric pain, nausea, and vomiting. His consciousness was unaffected. The pain did not radiate along his back and was without paroxysmal exacerbation. He denied chill, fever, cough, and expectoration.

History of past illness

He had no history of essential hypertension, diabetes mellitus, or relevant cerebrovascular disease. There was no previous history of gastrointestinal disease or biliary system symptoms. He had no history of smoking or alcohol consumption.

Physical examination upon admission

His temperature, blood pressure, heart rate, and respiratory rate were all normal on admission. There was mild upper abdominal tenderness, rebound tenderness, and no apparent muscle tension in physical examination.

Laboratory examinations

After admission, the patient completed a series of laboratory examinations. Blood tests revealed the following inflammation markers: White blood cell count, 26.71 × 10^9/L (4-10 × 10^9/L)(94.9% neutrophils); C-reactive protein, 35.5 mg/L (0-10 mg/L); hemoglobin, 152.0 g/L; platelet count, 433 × 10^9/L; glutamic-pyruvic transaminase, 15 μ/L; glutamic-oxalacetic transaminase, 13 μ/L; alkaline phosphatase, 121 μ/L; urea, 8.48 mmol/L; and creatinine, 127.0 μmol/L. The representative diagnostic markers of pancreatitis were as follows: serum amylase 1091 μ/L; serum sodium, 152.2 mmol/L; serum potassium, 4.01 mmol/L; serum calcium, 4.67 mmol/L; and serum PTH level, 95.8 pmol/L.

Imaging examinations

B-mode ultrasound showed mild fatty liver and no obvious gallbladder or pancreas abnormalities. Electronic gastroscopy revealed esophagitis, chronic superficial gastritis with bile reflux and duodenal bulb inflammation. Electrocardiography and contrast echocardiography showed no special heart symptoms. Computed tomography and magnetic resonance imaging of the abdomen revealed pancreatic tail contusion, exudative changes around the pancreas, and double kidney stones. Gallstones or correlative biliary system diseases were not observed (Figure 1).

Further diagnostic work-up

In order to confirm the diagnosis, thyroid ultrasound was undertaken in this patient. It showed bilateral thyroid nodules on the right neck mass originating from the inferior thyroid gland or parathyroid (Figure 2).

FINAL DIAGNOSIS

On the basis of biochemical parameters combined with his clinical manifestations, the patient was diagnosed with acute pancreatitis, hypercalcemia, and PHPT.

TREATMENT

During the first two days, the patient was treated with hydration consisting of 2 L isotonic saline every day, diuresis with the intravenous loop diuretic furosemide at 20 mg every 8 h, and small doses of glucocorticoids each day for depression of serum calcium concentration. However, the concentration of serum calcium was still maintained at a high level. Due to the high risk of cardiovascular and cerebrovascular disorders and deterioration of acute pancreatitis, following discussion with the patient and his relatives, he subsequently accepted parathyroid exploration and right parathyroidectomy. During surgery, the volume of the bilateral thyroid was normal. A 3.0 cm × 2.0 cm hyperplastic parathyroid mass on the inferior aspect of the right thyroid lobe was excised. After operation, the patient was admitted to the intensive care unit and treated with fasting, water deprivation, continuous gastrointestinal decompression, gastric acid suppression, inhibition of pancreatic secretion, fluid replacement, nutritional support, and anti-infectious agents.

OUTCOME AND FOLLOW-UP

In the postoperative period, the levels of serum PTH and calcium decreased slowly (Figure 3), and the patient gradually recovered over the next two weeks. Histopathological examination confirmed the diagnosis of right parathyroid adenoma (Figure 4). The patient was discharged with regular follow-up for six months.

DISCUSSION

PHPT is now a common endocrine disorder caused by the inappropriate over-production of PTH secreted by an overactive parathyroid gland[9]. Compared with young individuals, postmenopausal women over the age of 50 years have a greater probability of developing PHPT[10]. The most common pathogenesis of PHPT is parathyroid gland adenomas (80%-85%), and rare causes include parathyroid hyperplasia, carcinoma, multiple endocrine neoplasia type 1 and 2A, and parathyroid cysts[11]. Most patients with PHPT have mild symptoms or are asymptomatic. Despite the variety of PHPT clinical manifestations, hypercalcemia is the most common condition in most clinical cases[12].

Hypercalcemia is a common and potentially fatal metabolic disorder that is most often attributable to PHPT or malignancy-associated disease[13]. An elevation in PTH is one of the principal factors in the initiation of hypercalcemia. Excessive accumulation of serum calcium and decompensation of the renal system promote the development of hypercalcemia. Initially, the symptoms of hypercalcemia are mild or are not notable at the time of discovery. However, the developing symptoms that characterize a crisis are mental disturbance, metabolic encephalopathy, renal insufficiency, gastrointestinal symptoms, and cardiac dysrhythmia[14]. Early diagnosis and preoperative medical management are crucial, and surgical intervention is the optimal treatment for a hypercalcemic crisis.

Acute pancreatitis is an inflammatory process, which has sudden onset due to the

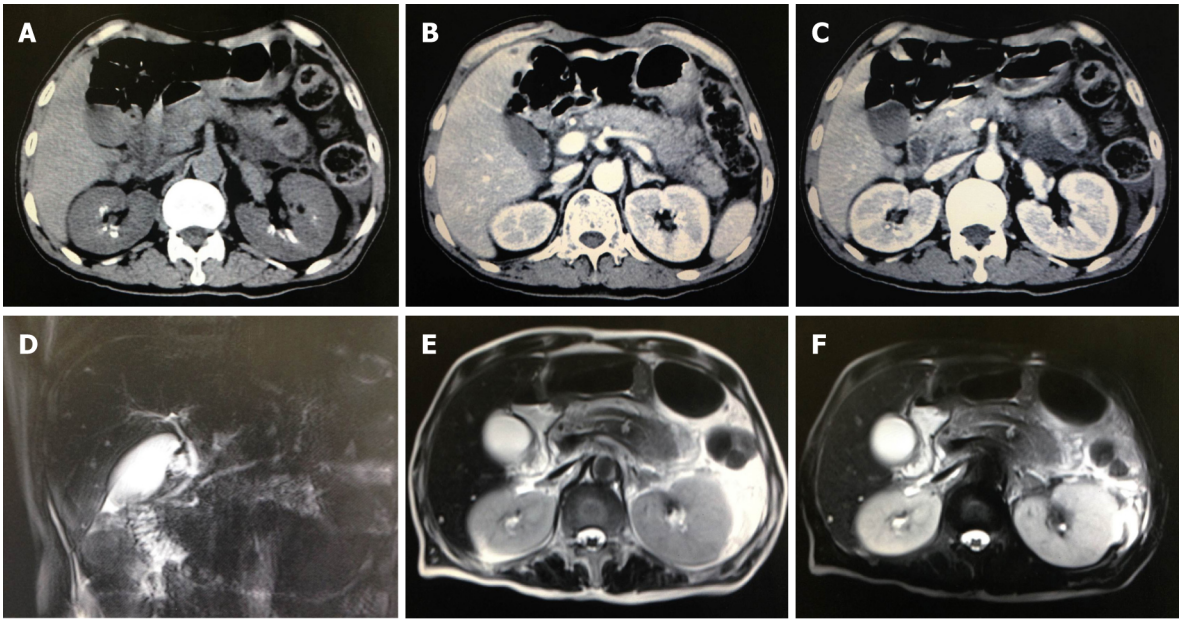

Figure 1 Abdomen computed tomography scan and magnetic resonance imaging. A-C: Abdomen computed tomography demonstrated double kidney stones and a swelling pancreas. The exudation is focused on the surrounding of pancreas body and tail; D-F: Abdomen magnetic resonance imaging similarly revealed pancreatic tail contusion and exudative changes around the pancreas. There were no obvious changes in the biliary system.

premature activation of proteolytic zymogens within the exocrine pancreas[15]. It can be severe with extensive morbidity and mortality[16]. The majority of cases of acute pancreatitis are caused by chronic alcohol consumption and biliary stones. The most frequent cause of acute pancreatitis is gallstone pancreatitis. Biliary stones induced pancreatitis is caused by duct obstruction by gallstone migration leading to temporary impaction of migrating stones at the duodenal ampulla, increased duct pressure, and unregulated stimulation of the digestive enzymes secreted by the pancreas. The second most common cause of acute pancreatitis is alcoholic pancreatitis. Alcohol may sensitize the pancreas to damage by external and environmental factors. The development and recurrence of acute pancreatitis are positively associated with chronic alcohol consumption.

Acute pancreatitis is an uncommon clinical manifestation of PHPT and the prevalence of PHPT-associated pancreatitis in patients were just 1%-8%[17]. The association between PHPT and acute pancreatitis has been debated for decades. Hypercalcemia may play a crucial role in this association and directly affect the severity and prognosis of the disease. Three mechanisms are involved in the development of PHPT-induced acute pancreatitis. One is PHPT-induced high serum calcium level, which can lead to acceleration of the conversion of trypsinogen to trypsin in the pancreas resulting in pancreatic autodigestion and subsequent acute pancreatitis[18]. Secondly, the accumulation of calcium can promote the formation of ductal obstruction, pancreatic calculi, and subsequent attacks of acute pancreatitis[19]. Thirdly, genetic variants in serine protease inhibitor Kazal type 1 and cystic fibrosis transmembrane conductance regulator genes in combination with hypercalcemia markedly increase the risk of developing acute pancreatitis in patients with PHPT[20].

The level of serum calcium is the key to early diagnosis, estimating disease severity, and treatment. When patients with acute pancreatitis are found to have no obvious causes and elevated serum calcium is observed, PHPT-induced pancreatitis should be suspected. The treatment of hypercalcemia includes hydration with saline, forced diuresis, bisphosphonates, calcitonin, oral phosphates, glucocorticoids, and dialysis. But surgical resection is still the most effective treatment. Parathyroidectomy may not only relieve the abdominal symptoms of acute pancreatitis but also prevent the recurrence of hypercalcemia. However, a severe parathyroid crisis caused by a parathyroid adenoma can lead to a series of secondary clinical manifestations, including gastrointestinal symptoms, electrolyte disturbance, renal dysfunction, and acute pancreatitis.

In our case, renal function reflected by creatinine and urea nitrogen increased, to some extent, before and after surgery. Therefore, we used continuous renal replacement therapy to maintain stable renal function. Continuous renal replacement therapy is a method of renal support that has the potential to avoid the development of electrolyte disturbance and deterioration of renal function. Renal replacement

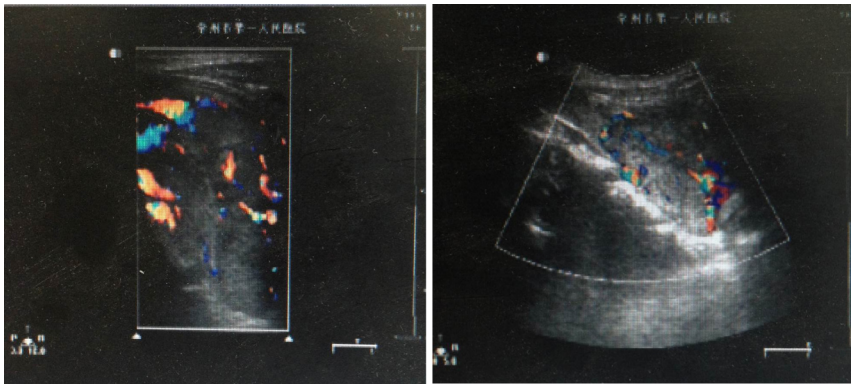

Figure 2 Ultrasonography of neck showed a 3.0 cm × 2.0 cm hypoechoic solid lesion posterior to right lobe of thyroid.

therapy was essential for controlling symptomatic hypercalcemia until medical therapy restored renal function and enhanced renal excretion of calcium. Therefore, PHPT-associated acute pancreatitis should be detected early and appropriate early treatment initiated. Establishing good cooperation between various hospital departments is also critical in treating this rare phenomenon of acute pancreatitis caused by PHPT-induced hypercalcemia.

CONCLUSION

The clinical features of PHPT-induced acute pancreatitis are nonspecific; thus, the diagnosis can be challenging. However, we should exclude patients with acute pancreatitis caused by chronic alcohol consumption and biliary stones before the diagnosis of PHPT-induced acute pancreatitis. Although the disease has a relatively good prognosis, complete surgical resection remains the optimal, safe and curative treatment option available. Early recognition and early-targeted treatment may be the best way to tackle the corresponding diseases.

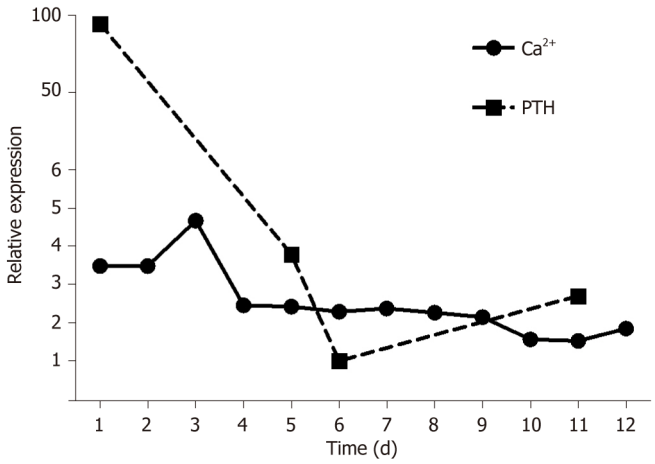

Figure 3 Changes of the concentration of parathyroid hormone and Ca^{2+}. The statistics reveals the expression changes of the concentration of parathyroid hormone (PTH) and Ca^{2+} during the period of admission. The operation was performed on the fourth day. Before the operation, the concentration of Ca^{2+} was maintained at a high-level. Both PTH and Ca^{2+} decreased dramatically and returned to normal levels after the operation.

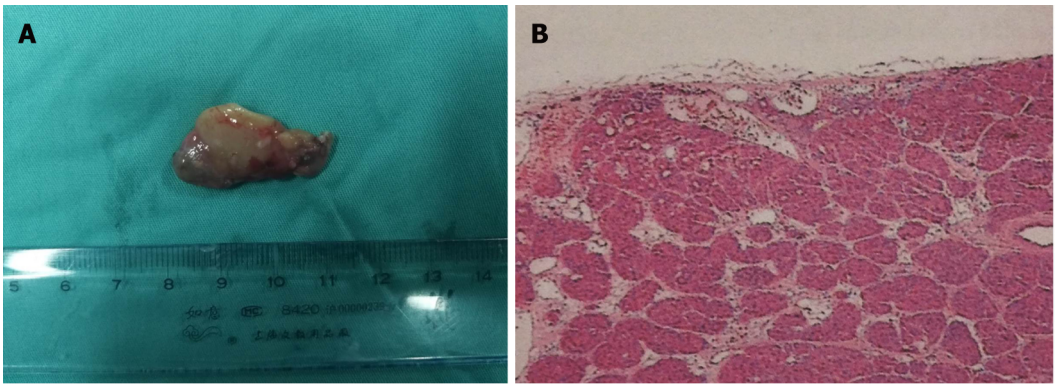

Figure 4 Resected specimen showed a 3.0 cm × 2.0 cm hyperplastic material parathyroid mass after excision (A) and histopathology: right parathyroid adenoma (B). Magnification, x 100.

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CASE REPORT

Treatment of invasive fungal disease: A case report

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Abstract

BACKGROUND

In recent years, the incidence of fungal infection has been increasing, often invading one or more systems of the body. However, it is rare for lymph nodes to be invaded without the involvement of other organs.

CASE SUMMARY

A 21-year-old man was admitted to hospital for repeated cough for 2 mo and abdominal pain for 1 mo. Physical examination revealed multiple lymph nodes enlargement, especially those in the left neck and groin. CT scan showed multiple lymph nodes enlargement in the chest, especially left lung, abdominal cavity, and retroperitoneum. The first lymph node biopsy revealed granulomatous lesions of lymph nodes, so intravenous infusion of Cefoperazone tazobactam combined with anti-tuberculosis drugs were given. Because fever and respiratory failure occurred 4 d after admission, mechanical ventilation was given, and Caspofungin and Voriconazole were used successively. However, the disease still could not be controlled. On the 11th day of admission, the body temperature reached 40° C. After mycosis of lymph nodes was confirmed by the second lymph node biopsy, Amphotericin B was given, and the patient recovered and was discharged from the hospital.

CONCLUSION

No fixed target organ was identified in this case, and only lymph node involvement was found. Caspofungin, a new antifungal drug, and the conventional first choice drug, Voriconazole, were ineffective, while Amphotericin B was effective.

Key words: Invasive fungal disease; Case report; Lymphadenectasis; Lymph node biopsy; Mycosis of lymph nodes; Amphotericin B

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Core tip: In this case, the results from cervical and supraclavicular lymph node biopsies were different. It is very difficult to diagnose lymph node mycosis quickly in the early stage. When conventional anti-infective treatment is ineffective, multi-stage and multi-site lymph node biopsy is particularly important. The new antifungal drug Caspofungin and the empirical antifungal agent Voriconazole were ineffective, and successful treatment was achieved with Amphotericin B.

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INTRODUCTION

Invasive fungal disease (IFD) is a common type of infection in daily clinical practice around the world. It is defined as fungus that invades body tissues, fluids, and blood, and its growth in these places causes inflammation reaction, leading to tissue damage and organ dysfunction. The incidence in patients with immunosuppression due to organ transplants, malignant tumors, *etc* is high (up to 20%-40%)[1]. In recent years, with increasing numbers of immunosuppression in patients with diseases (*e.g.*, malignant tumors and acquired immune deficiency syndrome) and those who use immunosuppressive drugs, IFD incidence has increased dramatically, and the proportion is higher in patients with chronic diseases[2-6]. Current estimates suggest that there are approximately 300 million life-threating fungal infections annually, resulting in 1.6 million deaths[7]. Health impacts worldwide include high morbidity, an overall mortality of 30%–80%, and a multibillion dollar annual economic burden[8].

Lung is the most common target organ of fungal infection. Some specific fungi also have corresponding sensory organs. For example, *Aspergillus* often diffuses in the brain, candida infection often appears in mucositis, and cryptococcal infection often involves the central nervous system[9]. However, it is not common that the main manifestation is lymph node invasion. Unlike previously reported cases, we report a case of invasive mycosis with lymph node fungal infection as the predominant manifestation in a non-immunodeficient patient.

CASE PRESENTATION

Chief complaints

A 21-year-old man presented to the emergency room department with the chief complaints of repeated cough and abdominal pain associated with multiple lymph nodes enlargement.

History of present illness

The patient began to cough and expectorate 2 mo ago, but he refused treatment at that time. These symptoms continued to appear repeatedly. One month ago, he felt pain in his abdominal region with persistence of colic and paroxysmal exacerbation. There were many lymph nodes on the left side of his neck and groin, but there was no fever over the course of disease. His appetite was poor, and his weight decreased approximately 20 kg in 2 mo.

History of past illness

There were no significant comorbidities at admission.

Personal and family history

The patient was unmarried and childless, lived in a good environment. He denied smoking or drinking and had no personal or family history of other diseases.

Physical examination upon admission

Clinical examination revealed the presence of multiple swollen lymph nodes, especially on the left side of his neck and groin. The lymph nodes looked like peanuts

with moderate hardness, and their borders were clear. There were no adhesions in the surrounding tissues, and an absence of tenderness. Lung auscultation revealed thick breathing sounds and dry and wet rales.

Laboratory examinations

Laboratory results including liver function, renal function, electrolytes, enzymology, and immunological tests, such as lymphocyte subsets, immunoglobulin, and immunoelectrophoresis, were normal. Blood culture, parasite detected, sputum acid fast staining, virology examination, rheumatoid factor tests, tuberculosis-antibody immunoglobulin G, tuberculosis-antibody immunoglobulin M tests, and human immunodeficiency virus (1+2) antibodies were negative. White cell count, neutrophil ratio, C-reactive protein, and erythrocyte sedimentation rate were elevated, and sputum culture showed *Klebsiella pneumoniae*.

Imaging examinations

The computed tomography showed there were many enlarged lymph nodes in the chest and abdominal cavity, with some distributed in the retroperitoneal space. We also found pulmonary atelectasis and infection in the left lung (Figure 1, Videos 1-3).

Other auxiliary examinations

In the first biopsy of the cervical lymph node, we found a few lymphocytes and multinucleated giant cells, with no tumor cells, and there tended to be lymph node granulomatous lesions (Figure 2).

In the second biopsy of the supraclavicular lymph node, we found lymph nodes with widespread degeneration and necrosis, and there were many spores and small quantities of hyphae in these tissues. There were many giant cell granulomas in the peripheral lymphoid tissues (Figure 3).

Bronchoscopy showed bilateral bronchial mucous that was uneven with hyperemia and edema. In addition, there were some small white ulcers. Blood samples as well as white glutinous secretions with filaments were seen in the airway.

FINAL DIAGNOSIS

Based on the imaging findings and the results of the secondary lymph node biopsy, the patient was finally diagnosed with mycosis of lymph nodes.

TREATMENT

After admission, he received regular antibiotic treatment and anti-tuberculosis treatment (Cefoperazone tazobactam 2 × 2 g/d,intravenous drip; Isoniazide 0.3 g; Rifampin 0.45 g; Pyrazinamide 3 × 0.5 g; Ethambutol 0.75 g/d, PO), but the treatment effect was not ideal. His temperature was raised gradually in the fifth day, and he started to present with respiratory failure (the oxygenation index less than 150 mmHg) and needed mechanical ventilation therapy. The general anti-infection and anti-tuberculosis treatment were invalid, so we stopped giving anti-tuberculosis drugs and switched to antifungal therapy using Caspofungin (50 mg/d, intravenous drip) for 7 d. The patient's temperature, however, was still not under control. Therefore, we added Voriconazole (2 × 0.2 g/d, intravenous drip) to his treatment. Four days later, this change appeared to be invalid, and the patient's temperature continued to rise. Then we conducted another lymph node biopsy (Figure 2), and at the same time, we began Amphotericin B (30 mg/d, intravenous drip) as the antifungal treatment and stopped using Caspofungin. As Amphotericin B was gradually added, Voriconazole was discontinued after 4 d of Amphotericin B. Figure 4 shows the timeline of drug intervention.

OUTCOME AND FOLLOW-UP

On the third day of Amphotericin B treatment, the patient's temperature gradually returned to normal, and respiratory failure relieved. On the 15th day after admission, the patient was evacuated from the ventilator, and his condition tended to improve. He was then transferred out of the intensive care unit. After continued antifungal treatment for 1 mo in the respiratory department, he went back to the local hospital for further antifungal treatment for 2 mo and recovered. Figure 5 represents the timeline from the patient's presentation to the final outcome.

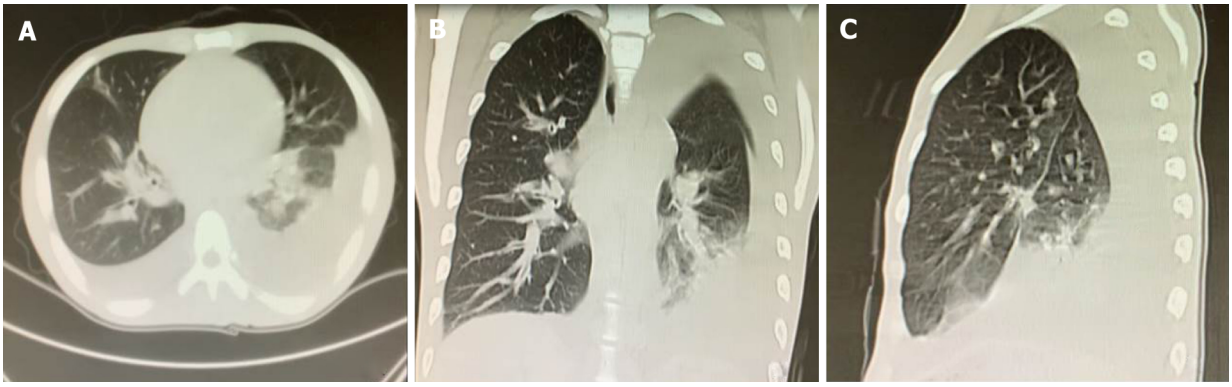

Figure 1 Radiographic findings. The computed tomography showed there were many enlarged lymph nodes in the chest, pulmonary atelectasis, and infection in the left lung. A: Transverse section; B: Coronal plane; C: Sagittal plane.

DISCUSSION

Clinical manifestations in fungal infection are various and lack of specificity, and they often appear in conjunction with other diseases and are easily concealed by the primary diseases. In general, the lung is the most common target organ in fungal infection. Some specific fungi also have corresponding target organs: Aspergillomycosis often spreads in the brain; mucosal inflammation is the most common manifestations in candidiasis; and cryptococcosis always involves central nervous system[9]. Onychomycosis is considered to be one of the hallmarks of human immunodeficiency virus[10]. However, swollen lymph nodes as the prominent manifestation are not common in fungal infections.

Many new antifungal drugs and dosage forms have been developed in recent years, but the incidence and mortality of IFD remains high[2,11-14]. It has been reported that the mortality rates exceed 30% in patients diagnosed with IFD[15]. In recent years, diagnostic testing has improved significantly, and the determination of some biomarkers, such as procalcitonin and presepsin, play an important role in the identification of fungal or bacterial infections[16-19]. However, accurate diagnosis of IFD remains challenging. Fungal infections lack specific characteristic clinical manifestations and laboratory indicators, making early diagnosis difficult and the rate of missed diagnosis and misdiagnosis high[11]. In this case, the patient was young and had no history of tumor or other immunodeficiency. The first lymph node biopsy indicated lymph node granulomatous lesions, where there is no specificity. Therefore, the implementation of empirical anti-bacterial and diagnostic anti-tuberculosis treatment was made. Obviously, there was no effect and the patient's condition gradually worsened, with onset of fever, shortness of breath, and the need for mechanical ventilation treatment. When conventional anti-infective treatment is ineffective or the disease advances progressively, the possibility of fungal infection should be taken into consideration. Antifungal treatment should be given appropriately, and lymph node biopsy should be performed again to find the pathogen.

Clarity and uniformity in defining these infections are important. At present, invasive fungal infection is mainly diagnosed by grading mode[1]. The diagnostic basis is composed of four parts: Host (risk) factors, clinical evidence, mycological evidence, and histopathological evidence[1]. The diagnostic level can be divided into three grades: Definite diagnosis, clinical diagnosis, and suspected diagnosis[1]. Diagnostic criteria are shown in Tables 1-3[1]. Infections caused by *Pneumocystis jirovec* are not included. The criteria for definite diagnosis and clinical diagnosis (Tables 1 and 2)[1] include indirect tests, whereas the level of suspected diagnosis (Table 3)[1] include fungal etiology, although mycological evidence is lacking. These definitions have been adopted by most practice guidelines for IFD. The most commonly identified fungal species associated with IFD are *Candida* species, *Aspergillus*, *Cryptococcus*, and *Pneumocystis*[20]. This case accorded with the grade of suspected diagnosis according to this standard. As there was no etiological basis, Caspofungin with relatively few side effects was given. In this case, Caspofungin was given first and then combined with Voriconazole. Voriconazole is the preferred antifungal drug for empirical antifungal therapy[21]. Unfortunately, the patient's condition was not effectively controlled, and fever occurred (the body temperature rose to 40° C). At this point, lymph nodes biopsy was again carried out, revealing lymph node mycosis. The diagnosis of fungal infection was clear, but empirical antifungal therapy was ineffective. At this point,

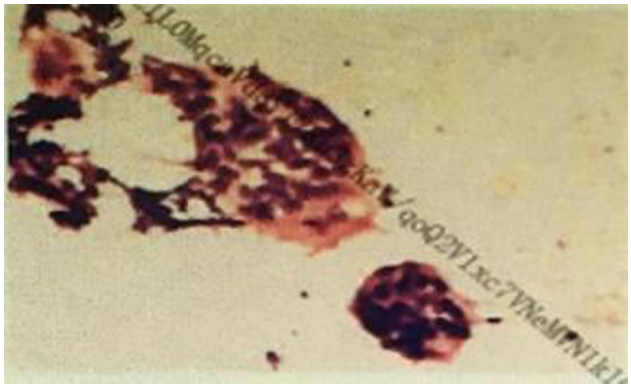

Figure 2 Biopsy of neck lymph node. There are a small number of lymphoid cells and multinucleated giant cells and no malignant cells. Pathological diagnosis: (the left neck lymph node fine-needle aspiration smear). Considering the lymph node granulomatous lesions.

Amphotericin B was resolutely replaced for treatment, and the patient eventually recovered. However, due to technical limitations, we failed to clear the specific type of the fungal infection. Detection and characterization of drug resistance *in vitro* could assist clinicians to select the best antifungal regimen[8]. Evidence supports therapeutic drug monitoring to optimize clinical efficacy[22,23], and our future research efforts will focus on optimization this strategy.

IFDs are characterized by insidious onset and lack of specificity of symptoms. Early neglect can cause delay of diagnosis and treatment, resulting in critical illness and life threatening complications. Therefore, effective antifungal therapy should be carried out once the definite diagnosis/clinical diagnosis is confirmed, and empirical antifungal therapy should also be carried out in the early stage for patients of suspected diagnosis with unclear pathogens. When empiric antifungal therapy is ineffective, it is important to change the antifungal drugs decisively. The patient eventually recovered and was discharged from the hospital, benefiting from early and timely empirical antifungal treatment, although ineffective, but winning the time and opportunity for the latter irrigation of changing antifungal drugs.

In summary, invasive mycosis is a common medical problem in the world. The positive rate of lymph node biopsy is not high. Once invasive fungal infection occurs, it is often accompanied by severe condition, long course, high medical cost, and poor prognosis. In addition, IFD has been shown to be a substantial financial burden to the health care system[24,25]. Therefore, multi-stage and multi-site lymph node biopsies are the key to the diagnosis of the disease. Timely and effective antifungal treatment is essential for curing the disease.

CONCLUSION

The possibility of fungal infection should be considered when both empirical anti-infection and diagnostic anti-tuberculosis treatments are ineffective. The new antifungal drug was not the best treatment, and the empirical antifungal drugs do not necessarily work for every patient. Precise individualized treatment is needed. When routine antifungal therapy is invalid, it is appropriate to change the drug. When replacing antifungal drugs, it is necessary to consider the overlap and continuity of drugs.

Table 1 Criteria for proven invasive fungal disease except for endemic mycoses

Analysis and specimen	Molds[1]	Yeasts[1]
Microscopic analysis: Sterile material	Histopathologic, cytopathologic, or direct microscopic examination[2] of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage	Histopathologic, cytopathologic, or direct microscopic examination[2] of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells - for example, *Cryptococcus* species indicated by encapsulated budding yeasts or *Candida* species showing pseudohyphae or true hyphae[3]
Culture; Sterile material	Recovery of a mold or "black yeast" by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine	Recovery of a yeast by culture of a sample obtained by a sterile procedure [including a freshly placed (< 24 h ago) drain] from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process
Blood	Blood culture that yields a mold[4] (*e.g.*, *Fusarium* species) in the context of a compatible infectious disease process	Blood culture that yields yeast (*e.g.*, *Cryptococcus* or *Candida* species) or yeast- like fungi (*e.g.*, *Trichosporon* species)
Serological analysis: CSF	Not applicable	Cryptococcal antigen in CSF indicates disseminated cryptococcosis

[1]If culture is available, append the identification at the genus or species level from the culture results.
[2]Tissue and cells submitted for histopathologic or cytopathologic studies should be stained by Grocott-Gomorri methenamine silver stain or by periodic acid Schiff stain, to facilitate inspection of fungal structures. Whenever possible, wet mounts of specimens from foci related to invasive fungal disease should be stained with a fluorescent dye (*e.g.*, calcofluor or blankophor).
[3]*Candida, Trichosporon*, and yeast-like *Geotrichum* species and *Blastoschizomyces capitatus* may also form pseudohyphae or true hyphae.
[4]Recovery of *Aspergillus* species from blood cultures invariably represents contamination. CSF: Cerebrospinal fluid.

Table 2 Criteria for probable invasive fungal disease except for endemic mycoses

Host factors[1]
Recent history of neutropenia [< 0.5 × 109 neutrophils/L (< 500 neutrophils/mm3] for > 10 d] temporally related to the onset of fungal disease
Receipt of an allogeneic stem cell transplant
Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/d of prednisone equivalent for > 3 wk
Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-*a* blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 d
Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)
Clinical criteria[2]
Lower respiratory tract fungal disease[3]
The presence of one of the following three signs on CT:
Dense, well-circumscribed lesions(s) with or without a halo sign
Air-crescent sign
Cavity
Tracheobronchitis
Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
Sinonasal infection
Imaging showing sinusitis plus at least one of the following three signs:
Acute localized pain (including pain radiating to the eye)
Nasal ulcer with black eschar
Extension from the paranasal sinus across bony barriers, including into the orbit
CNS infection
One of the following two signs:
Focal lesions on imaging
Meningeal enhancement on MRI or CT
Disseminated candidiasis[4]
At least one of the following two entities after an episode of candidemia within the previous 2 wk:
Small, target-like abscesses (bull's-eye lesions) in liver or spleen
Progressive retinal exudates on ophthalmologic examination
Mycological criteria

Direct test (cytology, direct microscopy, or culture)
Mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:
Presence of fungal elements indicating a mold
Recovery by culture of a mold (*e.g.*, *Aspergillus, Fusarium*, Zygomycetes, or *Scedosporium* species)
Indirect tests (detection of antigen or cell-wall constituents)[5]
Aspergillosis
Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF
Invasive fungal disease other than cryptococcosis and zygomycoses
β-D-glucan detected in serum

Probable IFD requires the presence of a host factor, a clinical criterion, and a mycological criterion. Cases that meet the criteria for a host factor and a clinical criterion but for which mycological criteria are absent are considered possible IFD.
[1]Host factors are not synonymous with risk factors and are characteristics by which individuals predisposed to invasive fungal diseases can be recognized. They are intended primarily to apply to patients given treatment for malignant disease and to recipients of allogeneic hematopoietic stem cell and solid-organ transplants. These host factors are also applicable to patients who receive corticosteroids and other T cell suppressants as well as to patients with primary immunodeficiencies.
[2]Must be consistent with the mycological findings, if any, and must be temporally related to current episode.
[3]Every reasonable attempt should be made to exclude an alternative etiology.
[4]The presence of signs and symptoms consistent with sepsis syndrome indicates acute disseminated disease, whereas their absence denotes chronic disseminated disease.
[5]These tests are primarily applicable to aspergillosis and candidiasis and are not useful in diagnosing infections due to *Cryptococcus* species or Zygomycetes (*e.g.*, *Rhizopus, Mucor*, or *Absidia* species). Detection of nucleic acid is not included, because there are as yet no validated or standardized Methods. TNF-*a*: Tumor necrosis factor-alpha; CT: Computed tomography; MRI: Magnetic resonance imaging; IFD: Invasive fungal disease.

Table 3 Criteria for the diagnosis of endemic mycoses

Diagnosis and criteria
Proven endemic mycosis
In a host with an illness consistent with an endemic mycosis, one of the following:
Recovery in culture from a specimen obtained from the affected site or from blood
Histopathologic or direct microscopic demonstration of appropriate morphologic forms with a truly distinctive appearance characteristic of dimorphic fungi, such as *Coccidioides* species spherules, *Blastomyces dermatitidis* thick-walled broad-based budding yeasts, *Paracoccidioides brasiliensis* multiple budding yeast cells, and, in the case of histoplasmosis, the presence of characteristic intracellular yeast forms in a phagocyte in a peripheral blood smear or in tissue macrophages
For coccidioidomycosis, demonstration of coccidioidal antibody in CSF, or a 2-dilution rise measured in two consecutive blood samples tested concurrently in the setting of an ongoing infectious disease process
For paracoccidioidomycosis, demonstration in two consecutive serum samples of a precipitin band to paracoccidioidin concurrently in the setting of an ongoing infectious disease process
Probable endemic mycosis
Presence of a host factor, including but not limited to those specified in Table [2], plus a clinical picture consistent with endemic mycosis and mycological evidence, such as a positive *Histoplasma* antigen test result from urine, blood, or CSF

Endemic mycoses include histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, sporotrichosis, and infection due to *Penicillium marneffei*. Onset within 3 mo after presentation defines a primary pulmonary infection. There is no category of possible endemic mycosis, as such, because neither host factors nor clinical features are sufficiently specific; such cases are considered to be of value too limited to include in clinical trials, epidemiological studies, or evaluations of diagnostic test. CSF: Cerebrospinal fluid.

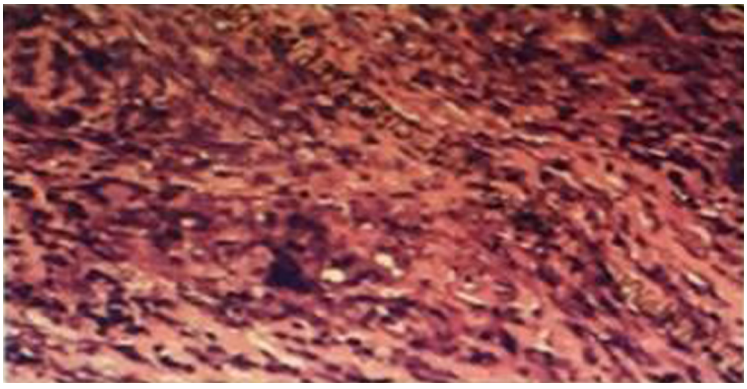

Figure 3 Secondary biopsy of supraclavicular lymph node. Lymph nodes with widespread degeneration and necrosis, and there are many spores and small quantities of hyphae in these tissues. There are many giant cell granuloma in the peripheral lymphoid tissues. Pathological diagnosis: (the left supraclavicular lymph

node fine-needle aspiration smear). The diagnosis conformed lymph nodes fungal disease.

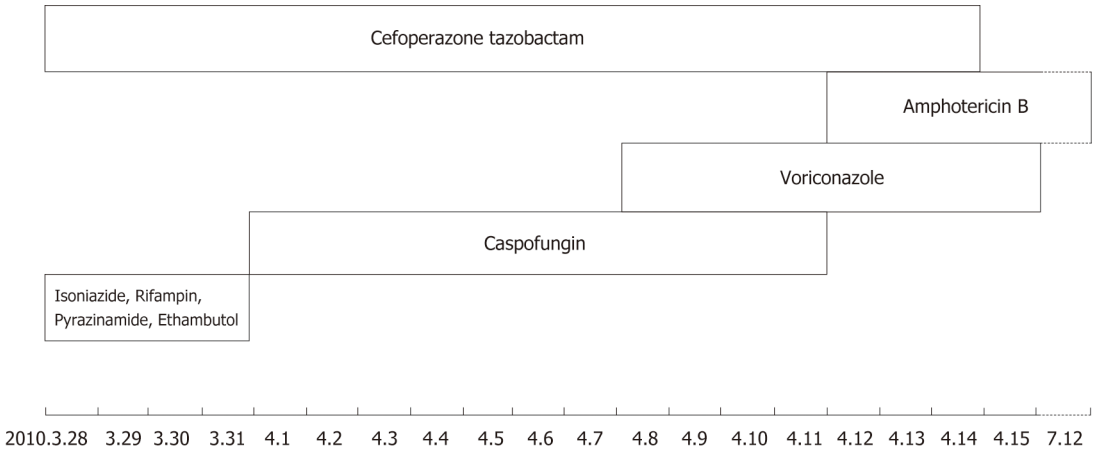

Figure 4 Timeline summarizing drug intervention.

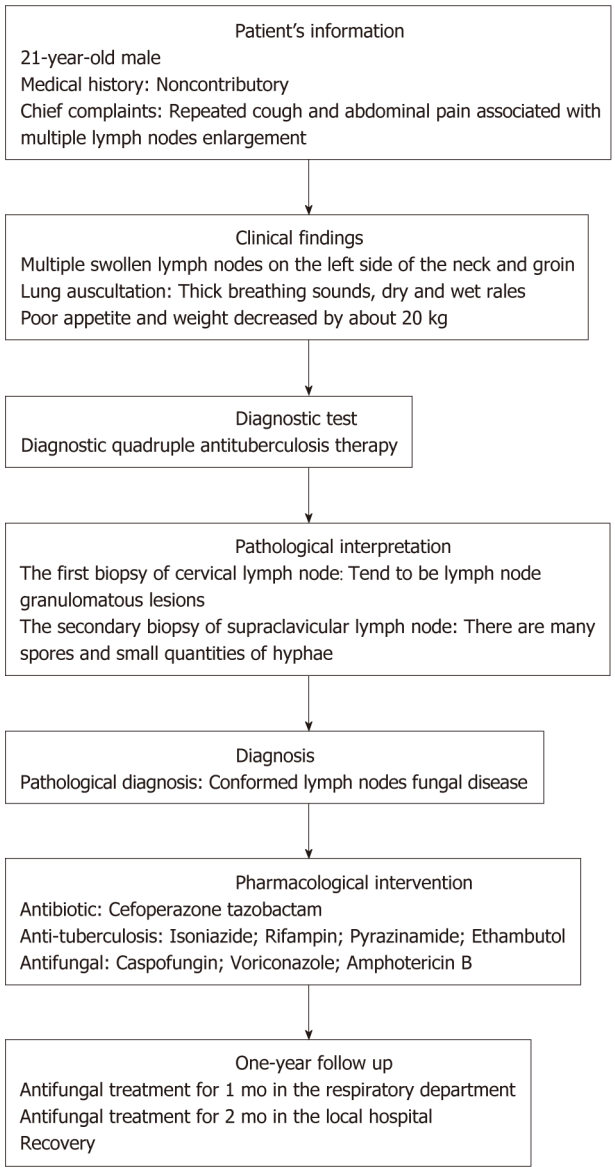

Figure 5 Timeline summarizing patient's information, clinical findings, diagnostic tests, diagnosis, pharmacological intervention, and follow up.

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CASE REPORT

Hepatocellular carcinoma successfully treated with ALPPS and apatinib: A case report

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Author contributions: Liu L conceived and coordinated the study, designed, performed, and analyzed the experiments, and wrote the paper; Li NF, Zhang Q, and Lin L carried out data collection and analysis and revised the paper; all authors reviewed the results and approved the final version of the manuscript.

Informed consent statement: Written informed 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.

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Abstract

BACKGROUND

Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) has becoming ever more recognized in the treatment of hepatocellular carcinoma (HCC). Nevertheless, long-term survival rate and postoperative complications are far from ideal, mainly since the majority of patients treated with ALPPS surgery have large or multiple lesions and microvascular tumor thrombus.

CASE SUMMARY

We present the case of a 47-year-old male patient with a huge right hepatic mass and an estimated insufficient residual liver, who was successfully treated with ALPPS surgery and apatinib. Postoperative pathology revealed HCC with several significant microvascular embolisms. Twenty months after operation, no tumor reoccurrence was observed.

CONCLUSION

Our case indicated that combined targeted drug therapy with ALPPS can lead to long-term survival for patients with large HCC.

Key words: Hepatocellular carcinoma; Associating liver partition and portal vein ligation for staged hepatectomy; Apatinib; Case report

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Core tip: Our aim was to explore the feasibility of associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) combined with targeted therapy in the treatment of advanced hepatocellular carcinoma (HCC). Herein, we present the case of a 47-year-old male patient with a huge right hepatic mass and an estimated insufficient residual liver, who was successfully treated with ALPPS surgery and apatinib.

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Postoperative pathology revealed HCC with several significant microvascular embolisms. Twenty months later, no tumor reoccurrence was observed, indicating that combined targeted drug therapy with ALPPS can lead to long-term survival for patients with large HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is among the most common causes of cancer-related deaths worldwide. Currently, HCC is the second most common malignancy in cities and the first most common malignancy in the countryside of China; it is the second cancer-related mortality in males and third in females, seriously affecting the health of Chinese people[1]. Currently, the treatments for HCC have greatly progressed due to the development of technology. In addition, a great variety of treatments have been applied so far, such as targeted drug therapy, microwave and radiofrequency ablation therapy, interventional radiotherapy and so on. Nevertheless, surgical resection remains the most effective treatment, preferred by patients with HCC, among whom only 20%-30% are eligible for surgical resection[2,3]. It has also been reported that the 5-year survival rate of HCC is 40%, while the rate of resectable early HCC can reach 60%-70%[4], which means that most patients cannot undergo radical resection but can only benefit from remedial treatments such as drugs and radiation interventions, which eventually leads to the poor prognosis of HCC treatment.

Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is a new surgical procedure that has emerged over the recent years. It was first applied to liver metastasis of colon cancer with good results[5]. ALPPS is directed at patients with large or multiple right liver tumors that cannot be resected at one time due to insufficient residual liver volume. The first step is the ligation of the affected side of the portal vein, which allows the blood supply of the portal vein to nourish the normal liver. In addition, the hepatic artery and vein of the affected side are retained to preserve parts of the function of the affected liver; the left and right livers are separated at the same time to prevent tumor invasion of the normal liver lobe. When the normal hepatic lobe volume increases to an ideal range, which usually takes 2-3 wk, the affected liver is resected to avoid the occurrence of postoperative liver failure. It has been reported that 55% of the patients with HCC in the world are Chinese[1]. Many large liver centers in China have applied ALPPS for the treatment of liver cancer over the recent years, achieving good results. The team of academician Fan Jia, Zhongshan Hospital of Shanghai Fudan University, has performed a retrospective analysis of 45 cases of hepatitis B-related HCC after ALPPS surgery from April 2013 to September 2017, showing that the long-term survival rate after ALPPS was better than that after transcatheter arterial chemoembolization (TACE), and that there was no significant difference between ALPPS and primary hepatectomy[6]. Yet, many scholars have questioned the high complication and mortality rates of ALPPS. Most of the patients who underwent ALPPS were advanced HCC. How to carry out postoperative comprehensive treatment to improve the survival rate of HCC patients is also a problem worthy of discussion. Herein, we present a case of giant HCC with cirrhosis of the right liver. Preoperative assessment of residual liver insufficiency made it difficult to perform one-off resection. Postoperative pathological examination revealed formation of multiple microvascular tumor thrombi. We performed ALPPS surgery which was subsequently combined with a targeted new drug (apatinib), eventually achieving good results. The main purpose of this study was to investigate the reasonable procedure of ALPPS in order to reduce postoperative complications and mortality, and to further improve the survival rate of patients undergoing ALPPS.

This study was approved by the ethic committee of Xiangya Hospital, Central South University and the patient has consented to the submission of the case report.

CASE PRESENTATION

Chief complaints

A 47-year-old male patient was admitted to the Department of Hepatobiliary and Pancreatic Surgery of Xiangya Hospital of Central South University in November 2016 due to right upper abdominal pain that lasted for one week.

History of present illness

He felt paroxysmal dull pain in his right upper abdomen for one week without fever, jaundice, or other symptoms.

History of past illness

He had a 10-year long history of hepatitis B that was not treated systematically, and a 10-year long history of gout.

Personal and family history

His brother had a history of hepatitis B, but his parents' histories of hepatitis B were not clear.

Physical examination upon admission

Physical examination revealed clear mind, moderate nutrition, and no yellow stains in the skin and sclera; no swollen lymph nodes; the abdomen was soft, and the liver and spleen were not palpable under the ribs with tapping pain in the liver area; there was no edema in lower limbs.

Laboratory examinations

Laboratory tests indicated the following: AFP 25.82 ng/mL; TBIL 19.2 μmol/L, DBIL 8.5 μmol/L, ALB 42.1 g/L, ALT 158.6 U/L, AST 231.6 U/L; HBsAg (+), HBcAb(+), HBV DNA 7.89E+04. Routine blood tests, routine urine tests, routine fecal tests, the renal and coagulation functions were all normal. Preoperative evaluation was performed, which revealed an indocyanine green (ICG) retention rate at 15 min (ICG15) of 6.9% and Child-Pugh grade A liver function.

Imaging examinations

Ultrasonography and hepatic contrast-enhanced computed tomography (CT) showed cirrhosis, splenomegaly, portal hypertension, and a solid mass about 135 mm × 124 mm close to the first and second hepatic hilum, commonly observed in massive HCC (Figure 1).

FINAL DIAGNOSIS

The final diagnosis of the presented case was primary HCC due to hepatitis B.

TREATMENT

According to CT imaging, 3D modeling revealed that residual liver volume/effective liver volume was 37.6%. Considering that the patient had large HCC that required right hemi-hepatectomy, and that hepatitis virus replication was in active phase and the patient had liver cirrhosis and mild damage of liver function, we performed ALPPS. The first step of ALPPS, *i.e.*, laparoscopic ligation of the right branch of portal vein and separation of the left and right liver, was performed after one week of treatment with magnesium isoglycyrrhizinate and entecavir. Briefly, a significant transaminase increase and albumin decrease were observed after the operation (Figure 2A). At the same time, the patient developed hepatorenal syndrome caused by oliguria, ascites, and renal dysfunction (Figure 2B). The validity of the ALPPS procedure was further confirmed by postoperative deterioration of liver function. The symptoms of ascites and oliguria were obviously improved after liver protection, diuresis, and albumin supplement, and the liver and kidney functions were close to normal. The indicators of liver and kidney function after operation are shown in Figure 2.

The liver volume increased rapidly after the first step of ALPPS. The residual liver volume/effective liver volume was 48.2% on the 9th day and 58.7% on the 16th day (Table 1 and Figure 3).

The second step of ALPPS, *i.e.*, right hepatectomy, was performed under general anesthesia on the 16th day after the first step of ALPPS. The patient was discharged on the 10th day after the operation.

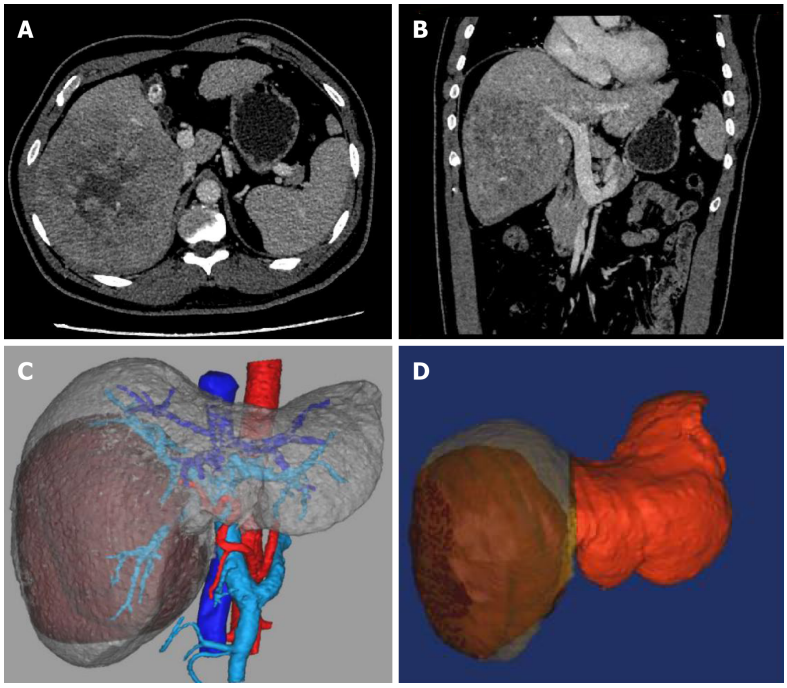

Figure 1 Preoperative computed tomography images and 3D reconstruction. A and B: Computed tomography (CT) transverse (A) and coronal (B) images before operation; C: 3D image showing the relationship between tumor, blood vessels, and the bile ducts; D: 3D image (red color for residual liver, gray color for tumor).

Postoperative pathological examination showed that high-grade differentiated HCC was found with more than five clear microvascular tumor thrombi adjacent to the tumor, and no tumor cells were found at the incisal margin (Figures 4 and 5).

After operation, the patient was treated with apatinib (500 mg daily) and oral administration of entecavir was continued. Mild hand-foot skin reaction and hypertension occurred during oral administration. The symptoms were improved after symptomatic treatment.

OUTCOME AND FOLLOW-UP

After discharge, the patient was regularly reexamined and returned to our hospital every 3 mo for systemic CT, AFP, second liver two half-and-half test, HBV DNA, liver and kidney function, blood routine test, and other related examinations. The latest follow-up time was August 2018. CT examination at the latest follow-up showed no recurrence or metastasis at 20 mo (Figure 6). The level of AFP was 5.48 ng/mL, which was significantly lower compared to 25.82 ng/mL, preoperatively.

DISCUSSION

There are three main reasons why HCC cannot be treated by resection: (1) patient's general condition is such that he/she cannot tolerate surgery, especially due to liver condition; (2) presence of extrahepatic metastasis; and (3) the volume of residual liver is insufficient. Japanese scholars advocate the use of portal vein embolization (PVE) to solve this problem, but residual liver volume growth is slower after PVE than ALPPS. The average interval between two resections is 28 d, and 20%-50% of patients cannot reach the standard of total resection[7-9]. From January 2015 to December 2017, 20 patients underwent ALPPS at our center. The average interval between the two steps was 14 d, and only two patients did not reach the ideal volume without total radical surgery. Currently, the feasibility of ALPPS for giant HCC remains controversial. The main focus is on postoperative liver failure, complications, and mortality, whose incidence rates are significantly higher than those of TACE + PVE. A multicenter retrospective study has found that the postoperative mortality rates of ALPPS and PVE are 15% and 6%, respectively. The mortality rates in the ALPPS group are significantly higher than those in PVE group[10]. However, 20 cases of ALPPS were performed at our center with no mortalities at 90 d after operation. Preoperative

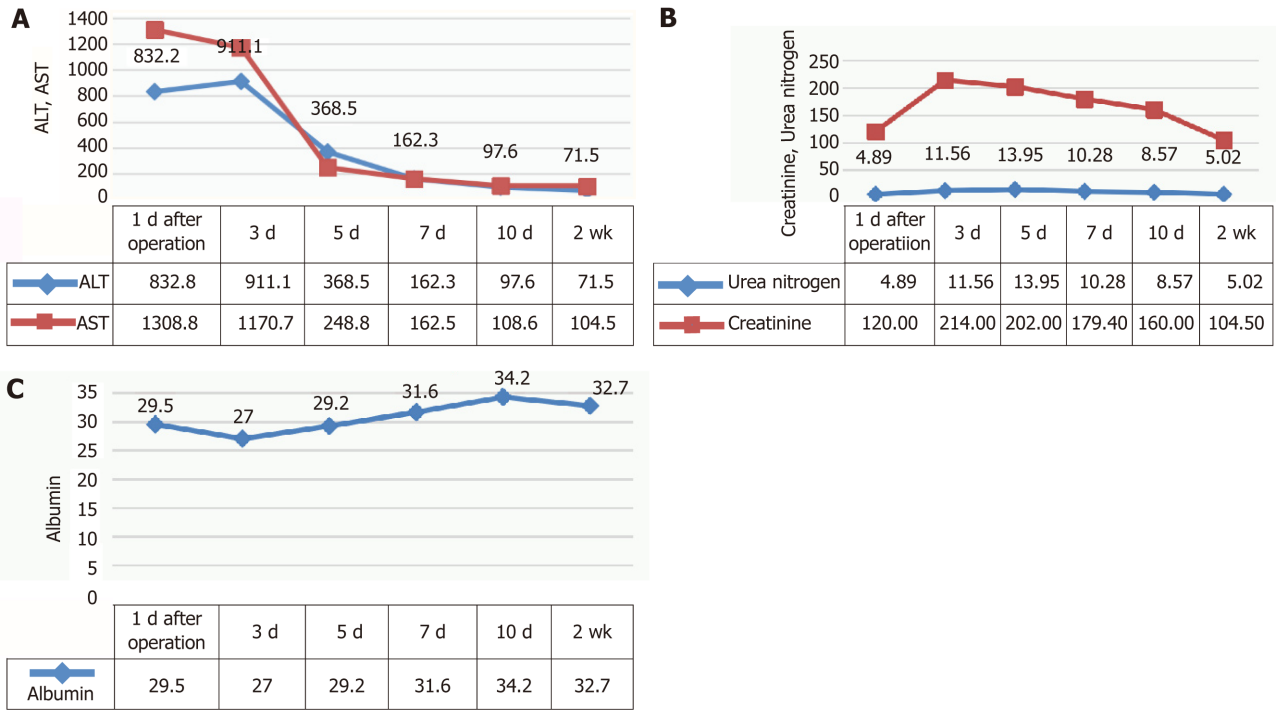

Figure 2 Changes of laboratory examinations after operation. A: Postoperative changes of transaminase (green line: ALT; yellow line: AST); B: Postoperative changes of renal function (green line: creatinine; yellow line: urea nitrogen); C: Postoperative albumin changes. ALT: Alanine transaminase; AST: Aspartate transaminase.

estimations of residual liver volume, ICG retention test, and Child-Pugh grading combined with 3D modeling provided accurate assessment for HCC resection, minimized the risk of operation, improved the resection rate, and significantly reduced the occurrence of postoperative complications. Thus, ALPPS is a safe treatment for patients with HCC who cannot be resected in one operation. From our experience, the best candidates were patients with Child-Pugh grade A, ICG15 < 10%, residual liver volume/effective liver volume > 40% with mild liver damage or liver cirrhosis, or residual liver volume/effective liver volume > 30% with normal liver for surgery. Although there is a lack of multicenter randomized trial data on ALPPS, current studies have successfully demonstrated the feasibility of ALPPS in the treatment of HCC.

As we all know, patients undergoing ALPPS are mostly large HCC or multiple HCC patients. Pawlik's study has shown that the occurrence of microvascular invasion (MVI) is positively correlated with tumor size. MVI rates were 25%, 40%, 55%, and 63% in patients with tumor diameter < 3 cm, 3.1-5 cm, 5.1-6.5 cm, and > 6.5 cm, respectively (P < 0.005). The size and quantity of HCC were important predictors of MVI[11]. Meanwhile, MVI was also closely related to poor prognosis (including high recurrence rate and low long-term survival rate) in HCC patients. Sumie *et al*[12] have classified the patients into MVI-free group, mild MVI group (1-5), and severe MVI group (> 5) according to the number of MVI. The results showed that the higher the classification, the shorter the disease-specific relapse-free survival. Patients with HCC undergoing ALPPS are at high risk for MVI, so it is necessary to treat them with systemic therapy. In the present study, pathological examination of the patient showed that there were more than five distinct microvascular invasions around the tumor. For the high-risk recurrence factors, we used targeted therapy after operation. The drug of our choice was apatinib (500 mg/d). Apatinib is a direct multi-target RTK blocker for HCC, which effectively inhibits the activity, proliferation, and metastasis of tumor cells and promotes the apoptosis of cells[13]. Its price is relatively cheap, and a number of clinical studies have confirmed its safety and efficacy in the treatment of HCC[14-17]. Mild hand-foot skin reaction and hypertension occurred in the course of oral administration, which were improved after symptomatic treatment. The patient's 20-mo tumor-free survival confirmed that apatinib associated with ALPPS resection greatly prolonged the survival of the HCC patient who could not have the tumor resected at one time and who had a vascular tumor thrombus. The patient had greatly benefited from this treatment, compared with patients with advanced HCC whose survival period was less than 10 mo.

Table 1 Changes of liver volume before and after operation

Time	Estimated residual liver volume
Pre-operation	37.6%
9 d after operation	48.2%
16 d after operation	58.7%

CONCLUSION

Based on this case and from our experience with ALPPS, ALPPS is an effective treatment for patients with HCC. Preoperative evaluation was based on the patient's general condition, Child classification of liver function, calculation of residual volume/effective volume of liver, and ICG retention test. The mortality rate was significantly reduced, and combination with systemic therapy, such as targeted therapy, is expected to prolong the survival of patients. Apatinib has shown good results in the treatment of HCC. In view of the huge number of patients with advanced HCC in China, future studies should further expand the sample size to demonstrate its efficacy that would benefit more patients.

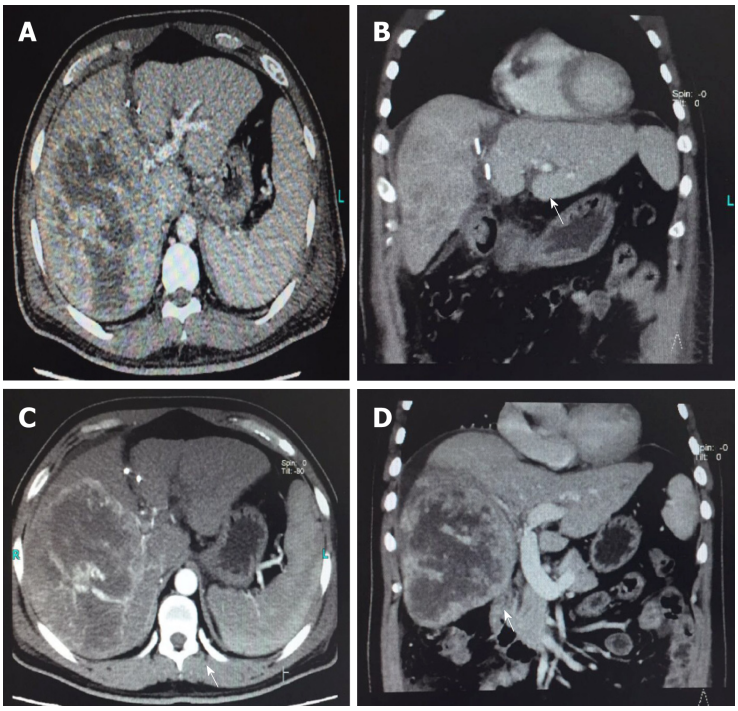

Figure 3 Postoperative computed tomography images. A and B: Computed tomography (CT) transverse (A) and coronal (B) images on the 9th day after operation; C and D: CT transverse (C) and coronal (D) images on the 16th day after operation.

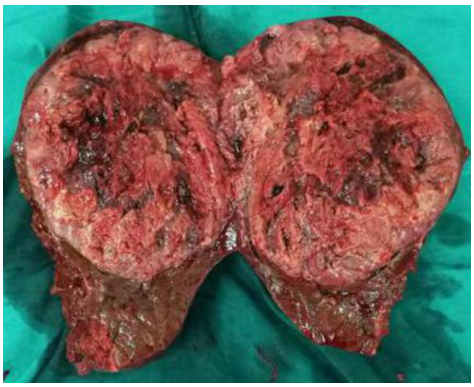

Figure 4 Postoperative liver specimen.

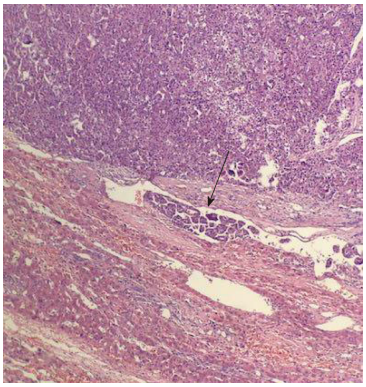

Figure 5 Pathological picture. The arrow in the picture shows a clear microvascular tumor thrombus.

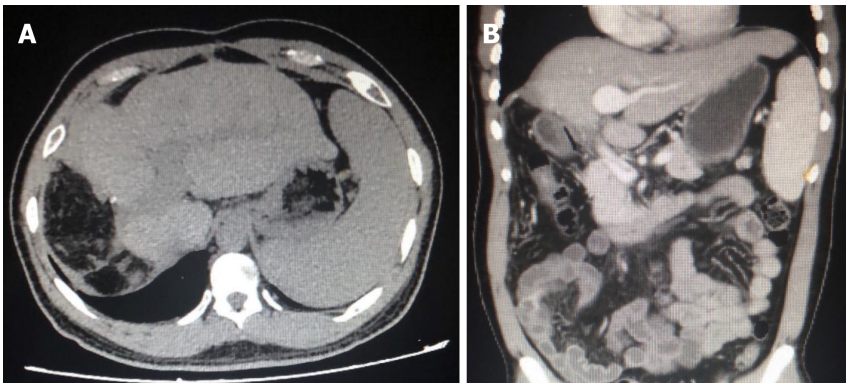

Figure 6 Twenty-month follow-up computed tomography images indicating no recurrence of tumor. A and B: Computed tomography (CT) transverse (A) and coronal (B) images obtained in August 2018.

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We wish to thank our patient for agreeing to share his case.

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CASE REPORT

Pseudothrombus deposition accompanied with minimal change nephrotic syndrome and chronic kidney disease in a patient with Waldenström's macroglobulinemia: A case report

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Abstract

BACKGROUND

Waldenström's macroglobulinemia (WM) is a rare lymphoid neoplasia, which can have renal complications. These rarely occur, and most common renal manifestations are mild proteinuria and microscopic hematuria. Herein we describe a case of WM that presented with pseudothrombi depositing in capillaries associated with minimal change nephrotic syndrome and chronic kidney disease (CKD).

CASE SUMMARY

A 52-year-old man presented with features suggesting nephrotic syndrome. Extensive workups were done, and there were elevated serum levels of interleukin-6 and vascular endothelial growth factor (VEGF), capillary pseudothrombus accumulation associated with minimal change nephrotic syndrome, CKD, and WM. Treatment was directed at the patient's WM with bortezomib, thalidomide, and dexamethasone whereby serum immunoglobulin M (IgM) decreased. The damage of IgM on the kidney was corrected; thus, the patient's proteinuria and serum creatinine had improved. The patient is still under clinical follow-up.

CONCLUSION

It is essential for clinicians to promptly pay more attention to patients presenting with features of nephrotic syndrome and do extensive workups to come up with a proper therapy strategy.

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Core tip: The occurrence of the association among plasma immunoglobulin M level, proteinuria, and plasma creatinine level has not been reported to our knowledge. The case presents an essential for clinicians to promptly pay more attention to patients presenting with features of nephrotic syndrome and do extensive workups to come up with a proper therapy strategy.

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INTRODUCTION

Waldenström's macroglobulinemia (WM) firstly described by J Waldenström in 1944, accounting for 2% of all hematological malignancies. As a rare lymphoid neoplasia, WM was interpreted primarily by bone marrow infiltration and immunoglobulin M (IgM) monoclonal gammopathy secreted in serum[1,2]. It affects three per million people per year and more prevalent in Caucasian males with an average age of 64 years at diagnosis[3]. The World Health Organization (WHO) classified WM as lymphoplasmacytic lymphoma (LPL) secreting IgM proteins, belonging to the non-Hodgkin B lymphomas (NHL)[4].

Clinical manifestations are linked to the deposition of IgM in various organs, which may result in hepatomegaly, splenomegaly, and lymphadenopathy. High titers of IgM results in hyperviscosity syndrome with associated complications[5]. Renal complications rarely occur, but the most common renal manifestations are mild proteinuria and microhematuria[5,6]. Among all patients, less than 3% develop end-stage renal disease. Moreover, nephrotic syndrome is seldom seen in WM. Few reports have described that the occurrence of nephrotic syndrome is less than 7% of patients with WM[7].

We herein present an unusual case of WM that presented with pseudothrombi depositing in capillaries associated with minimal change nephrotic syndrome and chronic kidney disease (CKD).

CASE PRESENTATION

Chief complaints and history of present illness

A 52-year-old Chinese man presented with a sudden onset of edema affecting the face and bilateral lower limbs, abdominal distension after eating, and weight gain (7 kg in 10 d).

History of present illness

His medical history was notable for nephrotic syndrome.

History of past illness

The patient denied a history of trauma. However, he had a kidney stone diagnosed in 2005.

Physical examination

The physical examination showed bilateral lower extremity edema in the absence of hepatosplenomegaly and lymphadenopathy. His blood pressure was 147/94 mmHg.

Laboratory examinations

Hemoglobin 14.2 g/dL [Normal (N) 13.5-17.5 g/dL], white blood cells (WBC) 7.76

G/L (N 4.5-11.0 G/L), red blood cells (RBC) 4.44 T/L (N 4.7-6.1T/L), lymphocytes 18.2% (N 20%-40%), neutrophils 74.6% (N 40%-60%), monocytes 5.9% (N), eosinophils 1% (N), platelets count 376 g/L 9 (N), urea 8.82 mmol/L (N 2.5-7.1 mmol/L), creatinine 211 μmol/L (N 60-110 μmol/L), glomerular filtration rate (GFR) 34.8 mL/min, calcium 2.12 mmol/L (N 2.2-2.7 mmol/L), uric acid 253.2 μmol/L (N), chloride 111.9 mmol/L (N 98-106 mmol/L), albumin 19.9 g/L (N 35-55 g/L), globulin 59.0g/L (N 20-35 g/L), urine glucose 1+, urine occult blood 3+, urine protein 3+, urine WBC 167/μL (N < 11/μL), urine RBC 147/μL(N < 14/μL), urine epithelial cells 19/μL (N < 6/μL), urine kappa 1140 mg/L (N < 19 mg/L), urine lambda 367 mg/L (N < 50 mg/L), and urine nitrates were negative. Blood glucose, liver function tests, lipids, coagulation, hepatitis B virus, and hepatitis C virus markers were normal.

Thyroid function parameters were: FT3 1.8 pmol/L (N 2.6-5.7 pmol/L), FT4 6.6 pmol/L (N 9-19.18 pmol/L), and thyroid stimulating hormone 10.78 μIU/mL (N 0.35-4.94 μIU/mL). Elevated serum cytokines included vascular endothelial growth factor (VEGF)-A 93.92 pg/mL (N 1.09-39.23 pg/mL), interleukin-6 (IL-6) 23.52 pg/mL (N 1.32-4.72 pg/mL), MIF 24.65 pg/mL (N 3.99-7.93 pg/mL), and MIP-1a 109.72 pg/mL(N 6.93-18.57 pg/mL); the rest were normal. Serum β-2 microglobulins 4.8 mg/L (N 1.0-3.0 mg/L), C3 0.739 g/L (N 0.790-1.520 g/L), and C4 were normal. Serum immunoglobulin and light chains were as follows: IgG 1.81 g/L (N 7.51-15.60 g/L), IgA 0.79 g/L (N 0.82-4.53 g/L), IgM 41.9 g/L (N 0.460-3.040 g/L), serum kappa 4.89 g/L (N 1.70-3.70 g/L), and serum lambda 0.32 g/L (N 0.90-2.10 g/L). Bence-Jones proteinuria was negative.

Imaging examinations

Renal imaging revealed that the left kidney was about 9 cm x 5.4 cm in size and right kidney about 9.5 cm x 4.8 cm. Renal perfusion decreased with no obstruction, and GFR decreased to 34.8 mL/min.

Echocardiographic findings suggested decreased left ventricular diastolic function in the ascending aorta.

Chest computed tomography (CT) showed left ventricular failure, mild edema in both lungs, and mild bilateral pleural effusion.

Whole body bone single photon emission CT showed a slight increase in the concentration of the bone-imaging agent in the upper and lower jaw. The left posterior sixth rib-imaging agent was less and evenly distributed. The other parts of the bone-imaging agent were evenly distributed, and no abnormal changes were noted in the double kidneys and bladder. These findings reflected enhanced bone metabolism limited to the mandible; bone metabolism in the sixth ribs was slightly decreased and no apparent changes were observed in the metabolism of other bones in the body.

Bone marrow histology showed active hematopoietic tissue proliferation with an increasing focal ratio of lymphocytes and plasmacytes.

Bone marrow fluorescence *in situ* hybridization test was normal. Bone marrow immunohistochemical staining results were: CD34 (-), CD117 (-), TDT (-), MP0 (+), CD3 (-) CD10 (-), CD20 (±), CD61 (+), κ (±), and λ (-).

Bone marrow immunophenotyping test did not detect any monoclonal lymphocytes or monoclonal plasmacytes with abnormal phenotype.

Renal biopsy

On light microscopy, the most extensive section showed 28 glomeruli that were non-lobulated and non-sclerotic, with one glomerular capillary loop shrunken, and their walls were slightly thickened with a small number of layers. The volume of residual glomeruli increased; generally, the number of cells was 80-120 per glomerulus, mesangial cells and mesangial matrix were slightly increased, capillary loops were open, and the number of infiltrating cells was < 3/glomeruli, mainly mononuclear cells. Red cells and "pseudothrombi" were seen in several capillary loops. One capillary loop was embedded into the urinary pole (Figure 1).

The periodic Schiff-Methenamine (PASM) and Masson staining showed that a large number of fuchsinophilic depositions were found in the basement membrane and under the endothelium. The tubulointerstitium presented moderate lesions. Diffuse turbidity, granular degeneration, and partial small and fine vacuolar degeneration were found in the tubular epithelial cells. Some small vessels were atrophic, and the basement membrane of tubules was thicker (Figure 2).

Alkaline Congo red staining was negative. Electron microscopy revealed diffused effacement of podocyte foot processes, and only mild mesangial hyperplasia and a few electron dense deposits (Figure 3).

There was no clear immunoglobulin or deposition of complement components under the immunofluorescence microscope.

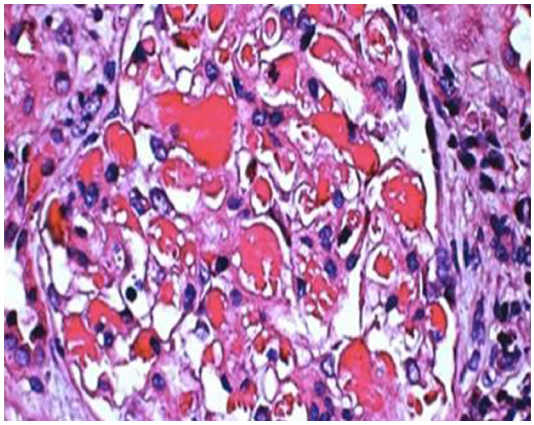

Figure 1 Light microscopy. The volume of residual glomeruli increased, the number of cells was 80-120 per glomerulus, mesangial cells and mesangial matrix were slightly increased, capillary loops were open, and the number of infiltrating cells was < 3 per glomeruli, mainly mononuclear cells. Red blood cells and "pseudothrombi" could be seen in several capillaries. One capillary loop was embedded into the urinary pole (HE staining; magnification, ×600).

FINAL DIAGNOSIS

A diagnosis of WM associated with minimal change nephrotic syndrome was made. Furthermore, the decreased GFR caused by capillary occlusion was diagnosed as CKD.

TREATMENT

The patient was treated with atorvastatin, human albumin, torsemide, alprostadil, levothyroxine, bortezomib, thalidomide, and dexamethasone. At the time of this report, the patient responded to the above therapy and has stabilization of renal function. Table 1 shows the chemotherapy treatment regimen used.

OUTCOME AND FOLLOW-UP

He is being on our required regular clinic follow-up since diagnosis. He has remained stable.

DISCUSSION

Renal involvement associated with WM is scarce compared to multiple myeloma, due to rare hypercalcemia and less Bence-Jones proteinuria. As few as 3.8%-7.4% of the patients advanced to renal failure in autopsies of WM patients. Its etiology is still unclear; however, the most recent theory is autoimmune sensitivity to self-antigens. Although proteinuria and microhematuria are not limited, the frequency of nephrotic syndrome is reported to be less than 7%. Minimal change nephrotic syndrome is a common complication of Hodgkin's lymphoma but rarely seen in patients with WM. To date, only four WM cases have been previously reported in the literature[3,8-10]. In WM, a T lymphocyte disorder that leads to a decreased CD4/CD8 ratio and abnormal secretion of lymphokines has been published and may be associated with the occurrence of minimal change nephrotic syndrome[11].

In this case, light microscopy showed a large number of pseudothrombi deposited in the capillaries of the patient's kidney, together with diffused effacement of podocyte foot processes observed by electron microscopy. In addition, our patient also presented with proteinuria, indicating that this was minimal change nephrotic syndrome accompanied with pseudothrombus deposition. We also believe that proteinuria was a result of pseudothrombus deposition in the glomeruli capillary. The pathophysiology of renal pseudothrombi associated with WM is unknown, but there are some mechanisms suggesting that WM patients are known to have an elevated risk of venous thrombosis[12]. Complications of WM include hyperviscosity and raised von Willebrand factor[13]. Additionally, the paraprotein may act as an antiphospholipid antibody; interactions of the paraprotein with the local renal

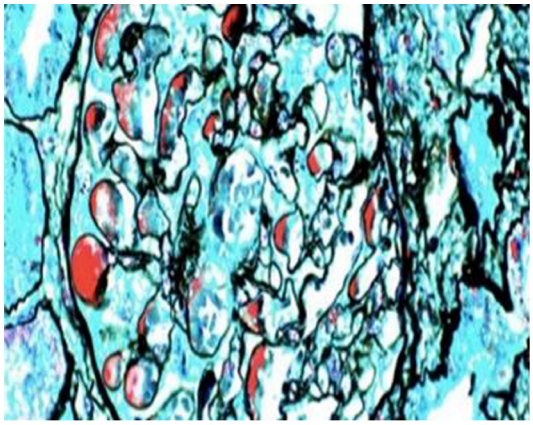

Figure 2 Light microscopy. Periodic Schiff-Methenamine (PASM) and Masson staining. Fuchsinophilic depositions were found in the basement membrane and under the endothelium. The tubulointerstitium exhibited moderate lesions, with acute lesions on chronic damage. There was diffuse turbidity and granular degeneration in the tubular epithelial cells. Partial tubular epithelial cells presented small and fine vacuolar degeneration, and the basement membrane of tubules became thicker. Brush border of the tubules was absent. Protein casts could be seen in some lumens. The renal interstitial region could be found to be focally enlarged, and fibrosis index was 1+. Individual arterioles presented segmental hyalinosis (PASM and Masson staining; magnification, ×400).

microvasculature or the complement system could all contribute to a prothrombotic state, thus damaging the endothelial cells, disrupting the glomerular microcirculation, and hence lowering GFR[14], whereby our patient's GFR was 34.8 mL/min, BUN was 8.82 μmol/L, and creatinine was 211 μmol/L. Besides, the patient had mild interstitial fibrosis, and we could not find monocyte, eosinophil, or plasmacyte infiltration in the interstitial area as compared with the results of other reports[15]. Thus, the declined GFR seen on this patient might have resulted from WM induced hyperviscosity.

One intriguing finding of our case was that serum IgM level correlates with proteinuria and serum creatinine. After the first circle of bortezomib, thalidomide, and dexamethasone therapy, the plasma IgM level decreased by 55%, plasma creatinine decreased by 29%, and plasma albumin increased by 23%. Thus, high levels of serum IgM are not only a diagnostic criterion for WM, but also a clinical parameter of WM associated kidney damage. Unlike the normal pathophysiology of podocyte injury, coagulation can also cause podocyte effacement and proteinuria. Immunofluorescence microscopy did not show immunoglobulin or deposition of complement components even though the patient's serum IgM was ten times more, which was 41.9 g/L. Some reports suggest that the absence of IgM deposition in the renal biopsy together with high serum levels of monoclonal IgM can be due to glomerular basement membrane being repaired as a result of the combined treatment, so renal IgM deposition will disappear[16]. In our case, the patient did not receive any treatment before his diagnosis, which suggested that kidney dysfunction was not a prerequisite for IgM deposition.

Electron microscopy only showed mesangial hyperplasia and a few electron dense deposits; however, others have shown significant proliferation in the mesangial, epithelial, and endothelial cells of the matrix, and a lot of dense depositions in the GBM, Bowman's capsule, and tubular basement wall[17].

We have shown here, for the first time, that a WM patient had elevated serum IL-6 and VEGF-A, and recent studies have shown that IL-6 and VEGF-A levels in the sera of WM patients were abnormal compared with nonmalignant subjects. Malignant B cells secrete detectable levels of these cytokines[18]. Nonetheless, it is important also to note that elevated plasma cytokines can be observed in CKD as well and mostly caused by increased production derived from oxidative stress, chronic inflammation, and fluid overload. Concurrently, decreased clearance of IL-6 due to impaired renal function also contributes to its accumulation[19]. Furthermore, elevated serum VEGF-A was linked to diabetic nephropathy and glomerular diseases in humans, and proteinuria, glomerulomegaly, glomerular basement membrane thickening, mesangial expansion, loss of slit diaphragms, and podocyte effacement were associated with increased kidney VEGF-A content in adult mice overexpressing VEGF164[20]. This appears to be the first case to report elevated serum levels of IL-6 and VEGF in a patient with WM and CKD associated with pseudothrombi. Therefore, it remains unclear whether IL-6 and VEGF elevation is caused by CKD or WM in this patient. To our knowledge, no study has yielded any suggestions regarding this association, so this question remains to be further determined.

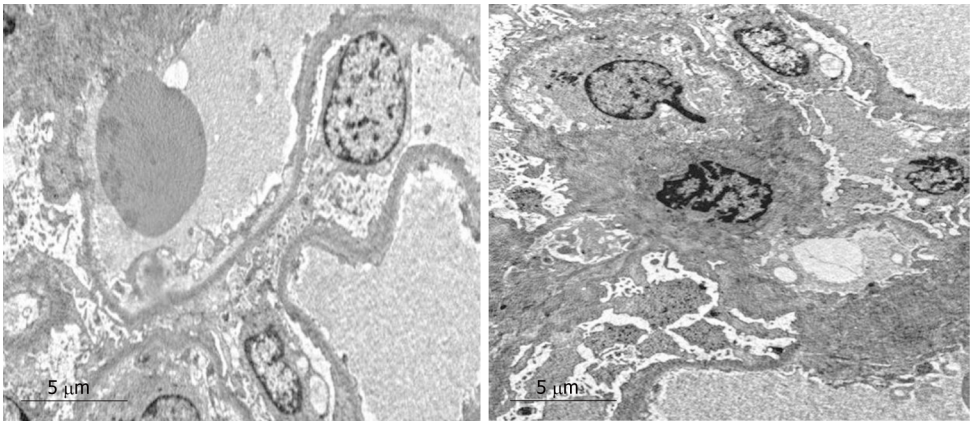

Figure 3 Electron microscopy. Extensive effacement of podocyte foot processes, slight hyperplasia of mesangial matrix, and small amounts of electron dense depositions were observed in the mesangial area. Interstitial fibrosis of the kidney was obvious, and inflammatory cell infiltration was seen.

Regarding the treatment of WM, chemotherapy with a combination of bortezomib, thalidomide, and dexamethasone as shown in Table 1 was a success, and complete remission was achieved in case of minimal change nephrotic syndrome occurring in the setting of WM. This treatment achieved hematological remission, thus suppressing serum IgM, hyperviscosity, and nephrotic range proteinuria, as observed in our case. Renal complications disappeared, podocyte efficiency was recovered, and proteinuria remission was ameliorated.

CONCLUSION

We present a rare case of WM with accumulation of lots of pseudothrombi deposited in the capillaries, associated with minimal change nephrotic syndrome and CKD due to capillary occlusion.

Table 1 Chemotherapy regimen

Chemotherapy cycle	Chemotherapy drugs used	Workups before chemotherapy	Workups after chemotherapy
First cycle; 3/5-17/5/2018	Bortezomib 2.5 mg, dexamethasone 20 mg, and thalidomide tablets 100 mg	CBC: WBC 7.03 × 10^9/L, Hb 114 g/L, PLT 360 × 10^9/L; LFT and RFT: Albumin 11.7 g/L, globulin 57.7 g/L, ALT and AST were normal, urea 10 mmol/L, creatinine 167.0 μmol/L, serum β-2 microglobulins; 2.54 mg/L; TFT: TSH 10.31 μIU/mL, FT3 2.11 pmol/L, FT4 7.7 pmol/L; Serum Igs: IgM > 66.42 g/L, IgG < 1.41 g/L, IgA, C3, and C4 were normal. ESR 114 mm/h; CRP < 5.00 mg/L; Urinalysis: Urine protein 2+, occult blood 1+, RBC count 181/μL, 24-h urine protein 1600.50 mg/24 h	CBC: WBC 26.37 × 10^9/L, Hb 104 g/L, PLT 112 × 10^9/L, neutrophils 9.8%; LFT and RFT: Albumin 24.9 g/L, globulin 47.5 g/L, ALT and AST were normal, urea 7.1 mmol/L, creatinine 118 μmol/L; ESR 123 mm/h; Serum Igs: IgM 44.66 g/L
Second cycle; 4/6-17/6/2018	Bortezomib 2.2 mg, dexamethasone 20 mg, and thalidomide tablets 100 mg	CBC: WBC 10.8 × 10^9/L, Hb 113 g/L, PLT 637 × 10^9/L; Urinalysis: Urine protein 1+; LFT and RFT: Albumin 30.1 g/L, globulin 33.8 g/L, ALT and AST were normal, urea 5.6 mmol/L, creatinine 112 μmol/L, serum β-2 microglobulins; 3.07 mg/L; Serum Igs: IgM 23.75 g/L, IgA 0.55 g/L, IgE was low; ESR 100 mm/h	CBC: WBC 24.2 × 10^9/L, neutrophils 83.7%, Hb 110 g/L, PLT 158 g/L; LFT and RFT: albumin 32 g/L, globulin 22 g/L, ALT and AST were normal, urea 5.4 mmol/L, creatinine 92 μmol/L, serum β-2 microglobulins 1.77 mg/L; Serum Igs: IgM 19.9 g/L; ESR 98 mm/h
Third cycle; 4/7-16/7/2018	Bortezomib 2.2 mg, dexamethasone 20 mg, and thalidomide tablets 100 mg	CBC: WBC 10.39 × 10^9/L, Hb 115 g/L, PLT 482 × 10^9/L; Urinalysis: Urine protein was negative; LFT and RFT: Albumin 38 g/L, globulin 29.8 g/L, urea, creatinine, and serum β-2 microglobulins were normal; Serum Igs: IgM 19.04 g/L; ESR 109 mm/h	CBC: WBC 15.38 × 10^9/L, neutrophils 80.7%, Hb 116 g/L, PLT 219 × 10^9/L; LFT and RFT: Albumin 37.8 g/L, globulin 32.7 g/L, urea, creatinine, and serum β-2 microglobulins were normal
Fourth cycle; 2018.1.8	Bortezomib 2.2 mg, dexamethasone 20 mg, and thalidomide 100 mg	CBC: WBC 8.5 × 10^9/L, Hb 115 g/L, PLT 461 × 10^9/L, Urinalysis: Urine protein was negative LFT and RFT: Albumin 37.9 g/L, globulin 34.6 g/L, urea, creatinine, and serum β-2 microglobulins were normal; Serum Igs: IgM 20.46 g/L; ESR 98 mm/h	

CBC: Complete blood count; LFT: Liver function test; RFT: Renal function test; ESR: Erythrocyte sedimentation rate; TFT: Thyroid function test; CRP: C-reactive protein.

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CASE REPORT

Ex vivo revascularization of renal artery aneurysms in a patient with solitary kidney: A case report

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Author contributions: Chen XY reviewed the literature, contributed to manuscript drafting, and acted as first assistant in the surgical procedure; Zhao JC was the patient's vascular surgeon and reviewed the literature; Huang B contributed to manuscript drafting and the surgical procedure; Yuan D and Yang Y were responsible for revision of the manuscript and interpreted the imaging findings; all authors gave final approval for the manuscript version to be submitted.

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Abstract

BACKGROUND

Multiple renal artery aneurysms (RAAs) involving multiple branches in a solitary kidney are rare and present a major challenge to surgeons. *Ex vivo* or *in situ* repair combined with renal artery revascularization is the classical procedure for these complicated cases, which are not suitable for endovascular repair. The choice of bypass graft remains controversial because of the risk of aneurysmal degeneration for autologous graft.

CASE SUMMARY

A 39-year-old female patient presented with left lumbar pain for more than 3 mo. Computed tomography angiography showed congenital absence of the right kidney and three left RAAs involving multiple distal branches. This patient met the criteria for surgical repair due to symptoms of threatened rupture. According to the anatomy and location of multiple RAAs, *ex vivo* revascularization with saphenous vein graft (SVG) was performed. At the 3-year follow-up, computed tomography angiography demonstrated the aneurysmal degeneration of the Y-shaped SVG. The patient remained asymptomatic and follow-up ultrasound showed no continuous growth of SVG aneurysm.

CONCLUSION

SVG aneurysm in RAA revascularization causes us to reflect on the choice of graft, especially for solitary kidney patients.

Key words: Renal artery aneurysm; Bypass; *Ex vivo* repair; Aneurysmal degeneration; Case report

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Core tip: This rare case of complicated left renal artery aneurysms (RAAs) with absence

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of right kidney presented a major challenge for the surgeon. From a technical aspect, most RAAs are treated by endovascular procedures, and such complicated surgical repair could give surgeons more confidence with complex renal artery revascularization. Although saphenous vein graft is considered the first choice for auto-renal bypass graft, the risk of restenosis and aneurysmal degeneration remains unresolved. For the RAAs without evidence of inflammation, prosthetic graft may be the alternative choice for patients.

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INTRODUCTION

Renal artery aneurysm (RAA) is uncommon with an estimated incidence around 0.1% in the general population. Although the incidence is low, once ruptured, the mortality can reach 80%. Isolated RAA is dominant, and multiple RAAs are seldom reported[1,2]. Treatment of RAA includes surgical repair with revascularization of the RA by *ex vivo* or *in situ* repair with or without cold preservation. As the development of percutaneous techniques, endovascular repair with coils and stents has increasingly been used[3-5]. This case of triple left RAAs with absence of the right kidney and involvement of multiple branches is extremely rare, and it was a major challenge for the vascular surgeon. We here report the details of the procedure and long-term follow-up results.

CASE PRESENTATION

Chief complaints

A 39-year-old female patient presented to the clinic of our hospital complaining of left lumbar pain for > 3 mo.

History of present illness

The patient's symptoms started 3 mo ago with left lumbar pain, which had worsened in the previous 2 wk.

History of past illness

The patient had no previous medical history.

Personal and family history

The patient had no personal and family history.

Physical examination upon admission

After admission to our department, the patient's temperature was 36.7 °C, heart rate 73 beats/min, respiratory rate 16 breaths/min, blood pressure 130/70 mmHg, and oxygen saturation in room air 98%. Physical examination revealed percussion tenderness over the left kidney region. No abdominal or rebound tenderness was detected.

Laboratory examinations

Blood analysis revealed normal white blood cell counts, neutrophils, hematocrit, and platelet count. Prothrombin and partial thromboplastin times were normal. Serum C-reactive protein, erythrocyte sedimentation rate, and other immunological tests were all negative. Creatine was 69 μmol/L (normal range 44-133 μmol/L), and estimated glomerular filtration rate was slightly decreased at 80 mL/min/1.73 m^2 (normal range > 90 mL/min/1.73 m^2). Electrocardiogram and chest X-ray were also normal.

Imaging examinations

Renography demonstrated that renal index was 38.06% (normal range > 45%). Computed tomography angiography (CTA) showed congenital absence of the right

kidney and three left RAAs. The first two aneurysms were located on the twisted main trunk of the left RA with a size of 3 cm and 4 cm, respectively, with a branch originating from the second aneurysm. Another distal small aneurysm of 1.8 cm was located on the distal bifurcation involving two branches (Figure 1A).

FINAL DIAGNOSIS

The final diagnosis of the present case was threatened rupture of left triple RAAs and congenital absence of right kidney.

TREATMENT

Left triple RAAs resection and *ex vivo* revascularization with saphenous vein graft (SVG) were performed. Surgical exposure was achieved with a wide left subcostal incision *via* a transperitoneal approach. As multiple distal branches were involved in the aneurysms, *ex vivo* repair was considered. Full mobilization and dissection of the left kidney, RAAs, and proximal and distal branches of the RA were performed. The renal pedicle and ureter were mobilized to the pelvic brim. After systemic heparinization (unfractionated heparin 0.5 mg/kg), the main left RA and vein were divided. The left kidney was then placed in ice slush with cold perfusion (4 °C Ringer's solution). After resection of the distal aneurysm, two residual distal branches were conjoined as a patch, and distal anastomosis was performed end-to-end between the patch and a reversed SVG (Figure 2A). Another branch originating from the second aneurysm was anastomosed end-to-side to the lateral wall of the SVG (Figure 2B). After branch reconstruction, the left kidney was put back into the orthotopic renal fossa. The proximal SVG was anastomosed end-to-end to the proximal main RA, and the renal vein was anastomosed with the original orifice of the inferior vena cava. Intraoperative renal ultrasound identified patency of the anastomosis and distal branches. Whole blocking time for renal vessels was 80 min.

OUTCOME AND FOLLOW-UP

The patient had an uneventful postoperative clinical course and was discharged from hospital 5 d after the operation. Three-year follow-up CTA demonstrated aneurysmal degeneration of the SVG (with a maximum graft diameter of 2.2 cm), and all distal branches were clearly visible (Figure 1B). We carried out further ultrasound follow-up every 6 mo that revealed no continuous growth of SVG aneurysm, and the patient remained asymptomatic. Update renography showed that the renal index of the left kidney increased to 72.06%.

DISCUSSION

Multiple RAAs involving multiple branches in a solitary kidney are rare and present a major challenge to surgeons. Open surgical repair has been the predominant method for treatment of these lesions; however, currently, endovascular techniques have offered less invasive treatment options in selected cases[4-6].

Currently accepted indications for RAA intervention include size > 2 cm; women within childbearing age; symptoms such as pain, hematuria, and medically refractory hypertension, including that associated with functionally important RA stenosis, thromboembolism, dissection, and rupture[5]. Several approaches are adopted for revascularization of RAA including *in situ* or *ex vivo* surgical repair and endovascular therapy.

It is reported truncal aneurysms can be treated by covered stent and intrarenal aneurysms by coil embolization. However, with RAAs involving multiple distal branches, current endovascular techniques do not warrant complete exclusion without renal infarction[7-10]. In the present case, endovascular repair was designed as isolation of the first two RAAs with covered stents, and the last small aneurysm was left behind as the size did not reach the indication for intervention. However, as a branch originated from the second aneurysm, isolation of the first two aneurysms could have blocked the blood flow of this branch, which may have caused partial infarction of the left kidney. Although the diameter of the third RAA involving distal branches did not reach the indication for intervention, we could not leave the risk of future aneurysm development as the patient was young, and it would be much more

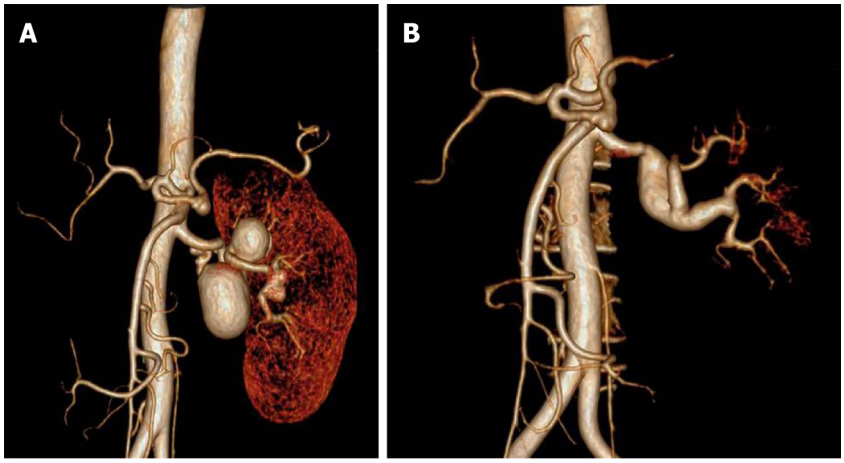

Figure 1 Computed tomography angiography. A: Preoperative computed tomography angiography. Triple complicated multiple renal artery aneurysms with a maximum size of 4 cm, distal renal artery bifurcation, and branches were involved; B: Three-year follow-up computed tomography angiography. The saphenous vein graft was patent with aneurysmal degeneration.

difficult to deal with the third RAA. The preliminary results of the endovascular treatment are not very informative. For the above reasons, surgical repair was performed on this patient.

It is reported that *ex vivo* repair ensures good protection of the renal parenchyma. In contrast, *ex vivo* surgical repair of RAA has the advantage of facilitating reconstruction of distal branches, especially those with multiple branches involved[10,11]. Several studies have already reported a satisfactory result of *ex vivo* surgical repair with low morbidity and patency rates of 82%–99%[12,13]. To date, there is no report of randomized controlled trial comparing *ex vivo* and *in situ* repair of RAAs. Considering the reconstruction of multiple distal branches, and *in situ* surgical repair is more challenging, *ex vivo* repair was performed for this patient.

For the choice of bypass graft, the saphenous vein is the most common conduit for revascularization. Most current studies have preferred to use SVG with good durability and patency. Follow-up patency (mean, 33 mo; range, 1–118 mo) was determined for 64 (91%) RA reconstructions. Product-limit estimate of primary patency at 48 mo was 96%[13,14]. Although the aneurysmal degeneration of SVG is widely reported in coronary artery disease, it is less frequently reported in the RAAs with revascularization by SVG, especially in noninflammatory RA disease. Besides SVG, branched and unbranched internal iliac artery autografts have been used as bypass grafts for the RA, and no aneurysmal degeneration of the internal iliac artery graft has been reported. To prevent risk of SVG aneurysmal degeneration, a tubular SVG supported by external Dacron mesh was adopted, presented to be a suitable graft material for renal reconstruction in pediatric population. With an average follow-up of 4.3 years, no SVG aneurysm was detected[15]. For RAAs without evidence of inflammation, considering the risk of aneurysmal degeneration of SVG, prosthetic grafts may be an alternative, as no aneurysmal degeneration was present in our patient with a solitary kidney, although more stable long-term results are needed.

Three-year follow-up CTA revealed aneurysmal degeneration of the SVG, with a maximum diameter of 2.1 cm. Because there was no growth of the aneurysm during follow-up under ultrasound surveillance, we chose to continue observing the size change.

CONCLUSION

This rare case of complicated RAAs with absence of the right kidney presented a major challenge to the surgeon. Successful results give us more confidence for *ex vivo* surgical repair of complicated RAA. Aneurysmal degeneration of SVG in RAA revascularization causes us to reflect on the choice of graft, especially for solitary kidney patients who need more stable long-term results.

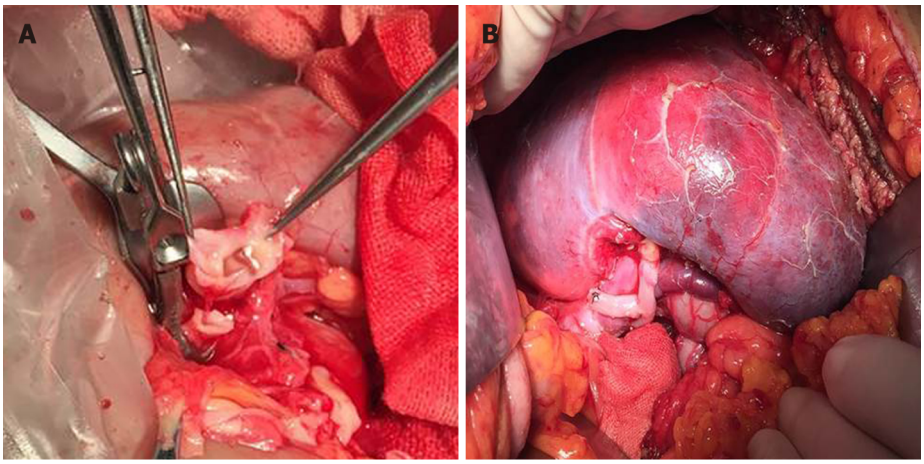

Figure 2 Surgical images. A: Two residual distal branches were conjoined, creating a common patch; the distal anastomosis was performed end-to-end between the common patch and a reversed saphenous vein graft; B: Another branch originating from the second aneurysm was anastomosed end-to-side to the lateral wall of the saphenous vein graft.

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CASE REPORT

Malignant syphilis accompanied with neurosyphilis in a malnourished patient: A case report

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Author contributions: Shi DM designed the case report; Ge G, Shi DM, and Li DM analyzed all the data and wrote the manuscript; Qiu Y, Fu HJ, and Zhang XY collected the information; Li DM made some modifications to the manuscript. All authors read and approved the final manuscript.

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Abstract

BACKGROUND

Syphilis is a common sexually transmitted disease caused by the *Treponema pallidum* (*T. pallidum*). Malignant syphilis is a rare presentation of secondary syphilis. Here, we present a case diagnosed with malignant syphilis accompanied with neurosyphilis.

CASE SUMMARY

A 56-year-old man present with a 2-mo history of spreading ulcerous and necrotic papules and nodules covered with thick crusts over the face, trunk, extremities, and genitalia. The patient was diagnosed with malignant syphilis accompanied by neurosyphilis based on the characteristic morphology of the lesions, positive serological and cerebrospinal fluid tests for syphilis, brain magnetic resonance imaging, and histopathology, along with resolution of the lesions following the institution of penicillin therapy. The lesions and neurological condition successfully resolved after a course of treatment with penicillin.

CONCLUSION

We suggest that neurosyphilis should be considered whenever people have psychiatric symptoms without cutaneous lesions or human immunodeficiency virus.

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Core tip: We present a 53-year-old malnourished man with a two-month history of spreading ulcerative and necrotic cutaneous lesions with psychiatric symptoms. The patient was diagnosed with malignant syphilis accompanied by neurosyphilis based on the characteristic morphology, positive serological and cerebrospinal fluid tests, and histopathology, with resolution of the lesions following penicillin therapy. We report the case to emphasize the diagnosis of the disease, and its association not only with human immunodeficiency virus (HIV), but also with other poor health conditions, specifically malnutrition. We suggest that neurosyphilis should be considered whenever people have psychiatric symptoms even in case of no cutaneous lesions or HIV infection.

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INTRODUCTION

Syphilis is a common sexually transmitted disease caused by *Treponema pallidum* (*T. pallidum*). The course of the disease normally comprises primary, secondary, latent, tertiary, and neurosyphilis stages. Malignant syphilis, also known as Lues malign or ulceronodular syphilis, an uncommon ulcerative variety of secondary syphilis, is an explosive form of syphilis that was first described in the 19th century. This rare form of syphilis is characterized by a prodrome of fever, headache, and muscle pain followed by a papulopustular eruption that soon becomes necrotic, resulting in sharply demarcated ulcers with a thick, rupioid crust[1,2]. Malignant syphilis most commonly affects individuals with human immunodeficiency virus (HIV) infection[3-5]. However, malignant syphilis has also been noted and described in immunocompetent patients[6-8] and is then commonly associated with alcoholism, malnutrition, hepatitis, pregnancy, and diabetes[9]. When untreated, syphilis can spread to the brain and nervous system (neurosyphilis) during any of the stages described above. Patients co-infected with *T. pallidum* and HIV are at significantly higher risk for developing neurosyphilis[3,5-6].

Because its range of manifestations is so vast, syphilis has earned the nickname "The Great Imitator"[10], making its diagnosis in the emergency room notoriously difficult. The clinical manifestations of malignant syphilis are different from classical secondary syphilis in that the former is characterized by pleomorphic pustules, nodules, and deep ulcers with thick crusts. This diagnosis is often forgotten or misdiagnosed, especially when an immunocompetent patient presents with spread skin lesions and cerebral manifestations[11]. Here, we present a case diagnosed as malignant syphilis accompanied with neurosyphilis, but first misdiagnosed as psoriasis/pyoderma gangrenosum and cerebral fracture. On the basis of clinical examination, serum and cerebrospinal fluid (CSF) *T. pallidum* particle agglutination (TPPA), rapid plasma regain (RPR), histological examination, and cerebral magnetic resonance imaging (MRI), the patient was re-diagnosed to be suffering from malignant syphilis and neurosyphilis. The patient recovered following a course of treatment with penicillin.

CASE PRESENTATION

Clinical summary

A 56-year-old male present with a 2-mo history of spreading ulcerous and necrotic papules and nodules covered with thick crusts over the face, trunk, extremities, and genitalia on March 20, 2018 (day 0). The skin lesions initially appeared as small erythematous papules over his back and gradually spread to the chest, face,

extremities, and genitals. The lesions progressed to ulcerous/necrotic lesions covered with thick yellowish and blackish crusts. There was no association with systemic manifestations such as fever, weight loss, or headache; however, the patient also suffered from a loss of coordination of movement, personality changes, and changes in speech. The patient was initially diagnosed with pyoderma gangrenosum and treated for 7 d (days -8 to 1) at a local hospital, but his loss of coordination and speech impairment worsened. The patient reported having unprotected sexual activity within the past 2 mo but denied ever having had sex with men. The patient had no other apparent underlying disease. Other possibly relevant habits included smoking 20-40 cigarettes each day for more than 30 years, but the patient denied ever drinking alcohol.

Pathological findings

His height and body mass index (BMI) of 18.36 kg/m² together indicated mild malnutrition. Examination revealed pleomorphic ulcers of varying sizes ranging from 1 to 6 cm, circular and oval in shape with sharp borders, covered by yellowish and blackish thick crusts. The lesions were distributed over the face, front and back of the trunk, extremities, and genitals (Figure 1). Neurological examination showed mental confusion, mania, paranoia, and mild motor dysphasia.

Laboratory examinations

Full blood cell count, CD4+ cell count, CD8+ cell count, HIV serotest, hepatitis B and hepatitis C, antineutrophil cytoplasmic antibody and anti-nuclear antibodies, and cultures for fungi and bacteria (including *Mycobacterium tuberculosis* and *Neisseria gonorrhoeae*) were all normal or negative. Other laboratory analyses showed slightly attenuated albumin (31.3 g/L), anemia (hemoglobin 117 g/L), and elevated C reactive protein (35.00 mg/L), with no other abnormalities. Serum TPPA was positive and RPR test was at a titer of 1:16 (at +1 d).

Imaging examinations

MRI revealed abnormal hyperintense lesions in the bilateral insular cortex and radial crown, and lateral anterior horn (at +1 d) (Figure 2). Histological examination of a biopsy sample from an ulcer on his abdomen indicated obliterative vasculitis as well as infiltration of cells (predominantly lymphocytes and plasma cells) in the dermis (Figure 3A). Staining for fungi, mycobacteria, and spirochetes was all negative. Immunohistochemical staining with a polyclonal antibody against *T. pallidum* was positive (Figure 3B).

Because the patient was suspected to have neurosyphilis, he was arranged to undergo a lumbar puncture, but at first he refused. After treatment with penicillin (at +11 d), the patient received a lumbar puncture, which showed elevated protein (5.9 mg/dL) and high white blood cell count (16 cells/μL; predominantly lymphocytes) in CSF. TPPA was positive and RPR was negative.

FINAL DIAGNOSIS

Based on the clinical findings, along with serum TPPA, RPR, histological examination, and MRI, the patient was diagnosed with malignant syphilis with neurosyphilis.

TREATMENT

The patient was initially treated with 60 million units of penicillin three times daily for 10 d (from +1 d to +11 d). He did not present with Jarisch-Herxhiemer reaction (JHR) possibly due to the corticosteroids prescribed earlier. The lesions regressed remarkably and quickly and he was discharged from the hospital. The patient continued to respond positively to treatment with 240 million units of penicillin intramuscularly once a week for three weeks, with a complete remission of lesions and neurotic systems.

OUTCOME AND FOLLOW-UP

In next follow-up, the ulcers had healed completely, although atrophic scars remained (Figure 4); the RPR titer dropped to 1:2 and HIV serotest remained negative.

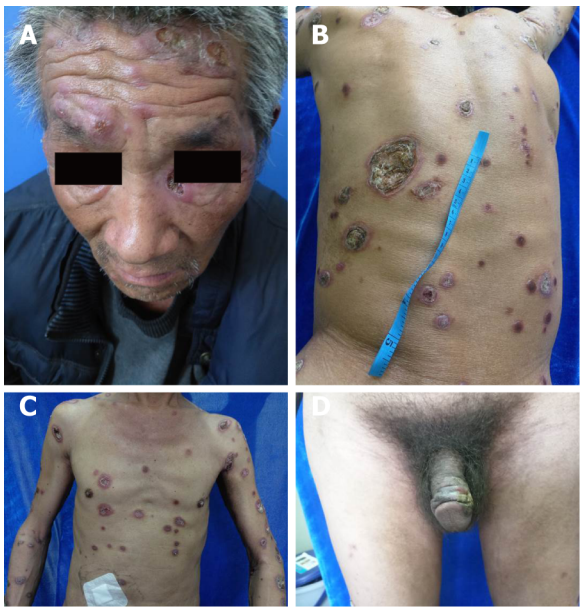

Figure 1 Widespread crusted skin ulceration. A: Face; B: Back; C: Chest; D: Genitals.

DISCUSSION

Malignant syphilis is a rare presentation of secondary syphilis, originally described by Bazin in 1859 as a nodular variant of syphilis. With the increase in the incidence of HIV infection, the morbidity as well as the frequency of this disease has increased[12]. It usually occurs from 6 weeks to 1 year after the primary symptoms manifest, or even earlier in people with HIV infection[13,14]. A few cases of malignant syphilis have been described, mostly associated with HIV infection, and it is rarely seen otherwise in patients with poor health conditions, diabetes, or malnutrition[9].

The occurrence of malignant syphilis together with neurosyphilis and malnutrition, as shown in the present case, is extremely rare. A few cases of malignant syphilis have been reported in the literature, most of which had no neurosyphilis or HIV infection[3,15]. In the present case, the patient was not HIV positive, and therefore one of the main risk factors for the development of both malignant syphilis and neurosyphilis was absent.

Typical lesions of malignant syphilis are initially papules that rapidly evolve into pustules and finally form ulcers with an elevated border and necrotic center[16]. The skin lesion mainly affects the trunk and extremities, although the face, scalp, mucous membranes, palms, soles, and genitals can also be involved. Because of the varied clinical manifestations and mimicking of several more common dermatoses, malignant syphilis is almost always misdiagnosed as another disease. In our case, at first the patient was misdiagnosed with pyoderma gangrenosum. When there is clinical suspicion of malignant syphilis, confirmation of the diagnosis will be supported by three criteria: Clinical and histopathological characteristics; presence of high-titer antibodies from Venereal Diseases Research Laboratory (VDRL) or a similar test; and intense and severe JHR and rapid resolution of lesions with adequate therapy. The diagnosis is typically established by strongly positive serological tests, a severe JHR, and an excellent response to antibiotic therapy[17]. In our case, the patient was prescribed with glucocorticoids prior to antibiotic therapy, in which case JHR might have been inhibited. Based on the circumstances of the present case, we believe that severe JHR should not be included as one of the mandatory criteria.

Syphilis can spread to the brain and nervous system (neurosyphilis) during any stage of the course of the disease. Neurosyphilis should be considered for any patients with syphilis accompanied by tabes dorsalis, general paresis, meningovascular neurosyphilis, or other unexplained neurological conditions. Early diagnosis and treatment are crucial due to potential persistent disabilities that can be easily treated or prevented if detected early. Brain MRI and CSF analyses are essential for treatment planning and management. In patients with a known syphilis infection who present with neurologic, ophthalmic, or tertiary syphilis symptoms, the United States Centers for Disease Control and Prevention (CDC) recommends a lumbar puncture with a CSF examination[18]. The possibility of neurosyphilis should be promptly investigated, followed by empiric treatment. In the present case, widespread lesions, tabes dorsalis,

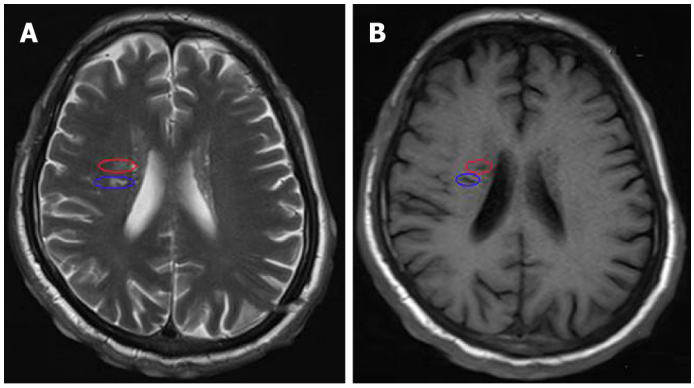

Figure 2 Magnetic resonance imaging revealing abnormal hyperintense lesions in the bilateral insular cortex and radial crown, and lateral anterior horn. The circles in blue and in red indicate lesions. A: T2 weighted imaging; B: T1 weighted imaging.

serum RPR test along with MRI all suggest neurosyphilis, even without brain fluid TPPA and RPR. In addition, CSF showed elevated protein and high white blood cell count with lymphocyte predominance. Also, CSF TPPA was positive and RPR was negative. We considered that RPR was negative due to the initial treatment with penicillin. After these considerations, the patient was diagnosed with malignant syphilis with neurosyphilis.

Pathological studies show that obliterative medium-sized vessel vasculitis and plasma cell infiltrates in the dermis can be valuable tools in a challenging diagnosis[19]. In the classical definition of malignant syphilis, the absence of spirochetes in tissue samples was cited as one diagnostic criterion. However, treponema can occasionally be identified by silver staining such as Steiner or Whartin-Starry techniques. Immunohistochemical staining using monoclonal antibodies against *T. pallidum* is sometimes helpful to identify microorganisms, especially in secondary syphilis, and has demonstrated a high sensitivity and specificity[2]. In the present case, treponema was not identified using silver staining, however, in agreement with other reports, spirochetes were detected using antibodies against *T. pallidum*. Therefore, we propose that immunohistochemical staining can be a useful tool for confirming the diagnosis along with the clinical and serologic findings.

Although penicillin is the treatment of choice, there is no special recommended treatment for malignant syphilis. Some authors recommend increasing the dose in cases of poor general health conditions. For resistant cases or relapses, prolonged therapy with high doses of penicillin is suggested[20]. In the present case, the patient was diagnosed with malignant syphilis accompanied with neurosyphilis, and he was initially treated with 60 million units of penicillin daily for 10 d, in view of the fact that crystalline penicillin was able to cross the blood-brain barrier. After he was discharged from the hospital, we chose a total dose of penicillin G benzathine (7.2 million units) for next three weeks. The lesions and neurological condition successfully resolved after the treatment, along with a fourfold decline in serum RPR.

CONCLUSION

The titers of VDRL or RPR were high in most published malignant syphilis cases, however, in our case the titer of RPR was not remarkably high, which suggests that the high titer of RPR is not always correlated to the occurrence of malignant syphilis. By presenting a variety of clinical manifestations and mimicking several common dermatoses, malignant syphilis should always be included in differential diagnoses, even though it might be rare in the absence of HIV infection. When accompanied by psychological or neurological symptoms, the physician should be alert for the possibility of neurosyphilis, and serological screening for syphilis should be routine for such patients.

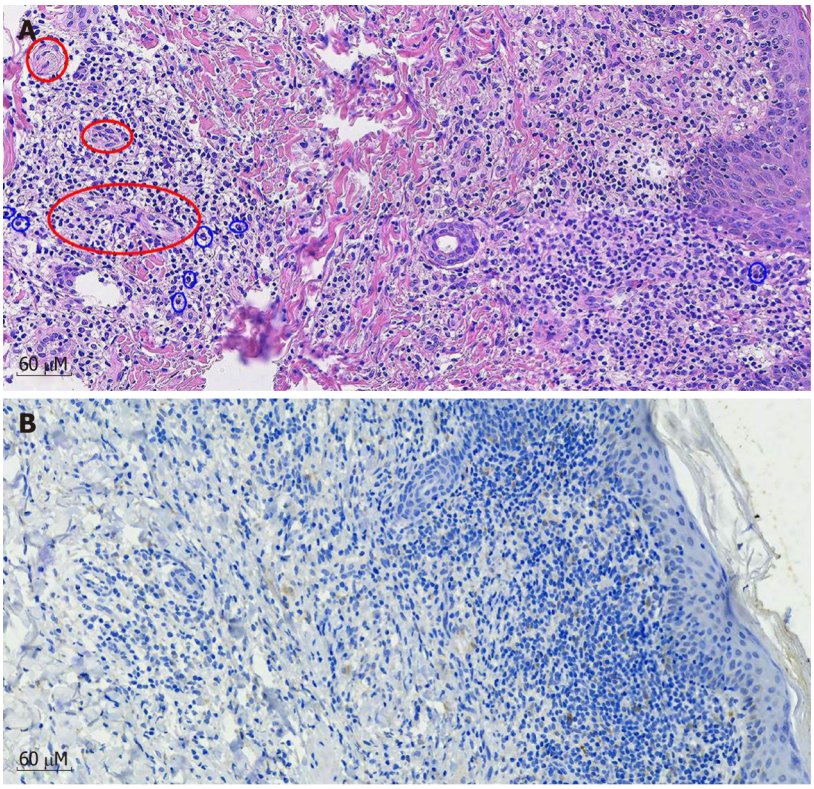

Figure 3 Photomicrograph of section of the skin biopsy from the abdomen lesion. A: The mixed infiltrate of lymphocytes, histocytes, and plasma cells (blue circle) accompanied by obliterative vasculitis in the dermis (red circle). (Hematoxylin-eosin staining, original magnification, ×200); B: Spiral and thread-like organisms, highlighted by the brown chromogen in the dermis, represent the spirochetes. (Immunohistochemical staining with anti-spirochetes, original magnification, ×200).

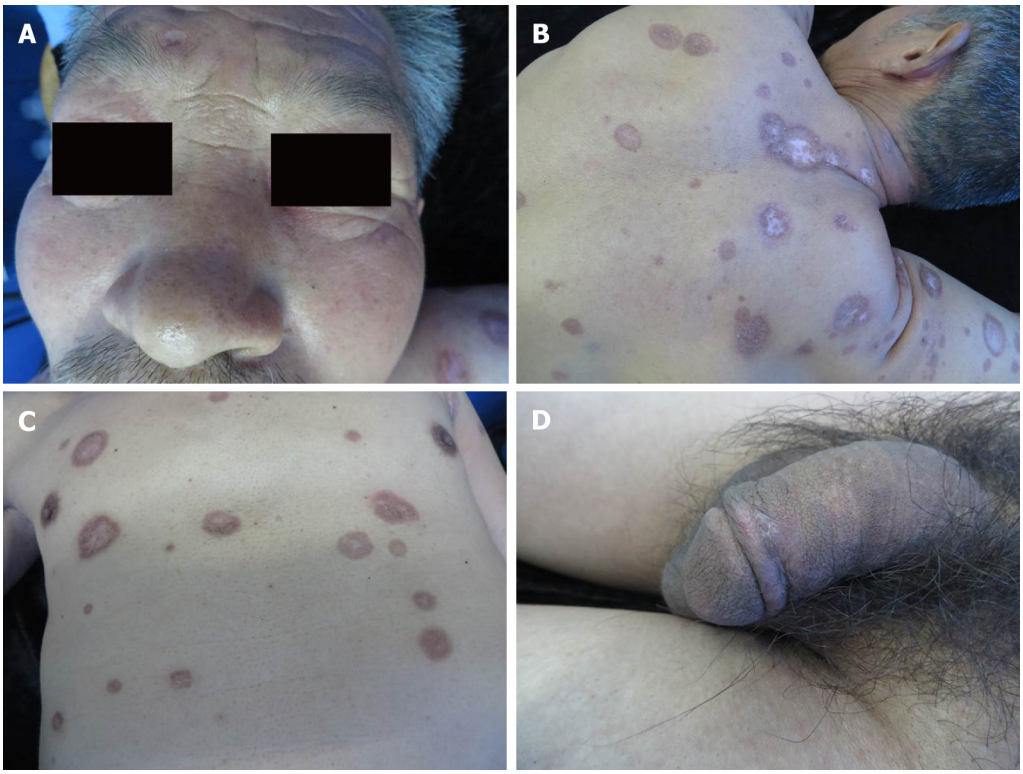

Figure 4 Pigmented and depigmented macules and atrophic scars 4 months after treatment. A: Face; B: Back; C: Chest; D: Genitals.

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