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EDITORIAL

Biomarkers for gastrointestinal adverse events related to thiopurine therapy

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Abstract

Thiopurines are immunomodulators used in the treatment of acute lymphoblastic leukemia and inflammatory bowel diseases. Adverse reactions to these agents are one of the main causes of treatment discontinuation or interruption. Myelosuppression is the most frequent adverse effect; however, approximately 5%-20% of patients develop gastrointestinal toxicity. The identification of biomarkers able to prevent and/or monitor these adverse reactions would be useful for clinicians for the proactive management of long-term thiopurine therapy. In this editorial, we discuss evidence supporting the use of *PACSIN2*, *RAC1*, and *ITPA* genes, in addition to *TPMT* and *NUDT15*, as possible biomarkers for thiopurine-related gastrointestinal toxicity.

Key Words: Thiopurines; Gastrointestinal adverse effects; Biomarkers; *PACSIN2*; *RAC1*; *ITPA*

Core Tip: Adverse reactions to thiopurines are one of the main causes of treatment discontinuation or interruption. In addition to myelosuppression, approximately 5–20% of patients develop gastrointestinal toxicity; the identification of biomarkers to prevent and/or monitor these adverse reactions is important for the proactive management of long-term thiopurine therapy. In this editorial, we discuss evidence supporting the use of *PACSIN2*, *RAC1*, and *ITPA* genes, in addition to *TPMT* and *NUDT15*, as possible

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INTRODUCTION

Mechanisms of action and adverse effects of thiopurine

Thiopurines, such as mercaptopurine (MP) and its prodrug azathioprine (AZA), are immunomodulatory drugs used in the treatment of pediatric acute lymphoblastic leukemia (ALL) and nonmalignant conditions, such as inflammatory bowel diseases (IBDs)[1,2]. These immunomodulators undergo a complex biotransformation that leads to the production of different thionucleotides (TGNs), such as thioguanosine mono-, di-, and triphosphate (tGMP, tGDP, and tGTP) and deoxythioguanosine mono-, di-, and triphosphate (tdGMP, tdGDP, and tdGTP) (Figure 1). These purine antimetabolites exert their cytotoxic activity through different mechanisms, such as inhibition of *de novo* purine synthesis, interference with the incorporation of guanosine nucleotides into DNA and RNA, and induction of apoptosis due to inhibition of the Ras-related C3 botulinum toxin substrate 1 (Rac-1) protein, a Rho-GTPase[3]. Under physiological conditions, Rac-1-GTP activates the MEKK/IκB/NF-κB and STAT3 survival pathways in activated lymphocytes, resulting in an increase in the antiapoptotic protein Bcl-xL, whereas during thiopurine treatment, the binding of tGTP to Rac-1 impairs these pathways, enhancing apoptosis[3]. Thiopurines are also processed through catabolic pathways, in which xanthine oxidase and thiopurine methyltransferase (TPMT) are the main enzymes involved, producing inactive metabolites such as thiouric acid and methylmercaptopurine, respectively. TPMT also catalyzes the S-methylation of intermediates resulting from MP conversion to TGN, leading to the production of secondary methylated nucleotides (MMPNs) (Figure 1). The role of MMPN metabolites is not fully characterized; however, they could contribute to the inhibition of *de novo* purine synthesis. Factors affecting the TGN/MMPN ratio could influence thiopurine efficacy and toxicity. For example, the amount of TGN in white blood cells is responsible for the immunosuppressive effects; when TPMT activity is compromised, TGN levels increase, leading to dangerous myelosuppression[3].

Thiopurines have a narrow therapeutic index, with an increased risk of severe toxicity and treatment discontinuation[4]. Direct cytotoxic damage can occur in proliferating cells of different tissues and organs. In particular, thiopurines have been associated with the dose-dependent hematological toxicity observed in approximately 80% of ALL cases; in IBD patients, the incidence of bone marrow toxicity is lower (approximately 10%)[5,6]. Neutropenia and leukopenia are the most frequent outcomes of myelosuppression, related to an increased risk of infection, and the main reasons for therapy discontinuation or interruption that can lead to disease aggravation in both ALL and IBD[7-9]. Thiopurine-induced gastrointestinal (GI) toxicity occurs in approximately 5%-20% of ALL and IBD patients; the main symptoms are nausea, vomiting, stomatitis, abdominal pain or cramping, gastritis, gastric ulcer, GI bleeding, and diarrhea[10,11]. Moreover, these immunosuppressors are associated with the risk of neurological complications, hepatotoxicity, pancreatitis, arthralgia, and skin rush[10,12-16].

In the clinic, white blood cell counting is commonly performed to monitor the immunosuppressive effects of these drugs; however, recently, pharmacogenetic biomarkers for predicting thiopurine-induced hematological adverse events have been identified. From a pharmacogenetic point of view, *TPMT* is one of the best characterized genes[17]. Both TPMT protein expression and enzymatic activity are affected by the presence of variants in the *TPMT* gene. More than 44 *TPMT* variant alleles have been described; *TPMT**2 (rs1800462, 238G>C, pAla80Pro), *TPMT* **3B* (rs1800460, 460G>A, p. Ala154Thr), *TPMT***3C* (rs1142345, 719A>G, p. Tyr240Cys), and *TPMT***3A* (rs1800460 and rs1142345 haplotypes) are the most frequent variants in Europeans and can explain up to 95% of TPMT deficiencies[18-20]. As reported above, decreased TPMT activity leads to higher TGN levels and lower MMPN in white blood cells; these

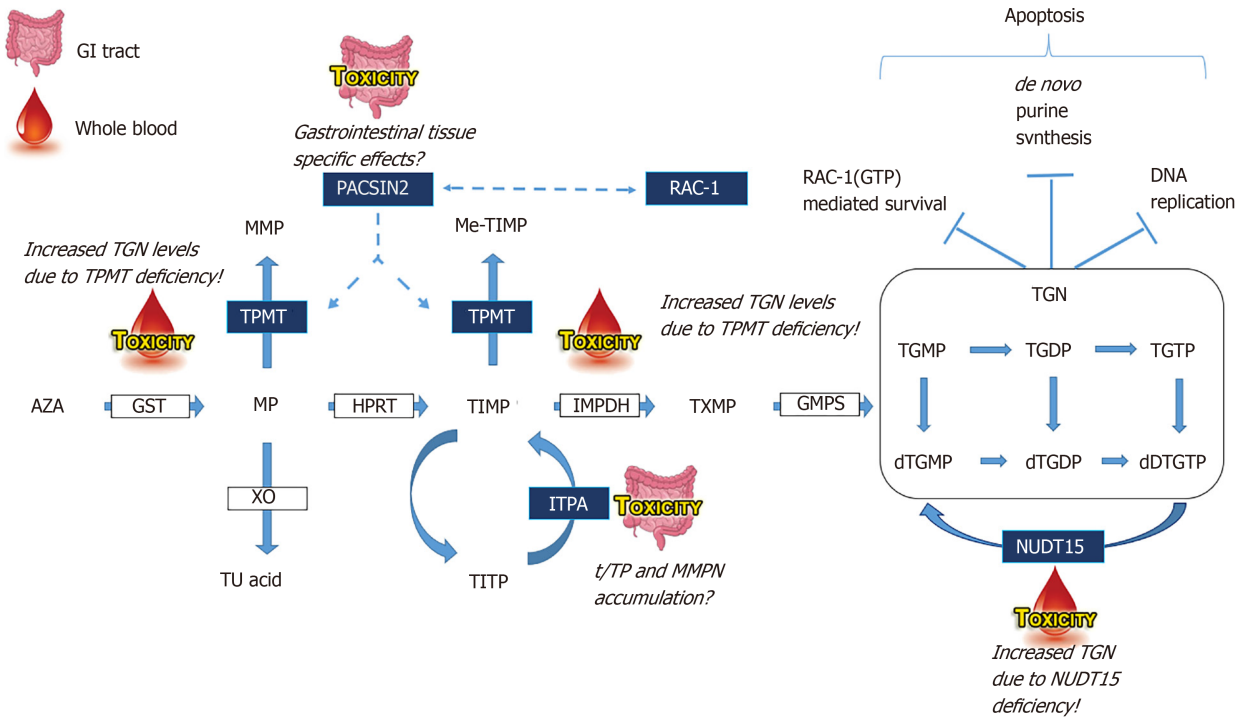

Figure 1 Thiopurine metabolic pathway and possible biomarkers for drug-related toxicity. Dashed arrows indicate the impact of PACSIN2 on TPMT activity and the interaction between PACSIN2 and Rac-1. AZA: Azathioprine; ITPA: Inosine triphosphate pyrophosphatase; IMPDH: Inosine-5'-monophosphate dehydrogenase; GMPS: GMP synthase; GST: Glutathione-S-transferase; Me-TIMP: Methyl-thioinosine monophosphate; Me-TITP: Methyl-thioinosinetriphosphate; MP: Mercaptopurine; MMP: Methyl-mercaptopurine; NUDT15: Nudix hydrolase 15; PACSIN2: Protein kinase C and casein kinase substrate in neurons protein 2; TGDP: Thioguanine diphosphate; TGMP: Thioguanine monophosphate; TGTP: Thioguanine triphosphate; TIMP: Thioinosine monophosphate; TITP: Thioinosine triphosphate; TNG: 6-Thioguanine nucleotide; TPMT: Thiopurine S-methyltransferase; TXMP: Thioxanthine monophosphate; 6-TU acid: Thiouric acid; XO: Xanthine oxidase.

variants are indeed associated with a higher risk of myelosuppression[21]. Variable number tandem repeats (VNTRs) in the *TPMT* promoter are associated with reduced *TPMT* expression levels and a higher risk of MP hematological toxicity[22]. Furthermore, genetic variants in nudix hydrolase 15 (*NUDT15*) have been identified as additional pharmacogenetic markers for the prediction of thiopurine-induced toxicities, especially in Asian individuals. NUDT15 removes a pyrophosphate group by canonical GTP and drug-derived tGTP active metabolites. The most studied *NUDT15* variants are rs116855232 (c.415C> T, p. Arg139Cys), rs147390019 (G>A, p. Arg139His), rs186364861 (G>A, p. Val18Ile), and rs746071566 (36_37insGGAGTC insertion, p.Val18_Val19insGlyVal). Variant alleles encode NUDT15 with compromised activity, leading to a higher tGTP/tGMP ratio and incorporation of TGN into DNA[23,24]. Indeed, these variants have been associated with MP and AZA intolerance[23,25]. On these bases, different guidelines for thiopurine dose adjustment based on *TPMT* and *NUDT15* genotypes have been released to reduce the occurrence of drug-related side effects[26].

In addition to pharmacogenetic markers, TGN levels could be monitored in erythrocytes to avoid severe myelosuppression during therapy. In particular, TGN levels higher than 450 pmol/8 × 10^8 red blood cells (RBCs) and higher than 1000 pmol/8 × 10^8 RBCs have been shown to be associated with myelotoxicity in IBD and ALL patients, respectively, while levels of MMPN above 5700 pmol/8 × 10^8 RBCs have been shown to be related to a higher hepatotoxicity risk in IBD patients[27,28].

BIOMARKERS FOR THIOPURINE-INDUCED GASTROINTESTINAL ADVERSE EVENTS

Although genome-wide association studies (GWAS) have indicated that TPMT activity is predominantly a monogenic trait[29], a percentage of wild-type *TPMT* carriers present reduced TPMT activity, suggesting the existence of other regulatory mechanisms able to modulate its function[30,31]. In 2012, Stocco *et al*[32] demonstrated

that the expression levels and the single nucleotide polymorphism (SNP) rs2413739 of the protein kinase C and casein kinase substrate in neurons 2 (*PACSIN2*) gene were associated with TPMT activity in HapMap cell lines and in a cohort of ALL pediatric patients enrolled at St. Jude Research Children Hospital (SJRCH, Memphis, United States), suggesting a possible role of *PACSIN2* as a TPMT modulator[32]. The authors found that the intronic variant rs2413739 (C>T) was associated with an increased risk of severe GI toxicity during consolidation therapy in two independent cohorts of ALL pediatric patients treated according to the SJRCH Total 13B protocol and to the Associazione Italiana Ematologia Oncologia Pediatrica/Berlin-Frankfurt-Münster (AIEOP-BFM) 2000 protocol[32]. Patients received 75 mg/m^2 MP daily and 2 g/m^2 high-dose methotrexate (HD-MTX) i.v. twice a week for 2 wk at SJRCH, whereas those undergoing the AIEOP-BFM 2000 protocol were treated daily with 25 mg/m^2 MP and received four HD-MTX (2-5 g/m^2) infusions once every 2 wk. To further validate these results, Franca *et al*[33] investigated the possible role of *PACSIN2* rs2413739 in an additional cohort of ALL pediatric patients treated according to the AIEOP-BFM 2009 protocol, with the same consolidation phase as AIEOP-BFM ALL 2000, and in a cohort of IBD pediatric patients undergoing AZA therapy. In the ALL cohort, the *PACSIN2* T allele was associated with decreased TPMT activity during maintenance therapy, particularly in patients heterozygous for *TPMT* rs1142345 and rs1800460. Moreover, the *PACSIN2* TT genotype was associated with a higher risk of GI toxicity during the consolidation phase. The latter association was borderline, likely because of the limited number of clinical data available (n = 81); however, it was in line with the findings of Stocco *et al*[32]. Far more complex to understand is thiopurine-induced GI toxicities in IBD patients, where the occurrence of adverse effects can overlap with clinical manifestations of the disease. Interestingly, Franca *et al*[33] showed that IBD patients carrying the *PACSIN2* T allele and undergoing AZA treatment presented a more active disease, measured as pediatric ulcerative colitis activity/pediatric Crohn's disease activity (PUCAI/PCDAI) indices > 10, according to standard clinical practice. No association between the rs2413739 variant and either TPMT activity or TGN/MMPN levels was found, suggesting a thiopurine-independent effect on the clinical phenotype [33]. Enzymatic activity was significantly higher in the ALL patients than in the IBD patients[33]. The different impact of *PACSIN2* SNP rs2413739 on TPMT activity could be partially explained by patient age: The ALL cohort comprised children under 10 years, while the IBD patients were mainly teenagers. The authors hypothesized that the *PACSIN2* genetic impact on TPMT activity could be more evident in younger patients, who seemed to have increased TPMT activity[34,35]. Moreover, concomitant treatment with MTX in the ALL cohort could contribute to discrepancies in the results; MTX could impact S-adenosyl methionine levels, a TPMT cofactor responsible for the stability of the protein[36]. Since Franca *et al*[33] did not detect significant changes in TGN levels in *PACSIN2* T allele carriers, they hypothesized a thiopurine-independent effect of PACSIN2 on GI toxicity and a tissue-specific role of *PACSIN2* in the intestine. Notably, the Genotype-Tissue Expression Portal (GTEx) shows that *PACSIN2* and *TPMT* expression levels are increased in blood and in the esophageal mucosa of healthy *PACSIN2* rs2413739 T allele carriers but not in the small intestine and colon of these subjects, supporting the idea that the enhanced GI toxicity observed in TT patients is not related to differential expression of *TPMT* in the GI tract[37]. Other evidence regarding PACSIN2 suggests its role as a regulator of intestinal mucosal homeostasis and inflammation. Intriguingly, an underinvestigated mechanism of IBD pathogenesis is VE-cadherin-directed vascular barrier disruption[38], and PACSIN2 has been recognized as a regulator of cell–cell adhesion in the endothelium through the inhibition of asymmetric VE-cadherin internalization from adherens junctions[39]. Stocco *et al*[32] performed an agnostic gene expression analysis in the human B leukemia cell line NALM6 and identified autophagy as one of the pathways significantly affected by *PACSIN2* knockdown, thus suggesting a possible role of this gene in autophagy, another mechanism involved in IBD pathogenesis[32,40,41]. Moreover, the human protein ATLAS report shows that lower levels of PACSIN2 are related to a reduced survival probability in colorectal adenocarcinoma patients, leaving open the question of whether PACSIN2 is a marker of therapeutic response or a contributing factor to intestinal cancer progression[42]. Dedicated studies to clarify the issue of PACSIN2 and GI pathology are needed; however, all this evidence supports the hypothesis that PACSIN2 could be a susceptibility factor for intestinal tissue damage.

Thiopurine-derived tGTPs are able to compete with GTP on Rac-1, a Rho-GTPase involved in cellular proliferation. It can be hypothesized that factors reducing Rac-1 expression or activity could influence cell susceptibility to cytotoxic stimuli, thus contributing to thiopurine efficacy and toxicity. Interestingly, Rac-1 was able to bind

PACSIN2 through a physical interaction[3]; this protein–protein interaction seemed to be responsible for reciprocal regulation: Rac-1 activity controlled PACSIN2 cellular distribution, whereas PACSIN2 could negatively modulate Rac-1 activity[43]. *In vitro* data showed decreased activity of Rac-1 in the presence of the rs34932801 (G>C) SNP in the *RAC1* promoter, and interestingly, this polymorphism was associated with MP hematologic toxicity in a cohort of European IBD patients[44]. Another study reported that Rac-1 expression levels decreased during thiopurine maintenance therapy in IBD patients and that MP responders presented lower Rac-1 expression and activity levels, whereas in nonresponders, these parameters were increased. On these bases, Rac-1 was proposed as a potential biomarker of thiopurine effectiveness in IBD[45]. Intriguingly, conditional disruption of Rac-1 in phagocytes of mice resulted in protection from colitis[46]. In contrast, Rac-1 and STAT3 signaling have been considered contributing factors to IBD development[47], and it was found that both the expression and activity levels of Rac-1 were directly related to colon inflammation grade[46]. Sustained Rac-1-GTP activity in lamina propria T lymphocytes could be more difficult to counteract by thiopurines and lead to resistance of T lymphocytes to apoptosis and thus to their unrestrained accumulation, which subsequently results in the amplification of the inflammatory response in the GI tract. In this sense, in IBD patients, Rac-1 could represent a biomarker of thiopurine-induced GI toxicity and of disease severity and progression, without a clear discrimination between the two clinical phenotypes.

Another potential biomarker for thiopurine GI toxicity is the inosine triphosphate pyrophosphatase (*ITPA*) gene. ITPA is one of the enzymes involved in the thiopurine metabolic pathway. By hydrolyzing inosine triphosphate (ITP) and xanthosine triphosphate nucleotides (XTP) into their monophosphate derivatives (IMP and XMP, respectively), ITPA prevents the accumulation of these noncanonical metabolites in cells and their incorporation into DNA or RNA, where they can interact with DNA/RNA polymerase activity[48]. The thioinosine analog (tIMP), an intermediate of MP conversion to TGN, is converted to tITP, which is also an ITPA substrate (Figure 1). A study performed on a large childhood ALL cohort (n = 511), treated according to the AIEOP-BFM-2000 protocol, showed that the missense variant rs1127354 (C>A, p. Pro32Thr) in *ITPA* was associated with a higher risk of severe GI toxicity during induction/consolidation therapy[10]. This missense variant partially reduces ITPA enzymatic activity in heterozygotes and completely reduces ITPA enzymatic activity in variant homozygotes[49,50], stimulating the accumulation of unusual tITP with the potential to cause adverse metabolic effects[51]. Other studies in pediatric ALL patients showed contradictory results on the *ITPA* rs1127354 association with myelotoxicity[52-54]. Stocco *et al*[53] found significantly higher concentrations of MMPN in patients with the nonfunctional *ITPA* allele. The association between the *ITPA* polymorphism and MP metabolism or neutropenia in ALL patients treated with an MP dose adjusted on the basis of the *TPMT* genotype underlined the important role of this gene in thiopurine toxicity.

CLINICAL IMPLEMENTATIONS

The *PACSIN2* rs2413739 SNP could be considered a potential biomarker for thiopurine-related GI toxicity, being associated with this clinical phenotype in three independent ALL cohorts and with increased active disease in a cohort of IBD patients. Further investigations are needed to understand the molecular basis of this genetic effect and the functional role of the PACSIN2 protein in the healthy and damaged GI epithelium before its possible translation into the clinic. Additionally, the contribution of *RAC1* and *ITPA* SNPs, as potential biomarkers for thiopurine-related GI toxicity, requires further validation in patients undergoing therapy with these drugs. Currently, there are no clinical trials focusing on the role of these genes/proteins in GI toxicity in ALL and IBD patients.

If these candidates would be confirmed as markers for GI toxicity, several applications could be speculated in clinical practice. For example, in patients treated with thiopurines, clinicians could be warned of the patients' genetic predisposition to GI damage (*e.g.*, patients carrying the *PACSIN2* rs2413739 or *ITPA* rs1127354 homozygous variant genotypes). Pharmacogenetic information could be used as an alert for physicians, identifying patients who need intensive monitoring for adverse effects or those who should undergo supportive care earlier, even when less severe episodes of toxicity occur.

CONCLUSION

While highly effective, thiopurines are responsible for serious toxicities in ALL and IBD. This scenario points out the importance of identifying predictive biomarkers for detecting and monitoring the tissue-specific side effects of thiopurine. Data reported in this editorial underline the complexity of thiopurine pharmacokinetic mechanisms, which could be influenced by multiple genes and nongenetic factors able to exert their function on the whole body or through a tissue-specific mechanism of action.

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REVIEW

Fully covered metal biliary stents: A review of the literature

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Abstract

Fully covered self-expandable metal stents (FCSEMS) represent the latest advancement of metal biliary stents used to endoscopically treat a variety of obstructive biliary pathology. A large stent diameter and synthetic covering over the tubular mesh prolong stent patency and reduce risk for tissue hyperplasia and tumor ingrowth. Additionally, FCSEMS can be easily removed. All these features address issues faced by plastic and uncovered metal stents. The purpose of this paper is to comprehensively review the application of FCSEMS in benign and malignant biliary strictures, biliary leak, and post-sphincterotomy bleeding.

Key Words: Fully covered self-expandable metal stents; Plastic stents; Endoscopy; Chronic pancreatitis; Biliary stricture; Biliary leak; Stent migration

Core Tip: Fully covered self-expandable metal stents (FCSEMS) are composed of a metal alloy tubular mesh with a synthetic covering to minimize tumor ingrowth. They have a broad range of biliary endoscopic applications, including the treatment of strictures, biliary leak, and post-sphincterotomy bleeding. Novel anchoring designs have been effective at addressing the common problem of stent migration with FCSEMS.

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INTRODUCTION

Recent advances in endoscopic stenting have enabled the treatment of a wide range of obstructive biliary pathologies previously requiring surgical and percutaneous intervention[1]. Early randomized control studies found that endoscopic stenting compared to surgical or percutaneous intervention was associated with decreased 30 d morbidity and mortality, decreased complications rates, lower overall cost, and higher quality of life[2-4]. Plastic stents were the earliest stent type used, and metal stents were introduced later in the 1980s[5]. The purpose of this report is to review the current applications of fully covered self-expandable metal stents (FCSEMS) for biliary strictures, biliary leak, and post-sphincterotomy bleeding.

PLASTIC STENTS

Plastic stents are commonly used because of their efficacy, easy insertion and removal, and low cost[1,6,7] (Figure 1A). Plastic biliary stents come in various shapes and can be made of Teflon, polyethylene or polyurethane materials. Standard diameters of plastic biliary stents are 7.0 Fr, 8.5 Fr, 10.0 Fr and 11.5 Fr, and standard lengths range from 5 cm to 18 cm. Appropriate stent length is determined using a graduated guide wire and guiding catheter. Initial placement is guided using a radio-opaque guidewire. Plastic stents are equipped with side holes that allow for drainage even when the ends are impacted against the biliary or digestive tract wall, or clogged with debris. Additionally, plastic stents may have anchoring flaps to prevent stent migration. Multiple plastic stents (MPS) can be inserted during each endoscopic retrograde cholangiopancreatography (ERCP) session.

A major disadvantage of plastic stents is the tendency for occlusion due to the formation of a bacterial biofilm, leading to recurrent jaundice and pruritis[8,9]. Acute cholangitis may develop in up to 20%-40% of cases requiring the need for stent exchange[10]. Stent occlusion rates and complications of acute cholangitis are lower with large stent diameters of 10 Fr and 11.5 Fr compared to 7 Fr or 8.5 Fr stents, likely due to a higher biliary flow rate[11]. However, a retrospective study of 33 patients comparing the efficacy and complications of 10 Fr biliary stents to 11.5 Fr stents for use in malignant and benign biliary tract disease found no significant differences in success rates or complications[12]. Median patency for 10 Fr plastic stents is an estimated 4-5 mo[13]. Moreover, a special therapeutic duodenoscope with a wide working channel of 4.2 mm diameter is needed to use 11.5 Fr plastic stents.

SELF-EXPANDABLE METAL STENTS

Self-expandable metal stents (SEMS) were designed with a larger luminal diameter than plastic stents to prolong stent patency and address occlusion complications faced by plastic stents[14-16]. The core structure of a SEMS comprises a 4-12 cm long tubular mesh made up of metal alloys, including stainless steel, Nitinol, Elgiloy and Platinol [17] (Figure 1B). Metal alloys allow adequate radial expansible force without compromising on flexibility inside the duct. Each metal stent is more expensive than a plastic stent. However, SEMS have a lower risk of recurrent biliary obstruction compared to plastic stents. Overall, SEMS is more cost-effective than plastic stents over time, especially when life expectancy exceeds 4 mo[18-22]. Comparisons between plastic stents and different types of SEMS are summarized in Table 1.

SEMS are constrained by an 8-8.5 Fr surrounding sheath while it is inserted through a channel in the duodenoscope. Deployment occurs when the sheath is retracted, expanding the stent to a maximal diameter of 10 mm.

Mechanisms of SEMS occlusion differ from plastic stents *via* the following: (1) Tissue in-growth through the stent mesh; (2) Tumor overgrowth around the proximal or distal end of the stent; (3) Mucosal hyperplasia into the stent because of a chronic inflammatory reaction to the stent mesh; and, less commonly; and (4) Biliary sludge [23-26]. In contrast to plastic stents, occluded SEMS with tumor or tissue in-growth or over-growth are often difficult to reposition or remove once deployed[27]. Treatment of a SEMS occlusion involves insertion of new plastic stents or deployment of another SEMS within the initial stent. Mechanical cleaning with an extraction balloon may also be helpful[28]. Additional complications of SEMS include duodenal wall erosion by the stent, which may lead to duodenal perforation and acute bleeding[29].

Table 1 Strengths and disadvantages of plastic stents and self-expandable metal stents

	Strengths	Disadvantages
Plastic stents	Easy placement, removal, and exchange; Cost-effective for use < 4 mo; Variety of shapes and sizes	Tendency for stent occlusion after several months
Uncovered self-expandable metal stents (USEMS)	Long stent patency due to large luminal diameter; Stent malposition is rare	Cost-effective for use > 4 mo; High risk for tissue ingrowth or tumor overgrowth; Risk for duodenal wall erosion; Biliary leakage possible; Difficult to reposition or remove
Partially covered self-expandable metal stents (PCSEMS)	Long stent patency due to large luminal diameter; Biliary leakage is rare	Cost-effective for use > 4 mo; Intermediate risk for tissue ingrowth or tumor overgrowth; Risk for duodenal wall erosion; Difficult to reposition or remove; Side branch obstruction possible
Fully covered self-expandable metal stents (FCSEMS)	Long stent patency due to large luminal diameter, covering inhibits tissue/tumor in-growth through mesh; No biliary leakage	Cost-effective for use > 4 mo; Risk for duodenal wall erosion; Difficult to reposition or remove; High risk of stent migration; Side branch obstruction possible

Stent designs continue to evolve to overcome these factors. Silicone, polyurethane, and expanded polytetrafluoroethylene coverings may be applied over a part (partly-covered) or entire (fully-covered) length of SEMS to prevent tumor in-growth through the mesh[27,30] (Figures 1C, 1D, and 1E). Additionally, the covering allows for easier SEMS removal compared to bare-metal stents[31].

BENIGN BILIARY STRICTURES

Benign biliary strictures (BBS) form after an initial insult to the biliary duct that leads to inflammation, collagen deposition, fibrosis, and bile duct narrowing[32]. Common causes include post-operative injury, chronic pancreatitis (CP), and chronic inflammatory cholangiopathies (Table 2)[33]. BBS may present with pain, jaundice, pruritis, and/or elevated liver function tests[34]. A combination of magnetic resonance cholangiopancreatography (MRCP) and/or endoscopic (ERCP) retrograde cholangiopancreatography may be used during the evaluation process to elucidate biliary ductal anatomy[35]. Tissue sampling *via* endoluminal biopsy or biliary brushings will confirm non-malignant tissue and determine the primary etiology. ERCP is the therapeutic modality of choice for BBS because of its efficacy, safety, and noninvasive nature[36-38].

Post-operative BBS

The most common causes of post-operative BBS occur after cholecystectomy and biliary duct surgery[39,40]. An effective means of therapeutic decompression using plastic stents can be achieved with the placement of MPS side-by-side across the stricture[41]. Plastic stents used for up to 1 year with interval replacement in 3-4 mo intervals have demonstrated excellent safety, high clinical success rates, and low rates of stricture recurrence[42]. Plastic stent occlusion within 4-5 mo requiring endoscopic stent exchange is a major limitation because of overall cost and requirement for patient compliance[13,43].

In contrast, FCSEMS can expand to a large stent diameter equivalent to three 10 Fr stents, and its chemical covering limits tissue hyperplasia and in-growth. A single FCSEMS can remain in place for a prolonged period without the need for repeated stent exchange prior to removal[44,45].

In a retrospective study of 69 patients comparing the efficacy and safety of FCSEMS and plastic stents for postsurgical BBS, findings revealed a similar technical success rate (100% in both groups) and stricture recurrence (16% in the PS group and 22% in the FCSEMS group)[46]. However, the median duration of stenting in the FCSEMS group (5.2 mo) was significantly lower than that of the PS group (10.7 mo), requiring multiple stent exchanges. In fact, the FCSEMS group had a shorter total operation and fluoroscopy time because only one metal stent needed to be deployed compared to 2-5 plastic stents in the PS group. The study highlighted the use of FCSEMS as a practical and effective alternative means to the endoscopic treatment of post-surgical BBS with plastic stents.

A prospective, nonrandomized study of 18 patients also evaluated the 6-12 mo temporary use of FCSEMS for symptomatic post-cholecystectomy BBS, including a 5 year follow-up time period[47]. Successful FCSEMS placement deployment and

Table 2 Etiology of benign biliary strictures

Extrinsic	Intrinsic
Chronic pancreatitis	Post-operative (*i.e.*, post-liver transplantation and post-cholecystectomy)
Pancreatic fluid collection	Primary sclerosing cholangitis
Cholecystitis	IgG Cholangiopathy

IgG: Immunoglobin G.

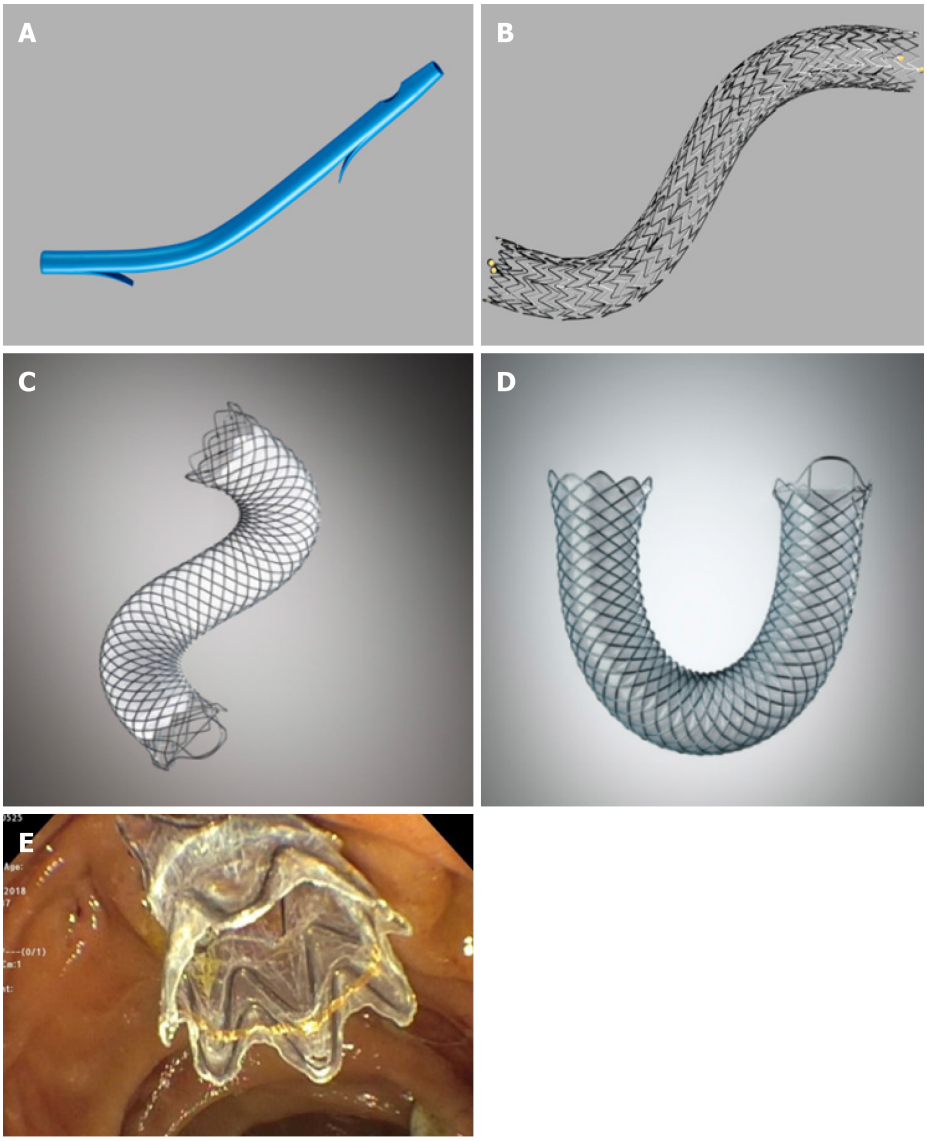

Figure 1 Various types of plastic and metal stents for endoscopic treatment. A: Plastic biliary stent (10 Fr with flaps); B: Uncovered metal biliary stent; C: Partially covered metal biliary stent; D: Fully covered self-expandable metal biliary stent; E: Fully covered self-expandable metal biliary stent with anti-migration fins (Images B-D courtesy of Boston Scientific Corporation, Boston, United States).

removal was achieved in 83% of patients with a median indwell time of 11 mo. As well, 72% of patients achieved stricture resolution at the end of the FCSEMS indwell period. Kaplan Meier analysis predicted 61% probability of remaining stent-free among patients who received FCSEMS. Complications included spontaneous stent migration (17%) and stent-related adverse events, including cholangitis (33%) and pancreatitis (6%). Despite a large sample size and absence of a control group, the study showcased that temporary 1-year placement of FCSEMS can maintain long-term stricture resolution. Table 3 highlights differences in study outcomes between plastic stents and FCSEMS for use in post-operative BBS.

Table 3 Comparison of study outcomes of plastic stents and fully covered self-expandable metal stents for treatment of post-operative benign biliary strictures

Ref.	Methods	Patients	Stent placement	Followup time(mo)	Clinical success[1] (%)	Recurrence (%)
Bergman *et al*[42]	Retrospective, single center	57	Two 10F plastic stents	24	77	20
Costamagna *et al*[41]	Retrospective, single center	154	Multiple plastic stents	108	96.7	12
Chaput *et al*[44]	Retrospective, single center	92	FCSEMS	12	84.9	21.9
Tringali *et al*[47]	Prospective, mult-center	187	FCSEMS	60	83.3	15.4

[1]Clinical success defined as removal of stent for resolution of benign biliary stricture. FCSEMS: Fully covered self-expandable metal stents.

Anastomotic biliary strictures from orthostatic liver transplantation

Anastomotic biliary strictures (AS) are responsible for nearly 80% of biliary strictures after orthostatic liver transplantation (OLT)[48,49]. They typically occur within 1-2 mo of surgery, and are characterized as single BBS short in length. Existing strategies to treat AS associated BBS include balloon dilatation and endoscopic stenting[50]. While the use of MPS is the gold standard similar to the treatment post-cholecystectomy BBS, premature stent occlusion is a major limitation[51-53].

In the only meta-analysis to date comparing FCSEMS to plastic stents in the use of post-OLT AS, there was no significant difference in stricture resolution, complications, and recurrence[54]. However, FCSEMS use was associated with reduced treatment time (mean difference of -3.58 mo). Complications of pancreatitis and post-procedural pain were more common in FCSEMS. While prior studies have identified FCSEMS with stent migration rates as high as 33%, this adverse event could not be compared in the metanalysis because it was inconsistently reported across the 7 included studies [55,56].

Novel FCSEMS designs with antimigration properties have been developed to address shortcomings with stent migration. A retrospective multicenter Australian study evaluated the use of a novel FCSEMS in 36 patients for treatment of AS after OLT. Some of the patients within the cohort had failed the MPS approach[57]. The Niti-S FCSEMS biliary stent (Taewoong Medical Co Ltd., South Korea) used had an antimigration waist, short stent length, and a removable string for easy removal. Average duration of indwell treatment time was 3.8 mo. Stricture resolution was achieved in 100% of patients and all stents were removed without any difficulty. Only 2.8% of patients experienced stent migration. Nearly 75% of patients remained free of AS recurrence. Excellent clinical and technical success in this study with low rates of stent migration was a major breakthrough in the use of FCSEMS with anti-migration features for AS.

A more recent British retrospective study also evaluated the same type of antimigration FCSEMS (Taewoong Medical Co Ltd., South Korea) in a cohort of 62 patients[58]. Approximately 96% of patients had immediate stricture resolution after 12 wk indwell time. Furthermore, 72% of patients continued to maintain long-term resolution of the AS with a mean follow up period of 548 d. No stent migration occurred in any of the patients. Complication rates were low at 15%, and were primarily stent and procedure-related, such as pancreatitis, cholangitis and wire-guided perforation.

Overall, study findings using FCSEMS with new antimigration designs have shown promise in their application for post-OLT AS treatment. Larger cohort and randomized controlled studies should compare these novel FCSEMS to plastic stents. Alternative antimigration designs should be considered to maximize safe indwell time of stents.

CP

CP-associated BBS are a consequence of recurrent inflammation and subsequent fibrosis. Stricture formation occurs in up to 30% to 40% of cases[59]. Endoscopic intervention is focused on draining the main pancreatic duct to provide pancreatic decompression[60]. Historically, the strictures were treated *via* balloon dilation and endoscopic plastic stent placement, requiring up to 3 or more ERCP sessions[61].

Current recommendations from the Asia-Pacific guidelines recommend the use of FCSEMS or MPS for the endoscopic treatment of CP-associated BBS[62]. To date, the WallFlex Biliary RX Fully Covered Stent (Boston Scientific, United States) is the only FCSEMS approved by the United States Food and Drug Administration for treatment of BBS due to CP for indwell time up to 12 mo[63].

Existing retrospective and case series studies have supported the use of FCSEMS for the treatment of refractory pancreatic duct strictures: clinical and technical success rates have been as high as 90%-100% with a shorter indwell time compared to plastic stents[64-66]. The largest and longest prospective study to date investigated long-term outcomes after 10-12 mo indwell of a single FCSEMS for CP-associated BBS[67]. The cohort consisted of 118 patients, most of whom had failed previous treated with plastic stents. Patients were regularly followed for 5 years after FCSEMS removal. Approximately 80% of patients had stricture resolution at the time of stent removal. Nearly 62% of patients remained stent-free following 75 mo of FCSEMS placement. The success rate was relatively high compared to traditional outcomes with a single plastic stent, which faces issues of short occlusion time[68,69]. In fact, 78% of patients who had a resolved BBS post-indwell continued to remain stent free for 5 years. Of note, 23% of patients experienced serious adverse events that were mostly stent-related, including cholangitis, pancreatitis, and cholecystitis. This study was important in further demonstrating the single-use application of FCSEMS for CP BBS with long term stricture-free durability. This is notable in CP BBS, which notoriously has a poor response to endoscopic intervention with high risks of recurrence.

MALIGNANT BILIARY STRICTURES

Malignant biliary strictures (MBS) are most commonly caused by pancreatic cancer, but can also be caused by cholangiocarcinoma and metastatic disease[70,71]. Clinical presentation is consistent with biliary duct obstruction, including jaundice and cholestatic pattern of transaminase elevation. While MBS shares the same diagnostic evaluation as BBS with MRCP and/or ERCP modalities, tissue sampling will be the differentiating factor with findings of malignancy. Prognosis is poor 5-year survival rates for pancreatic cancer and cholangiocarcinoma are 8% and 10% respectively[72,73].

Non-hilar MBS

Among the 30% of MBS cases which are surgically resectable, endoscopic intervention may be considered to provide preoperative biliary decompression (PBD) (Figures 2A and 2B). PBD decompression is an area of great controversy because of the risks for serious complications, including pancreatitis and cholangitis. A landmark randomized control trial of 202 patients with a pancreatic head cancer compared the outcomes of preoperative biliary drainage for 5 wk to 6 wk preceding surgery to surgery alone. The biliary drainage group had significantly higher (74%) rates of complications than the surgery alone group (39%). Mortality or hospital length of stay did not differ between both groups[74]. Metanalyses investigating the benefits of preoperative biliary drainage has found similar results–length of hospital stay and mortality rate are comparable in preoperative biliary drainage and surgery alone groups[75,76]. Currently, preoperative biliary drainage is indicated for patients with MBS with concurrent severe symptomology (*i.e.*, severe pruritis, cholangitis), elevated bilirubin > 250 μmol/L, or patients undergoing neoadjuvant chemotherapy[77,78].

Nearly 70% of MBS are surgically unresectable at the initial presentation[79]. Endoscopic palliative stent placement for symptom relief is one approach to management. SEMS placement is preferred over plastic stents, especially when life expectancy is greater than 4-6 mo – SEMS have lower rates of stent dysfunction, reintervention, and cholangitis, and higher survival and stent patency rates[80-82].

Outcomes of FCSEMS compared to uncovered self-expandable metal stents (USEMS) in patients with unresectable MBS have also been extensively studied. A randomized controlled trial (RCT) of 749 patients with MBS investigated the outcomes of FCSMES *vs* USEMS placement. No significant difference was found between both groups for patency or survival rates[83]. Tumor ingrowth was higher in the USEMS group compared to the FCSEMS group. However, patients in the FCSEMS were more prone to stent migration and acute pancreatitis. Another recent RCT compared the use of FCSEMS to USEMS in 158 patients with nonresectable MBS. Surprisingly, median stent patency was lower for the FCSEMS (240 d) than the USEMS (541 d) group[84]. Consistent with the other study, FCSEMS had a significantly higher rate of migration,

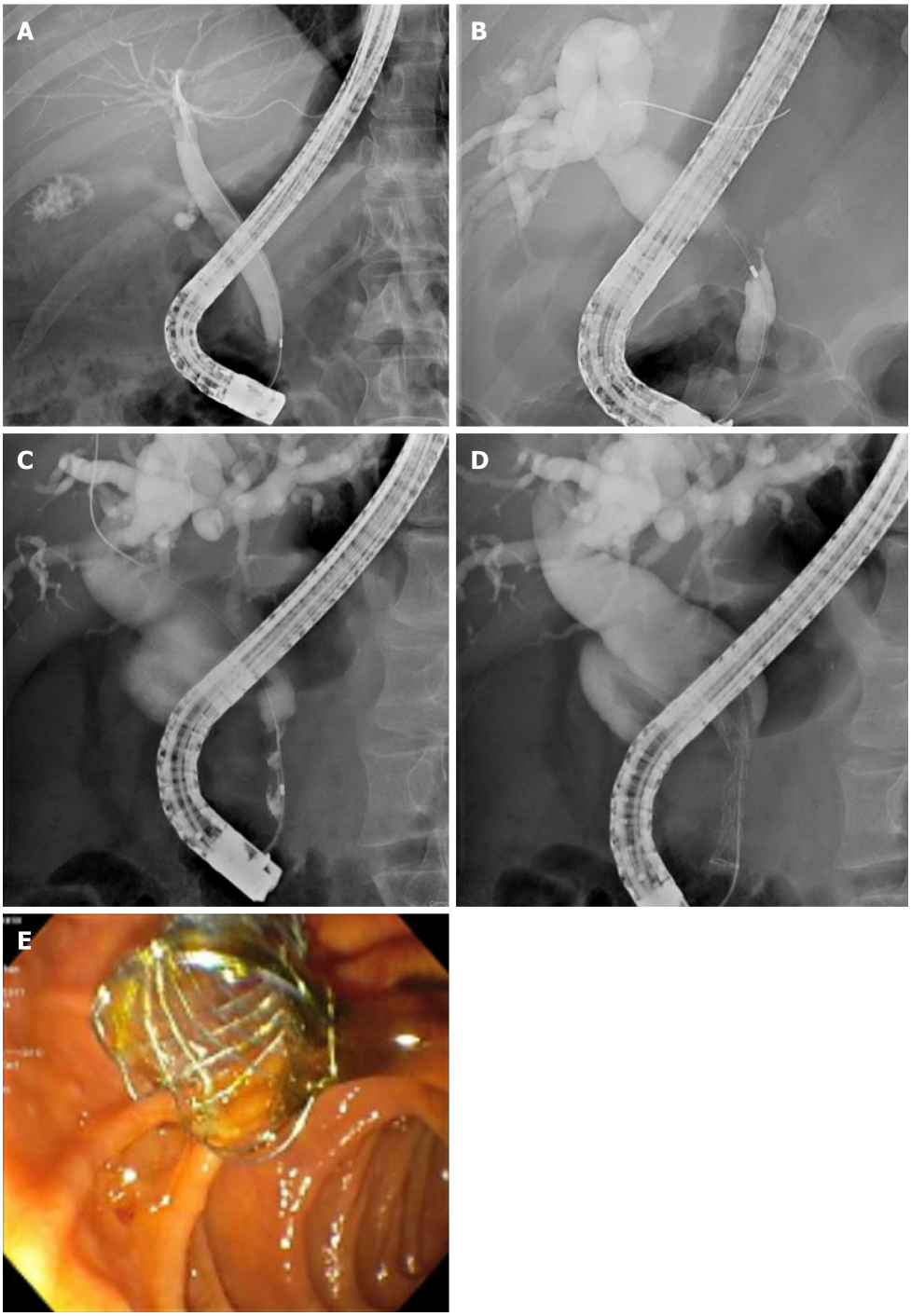

Figure 2 Endoscopic retrograde cholangiopancreatography showing cholangiocarcinoma in mid and distal common bile duct compared to normal anatomy. A: Normal anatomy demomonstrating patent cystic, common bile, and intrahepatic ducts; B: Mid common bile duct biliary stricture with dilated common bile duct and intrahepatic ducts; C: Distal common bile duct biliary stricture with dilated common bile duct and intrahepaptic ducts; D: Fully covered self-expandable metal stent (FCSEMS) placement in the common bile duct; E: Endoscopic view of FCSEMS placed in the distal common bile duct.

with no difference in overall survival between both groups[84]. To explain the stent patency finding in the FCSEMS group, the study proposed that occlusion may not be due to tumor ingrowth per se. The specific NitiS FCSEMS stent chosen for the study may promote an inflammatory reaction causing tissue hyperplasia. Thus, this finding may not be generalizable to all FCSEMS.

Metanalyses have also investigated this topic with mixed results, including studies with partly covered SEMS. A metanalysis involving 5 RCTs with 781 patients showed that CSEMS had superior patency rates compared with UCSEMS for MBS[85]. However, another metanalysis involving 9 RCTs with 1061 patients found no difference in stent patency between both groups[86].

Overall, there is no consensus regarding the superiority of FCSEMS or USEMS for palliative decompression of MBS. FCSEMS may be preferred for ease of removal or in circumstances where tumor ingrowth risk is high, such as in pancreatic cancer or cholangiocarcinoma. However, the problem of stent migration should be factored into the management decision. UCSEMS should be considered when there is short survival time or where likelihood of tumor in growth is low.

Hilar MBS

Malignant Hilar Strictures (MHS) are most commonly unresectable at presentation[87] (Figure 2C). MHS are classified by the Bismuth-Corlette classification, which accounts for the extent of involvement of the hilum and/or left or right main hepatic ducts[88]. The goal of management is to provide palliative decompression to improve quality of life, reduce jaundice, and decrease risk of cholangitis[89]. As with other MBS, metal stents are preferred over plastic ones because of superior patient survival, drainage rate, and stent patency[90]. Stent placement in the hilar region is technically difficult, and can be managed by unilateral or bilateral stenting (Figures 2D and 2E). Bilateral stenting can be approached *via* stent in stent or side by side approach[91].

A RCT involving 159 patients with malignant hilar biliary obstruction showed that patients with unilateral stenting (88.6%) had significantly higher rate of technical success than bilateral stenting (76.9%)[92]. Additionally, the bilateral stent group (16.6%) had significantly higher rates of early cholangitis. Study findings suggested that unilateral drainage was superior to bilateral stenting with lower risk of adverse complications. However, a more recent multicenter RCT found no significant difference in the technical success rate between bilateral (95.5%) and unilateral groups (100%). The median stent patency was higher in the bilateral group (252 d) than the unilateral (139 d), with fewer reinterventions needed for the bilateral group[93]. A metanalysis with 1292 patients found higher success rate in the unilateral group (97%) than the bilateral group (89%)[94]. Other retrospective studies have found comparable rates of technical success between both groups[95-98]. Overall, there is no clear consensus whether the unilateral or bilateral approach is superior. Unilateral approach may be preferred given the technical difficulty of bilateral placement.

Y-shaped bilateral SEMS devices have also been designed, which offers an effective and safe approach for unresectable MHS[98,99]. However, they are yet to be practiced routinely across endoscopic centers.

BILIARY LEAK

Biliary leak occurs due to traumatic injury or iatrogenic injury to the biliary duct from cholecystectomy, ERCP, liver resection, or orthostatic liver transplantation[100-103]. Biliary leak tends to present within 1 wk postoperatively, and are commonly located at the end of the cystic duct stump or duct of Luschka[104]. Minor bile duct leaks are characterized by low drain output and self-resolve without intervention[105]. In contrast, complex bile leaks are characterized by the persistent biliary discharge from an open T-tube or drain[106,107]. Standard of care involves plastic stent placement with or without sphincterotomy to reduce transpapillary pressure gradient[108]. As a result, bile drainage will preferentially drain into the duodenum and bypass the site of bile leak. Complex bile leaks commonly occur in anastomotic bile leaks following orthostatic liver transplantation or cholecystectomy, and may be refractory to plastic stent intervention[103,109]. FCSEMS have been investigated for use in these situations because of the larger expansile diameter and membrane coating, which theoretically provides an effective seal at the site of the leak (Figures 3A and 3B).

In a retrospective study investigating patients with complex post-OLT bile leaks treated with CSEMS placement, successful deployment and long-term leakage control was achieved using FCSEMS with fins (77.8% of patients) and FCSEMS with flared ends (70%)[110]. A concerning finding was that large number of patients who received FCSEMS with fins (35%) experienced post-stent removal biliary strictures. This likely occurred due to stent-induced biliary duct compression and ischemia, which highlights one of the major risks of FCSEMS use for biliary leak.

Another US-based study evaluated the use of Viabil FCSEMS (Gore, United States) placement for 5 patients with bile leaks[111]. All patients had successfully sealed bile leaks confirmed with cholangiogram. Most patients did not have any complications. However, 1 patient developed a hepatic abscess and sepsis. Another patient had stent occlusion after being lost to followup for 8.5 mo, but post-ERCP removal of the occluded stent revealed a sealed bile leak on cholangiogram.

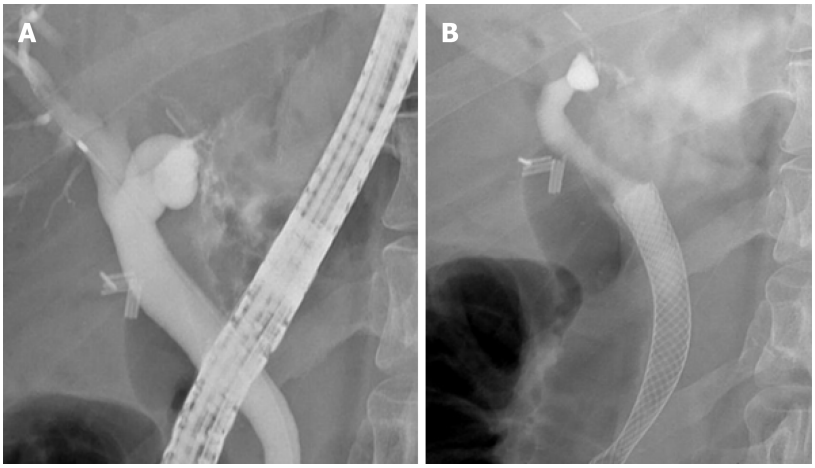

Figure 3 Endoscopic treatment of biliary leak after donor liver transplantation. A: Extravasation of contrast at site of biliary leak; B: Resolution of biliary leak after placement of a fully covered self-expandable metal stent.

A pilot study consisted of 16 patients who underwent CSEMS placement for a biliary leak. These patients either failed to respond to conventional ERCP or had severe comorbidities that prevented multiple procedures[112]. Nearly all (15/16 patients) of the patients had complete resolution of the leak confirmed with cholangiogram. Only 1 proximal and distal migration complication was documented.

In summary, existing studies have demonstrated proof of successful bile leak closure using FCSEMS (Table 4). However, complications, including stent migration and post-removal strictures, make the widespread use of FCSEMS for complex biliary leaks debatable. Future studies should investigate this topic using larger cohort sizes.

POST-SPHINCTEROTOMY BLEEDING

Biliary endoscopic sphincterotomy (ES) refers to the resection of the biliary sphincter and intraduodenal segment of the common bile duct. It is an essential procedure for ERCP in the treatment for a variety of biliary and papillary disease, including choledocholithiasis, bile leak, and biliary strictures[113]. Post-endoscopic bleeding is a major complication of biliary sphincterotomy[114]. Most post-ES bleeds are small and resolve spontaneously[115]. Other cases present with significant bleeding requiring blood transfusion support and urgent endoscopic intervention. While existing methods include injection, balloon tamponade, and mechanical (*e.g.*, embolization) approaches, endoscopic hemostasis can also be achieved by tamponade effect *via* FCSEMS deployment[113].

The largest retrospective study to date evaluated 67 patients with post-ES bleeding, and compared the use of FCSEMS treatment *vs* non-FCSEMS treatment (*e.g.*, balloon tamponade, sclerotherapy, epinephrine injection, thermal cautery or clip placement) [116]. At 72 h post intervention, the study found that the FCSEMS group (0.66 g/dL) had a significantly lower bleeding rate than the non-FCSEMS group (1.98 g/dL) even though the proportion of patients determined to be at high risk for post-ES bleeding was higher in the FCSEMS treatment group (40%) *vs* non-FCSEMS treatment group (9%). All patients in the FCSEMS group achieved hemostasis, and no adverse events were documented. The study findings supported the use of FCSEMS in patients with severe or immediate bleeding not responsive to initial endoscopic treatment.

Smaller-sized retrospective and case studies have demonstrated similar success with complete hemostasis using FCSEMS for difficult-control post-ES bleeding[117,118]. No studies to date have investigated the use of FCSEMS as primary post-ES treatment therapy. Larger perspective and randomized controlled studies should be conducted to evaluate the optimal timing for FCSEMS removal after achieving hemostasis.

COMPLICATIONS

Stent migration is the most frequent complication of FCSEMS, which occurs in 20%-40% of cases[119,120]. Proximal stent migration refers to FCSEMS migration into the

Table 4 Comparison of study outcomes usng fully covered self-expanding metal stents for treatment of biliary leak

Ref.	Methods	Patients	Type, duration of stent placement (mo)	Follow-up time (mo)	Clinical success rate[1] (%)
Martins *et al* [110]	Restrospective, single center	31	PCSEMS (3), FCSEMS with fins (3.3), FCSEMS with flare ends (3)	PCSEMS (44), FCSEMS with fins (27), FCSEMS with flare ends (6.6)	PCSEMS (100), FCSEMS with fins (77.8), FCSEMS with flare ends (70)
Lalezari *et al*[111]	Retrospective, single center	5	FCSEMS with fins (3)	26	100
Kahaleh *et al*[112]	Retrospective single, center	16	FCSEMS (3)	9	94

[1]Clinical success rate defined as resolution of biliary leak confirmed with imaging. PCSEMS: Partially covered self-expandable metal stents; FCSEMS: Fully covered self-expandable metal stents.

biliary duct, while distal stent migration refers to migration to the duodenum or bowel. Endoscopic intervention is needed to remove and replace the displaced stent. A prospective study comparing the temporary use of FCSEMS with unflared ends to flared ends in patients with CP-related BBS revealed the migration rate was 100% for unflared FCSEMS compared to 40% of flared FCSEMS[121]. FCSEMS designed with anchoring flaps or flared ends are preferred as they reduce the risk of migration. A multicenter, prospective study of compared the antimigration effects and efficacy of FCSEMS with an anchoring flap to a flared end at the proximal end. After a median followup time of 6 mo, none of the patients in the anchoring flap had stent migration compared to 33% of patients in the flared end, suggesting that the anchoring flap design was superior to the flared end design. Both types of FCSEMS were able to be removed in all of the patients[122]. Overall, new stent designs including anchoring flaps, flared ends, and additional anchoring plastic stents have been validated in studies to reduce migration risk[123-125].

Cholecystitis is another complication associated with FCSEMS use most commonly in MBS. The pathophysiological mechanism is thought to be obstruction of biliary flow if the cystic duct orifice is obstructed by the synthetic covering of the FCSEMS[126, 127].

Stent occlusion may occur from tumor ingrowth, tissue overgrowth, or sludge, predisposing to the development of cholangitis[23-26]. However, time to and rate of occlusion is considerably less compared to plastic stents[128]. Factors such as FCSEMS location and treatment indication dictate different levels of risk[129].

CONCLUSION

FCSEMS represents the latest development of SEMS used by endoscopists treat a variety of benign and malignant biliary pathology, including biliary leak, post-sphincterotomy bleeding, and strictures. A chemical covering reduces the risk of tissue hyperplasia and tumor ingrowth, prolonging stent patency. The studies examined in our review have shown that FCSEMS can be clinically effective for a variety of endoscopic applications. However, FCSEMS commonly face problems of stent migration, which can be addressed with modifications including flared ends and anchoring flaps. Future areas of research will continue to expand the interventional applications using FCSEMS, and design new modifications to reduce complications.

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REVIEW

Intraoperative use of indocyanine green fluorescence imaging in rectal cancer surgery: The state of the art

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Abstract

Indocyanine green (ICG) fluorescence imaging is widely used in abdominal surgery. The implementation of minimally invasive rectal surgery using new methods like robotics or a transanal approach required improvement of optical systems. In that setting, ICG fluorescence optimizes intraoperative vision of anatomical structures by improving blood and lymphatic flow. The purpose of this review was to summarize all potential applications of this upcoming technology in rectal cancer surgery. Each type of use has been separately addressed and the evidence was investigated. During rectal resection, ICG fluorescence angiography is mainly used to evaluate the perfusion of the colonic stump in order to reduce the risk of anastomotic leaks. In addition, ICG fluorescence imaging allows easy visualization of organs such as the ureter or urethra to protect them from injury. This intraoperative technology is a valuable tool for conducting lymph node dissection along the iliac lymphatic chain or to better identifying the rectal dissection planes when a transanal approach is performed. This is an overview of the applications of ICG fluorescence imaging in current surgical practice and a synthesis of the results obtained from the literature. Although further studies are need to investigate the real clinical benefits, these findings may enhance use of ICG fluorescence in current clinical practice and stimulate future research on new applications.

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Core Tip: There is growing interest in real-time fluorescence-guided surgery. The intraoperative use of indocyanine green (ICG) during rectal cancer surgery has found many applications over time. Given the wide availability in current practice, it is important for clinicians to be aware of all potential uses of ICG fluorescence technology in order to facilitate the procedures, limit injuries, and improve outcomes. Herein, we provide a concise overview of the literature regarding the use of ICG fluorescence imaging in this setting.

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INTRODUCTION

Colorectal carcinoma is the third most common cancer for both men and women and the second leading cause of cancer-related deaths[1]. Primary rectal localization occurs in 35% of cases[2]. Although multidisciplinary management of rectal cancer is the standard of care, surgery remains the cornerstone of curative treatment. Total mesorectal excision (TME), described for the first time by Heald *et al*[3,4], should now be performed routinely in cases of middle and lower rectal tumors after neoadjuvant therapy for locally advanced cancer (T3-4 and/or N+)[5]. Respect for the principles of surgical oncology, including complete TME, and negative distal and circumferential resection margins is mandatory to achieve improved survival. Also, much effort has been made to achieve faster and enhanced recovery[6,7] and better quality of life after rectal cancer surgery[8] over time. Advances in oncology research[9] have resulted in increasing roles for the latest technologies in surgery. Enhanced video/camera systems [10], robotic technology[11], powered staplers[12], and specialized operating platforms for natural orifice transluminal endoscopic surgery[13] are just a few examples of surgical innovations in colorectal surgery introduced in the last decades. In this setting, laser fluorescence using indocyanine green (ICG) dye is a promising and widespread real-time technology because of its easy accessibility, accuracy and cost-effectiveness.

The concept of ICG intraoperative angiography is based on the ability of ICG to absorb near-infrared light (NIR) at 800 nm and to emit fluorescence at a wavelength of 830 nm. Albumin is the most important intravascular binding-protein for ICG so that tissue microperfusion is revealed by the presence of fluorescence. In brief, a bolus of ICG is injected into the patient intravenously, NIR light is then absorbed by ICG in the tissue, and the resulting fluorescence is a reflection of perfusion. After the introduction of ICG angiography in clinical practice in 1989 to evaluate choroidal circulation[14], fluorescence imaging technology has been used in hepatobiliary[15,16], gastric cancer [17], gynecologic cancer[18], breast cancer[19] and transplantation surgery[20]. Furthermore, many colorectal surgeons routinely use ICG imaging to assess bowel viability during colorectal anastomosis. However, several applications of ICG angiography have been described in colorectal surgery with increasing interest for rectal cancer resection. In fact, the implementation of minimally invasive rectal surgery using new approaches (*e.g.*, robotic, and transanal) required the improvement of optical systems. In that regard, ICG fluorescence has optimized the intraoperative vision of anatomical structures by the enhancement of blood and lymph flow. The aim of this review is to identify and synthesize data from original research evaluating any possible application of ICG fluorescence imaging in rectal cancer surgery. In the last 5 years, the use of ICG fluorescence in rectal cancer surgery has attracted great interest.

The items regarding different intraoperative applications are summarized below.

ASSESSMENT OF VASCULAR PERFUSION AT THE ANASTOMOTIC SITE

Anastomotic leakage (AL) is the most feared complication after TME because it is associated with increased mortality, reoperation, and definitive stoma formation[21,22]. Additionally, the relationship between AL and local recurrence has been found to lead to significant differences in long-term outcomes[23]. It has a significant impact on postoperative functional outcome[24] and increases the economic burden of public health systems[25]. That easily explains the importance of developing prevention strategies in order to reduce the amount of related complications affecting survival and quality of life. The AL rate after anterior rectal resection ranges from 3% to 23%[26,27]. AL is defined by the International Study Group of Rectal cancer as a defect at the level of the anastomotic site that allows communication between the intraluminal and extraluminal compartments identified through clinical evaluation with digital rectal exploration, endoscopic examination, computed tomography radiological evidence of contrast leakage through the suture gap or the presence of perianastomotic hydro-aerial collection. It is also classified as grade A if it does not affect the postoperative course, grade B when conservative management antibiotic therapy or percutaneous/transanal drainage is required, and grace C when surgical revision is required[26,28].

Various risk factors are associated with AL, but insufficient blood perfusion is generally considered as one of the main causes[29,30]. ICG is a water soluble, tricarbocyanine dye that provides real-time visualization of vascular structures by emitting fluorescence when stimulated by polarized light[31]. The evaluation of perfusion of the supposed proximal section line starts within 60 s of an intravenous bolus injection of ICG. An NIR camera detects the fluorescence of the microcirculation of the colonic wall receiving adequate vascularization. Therefore, ICG fluorescence reflects the colonic stump perfusion allowing the choice of the most appropriate site for the final section and then for the anastomosis (Figure 1). A second check can be also performed after the anastomosis construction both by injecting a second bolus of ICG to verify fluorescence of the stumps and by transanal endoscopic suture line visualization. Some limitations of the technique are related to its being, once again, a subjective evaluation by the surgeon of the intensity of the emitted infrared light. Furthermore, it is not a completely standardized technique as there are variables related to the dose of injected ICG (0.013-0.89 mg/kg), the proximity of the laparoscope to the colic wall, the number and type of checks performed, and differences in the equipment available on the market[32-34].

Kudszus was the first to show the usefulness of ICG fluorescence angiography (FA) in colorectal surgery[35]. The study reported that ICG FA led to a change in the location of the planned proximal resection line in 13.9% of patients. ICG FA significantly reduced AL by 4% compared with the control group. Furthermore, Jafari *et al*[36] assessed the utility of ICG FA in left colectomy and anterior resection in the PILLAR II study. The incidence of AL in their study was 1.4%, with a change in the surgical strategy in 8% of patients. Boni *et al*[37] reported that ICG FA could be safely and effectively performed in rectal surgery. The use of IGC-FA changed the surgical plan in 4.7% of their patients and AL did not occur in the ICG group, compared with an incidence of 5.2% in the control group. However, the differences were not statistically significant. The studies agree on the safety and feasibility of the technique and demonstrate its usefulness in the assessment of tissue perfusion.

Several comparative retrospective and prospective studies investigated the relationship between the use of ICG and the 30-d AL rate as the primary outcome[31,33,38-40]. They showed a statistically significant correlation between ICG FA and reduction in the risk of AL associated with the modification of the proximal section line. In a propensity score-matched analysis[41], AL rates of Clavien-Dindo grade ≥ II and ≥ III were 10.4% (22/211) and 9.5% (20/211) in the non-ICG FA group and 4.7% (10/211) and 2.8% (6/211) in the ICG FA group, respectively. Similarly, Foo *et al*[42] demonstrated that the use of ICG FA was significantly associated with a lower AL rate in TME (4.7% *vs* 11.6%; P = 0.043) but not non-TME resections. However, not all studies agree on the results. Two propensity score-matched studies failed to demonstrate a statistically significant difference in the rate of AL between the two compared groups, although both reported a change in the proximal section after ICG injection of 27.1% and 18.18%, respectively[43,44]. Most of the studies had limitations such as a small sample size or their retrospective nature. Only two randomized

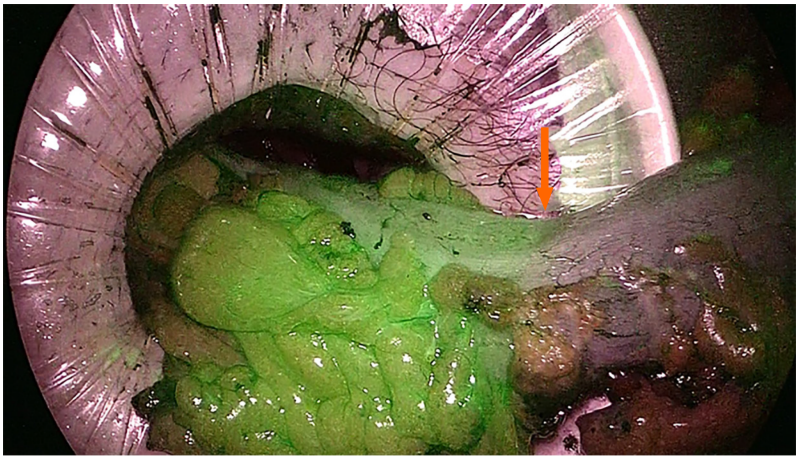

Figure 1 Evaluation of intestinal perfusion after intravenous injection of indocyanine green during total mesorectal excision allows identification of the section line before anastomosis construction (orange arrow).

controlled trials (RCTs) were published. In the first one[45], differences in AL or reoperation rates between ICG and the control group (5% *vs* 9% and 6.7% *vs* 6.5%) were not significant. Although the authors confirmed the efficacy of ICG in bowel viability assessment during left colectomy or anterior resection, a real advantage related to AL was not demonstrated. The FLAG trial[46] involved 377 patients who underwent anterior rectal resection, 187 in the ICG FA group and 190 in the control group. The results showed that changes in the transection line were performed in almost 20% of patients. A decrease in AL was achieved using ICG FA, but the difference was statistically significant only in cases with low rectal anastomosis (14.4% with ICG FA *vs* 25.7% without ICG FA; $P = 0.04$). Two recent meta-analyses assessed the role of ICG FA imaging on the incidence of AL after rectal cancer surgery[34,47]. In a pooled analysis of 2088 patients, the AL rate in the ICG group was significantly lower than that in the control group, and the intraoperative use of ICG was associated with a decreased overall complication rate and reduced reoperation rate. However, both analyses suffer from the same limitations as the studies taken into consideration and the lack of RCTs in the analysis.

Finally, evaluation of bowel perfusion by ICG FA was reported exclusively after transanal total mesorectal excision (TaTME) by Mizrahi *et al*[48]. In their retrospective cohort of 54 patients who received a very low anastomosis, a check was performed before proximal transection and at the completion of anastomosis. In 18.5% of the cases the surgeon changed the proximal resection margin because of impaired fluorescence. All those anastomoses were shown to be successful at the second control. Two patients (3.7%) suffered from AL, and in neither of them was the splenic flexure mobilized. Furthermore, ICG FA improved clinical outcomes also after robotic sphincter-saving rectal resections[49]. In conclusion, the use of fluorescent angiography with ICG injection has proven to be a safe and feasible method to evaluate bowel perfusion whatever the surgical approach for rectal resection. At the time of the construction of the anastomosis, ICG FA can influence decision making by reconsidering the resection line. Several studies have found a significant decrease in the rate of AL after ICG FA imaging, and that has had a large impact on recovery. Future high quality trials should confirm the impact on AL rates and standardize the technique.

URETHRA VISUALIZATION DURING TRANSANAL TME

TaTME is a relatively new procedure[50] for the curative resection of rectal tumors. It was developed to overcome the difficult dissection at the lower third of the rectum, especially in obese male patients and/or bulky tumors. Some retrospective series have reported that enhanced visualization of the dissection plane allowed better nerve preservation, improved resection margins, and improved functional outcomes compared with laparoscopic TME[51,52]. However, the bottom-up transanal approach is not without complications. Incidence of iatrogenic urethral injuries has been reported, ranging from 1% to 6.7% during TaTME procedures[53]. An international inquiry reported 34 urethral injuries from 32 surgical teams worldwide between 2010

and 2017, resulting in a significant postoperative morbidity rate of 26%[50]. However, there is still concern that urologic injuries during TaTME may be underreported and that their incidence might be related to surgeon experience. Therefore, enhancement of urethral visualization should be considered useful and advantageous in the early learning experience. Several bioimaging modalities exist that can improve urethral identification, including ICG NIR fluorescence[54]. Different systems have been successfully used to detect the urethra by ICG fluorescence imaging such as the IRIS ureteral kit (Stryker, Kalamazoo, MI, United States)[55] or the PINPOINT laparoscopic system (Stryker, United States)[56] with intraurethral ICG injection or infiltration adjacent to the catheter in the urethra, respectively. Experimental studies demonstrated that direct ICG instillation into the urethra or through a urine catheter for NIR fluorescence imaging seem to be easily applicable and clinically reproducible during TaTME[55-57]. Although an open-label clinical feasibility study (NCT03204201) with intraoperative direct instillation of ICG into the urethra for low rectal cancers was terminated because of technique failures, implementation of the technique has been described by some authors. Barnes *et al*[58] evaluated the efficacy of two novel methods in cadaveric models. In the first, ICG mixed with silicone was infiltrated into 10-Fr one-way Foley catheter and allowed to set for 1 wk. In the second, new preclinical IRDye 800BK (LI-COR Biosciences®, Lincoln, Nebraska, United States) was infiltrated directly into the urethra *via* the urethral meatus prior to dissection. Both methods were effective in identifying the fluorescence located only within the urethra. IRDye 800BK provided a greater depth of penetration than the ICG-silicone mix, suggesting it could be a more satisfactory alternative to ICG. In addition, Barberio *et al*[59] demonstrated the superior brightness of near-infrared coating of equipment (NICE) coated catheter compared with ICG-based solutions in cadaveric experiments by exhibiting a higher fluorescence intensity than urinary catheters filled with ICG. In conclusion, no final specific recommendations can be drawn from the clinical use of ICG fluorescence imaging to identify and prevent urethral injuries during TaTME procedures because of the very limited data available. However, future studies will have to take into account that fluorescence technology plays a major role in this setting.

URETER IDENTIFICATION

The incidence of iatrogenic urethral injuries (IUI) ranges from 0.24% to 1.95% in colorectal surgery, and rectal cancer is considered a risk factor for IUI because of the close proximity of the ureters to the dissection plane[60], similar to the risk with deep pelvic endometriosis[61,62]. Despite its low incidence, IUI significantly affects postoperative morbidity, mortality, length of stay and hospital charges[60]. Visualization of the ureters is thus advocated during pelvic surgery by the visible peristalsis that occurs when the ureter is gently pressed (Kelly's sign). However, adhesions, obesity, and an incorrect plane of dissection contribute to the lack of or incorrect recognition of the ureter, which can jeopardize its integrity. For that reason, a selective use of prophylactic urethral stents in high risk procedures is commonly accepted, but there is no sufficient evidence to support a decrease in IUI or intraoperative identification[63,64]. In that setting, interest in fluorescence imaging has been increasing over time. While contrasting results were found for intravenous administration of methylene blue dye to urethral detection in colorectal surgery[65,66], ICG FA proved to be a viable alternative to real-time ureter identification and IUI prevention. Before surgery, a 6-Fr catheter is placed into the urethral orifice by cystoscopy. As ICG binds to the proteins of the ureteric epithelium[67], a retrograde injection of 5 mg ICG diluted in 2 mL of distilled water is made, and infrared emission is captured by the filtered lens system and electronically converted into green color visualizing ureter location. The technique has proven to be safe and helpful to identify the ureter in several small case-series who underwent minimally invasive pelvic surgery[68-72]. As a catheter insertion of only 1 cm is required, there is a lower risk of IUI during catheterization than during conventional endoscopic stenting procedures, thus avoiding additional cystoscopies to remove the catheter. Furthermore, ICG urethral instillation is less expensive than other fluorescence-based systems such as illuminated catheters [68].

White *et al*[73] recently evaluated the safety and efficacy of intraurethral ICG FA along with any potential benefit related to the technique during colorectal robotic surgery. In their experience involving 16 patients, there were short procedure times, low morbidity, and reliable urethral identification and avoidance. The United States

Food and Drug Administration approval of ICG is limited to intravenous use[74]. Therefore, disclosure of intraurethral off-label use would be needed. In contrast, new intravenous fluorescent dyes with renal clearance, such as fluorescein sodium[75] and IRDye® 800-BK[76] have been used in experimental models to test the penetration of fluorescence in the ureters, with promising results for surgical practice. Additionally, the formulation of ICG in a liposome-based delivery system allows its excretion in urine in animal models and seems a promising fluorophore solution[77,78]. In conclusion, evidence supporting the use of intraurethral ICG instillation in order to improve intraoperative ureter detection is based on few noncomparative feasibility studies involving mixed pelvic surgeries. Despite the efficacy demonstrated in ordinary or complex situations, no study exclusively focused on rectal cancer resections exists to date. It remains to be proven whether this innovation significantly affects surgical procedures and provides clinical benefits by reducing IUI.

LYMPH NODE MAPPING

Lateral pelvic lymph node dissection (LLND) allows the removal of the nodal compartment along the common iliac, internal iliac, and obturator arteries. The lymphatic stations are considered a major cause of locoregional recurrence in rectal cancer and are treated with preoperative chemoradiotherapy and curative resection [79]. While it is widely accepted to perform LLND in selected patients with rectal cancer and lateral lymph nodes that are clinically positive[5], the Japanese Society for Cancer of the Colon and Rectum guidelines[80] recommend LLND even when lateral lymph node metastasis is not detected by preoperative or intraoperative diagnosis. Indeed, LLND is associated with a lower rate of local recurrence compared with TME alone despite no significant differences in either overall survival or local recurrence-free survival[81].

As ICG fluorescence imaging has proven to be a useful tool for identifying lymphatic drainage in colorectal surgery[82,83], ICG-enhanced NIR fluorescence-guided imaging has been used to improve the accuracy and the completeness of LLND. In such cases, ICG fluorescence imaging is carried out the injection of ICG dye into the submucosal layer on the distal side of the tumor through the anus immediately before surgery. In a comparative retrospective series of 42 mid and low rectal cancer patients, the ICG group experienced a significantly lower intraoperative blood loss and a larger number of harvested lateral pelvic lymph nodes[84]. The use of ICG may improve the safety of LLND that is affected by the technical difficulties of the procedure, complicated pelvic wall anatomy, and the effects of preoperative radiation on the tissues. In that setting, real-time identification of lateral pelvic nodes could help to distinguish lymphatic tissue from vascular and nervous structures, thus avoiding postoperative genitourinary dysfunction and providing better surgical staging. However, evidence is still limited[85-87] and additional studies are needed to address the real clinical advantages and standardization of this technique.

A sentinel node (SN) is defined as the first node in the regional peritumoral area that drains the tumor. SN biopsy, in addition to conventional resection, may add clinically significant prognostic information in colorectal surgery[88-90]. NIR laparoscopy with ICG mapping allowed easy intraoperative identification of mesocolic lymphatic drainage and SN during colorectal oncologic resections[91]. Similarly Noura *et al*[92] described the detection of SN by ICG with an NIR system in 25 patients who had no preoperative diagnosis of metastatic lateral pelvic lymph nodes. The success rate of detecting the lateral SN was 92%, and 100% concordance was observed between SN and dissected lateral lymph nodes status. That preliminary study highlighted the feasibility and reliability of lateral SN biopsy as a potential discriminator to perform LLND, but the sensitivity may be compromised by preoperative neoadjuvant chemoradiotherapy[93,94].

TUMOR LOCALIZATION

Several reports have described the intraoperative identification of colonic tumors by NIR with ICG fluorescence imaging, with satisfactory results[95-98]. Accurate identification of the location of colorectal tumors is crucial in minimally invasive surgery because of the lack of tactile perception, especially for cancer at an early stage because of its small size or location on a movable part of the colon. As for rectal cancer, precise tumor site localization allows achieving a clear and safe distal resection margin, which

may affect not only oncological outcomes but also bowel function and quality of life. In that setting, endoscopic tattooing of rectal tumors, both with a high-definition fluorescence imaging system (Karl Storz GmbH & Co. KG, Tuttlingen, Germany)[93] and the PINPOINT® endoscopic fluorescence imaging system (PINPOINT system; Novadaq Technologies Inc., Mississauga, ON, Canada)[99], is feasible and has clinical advantages. In a comparative retrospective series, 342 patients scheduled for laparoscopic colorectal resection were enrolled after propensity score matching[100]. The tumor was tattooed in 114 patients. In a subgroup analysis of 160 patients who underwent anterior resection, the tattooed group had a significantly shorter operative time (unlike right and left colectomy), less blood loss, and a shorter hospital stay than the non-tattooed group. In addition, Goo *et al*[101] compared 200 tattooed colorectal cancer patients (44 rectal cancers) with 879 non-tattooed patients (300 rectal cancers) to evaluate the effect of preoperative colonoscopic tattooing with ICG on adequate lymph node harvest in colorectal cancer. They found that preoperative tattooing in T1 colorectal cancer significantly improved adequate lymph node harvest, with a higher number of retrieved lymph nodes in rectal cancer than in colon cancer.

Fluorescence technology to localize rectal tumors has been developed not only in the field of imaging systems[99], but also by ICG formulation. Fenestrated peritumoral capillaries and impaired lymphatic drainage delay the washout of large molecules from tumors, which has been described as the enhanced permeability and retention effect[102]. The formulation of ICG as a liposome-based delivery system improved tumor-specific localization in experimental models, with the advantages of intravenous injection and better results than free ICG[103,104]. Finally, there are limited data on the role of ICG in the detection of peritoneal carcinomatosis of colorectal origin. Cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy is the only potentially curative option in patients with limited peritoneal metastases[105]. Intraoperative injection of ICG seems a useful tool to identify peritoneal metastases and detect additional subclinical malignant peritoneal nodules, resulting in modification of the planned surgery in 29% of patients[106]. However, further investigations are required to draw firm conclusions.

OTHER USES

Peritumoral injection of ICG may help the surgeon to perform an adequate dissection along the embryological surgical planes and visualize the relationship with surrounding structures during TaTME[107], conventional laparoscopic TME[108], and abdominoperineal resection (APR)[109]. Omentoplasty is a well-known method to fill the pelvic cavity after APR or in case of complications after rectal cancer surgery. A pilot study of the intraoperative value of NIR fluorescence imaging with ICG to assess omental perfusion after the creation of a pedicled omentoplasty found that a change in decision making occurred in 80% of the cases[110], and a positive impact on the nonhealing rates of patients undergoing salvage surgery for chronic pelvic sepsis was also observed[111].

CONCLUSION

The adoption of ICG fluorescence imaging in rectal cancer surgery and its multiple applications has increased over time. The major field of application is the evaluation of bowel perfusion at the time of anastomosis construction along with a better intraoperative identification of anatomical structures such as the ureter, urethra, lymph nodes, and tumor location. These objectives are relevant because they aim to improve patient safety by avoiding or reducing the risk of complications. However, further investigations are needed to assess the impact of intraoperative ICG fluorescence imaging on clinical, oncological and cost-effective outcomes.

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MINIREVIEWS

Transcription factors specificity protein and nuclear receptor 4A1 in pancreatic cancer

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Abstract

Specificity protein (Sp) transcription factors (TFs) Sp1, Sp3 and Sp4, and the orphan nuclear receptor 4A1 (NR4A1) are highly expressed in pancreatic tumors and Sp1 is a negative prognostic factor for pancreatic cancer patient survival. Results of knockdown and overexpression of Sp1, Sp3 and Sp4 in pancreatic and other cancer lines show that these TFs are individually pro-oncogenic factors and loss of one Sp TF is not compensated by other members. NR4A1 is also a pro-oncogenic factor and both NR4A1 and Sp TFs exhibit similar functions in pancreatic cancer cells and regulate cell growth, survival, migration and invasion. There is also evidence that Sp TFs and NR4A1 regulate some of the same genes including survivin, epidermal growth factor receptor, PAX3-FOXO1, α5- and α6-integrins, β1-, β3- and β4-integrins; this is due to NR4A1 acting as a cofactor and mediating NR4A1/Sp1/4-regulated gene expression through GC-rich gene promoter sites. Several studies show that drugs targeting Sp downregulation or NR4A1 antagonists are highly effective inhibitors of Sp/NR4A1-regulated pathways and genes in pancreatic and other cancer cells, and the triterpenoid celastrol is a novel dual-acting agent that targets both Sp TFs and NR4A1.

Key Words: Specificity protein; Nuclear receptor 4A1; Pancreatic cancer; Transcription factors; Ligand inhibitors; Nuclear receptor 4A antagonists

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Core Tip: Specificity protein (Sp), transcription factors (TFs), Sp1, Sp3 and Sp4, and nuclear receptor 4A1 (NR4A1, Nur77) are highly expressed in pancreatic cancer cells and tumors. Results of gene silencing studies show that Sp TFs and NR4A1 are pro-oncogenic and regulate pathways/genes associated with cell proliferation, survival and migration/invasion. Bis-indole derived ligands (CDIMs) that bind NR4A1 act as NR4A1 antagonists and we discuss an important mechanism of gene regulation by NR4A1/Sp complexes which can be inhibited by NR4A1 antagonists.

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INTRODUCTION

Pancreatic cancer is a devastating disease and it is estimated that in 2020, the number of diagnosed cases in the United States will be 57600 and 47050 will die because of this disease[1]. Despite advances in treating pancreatic cancer with surgical intervention and various therapeutic regimens, the five-year survival rate from pancreatic cancer is < 10%[1] and this is due, in part to the late detection of the tumors due to lack of symptoms and lack of early biomarkers of this cancer. There are many risk factors for pancreatic cancer and this includes pancreatitis, obesity and metabolic syndrome; due to the obesity crisis in many countries, it is estimated that by 2030, pancreatic cancer will be the second leading cause of cancer deaths[2]. Development of pancreatic cancer is associated with the temporal increases in expression of various oncogenes and inactivation of tumor suppressor genes resulting in enhanced cancer cell proliferation, survival, migration, invasion and metastasis[3-6]. Gemcitabine has largely replaced 5-fluorouracil as the major cytotoxic drug for treatment of pancreatic cancer and many other mechanism-based drugs targeting critical genes associated with various pro-oncogenic pathways in pancreatic cancer are being developed or are in clinical trials[7, 8]. The success of these agents in combination therapies targeting one or more pro-oncogenic pathways has been limited but promising. For example, one study using the vascular endothelial growth factor inhibitor bevacizumab in combination with leucovorin, nab-paclitaxel, oxiplatin and 5-fluorouracil and this resulted in a one-year survival of 82% for the treated patients[9]. In this review, we will focus on three transcription factors (TFs), namely specificity proteins (Sps) Sp1, Sp3 and Sp4, and nuclear receptor 4A1 (NR4A1, TR3, Nur77) and their overlapping pro-oncogenic roles in pancreatic cancers. We will also highlight an interesting convergence of these TFs and outline approaches for simultaneous targeting NR4A1 and Sp TFs by mechanism-based agents resulting in inhibition of cancer cell growth, survival, migration and invasion.

Sp and NR4A1: Background

Sp TFs are members of the Sp-Kruppel-like factor family (Sp/KLF) of TFs which includes 9 Sp TFs and 17 KLF proteins[10-14]. Sp1-Sp4 genes have a similar domain structure, however, it is evident that among other Sp/KLF family of TFs, their domain structures are highly diverse with the most common features being the zinc finger DNA binding domain and its subsequent interactions with GC/GT-rich gene promoter sequences[10-14]. Sp1 was the first TF identified and characterized[15] and Sp1 knockout mouse embryos exhibited retarded development and other abnormalities, and embryolethality is observed (day 11)[11]. Comparable studies on other Sp family members indicate that these TFs play important roles in early development and differentiation by their regulation of key genes involved in these functions. A detailed analysis of the age-dependent expression of individual Sp TFs is not available, however, there is evidence that for Sp1, there is an age-dependent decrease in expression in both rodent and human tissues[16-19]. Presumably the functions of Sp1 in aging animals are replaced by other TFs. The concern and subsequent focus on Sp1 and other Sp TFs (Sp3 and Sp4) in cancer emerged from studies showing that Sp1, Sp3 and Sp4 were overexpressed in cancer cells and tumors

[20]. These TFs were also identified as negative prognostic factors for cancer patient survival from several cancers and functional studies demonstrated the pro-oncogenic activity for Sp TFs[20]. The overexpression and prognostic value of Sp TFs have primarily focused on Sp1 and one of the first studies showed that Sp1 was overexpressed in patients with pancreatic tumors and was a negative prognostic factor for patient survival[21]. Most studies on cancer patients have reported that Sp1 or Sp3 is overexpressed and/or is a negative prognostic factor for glioma, astrocytoma, colon, gastric, liver, prostate and head and neck cancers; in lung and breast cancers; in addition, there are some conflicting results[22-40]. An important linkage between Sp1 and cancer was observed in studies showing that carcinogen- or oncogene- induced transformation of human fibroblasts into fibrosarcomas was accompanied by an 8-18-fold increase in expression of Sp1. Moreover, the ability of fibrosarcoma cells to form tumors in athymic nude mice was abrogated after Sp1 knockdown[41]. Subsequent studies show that Sp TFs are pro-oncogenic and regulate pathways and genes associated with cell growth, survival, migration and invasion (Figure 1).

NR4A1 and two related receptors NR4A2 (Nurr1) and NR4A3 (Nor1) are orphan nuclear receptors with structures and endogenous functions that differ significantly from Sp TFs[42,43]. NR4A1 and NR4A3 knockout mice are viable whereas NR4A2$^{-/-}$ mice exhibit early mortality due to dopaminergic and other neuronal deficits[44-48]. Endogenous ligands for NR4A have not been identified, however, NR4A bind structurally diverse synthetic compounds and these receptors interact with nerve growth factor β response elements (NBREs: AAAGGTCA) and Nur response elements [NuRE: AAAT(GA)C/T/CA] as monomers and dimers respectively[49-51]. In addition, NR4A1 and NR4A2 form a heterodimer with retinoid X receptor and bind a DR5 motif[52,53]. NR4As are immediate early genes induced by diverse stressors and they play important roles in maintaining cellular homeostasis and in pathophysiology [42,43,54]. NR4A1 and Sp TFs are remarkably distinct in their structures and functions in maintaining cellular homeostasis, however, in pancreatic cancer and other tumor types, their functions are similar. Like Sp1, NR4A1 is overexpressed in solid tumors from patients with pancreatic, breast, liver, glioblastoma, ovarian, colon, melanoma, endometrial, cervical, rhabdomyosarcoma and gastric cancer[55-64]. Moreover, high expression of NR4A1 in tumors is a negative prognostic factor for lung, breast, colon and ovarian cancer patients[58-60,63]. The negative prognostic significance of NR4A1 overexpression is paralleled by studies showing that like Sp TFs, NR4A1 is pro-oncogenic and regulates pathways comparable to that described for Sp TFs (Figure 1). Thus, there is an interesting parallel in the expression, functions and prognostic values of Sp TFs (primarily Sp1) and NR4A1 in multiple tumor types. This review will focus on the individual roles and interactions of Sp TFs and NR4A1 in pancreatic cancer and this will include genes commonly regulated by NR4A1/Sp complex in which NR4A1/Sp1 and NR4A1/Sp4 interact at GC-rich gene promoter sites that bind Sp.

Sp TFs and pancreatic cancer

Shi *et al*[65] first reported the overexpression of Sp1 in pancreatic cancer cell lines and in pancreatic tumors compared to non-tumor tissue and subsequent studies showed that Sp1, Sp3 and Sp4 were co-expressed in most pancreatic cancer cells[66]. The three structurally related TFs target similar GC-rich sequences in gene promoters and there is evidence for some genes, that Sp3 acts as a transcriptional repressor. A systematic study of the functions of Sp1, Sp3 and Sp4 was investigated in Panc1, MiaPaCa2 and L3.6pL pancreatic cancer cells and also in cell lines derived from lung, colon, kidney and breast tumors[66]. Figure 1 illustrates the functional effects of Sp1 knockdown which resulted in decreased pancreatic cancer cell growth, induced Annexin V staining (apoptosis) and decreased migration and this was accompanied by PARP cleavage, another marker of apoptosis. These results were somewhat surprising since it might be expected that the loss of Sp1 would be compensated for by Sp3 and Sp4. The functional properties of Sp3 and Sp4 were also determined in Panc1, L3.6pL and MiaPaca2 cells by knockdown and the results showed that like Sp1, knockdown of Sp3 or Sp4 decreased cell proliferation, induced apoptosis (increased Annexin V staining and cleaved PARP) and decreased cell invasion and similar results were observed in lung, breast, kidney and colon cancer cell lines[66]. These results indicated the importance of all three Sp TFs as independent pro-oncogenic factors that regulate pancreatic cell growth, survival and invasion, and related genes and it was suggested that Sp TFs were non-oncogene addiction genes[66].

Genomic analysis of altered gene expression after individual knockdown of Sp1, Sp3 and Sp4 in Panc1 pancreatic cancer cells further demonstrated their pro-oncogenic functions and unique effects of Sp1, Sp3 and Sp4 in this cell line. Figure 2A illustrates the total number of genes that are changed after individual knockdown of Sp1, Sp3

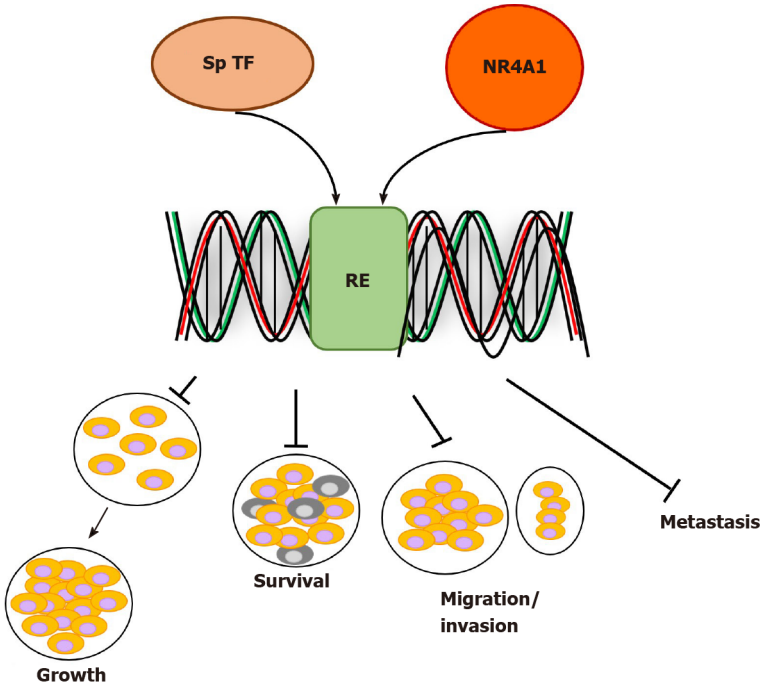

Figure 1 Common functional properties of specificity protein transcription factors and nuclear receptor 4A1 in pancreatic and other cancer cells. Specificity protein (Sp) transcription factors (TFs) (Sp1, Sp3 and Sp4) bind GC-rich gene promoter sequences to activate gene expression associated with enhanced cell proliferation, survival, migration, invasion and metastasis. Nuclear receptor 4A1 (NR4A1) binds as monomer or homodimers to NBRE and NuRE promoter elements and NR4A1 also acts as a cofactor for Sp1 and Sp4-regulated genes and NR4A1/Sp binds GC-rich promoter sequences. Sp: Specificity protein; TFs: Transcription factors; NR4A1: Nuclear receptor 4A1.

and Sp4, the number of uniquely modified genes and the number of genes commonly repressed or induced by Sp1-Sp3, Sp1-Sp4 and Sp3-Sp4. Knockdown of Sp3 and Sp4 resulted in the highest number (and percentage) of commonly induced or repressed genes. Causal Ingenuity Pathway Analysis (IPA) of the genes affected by knockdown were sorted into functional groups and Figure 2B-D illustrate the overlap in affected genes in the Sp1-Sp3, Sp1-Sp4 and Sp3-Sp4 groups associated cell growth, survival and cellular movement (migration and invasion). The overall number and percentage of commonly induced or repressed genes was highest for Sp3 and Sp4 in each functional category and this was similar to that observed for the total number of commonly induced or repressed genes (Figure 2A). Quantitative results from the causal IPA confirmed that the genomic changes observed in the array data were consistent with the functional changes observed after individual knockdown of Sp1, Sp3 and Sp4. This study also confirmed that knockdown of Sp1 alone or Sp1/3/4 (combination) in L3.6pL pancreatic cancer cells also decreased the growth of tumors in athymic nude mice bearing L3.6pL cells as xenografts[66].

In addition to the array studies noted above, there are a number of studies on Sp knockdown or overexpression in pancreatic cancer cells that have identified other Sp-regulated genes that contribute to the pro-oncogenic functions of Sp1, Sp3 and Sp4, and this includes regulation of pro-oncogenic long non-coding RNAs (MALAT1) and microRNAs (Table 1)[20,66-69]. These data were obtained only in pancreatic cancer cells; however, similar results for many of these genes have also been observed in other cancer cell lines[20]. Results summarized in Table 1 were observed in multiple pancreatic cancer cell lines and for some genes, their regulation by Sp TFs is cell context-dependent and there are also differences in the roles of Sp1, Sp3 and Sp4. Some studies used a combination of oligonucleotides targeting the 3 TFs (siSp1/3/4) and the combined knockdown of Sp1, Sp3 and Sp4 by RNA interference in Panc28 and L3.6pL pancreatic cancer cells decreased expression of NFkB-p65 and NFkB-p50 proteins[70]. In the former cell line, individual knockdown of Sp1, Sp3 or Sp4 partially decreased expression of both NFkB subunits. In L3.6pL cells, Sp1 and to a lesser extent Sp3, but not Sp4 knockdown decreased NFkB-p65 whereas Sp4 knockdown was primarily responsible for decreased expression of NFkB-p50 protein.

NR4A1 and pancreatic cancer

The pro-oncogenic role of NR4A1 has been investigated in multiple solid tumor

Table 1 Specificity protein regulated genes in pancreatic cancer cells

Functional	Sp-regulated gene
Cell proliferation and cell cycle progression	*EGFR, IGFR, cyclin D1, PIK3R1, pmTOR, 4-EBP, pS6RP, STAT3, pSTAT3, NFkB-p65/950*
Survival and apoptosis	*Survivin, bcl-2, PARP cleavage, APAF1, ATM, TNFRSF25, COX2, NFkB-p65/5*
Cell migration/movement EMT	*B1-integrin, α2-integrin, MTSS1, vimentin*
Angiogenesis	*VEGF, VEGFR1, VEGFR2, COX2*
Other pro-oncogenic factors	*TNFα, BRCA1, ARHGEF2, Ajuba, MALAT-1, RAS-6TP, FAS*

A detailed analysis of specificity protein (Sp)-regulated genes (Sp1, Sp3 and Sp4) in Panc1 cells based on RNAseq after Sp knockdown has been reported [66] and reviewed[20]. Sp: Specificity protein; EMT: Epithelial-mesenchymal transition.

derived cancer cell lines (rev. in 70), however, the number of publications on the functions of NR4A1 in pancreatic cancer is limited[55,71,72]. Knockdown of NR4A1 in pancreatic cancer cells inhibits cancer cell and tumor growth in athymic nude mouse xenograft studies, induces apoptosis and inhibits migration/invasion. Thus, the pro-oncogenic functions of NR4A1 and Sp TFs in pancreatic cancer cell lines are similar (Figure 1) and comparable results were obtained after their knockdown. For example, knockdown of NR4A1 by RNA interference in pancreatic cancer cells decreased expression of bcl-2 and survivin and this was accompanied by markers of apoptosis including increased TUNEL staining, caspase-3 and PARP cleavage[55,71]. Proteome analysis confirmed that loss of NR4A1 enhanced cell death and decreased gene products associated with cell proliferation and this was accompanied by increased reactive oxygen species (ROS) and ROS-induced endoplasmic reticulum (ER) stress genes including CHOP, Grp78, ATF4 and XBP-1s[71]. In addition, genes such as thioredoxin domain containing 5 (TXNDC5) and isocitrate dehydrogenase 1 (IDH1) that maintain high reductant levels are also NR4A1 regulated genes and their loss after receptor knockdown triggers the ROS-ER stress response. Knockdown of NR4A1 also decreased cell migration and expression of β1- and α5-integrins which play a role in NR4A1-regulated pancreatic cancer cell migration[72]. Thus, there is overlap between the function of Sp TFs and NR4A1 in pancreatic cancer cell lines and studies in other cancer cell lines show that many of the Sp-regulated genes summarized in Table 1 are also regulated by NR4A1[70]. Regulation of the same genes by Sp TFs and NR4A1 was recently reported in breast cancer cells where knockdown of Sp4 or NR4A1 decreased expression of α6- and β1-integrins in MDA-MB-231 and SKBR3 breast cancer cells. The effects of Sp3 and Sp1 knockdown on expression of these integrins was gene- and cell context-specific.

Mechanistic convergence of NR4A1 and Sp TFs

Both NR4A1 and Sp TFs are nuclear proteins that interact with defined cis-elements in their target gene promoters. Mechanistic studies showing cooperative activation of common genes by NR4A1 and Sp1 was first observed in pancreatic cancer cells and subsequently investigated and confirmed in other cancer cell lines[55,72]. The survivin gene has a GC-rich promoter that binds Sp TFs and has been extensively characterized as an Sp-regulated gene[73,74]. Knockdown of NR4A1 or treatment with NR4A1 antagonists decreased expression of survivin protein and mRNA and also decreased luciferase activity in pancreatic cancer cells transfected with GC-rich survivin promoter-luciferase constructs[55]. Further analysis of survivin regulation in pancreatic cancer cells showed that survivin is regulated by a p300/NR4A1/Sp1 complex where Sp1 binds the GC-rich survivin promoter, NR4A1 acts as a cofactor/coactivator and p300 is also part of the complex. The identification of this functional NR4A1/Sp complex is consistent with previous reports showing many other nuclear receptors also activate gene expression through interactions with Sp1. Studies in other cancer cell lines have identified other NR4A1-regulated genes that are regulated by NR4A1/Sp1, NR4A1/Sp4 or NR4A1/Sp1/4 and these include β1-, β3- and β4-integrins, α5- and α6-integrins, TXNDC5 and the PAX3-FOX01 fusion oncogene and the checkpoint gene PD-L1 (rev. in 70). Thus, like Ajuba[75], NR4A1 acts as a cofactor for Sp TFs in pancreatic and other cancer cell lines and they coregulate some of the same genes (EGFR, survivin and IGF1R). The possible interactions of NR4A1 and Ajuba, a coregulator of Sp-regulated genes needs to be further investigated.

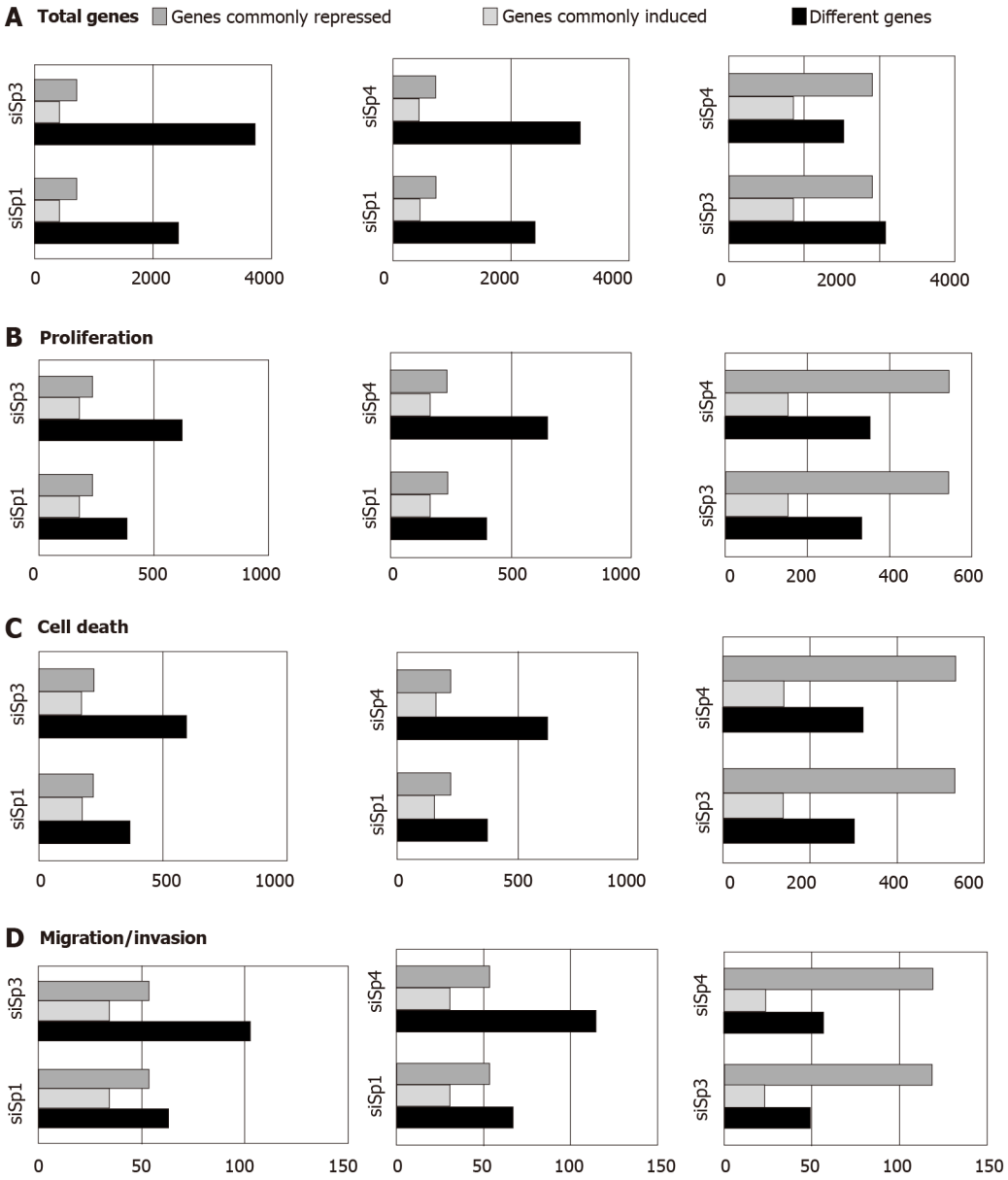

Figure 2 Genomic analysis of Sp1, Sp3 and Sp4 regulated genes in pancreatic cancer cells. A: The number of total genes regulated by specificity protein (Sp) transcription factors (TFs) in Panc1 cell was determined by individual Sp knockdown (siSp1, siSp3 and siSp4) and the number of unique (Different genes) and commonly repressed (Genes commonly repressed) and induced (Genes commonly induced) genes are indicated; B-D: Ingenuity Pathway Analysis analysis of genes that regulate cell proliferation (B), cell death (C) and migration/invasion (D) compares the number of unique and commonly repressed/induced genes by Sp1 and Sp3, Sp1 and Sp4 and Sp3 and Sp4 as described[66].

DRUGS THAT TARGET SP TFS AND NR4A1

Several drugs that target Sp TFs in pancreatic cancer cells have been identified and these include tolfenamic acid and structurally related compounds, COX2 inhibitors curcumin, metformin, piperlongumine, methyl 2-cyano-3,12-dioxooleana-1,9-dien-28-oate (CDDO-Me), benzylisothiocyanate (BITC) and phenethylisothiocyanate (PEITC) (Figure 3)[20]. All of these compounds downregulate Sp1, Sp3 and Sp4 through different pathways. For example, tolfenamic acid and COX2 inhibitors induce proteasome-dependent degradation of Sp1, Sp3 and Sp4 whereas metformin induces the dual specificity phosphatases MPK-1 and MPK-5 which are important for Sp downregulation. The phosphatases downregulate miR-27a which results in upregulation of ZBTB10, a Sp repressor which competitively displaces Sp TFs from GC-rich sites resulting in gene inactivation since ZBTB10 Lacks a transactivation domain. ROS-inducers including PEITC, BITC, piperlongumine, CDDO-Me and curcumin also decrease Sp-regulated gene expression through an ROS-mediated pathway which also involves induction of Sp repressors namely ZBTB4, ZBTB10 and ZBTB34[20] (Figure 4). This pathway involves ROS-dependent remodeling of repressor complexes

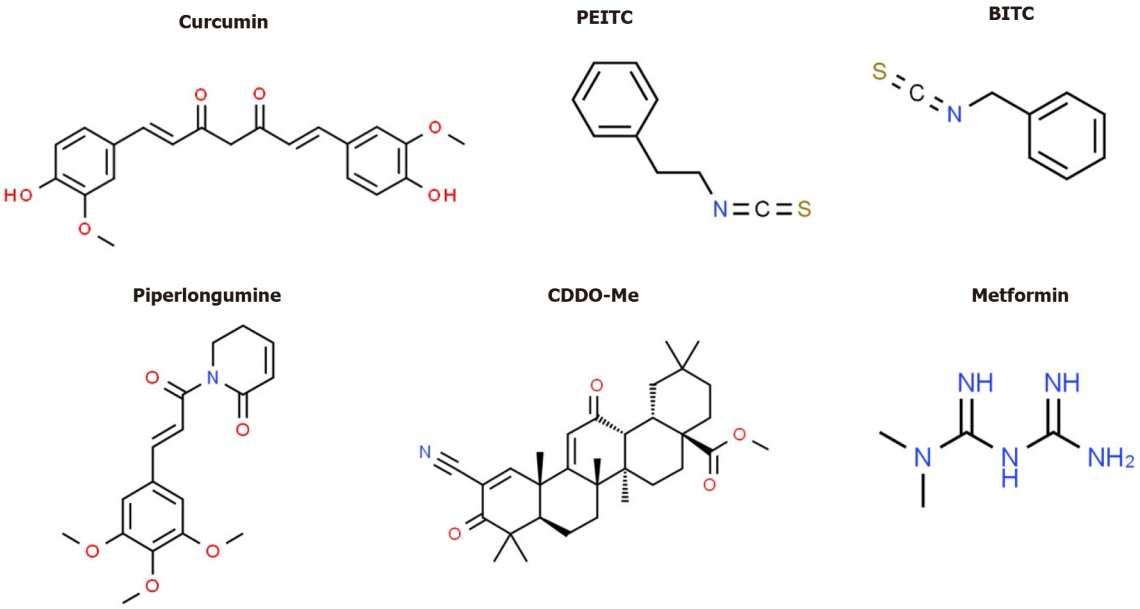

Figure 3 Structures of agents that target downregulation of Sp1, Sp3 and Sp4 in pancreatic and other cancer cell lines[68]. The structurally diverse compounds act through multiple pathways in different cancer cell lines.

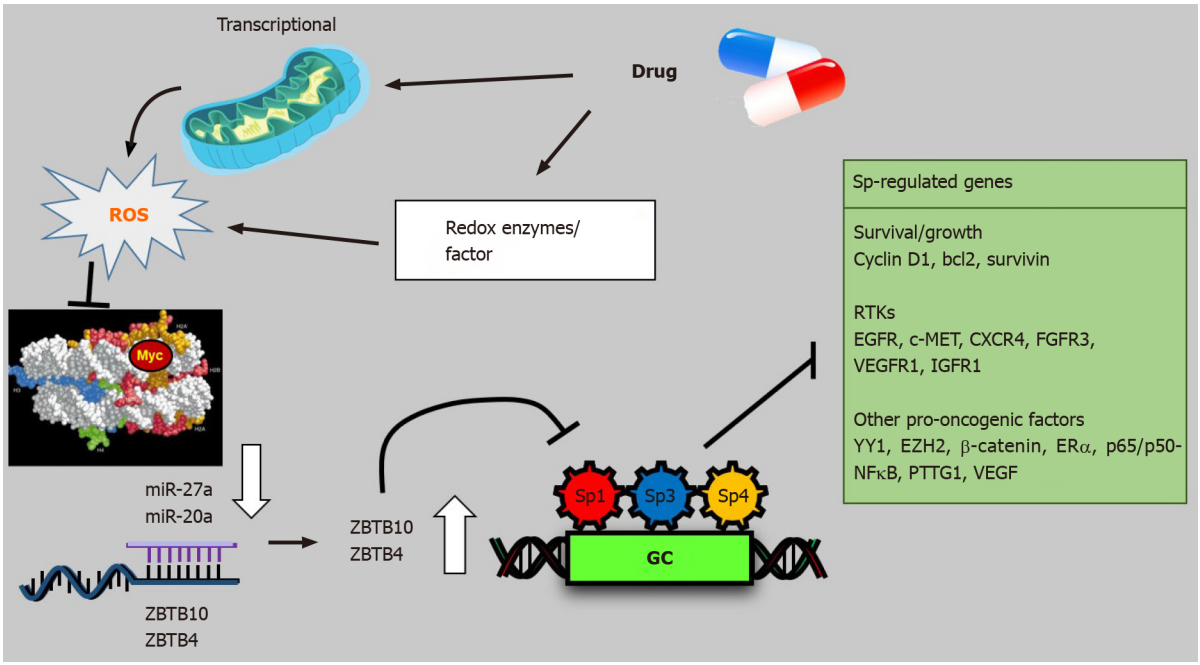

Figure 4 Mechanism of reactive oxygen species-dependent downregulation of specificity protein transcription factors. Induction of reactive oxygen species results in epigenetic suppression of Myc, downregulation of microRNAs, induction of ZBTB suppressors and decreased expression of specificity protein (Sp) transcription factors and Sp-regulated genes[20]. ROS: Reactive oxygen species; Sp: Specificity protein.

which results in downregulation of cMyc and cMyc-dependent microRNAs (miR-27a, miR-17-92) and induction of ZBTBs. These compounds (Figure 4) and others induce similar patterns and mechanisms of Sp downregulation in other cancer cell lines[20], however, there are compound-specific differences in mechanisms that are cell context-dependent.

Bis (3'-indolyl)-1-(p-hydroxyphenyl) methane (DIM-C-pPhOH) (Figure 5) was identified as an NR4A1 Ligand that acts as a receptor antagonist and mimics the effects of NR4A1 knockdown in pancreatic and other cancer cell lines[70]. DIM-C-pPhOH and several 3,5-disubstituted phenyl analogs are inhibitors of cell proliferation, survival and migration/invasion and they inhibit expression of NR4A1/Sp- and other NR4A1-regulated genes. Moreover, many of the 3,5-disubstituted analogs of

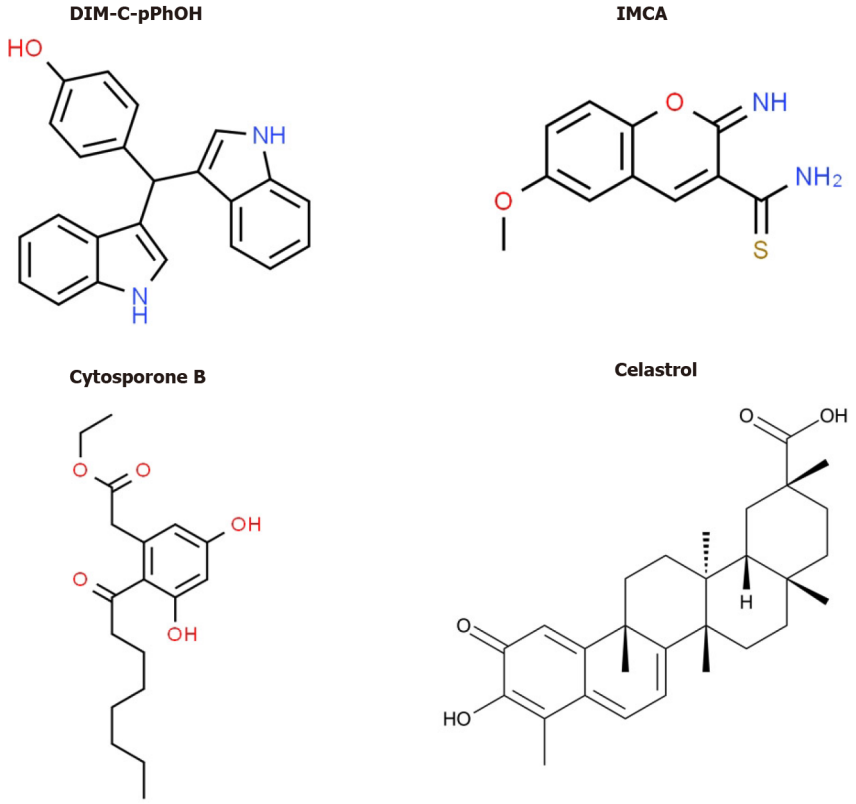

Figure 5 Nuclear receptor 4A1 ligands. Structures of compounds that directly bind nuclear receptor 4A1 include DIM-C-pPhOH, IMCA, cytosporone B and celastrol[76-80].

DIM-C-pPhOH inhibit tumor growth in mouse xenograft models at doses < 5 mg/kg per day and ongoing studies have identified ligands that inhibit tumor growth at doses < 1mg/kg per day. Interestingly, several other classes of compounds bind and modulate NR4A1-dependent pathways/genes and these include cytosporone B and structurally related compounds, IMCA (2-imino-6-methoxy-2H-chromene-3-carbothiomide) and the triterpenoid celastrol and analogs[76-80] (Figure 5). Fascinatingly, celastrol also activates ROS-dependent downregulation of Sp1, Sp3 and Sp4 in bladder cancer cells[80] (*e.g.*, Figure 4) and uniquely acts as a bifunctional agent targeting both Sp TFs and NR4A1. Ongoing studies in this laboratory have identified other dual targeting agents with potential for clinical applications for pancreatic cancer therapy.

CONCLUSION

In summary, it is evident that both Sp TFs and NR4A1 are pro-oncogenic factors that regulate pancreatic cancer cell and tumor growth and interactions of NR4A1 and Sp TFs are also important for regulating many critical genes (Figure 1) involved in pro-oncogenic functions. Although the functional importance of NR4A1/Sp regulated genes in cancer cell growth, survival, migration and invasion has been established in pancreatic and other cancers, clinical applications of drugs targeting Sp and NR4A1 are lacking. Several compounds have been identified that induce Sp downregulation or inhibit/antagonize NR4A1. Current studies in our laboratories are focused on identifying agents like celastrol that act simultaneously as NR4A1 antagonists and Sp downregulators as a new class of drugs that will enhance the effectiveness of current chemotherapies used for clinical treatment of pancreatic and other cancers.

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MINIREVIEWS

Artificial intelligence for the early detection of colorectal cancer: A comprehensive review of its advantages and misconceptions

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Abstract

Colorectal cancer (CRC) was the second-ranked worldwide type of cancer during 2020 due to the crude mortality rate of 12.0 per 100000 inhabitants. It can be prevented if glandular tissue (adenomatous polyps) is detected early. Colonoscopy has been strongly recommended as a screening test for both early cancer and adenomatous polyps. However, it has some limitations that include the high polyp miss rate for smaller (< 10 mm) or flat polyps, which are easily missed during visual inspection. Due to the rapid advancement of technology, artificial intelligence (AI) has been a thriving area in different fields, including medicine. Particularly, in gastroenterology AI software has been included in computer-aided systems for diagnosis and to improve the assertiveness of automatic polyp detection and its classification as a preventive method for CRC. This article provides an overview of recent research focusing on AI tools and their applications in the early detection of CRC and adenomatous polyps, as well as an insightful analysis of the main advantages and misconceptions in the field.

Key Words: Artificial intelligence; Machine learning; Deep learning; Medical images; Colorectal cancer; Colorectal polyps

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Core Tip: Artificial intelligence-based (AI) methods have demonstrated high performance in classification, object detection, and segmentation tasks. Through multidisciplinary and collaborative work between clinicians and technicians, the advantages of AI have been successfully applied in automatic polyp detection and classification. The new AI-based systems present a better polyp detection rate and contribute to better clinical decision-making for preventing colorectal cancer (CRC). This article provides an overview of recent research focusing on AI and its applications in the early detection of CRC and adenomatous polyps.

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INTRODUCTION

Colorectal cancer (CRC) is a common malignancy. Worldwide, in 2020, it ranked third among neoplasms, with incidences of 1931590 cases, representing 10% of neoplasms. However, in terms of mortality, it ranked second for the same year after lung cancer due to the crude mortality rate of 12.0 per 100000 inhabitants, prevailing in the male population[1].

Although CRC remains among the ten most frequent cancers, in a retrospective description, it was observed that at a global level, between 2000 and 2019 its ranking was stable in high-income countries, in which it maintained second place as a cause of death from neoplasia. However, in the remaining countries, it gradually increased; hence, in 2019, CRC was the 3rd leading cause of cancer death in upper-middle-income countries, the 4th leading cause in lower-middle-income countries, and the 5th leading cause in countries where income was low[2]. It is expected that by 2035, in those countries where it remains stable, the CRC mortality rate will decrease due to the application of early detection programs that are being implemented, the active participation of the population, and the prioritization of education on this matter. Nevertheless, it is expected that by 2035, in countries with low incomes, the mortality rate will continue to increase due mainly to late diagnosis and limited access to treatment if these indicators are not strategically addressed in time[3].

The risk of CRC in the general population is not uniform, and it is associated with factors such as a family history of CRC, lifestyles and eating habits and above all, the presence of polyps, either isolated or associated with genetic polyposis syndromes[4]. CRC can then be prevented by modifying diet and lifestyle, as well as early detection and timely treatment. Various studies have shown that screening tests facilitate the detection of precursor lesions in early stages. This, added to their subsequent elimination, promotes a reduction in CRC incidence and mortality[5-7].

Colonoscopy is the gold standard procedure for the diagnosis of large intestine (colon) and rectal diseases. The World Gastroenterology Organization establishes that both the sensitivity and the specificity of colonoscopy for the detection of polyps and colon cancer is 95%[4]. The United States Preventive Services Task Force determined that colonoscopy has a sensitivity between 89% and 98% for detecting adenomas of 10 mm and larger. For adenomas of 6 mm or more, the sensitivity ranges from 75% to 93%, while the specificity found was 89%[8], in which case a screening test for CRC is recommended. Additionally, in joint work between the American Cancer Society, the United States Multisocial Working Group and the American College of Radiology in 2008, colonoscopy was strongly recommended as a screening test designed to detect both early cancer and adenomatous polyps if resources were available and patients were willing to undergo an invasive test[5]. Similarly, the National Comprehensive Cancer Network recommends and promotes the application of colonoscopy for the detection of adenomatous polyps and early stages of CRC[9]. It should be noted that colonoscopy is a procedure that depends fundamentally on physician observation. In recent decades, technology has been incorporated into the inspection procedure, known as computer-aided systems.

Computer-aided detection/diagnosis systems (CADe/CADx) have been proposed, developed, and clinically used since 1966, especially in thoracic and breast imaging as well as in the cancer risk assessment[10]. The progress of computational resources and medical imaging devices has enabled CADe/CADx systems to support tasks in other areas, such as endoscopic examination[11]. CADe aims to find or localize abnormal or suspicious regions, increasing the detection rate while reducing the false negative rate (FNR). Additionally, CADx provides a second objective opinion regarding the assessment of a disease from image-based information. In the early stages of both systems, their algorithms were predominantly based on feature extraction methods engineered by domain experts[12]. However, the widespread progress of diseases and variability of cases have rendered these methods obsolete and have opened the research to new and improved methods. In particular, artificial intelligence (AI) has provided tools and algorithms capable of achieving high performance in terms of accuracy, sensitivity, and specificity to face tasks related to feature extraction, classification, detection, and region segmentation.

This work focuses on the main contributions of AI in gastroenterology, in particular, to the early detection of CRC through polyp detection and classification. We focus on those works that enhance the performance of endoscopic tests, which allow for direct visualization of existing lesions in the mucosa of the colon and rectum. With the numerous applications of AI and the growing interest in AI-related topics, some misconceptions have been discovered that are worth analysing.

WORLD OF AI

Since the term AI was first used in 1956, it has been a thriving field with relevant applications in several areas, including medicine[10]. The term AI refers to technology that allows computer systems to perform tasks that normally require human skills. The field of AI is broad and includes different fields, such as robotics, computer vision, natural language processing and machine learning, as shown in Figure 1. Often, such areas overlap to deliver more advanced features and capabilities. In medicine, robotic devices are increasingly being used in minimally invasive surgical procedures, such as robotic-assisted surgery for patients with CRC[13]. Natural language processing is another crucial AI area used to make the machine read, understand, and interpret human language. In the treatment of CRC, natural language processing has been useful for extracting relevant clinical information from scanned colonoscopy and pathology reports that would otherwise have to be extracted manually[14]. Computer vision and image processing have also been helpful in colonoscopy exploration, enhancing the visualization of lesion tissues[15]. However, from all AI fields, machine learning is the most widely used in three areas of medicine: Early detection and diagnosis, treatment, and outcome prediction and prognosis evaluation[16]. Gastrointestinal endoscopy has advanced in all three areas, but there is a clear trend in the detection and classification of polyps (see Wang *et al*[11], Nogueira-Rodríguez *et al*[17] and the references therein).

The following subsections focus on analysing the most prominent AI-based works on endoscopic tests without ignoring a brief review of the most commonly used machine learning algorithms (including deep neural networks) and evaluation metrics.

MACHINE LEARNING

Machine learning, a subset of AI, refers to a set of computer algorithms that learn from the input data provided, adjust a model through a training process, and perform predictions in novel situations by using the trained model[18]. According to the type of learning strategy, machine learning algorithms can be classified into two categories: Supervised and unsupervised learning. In supervised learning, a training set that contains the input data with the correct response (targets) must be previously available. The model is trained using the training set until it is generalized to respond correctly to all possible inputs. In the case of unsupervised learning, the correct answers are not provided, and the model attempts to group the data into categories identifying similarities between such data[19,20]. In medicine, supervised learning is the most commonly applied strategy because the goal is predicting a known outcome by mimicking a physician or health professional.

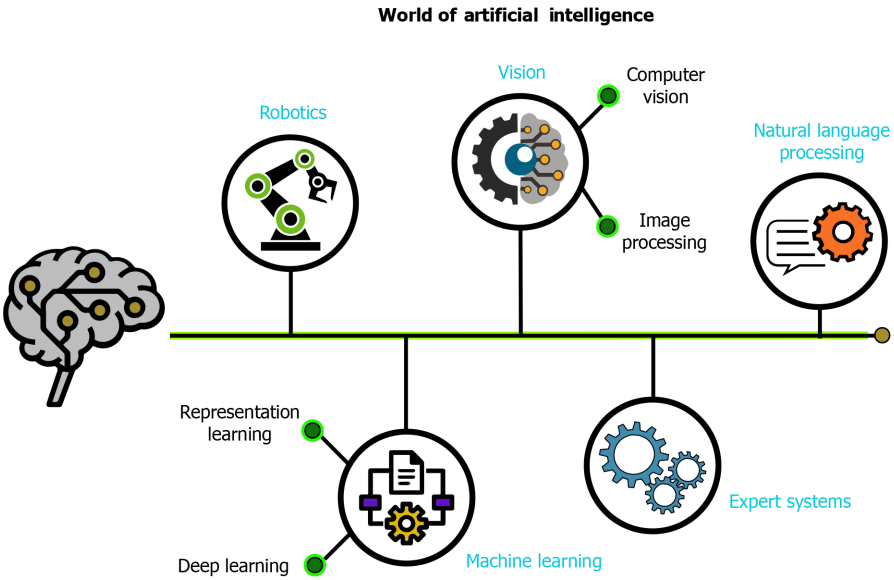

Figure 1 Artificial intelligence is a set of fields that are combining to improve tasks that involve human cognitive functions such as learning, reasoning and self-correction.

In the context of machine learning, healthcare data can be categorized as structured and unstructured. Imaging, genetic data, and electrophysiological data are some examples of structured data, whereas physical examination notes or clinical laboratory results that contain large portions of narrative texts are unstructured data[19]. The major digital data sources in medicine are images resulting from the development and improvement of different medical imaging techniques (*e.g.*, computer tomography, magnetic resonance imaging, ultrasound, X-ray and endoscopy)[10].

In gastroenterology, most computer-aided diagnosis/detection systems use images or videos, enabling the use of machine learning techniques to enhance their outcomes. In early works, CADe/x systems combined feature extractor methods and classical machine learning techniques such as random forest, decision trees, and support vector machines[21-23]. More recent works have shown applications that use deep learning algorithms such as convolutional neural networks (CNNs), in part due to the high performance and low latency of the systems[20].

The selection of one machine learning algorithm over another should be guided by analysing the available data as well as the task to be performed with it. Table 1 summarizes the main characteristics of the algorithms used in the last 5 years of state-of-the-art polyp detection and/or classification. We narrowed the analysis to the four most commonly used methods according to the Scopus and PubMed databases applied to medicine namely, support vector machines, random forest, decision trees and deep neural networks. Both support vector machines and random forests present high performance even if the data have high dimensionality. However, support vector machines are not recommended when the database is large because they increase training and inference time without improving performance[24]. Conversely, random forest presents high performance when working with large databases[25]. Deep neural networks outperform classical machine learning algorithms in almost all criteria but require large quantities of labelled data that may not be available, or the acquisition and labelling process may be very expensive or time consuming.

EVALUATION METRICS OF MACHINE LEARNING ALGORITHMS

Evaluation metrics are tied to the tasks (*e.g.*, classification, detection, localization, and segmentation) performed by the machine learning models. In gastroenterology in applications such as automatic polyp detection or classification, the evaluation metrics can be computed considering different levels: Video sequence, image, or region (pixel level).

Table 2 summarizes the terms and formulation of metrics commonly used for performance evaluation of machine learning models. In particular, those used in AI-based applications for colonoscopy. Some terms are key to understanding the

Table 1 Comparison between different types of machine learning approaches used in studies focused on polyp detection and classification

Characteristics	Support vector machine	Random forest	Decision trees	Deep neural networks	Context	Ref.
High dimensional data	High	High	Moderate	High	Performance	Shen *et al*[12]; Goodfellow *et al*[26]
Overlapped classes	Low	Low	Low	High		
Imbalance datasets	Moderate	High	Low	Moderate		
Non-linear data	Moderate	High	Moderate	High		
Larger dataset	Moderate[1]	High[1]	Low	High		
Outliers	Moderate	Moderate	Low	High	Robustness	Shen *et al*[12]; Yu *et al*[20]
Over-fitting	Moderate	High	Low	High		
Handling of missing values	Poor	Good	Good	Good		
Reproducibility	High	High	High	Moderate	Complexity	Yu *et al*[20]
Interpretability	Moderate	Moderate	High	Low		

[1]Consider that it leads to slow response time.

Table 2 Most common evaluation metrics found in the state of the art for detection, segmentation and classification tasks

Term	Symbol	Description
Positive	P	Number of real positive cases in the data
Negative	N	Number of real negative cases in the data
True positive	TP	Number of correct positive cases classified/detected
True negative	TN	Number of correct negative cases classified/detected
False positive	FP	Instances incorrectly classified/detected as positive
False negative	FN	Instances incorrectly classified/detected as negative
Area under curve	AUC	Area under the ROC plot
Term	**Task**	**Formulation**
Accuracy	C, D, S	(TP + TN)/(TP + TN + FN + FP)
Precision/PPV	C, D, S	TP/(TP + FP)
Sensitivity/Recall/TPR	C, D, S	TP/(TP + FN)
Specificity/TNR	C, D, S	TN/(TN + FP)
FPR	C, D, S	FP/(TN + FP)
FNR	C, D, S	FN/(TP + FN)
f1-score/DICE index	C, D, S	2 · (precision · recall)/(precision + recall)
f2-score	C, D, S	4 · (precision·recall)/(4·precision + recall)
IoU/Jaccard index	D, S	(target ∩ prediction)/(target ∪ prediction)
AAC	D, S	(detected area ∩ real area)/(real area)

C: Classification; D: Detection; S: Segmentation. PPV: Positive predictive value; TPR: True positive rate; TNR: True negative rate. AAC: Annotated area covered.

evaluation metrics in algorithms used for automatic polyp detection and/or classification. There are two well-defined cases: Images with polyps (positive cases) and images without polyps (negative cases). In both cases, some authors[15,27,28] define a true positive (TP) when the algorithm output finds the correct region of the polyp

(detection) or labels the image as a polyp (classification). In the case of detection, only one TP is considered per polyp, avoiding over-detection. Any detection or classification as a positive case outside the region of polyp or images without polyps is considered false positive. The absence of positive output in detection or classification in images with a polyp is considered a false negative. If the algorithm does not provide any positive output in images without polyps, it is considered a true negative. Positive polyp detection is a common evaluation metric that can be computed as a true positive rate (see Table 2) or polyp-based analysis by defining a threshold of the positive frame-level predictions[29].

The most widely used evaluation metric is accuracy (see formulation in Table 2). It works well in datasets with an equal number of samples belonging to each class (*i.e.*, balanced dataset), but it is not recommended for imbalanced datasets[30]. Evaluation metrics such as sensitivity, specificity, and positive predictive value are not dependent on the class distribution; therefore, they are not biased by imbalanced datasets[31]. The use of evaluation metrics also depends on the task to be performed. In detection tasks, metrics such as the f1-score (or DICE index) and f2-score of the Jaccard index are widely used[17]. We analyse each evaluation metric below.

Accuracy represents the overall effectiveness of the algorithm when comparing the number of correctly classified/detected samples with the total number of samples[17].

Precision (positive predictive value) represents the proportion of predicted positive cases that are real positives[17].

Sensitivity (recall or true positive rate) measures the ability of the algorithm to correctly identify the positive cases[17].

Specificity (true negative rate) measures the ability of the algorithm to correctly identify the negative cases[17].

The false positive rate (FPR) represents the proportion of negative cases incorrectly identified as positive cases in the data. In statistics, the FPR is equivalent to the type I error[26].

The FNR represents the proportion of positive cases incorrectly identified as negative cases in the data. In statistics, the FPR is equivalent to the type II error[26].

The DICE index (f1-score) determines the similarity between two different areas whether the algorithm is performing segmentation or detection tasks. In classification, the f1-score is a metric to evaluate a trade-off between precision and recall[32].

The f2-score is a metric to evaluate a trade-off between precision and recall but lowers the importance of precision and increases the importance of recall[17].

The Jaccard index (IoU) is a metric mostly used in detection/segmentation algorithms and quantifies overlap between the target area and the area predicted by the algorithm[32].

Annotated area covered is an evaluation metric mostly used in detection or segmentation tasks. It represents the proportion of the real area detected/segmented by the algorithm[33].

The area under the curve (AUC) is a metric obtained from the receiver operating characteristic curve that relates to the sensitivity *vs* specificity of a binary classifier[31]. The best classifier is the one with the AUC closest to 1.

ENHANCING COLONOSCOPY OUTCOMES *VIA* AI

Colonoscopy exploration is performed through a flexible tube (endoscope) that contains a tiny video camera at the tip. The camera allows the physician to see the inside of the entire colon by displaying the image on a digital screen, as shown in Figure 2. During the process, the physician detects (or not) the presence of lesions on the colon and polyps, and then, depending on the shape, colour and texture of the polyp, determines whether to remove it[15]. The outcome of colonoscopy exploration depends on several factors. On the one hand, the procedure is intrinsically dependent on the technology used, such as the camera resolution, screen size and resolution, frame rate, and capability to deal with blurriness, among other issues[15]. On the other hand, the results can be affected by the cognitive capabilities of the physician (*e.g.*, tiredness, fatigue or concentration) during the exploration procedure[34]. Other factors, such as bowel preparation and the percentage of the colon explored, can also affect the outcome of the exploration[35].

Traditional colonoscopy has been shown to be successful when detecting polyps larger than 10 mm, which are easily detected by physicians during inspection. However, the miss rate of polyp detection increases with smaller sized and/or flat polyps[36]. There are both clinical and technical efforts to improve colonoscopy

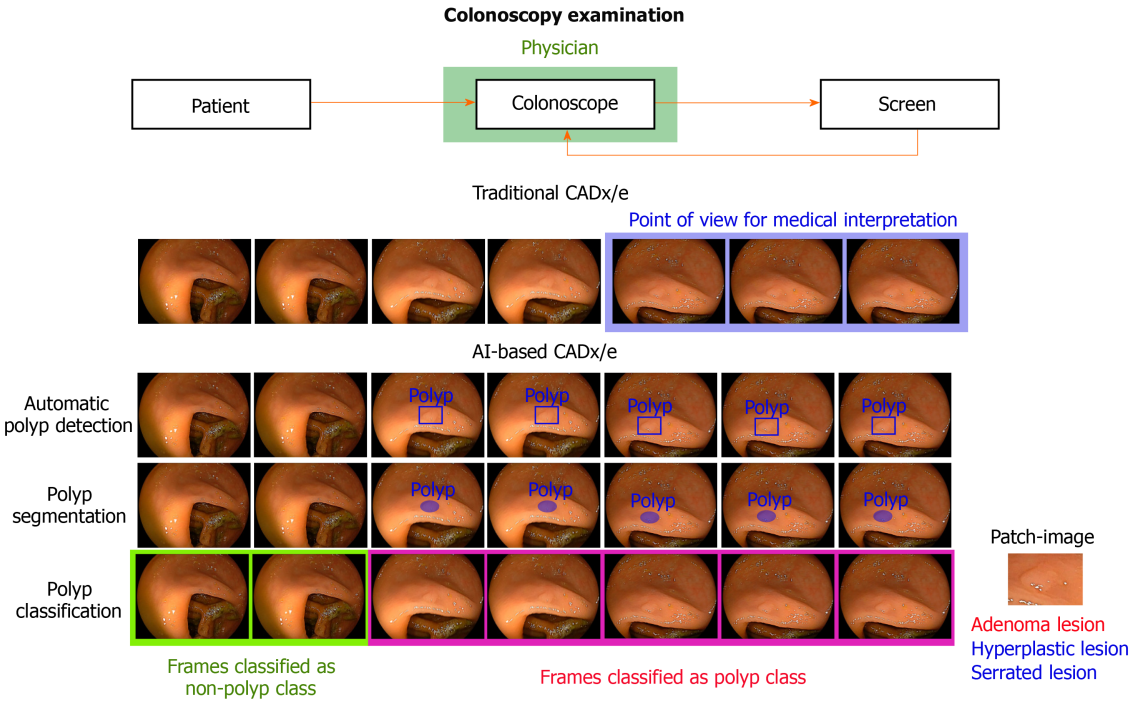

Figure 2 Comparison of traditional and AI-based computer-aided systems on colonoscopy examination.

results. For example, continuous improvement of the skills of physicians through training and practice[37] and the improvement of image/video acquisition devices combined with the development of clinically applicable CADx/e systems have been reported. Another technique used to make fine details of the mucosal surface more visible (evidencing small or slight lesions) on endoscopy tests is chromoscopy (also known as chromoendoscopy or chromocolonoscopy)[38].

Initially, chromoscopy consisted of spraying contrast dyes on the mucosa with the aim of outlining the mucosal morphology (dye-based chromoendoscopy DCE)[39]. The most frequent contrast dye used is indigo-carmine in concentrations varying from 0.2% to 2%[38]. DCE has been demonstrated to be a useful tool for endoscopists to detect and characterize lesions more accurately. A study presented by Brown *et al*[38] found that the rate of detection of small polyps was improved by DCE by approximately 90%. Such analysis was conducted on 2727 patients and showed that the detection of small polyps that could potentially develop into cancer was increased by 30% when chromoscopy was used. Although the DCE technique is simple to use and safe, it is labour intensive and time consuming, and the outcomes highly depend on bowel preparation[38,39]. Over the years, with the introduction of electronics and the improvement in technology, a new era of chromoscopy called virtual chromoendoscopy (VCE) has been adopted. VCE includes pre-processing optical imaging techniques, such as Olympus' narrow-band imaging (NBI) and autofluorescence imaging, as well as the post-processing techniques Pentax's i-SCAN and Fujinon intelligent chromoendoscopy[15]. Of all VCE techniques, NBI has been studied most frequently for assessing gastroenterology diseases[40]. This pre-processing technique uses light of specific wavelengths (green - 540 nm and blue -415 nm) to enhance detail on the mucosal surface. Although VCE techniques such as NBI can detect small-size or flat polyps, they suffer from some drawbacks, such as interobserver and intraobserver variability[41]. Such drawbacks refer to expertise, levels of distraction, or stress. However, the use of CADx/e systems may increase standardization in the process and, perhaps most importantly, more widespread adoption by non-experts in the field [41].

In this context, the new developments of CADx/e systems have focused on systems to assist in the detection and/or localization of polyps and the classification of the different types of polyps, both fundamental tasks to help clinicians at all stages of CRC diagnosis. AI has emerged as a powerful tool in two well-differentiated tasks: Polyp detection (including localization and segmentation) and polyp classification. By including an AI-based algorithm in the CADx/e systems that attend colonoscopy exploration, they can predict whether there are one (or more) polyps in a given video frame using white light alone, without the aid of advanced endoscopic imaging

modalities. If the purpose is also to locate the polyp, the algorithm predicts the position of the polyp in the image, as shown in Figure 2. If the physician requires a finer analysis, segmentation tools can allow isolation of the polyp region at the pixel level on the image. Once a polyp is detected, polyp classification aims to catalogue the type of polyp. The latter is particularly important because it allows the clinician to make a better decision as to whether to remove the polyp depending on whether it seems to be a benign, pre-malignant, or malignant polyp. Currently, to confirm if a polyp is malignant, the suspected polyp must be removed, and then a pathology test must be performed. However, an expected advance in the future is that AI can assist clinicians in differentiating polyps.

Colorectal polyps are anatomically pathologically classified as adenomatous, hyperplastic, or serrated and inflammatory, hamartomatous, juvenile, the latter being synthesized terminologically as miscellaneous, given their low prevalence (10%-20%) [42]. Adenomatous polyps are the most frequent (60%-70%). Depending on their histological characteristics, they can be tubular, villous, or tubulovillous. They can be of different degrees of dysplasia, which constitutes one of the elements for the diagnosis or the presumption of CRC[8,42]. Hyperplastic polyps have a prevalence between 10% and 30%. Although they are not usually neoplastic, there is a type of serrated polyp, the sessile serrated adenoma, which is considered a CRC precursor lesion through what is known as the serrated pathway of carcinogenesis[8,42,43].

ADVANCES IN AI FOR DETECTING AND CLASSIFYING COLORECTAL POLYPS

Automatic polyp detection—including classification and segmentation—in colonoscopy videos has been an active research topic during the last two decades. After analysing approaches reported in the literature, there are three well-defined methods: Hand-crafted, feature-based machine learning, and end-to-end learning approaches. Each method is discussed in more detail below. At the end of the analysis, we summarize the works in Table 3, showing the screening test, imaging modality and contribution for each one.

Hand-crafted approach

Hand-crafted methods refer to those based on exploiting low-level image processing techniques to obtain candidate polyp boundaries. This method considers the polyp as a protruding surface, with its boundaries detected using intensity valleys[37], Hessian filters[44], or the Hough transform[45].

Feature-based machine learning approach

Feature-based methods encompass the first era of machine learning: Designing a feature extractor and then training a classifier to predict a given class (*e.g.*, polyps or non-polyps). In early works, a texture descriptor was used to provide relevant features about the region of the image containing polyps using wavelet sub-band information in the work of Wimmer *et al*[46], Haralick co-occurrence matrix in the work of Hu *et al* [21], or Gaussian-kernel low pass filtering in the work of Mamanov *et al*[47]. Other features, such as shape, colour, and edge geometry, have also been used to create more robust detection systems that include polyp segmentation[32]. Glasmachers[33] proposed a CAD system that combines context-based image information to remove non-polyp information and shape features to reliably localize polyps.

After generating a feature vector using descriptors of texture, colour and/or shape, a classifier is required to predict whether polyps are present in the colonoscopy image, distinguishing between the different types of polyps or whether the region characterized on the image is a polyp (localization). The most commonly used classifiers are k-nearest neighbours[46], decision trees[22], random forests[22,32], and support vector machine[23].

End-to-end learning approach

The end-to-end (E2E) learning approach refers to training a learning system represented by a single model (generally a deep neural network)[33]. As the technology evolved and the computational capabilities increased, the use of convolutional neural networks as a key part of CADx/e systems is increasingly frequent in automatic polyp detection[48] and/or polyp classification tasks[49]. The advantage of the E2E approach is the possibility of designing more complex multitasking systems:

Table 3 Summary of studies focused on artificial intelligence applications for automatic polyp detection, classification, and segmentation

Study	Screening test	Imaging modality	Data type	AI-based algorithm	Contribution	Acc	Sen	Spe
Wimmer *et al* [46]	Colonoscopy	WL, NBI	Images	k-nearest neighbours	Polyp classification: non-neoplastic, neoplastic	80%	-	-
Tajbakhsh *et al* [22]	Colonoscopy	WL	Images	Decision trees; Random forest	Automatic polyp detection	-	88%	-
Hu *et al*[21]	CT Colonography	Greyscale	Images	Random forest	Polyp classification: non-neoplastic, neoplastic	-	-	-
Zhang *et al*[50]	Colonoscopy	WL, NBI	Images	CNN: Caffenet	Polyp detection and classification: benign from malignant	86%	88%	-
Shin *et al*[23]	Colonoscopy	WL	Images	Support vector machine	Whole image classification: polyps from non-polyps	96%	96%	96%
Sánchez-González *et al*[32]	Colonoscopy	WL	Images	Random forest; CNN: Bayesnet	Polyp segmentation	97%	76%	99%
Tan *et al*[52]	CT Colonography	Greyscale	Images	Customized CNN	Polyp classification: adenoma from adenocarcinoma	87%	90%	71%
Fonolla *et al*[51]	Colonoscopy	WL, NBI, LCI	Images	CNN: EfficientNet	Polyp classification: benign from pre-malignant	95%	96%	93%
Hwang *et al*[46]	Colonoscopy	WL	Images	Customized CNN	Polyp detection and segmentation	-	-	-
Park *et al*[53]	Colonoscopy	WL	Images	Customized CNN	Whole image classification: normal, adenoma and adenocarcinoma	94%	~94%	-
Viscaino *et al* [54]	Colonoscopy	Greyscale	Images	Support vector machine; Decision treesk-nearest neighbours; Random forest	Whole image classification: polyp and non-polyp	97%	98%	96%

WL: White light; NBI: Narrow-band imaging; LCI: Linked colour imaging; Acc: Accuracy; Sen: Sensitivity; Spe: Specificity.

Detecting polyps and then identifying whether the detected polyp is hyperplastic or adenomatous[50]. Information about whether a polyp can be malignant will assist the clinician in making a better clinical decision (to remove or not the polyp)[51].

There are other alternatives to colonoscopy; colonography or wireless capsule endoscopy are also used as screening techniques to detect polyps. Both alternatives are less invasive and do not present a risk of perforation to patients as colonoscopy does. AI-based algorithms have also been used to enhance the analysis *via* CT colonography. In particular, the use of greyscale information in the image (using a grey-level co-occurrence matrix in the work of Tan *et al*[52] or texture information in the work of Hu *et al*[21]) combined with CNN has been useful for differentiation of polyps: Adenoma from adenocarcinoma[53], non-neoplastic from neoplastic polyp[21], or images with polyps and without polyps[54].

MICCAI 2015 POLYP DETECTION CHALLENGE

MICCAI proposes a common validation and evaluation framework of new algorithms published in the field of biomedical image analysis (Bernal *et al*[15] and the references therein). Each year MICCAI launches international competitions (challenges) that allow for benchmarking algorithms on publicly released datasets and offers a basis to discuss validation strategies[22]. In 2015, the MICCAI sub-challenge on automatic polyps was launched and represented a significant advance in the area. As a result of this competition, three large endoscopic image databases were published, establishing a benchmark for new algorithms[22,37,55].

Colonoscopy datasets

To successfully train classical machine learning models, it is necessary to have reasonably sized databases[22]. However, to train deep learning models, large databases are required because the quantity of data is related to the network

performance. The most famous public databases used in computer science are ImageNet[56], with more than 14 million natural images hand-annotated in 20000 categories, or Microsoft's COCO[57], with more than 2500000 images. The state-of-the-art reports better than 90% accuracy in classification, object detection and localization tasks with deep neural networks pre-trained with these databases. In medicine, creating large databases represents a challenge because the data and the expertly annotated ground truth are required. In the case of colonoscopy, some publicly available datasets for polyp detection and classification have been released in the last few years. In particular, efforts such as the MICCA 2015 sub-challenge have prompted different groups to create and make available the databases summarized in Table 4.

Three datasets annotated for automatic polyp detection have been very popular in the scientific community: CVC-ClinicDB[37], ETIS-Larib[55], and ASU-Mayo Clinic Colonoscopy Video[22]. Both datasets CVC-ClinicDB and ETIS-Larib are composed of annotated frames, whereas the ASU-Mayo Clinic dataset is composed of 38 fully annotated videos selected to show maximum variation in colonoscopy procedures. All public databases are summarized in Table 4, as well as their characteristics.

MISCONCEPTIONS IN AI

Deep learning-based AI models offer promising results for medical image analysis. Nevertheless, a thorough understanding of the available data and its limitations and proficient curation of suitable training, testing, and validation subsets are required to successfully train these models responsibly and use them in a clinical setup, *e.g.*, as a diagnostic support tool. Following are some of the most common misconceptions.

Imbalanced datasets

In medicine, obtaining samples to create datasets can be a time consuming and expensive process[15]. This becomes even more complicated when samples are obtained from invasive procedures such as colonoscopy. Another important aspect is that as a result of data scarcity (*e.g.*, due to the low incidence of a medical condition), an intrinsic imbalance in the data can occur. Therefore, the available colonoscopy datasets mostly do not contain the same number of samples per class (also known as an imbalanced dataset)[29,63,64]. If a deep learning model is trained on such a dataset, the result will present a high risk of exhibiting bias against the minority classes and, in extreme cases, ignoring it altogether.

Moreover, the dataset structure needs to be considered when studying the performance metrics of deep learning models, such as accuracy and/or error rate, which are the most frequently used metrics when evaluating classification results. However, both are insufficient when working with an imbalanced dataset, as the relative contribution of the minority classes to these metrics is negligible[30]. Best practice is to be aware of the limitations of each metric and evaluate the performance of the algorithm with a set of complementary metrics (see Table 2).

The time and effort required to curate balanced datasets for intrinsically imbalanced problems have led researchers to develop techniques to enable AI models to be successfully trained on an imbalanced dataset[30]. Currently, the proposed methods can be grouped into data-level techniques and algorithm-level methods, which can be combined in hybrid approaches.

Data-level techniques aim to decrease the level of imbalance by modifying the class distribution within the available dataset. On the one hand, under-sampling methods voluntarily discard data from the majority classes, reducing the total information available to train the model. The simplistic approach to under-sampling is random under-sampling, which discards random samples from the majority classes. Notwithstanding, valuable information might be lost in the process. Intelligent under-sampling methods select removal candidates using more elaborated criteria, such as redundancy within each class in the majority group, known as one-sided selection[65], or their distance from minority samples, known as near-miss algorithms, as the several alternatives presented in Mani *et al*[66]. On the other hand, over-sampling methods artificially increase the quantity of available data in the minority classes. One technique, random over-sampling (ROS), which randomly duplicates samples from the minority classes, is the naive approach to over-sampling and is known to cause overfitting[67]. The model memorizes particular training samples instead of learning the underlying characteristics of the corresponding class and is then unable to generalize to novel data [26]. Several methods have been proposed to reduce over-fitting while over-sampling, such as the synthetic minority over-sampling technique introduced in Chawla *et al*[68]

Table 4 Summary of publicly available colonoscopy datasets

Dataset	Year	Description	Data type	Ground truth
CVC-ColonDB[29,58]	2012	380 sequential WL images from 15 videos	Images (574 × 500 pixels)	Binary mask to locate the polyp
CVC-PolypHD[58,59]	2012	56 WL images	Images (1920 × 1080 pixels)	Binary mask to locate the polyp
ETIS-Larib[55]	2014	196 WL images from 34 video sequences (44 different polyps)	Images (1125 × 966)	Binary mask to locate the polyp
CVC-ClinicDB[37]	2015	612 sequential WL images from 31 videos sequences (31 different polyps)	Images (388 × 284 pixels)	Binary mask to locate the polyp
ASU-Mayo[22]	2016	38 short video sequences (NBI, WL)	Video (SD and HD video)	Binary mask for 20 training videos
Colonoscopic dataset[49]	2016	76 short video sequences (NBI, WL)	Video	Labels: hyperplastic, adenoma and serrated
Kvasir-SEG[60]	2017	1000 images with polyps	Images	Binary mask to locate the polyp
CVC-ClinicVideoDB[61]	2017	18 sequences	Video (SD video)	Binary mask to locate the polyp
CP-CHILD-A, CP-CHILD-B[62]	2020	10000 images	Images (256 × 256)	Labels: polyp and non-polyp

WL: White light; NBI: Narrow-band imaging.

and its variants Han *et al*[69], Jo *et al*[70], or the cluster-based over-sampling method proposed in Jo *et al*[70].

Algorithm-level methods comprise cost-sensitive learning algorithms[71], which assign penalties to each majority class, increasing the importance of the minority classes, and decision process adjustments, which shift the decision threshold such that bias towards the minority classes is reduced.

Correlated data

Another dataset structure aspect to consider when inspecting performance metrics is the presence of correlation between dataset splits (most commonly training, testing and validation)[26]. We consider the task of analysing images obtained from a recorded colonoscopy to classify detected colorectal polyps as malignant or benign. If the training and validation dataset splits contain frames from the same video or patient, the correlation introduced by this situation will affect validation metrics, resulting in over-optimistic results and the risk of hidden generalization or over-fitting problems.

Interpretability

Machine learning, and more broadly AI, are essentially statistical models. During the training process, a set of parameters that define the specific behaviour of the base model is adjusted so that model predictions match expert annotations for elements in the database. Notwithstanding, commonly used models do not consider domain-specific expert knowledge in their predictions. Hence, it is possible for the trained model to learn features that are undesirable or incorrect, such as unintended patterns or visual artefacts present in the database, instead of constraining the feature space to medically relevant features only. To avoid this problem, manual assessment of the database elements is advised, along with internal feature visualization techniques[72]. See Deng *et al*[73] for a review of different strategies proposed to incorporate expert knowledge as prior information to machine learning (ML)/AI models.

FUTURE PROSPECTS

The results obtained from AI-based models are promising and establish an advantage compared to traditional methods. However, there are some limitations to be overcome by future research to propose clinically useful methods.

Overcoming real-time constraints: Videos in a colonoscopy exploration are generally acquired at 25 frames per second[15], which means that the maximum time

available to process each image (frame) must be less than 40 ms.

Increasing the variability of polyp cases, including studies with data from multiple medical centres if possible. However, given the scarcity of data on less common lesions (serrated adenomas) and knowing that deep learning approaches require vast numbers of labelled training samples, new research may include techniques such as few-shot learning introduced by Vinyals *et al*[74]. This technique focuses on learning a class from one or a few labelled samples and has been successfully applied in other medical areas, such as cervical cancer cell classification[75], breast cancer classification [76], and metastatic tumour classification[77].

Including in the polyp detection scheme the ability to detect other elements such as folds or blood vessels that can appear in a real exploration and can affect current methods' performance.

Tests were performed on complete video sequences to analyse the performance of the model under temporal consistency constraints and high variability in polyp appearance due to camera progression. Both conditions might impact the models' performance in a real clinical environment.

The ability to obtain uncertainty estimates from ML/AI model predictions is key to a responsible adoption of these techniques in clinical setups, as biased recommendations from CADx/e can have adverse effects on the final diagnosis. Bayesian deep learning has been proposed as a framework to address this problem, where deep learning models can deliver uncertainty information along with classification results [78] at the expense of an increased number of training parameters (and hence more training data required) or a more restricted model structure, *e.g.*, the need to incorporate dropout units within the mode architecture, as in Gal *et al*[79] 2016. Both of the abovementioned techniques have been successfully combined with active learning algorithms that enable incremental dataset labelling and/or training of the model parameters as new data become available (see Gal *et al*[80], 2017, and Woodward *et al* [81], 2016).

CONCLUSION

AI is a promising area in gastroenterology. With the processing power and high performance of algorithms such as deep learning, a new era of AI-based computer-aided systems can assist physicians in essential tasks such as colorectal polyp detection and classification. To achieve clinically useful systems, both clinicians and technicians must cooperate to mitigate AI drawbacks. Although most of the current technological effort has been focused on creating more precise polyp detection and classification tools, it remains a long path to be covered before adopting AI-based technology into the physician's daily work as an assistive tool for diagnosis decisions.

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MINIREVIEWS

Faecal immunochemical test outside colorectal cancer screening?

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Abstract

Faecal immunochemical tests (FITs) are the most widely colorectal cancer (CRC) diagnostic biomarker available. Many population screening programmes are based on this biomarker, with the goal of reducing CRC mortality. Moreover, in recent years, a large amount of evidence has been produced on the use of FIT to detect CRC in patients with abdominal symptoms in primary healthcare as well as in surveillance after adenoma resection. The aim of this review is to highlight the available evidence on these two topics. We will summarize the evidence on diagnostic yield in symptomatic patients with CRC and significant colonic lesion and the different options to use this (thresholds, brands, number of determinations, prediction models and combinations). We will include recommendations on FIT strategies in primary healthcare proposed by regulatory bodies and scientific societies and their potential effects on healthcare resources and CRC prognosis. Finally, we will show information regarding FIT-based surveillance as an alternative to endoscopic surveillance after high-risk polyp resection. To conclude, due to the coronavirus disease 2019 pandemic, FIT-based strategies have become extremely relevant since they enable a reduction of colonoscopy demand and access to the healthcare system by selecting individuals with the highest risk of CRC.

Key Words: Adenoma; Colorectal cancer; Diagnostic performance; Faecal biomarkers; Faecal haemoglobin; Faecal immunochemical test; Primary healthcare

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biomarker used widely in CRC screening programmes. In recent years, a large body of evidence has appeared that enables recommending its use in different scenarios. For the evaluation of symptomatic patients in primary healthcare, FIT improves use of available endoscopic resources, avoiding unnecessary colonoscopies, predicts the risk of CRC and may have an impact on prognosis. Furthermore, although endoscopic surveillance after adenoma resection is widely extended, there are relevant doubts over its efficiency in the context of high-quality baseline colonoscopies and a FIT-based surveillance strategy could be an alternative.

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INTRODUCTION

Colorectal cancer (CRC) is a relevant health problem in the Western world. In 2018, almost half a million new cases were diagnosed in Europe and 250000 patients died due to CRC[1]. Health authorities have devised two main strategies to reduce the impact of CRC: screening in average- and high-risk populations and early detection in symptomatic patients[2-4].

All preventive and diagnostic strategies are based either on invasive or non-invasive techniques. Colonoscopy is the cornerstone of all techniques, due to its diagnostic yield, the capacity of histological sampling, and especially, its ability to perform therapeutic procedures. However, colonoscopy is time and resource consuming, there is limited capacity with potential waiting lists and it is associated with side effects. Among the non-invasive techniques either imaging techniques or several diagnostic biomarkers have been evaluated[5].

Faecal occult blood tests (FOBTs) are the most widely CRC diagnostic biomarker available. FOBTs detect either blood or blood products (such as globin) in faeces with different methods. There are two main types of FOBTs: Chemical (cFOBT) or immunological (faecal immunochemical test, FIT). cFOBTs demonstrated its effect on reducing CRC mortality in CRC screening in large-scale randomized controlled trials[5]. However, they have been gradually replaced by semiquantitative FITs due to their advantages. FITs are based on the reaction of monoclonal or polyclonal antibodies specific for human faecal haemoglobin (f-Hb), albumin, or other faecal blood components. Thus, they do not require any dietary or pharmacological restriction, as long as they do not react with blood from the upper digestive tract or with any food component. The greatest advantage is determined by its ability to detect and quantify f-Hb concentrations 7 to 15 times lower than those detected by chemical tests. This significantly improves CRC and advanced adenoma detection sensitivity. Moreover, FITs enable reliable and accurate automated analyses, which prevents subjective interpretation[6]. For this reason, most CRC mass screening programmes have opted for FIT. Moreover, there is a large amount of evidence on the diagnostic accuracy of FITs for CRC and adenoma detection in asymptomatic patients[7].

In recent years, a large amount of evidence has been produced on the use of FITs to detect CRC in patients with abdominal symptoms[8-10] as well as in the surveillance of high-risk subjects (surveillance after adenoma resection)[11,12]. Furthermore, due to the coronavirus disease 2019 (COVID-19) pandemic, FIT has become extremely relevant due to the limited endoscopic resources available and access to healthcare systems[13]. The aim of this review is, therefore, to update the evidence and recommendations available on use of FIT outside the scope of CRC screening. This topic is structured into the following sections: Evidence on FIT in the evaluation of symptomatic patients; risk prediction models for CRC incorporating FIT in symptomatic patients; a combination of FIT with other non-invasive biomarkers in patients with abdominal symptoms; recommendations on the use of FIT in primary healthcare; effect of FIT on CRC prognosis; and FIT for surveillance after adenoma resection.

EVIDENCE ON FIT IN THE EVALUATION OF SYMPTOMATIC PATIENTS

Despite the implementation of CRC screening programmes, most CRC cases are still diagnosed after symptomatic presentation[14]. A large number of studies have been performed on the effectiveness of FIT for triaging referrals; also in symptomatic patients, which have been summarized in a number of recent systematic reviews[8-10, 15]. The first systematic review assessing the value of immunochemical-based FOBTs as first-line investigation in symptomatic patients was completed in 2008[15]. This review included nine studies evaluating several FIT assays using different methods and reported that FIT had better diagnostic performance than cFOBTs. However, the studies included had a small sample size and a high degree of clinical heterogeneity (*i.e.* different populations, use of quantitative and qualitative FITs), which limits the conclusions of the meta-analysis.

This work was supplemented by another systematic review[10], conducted to inform the development of a new National Institute for Health and Care Excellence (NICE) diagnostics guidance (DG) 30[16]. This review included nine studies, which provided data about clinical evaluations of three quantitative FIT assays and showed that when the FIT result is based on a single sample with a cut-off point of 10 μg Hb/g of faeces, the sensitivity for detecting CRC was 92.1% (95% confidence interval [CI]: 86.9-95.3) and 100% (95%CI: 71.5-100) for OC-Sensor (Eiken Chemical Co. Ltd., Tokyo, Japan) and HM-JACKarc (Kyowa-Medex Co. Ltd., Tokyo, Japan) FIT assays, respectively, indicating that both could be useful to rule out CRC. In that review, specificity was estimated to be 85.8% (95%CI: 78.3-91) and 76.6% (95%CI: 72.6-80.3) for OC-Sensor and HM-JACKarc FIT assays, respectively. The systematic review also included results from a study evaluating the diagnostic accuracy of the FOB Gold (Sentinel Diagnostics, Milan, Italy) FIT assay to detect a significant colonic lesion (SCL) using a f-Hb cut-off of 9 μg Hb/g faeces, defined as bleeding, cancer or polyp (sensitivity 45.2%; specificity 92.3%). As a result of this evidence,[10] NICE recommended use of the OC Sensor®, HMJACKarc®, and FOB Gold® FIT assays in primary healthcare to assess people who have accounted low risk symptoms (without rectal bleeding) who do not meet the criteria for a suspected cancer pathway referral, using a threshold of 10 μg Hb/g faeces[16,17]. However, important clinical concerns have been raised related to this recommendation[18].

Applicability of quantitative FITs in the assessment of patients with abdominal symptoms in primary healthcare

The systematic review supporting DG 30 only included one study performed in a primary healthcare setting[19], and none of the reviewed studies assessed FIT accuracy in real practice. Hence, the assessment of FIT accuracy to detect CRC in a cohort of symptomatic patients recruited from primary healthcare could lead to different results when compared to another comprised by symptomatic patients referred to secondary healthcare[8]. This could happen for reasons unrelated to CRC prevalence[20]. In this sense, high-risk symptoms (*i.e.* rectal bleeding) are shared between CRC and other benign conditions (*i.e.* anorectal disease, diverticular disease, colorectal polyps, inflammatory bowel disease or non-steroidal anti-inflammatory drug related enteropathy) which are more prevalent, thus reducing FIT specificity to detect CRC[21].

A subsequent meta-analysis was aimed at solving these problems[8]. The authors performed subgroup analyses according to the characteristics of the studies (100% symptomatic cohorts/mixed cohorts of symptomatic/asymptomatic subjects) and CRC prevalence. In this respect, the pooled estimates of sensitivity for studies comprised solely by symptomatic patients and studies made up of mixed cohorts was 94.1% (95%CI: 90.0-96.6) and 85.5% (95%CI: 76.5-91.4), respectively. Conversely, there were no statistically significant differences between the pooled sensitivity of studies with CRC prevalence < 2.5% (84.9%, 95%CI: 73.4-92.0) and ≥ 2.5% (91.7%, 95%CI: 83.3-96.1).

Since then, many studies assessing the diagnostic accuracy of FIT have been performed in primary healthcare. However, some have limitations, mainly due to their retrospective nature[9] or the criteria used for CRC diagnosis. Several studies have not used colonoscopy or a sufficient follow-up time (at least 2 years) to detect incident CRC. These two criteria have been used as "reference tests" in the systematic reviews available to select high-quality studies[8,10,15,22]. Furthermore, they have been confirmed in a different meta-analysis which detected similar diagnostic accuracy among the studies that used these criteria[7]. This evidence has been summarized in a recently published meta-analysis that has evaluated the diagnostic accuracy of FIT in patients presenting lower abdominal symptoms in primary healthcare. The results

confirmed previous findings. Twenty-three studies (69536 participants) were included with a CRC prevalence ranging between 0.3% and 6.2%. Six studies (n = 34691) evaluated FIT as rule in test (threshold of ≥ 150 μg Hb/g faeces) showing moderate sensitivity (64.1%, 95%CI: 57.8-69.9) and high specificity (95.0%, 95%CI: 91.2-97.2). A threshold of 10 μg/g (15 studies; n = 48872) resulted in sensitivity and specificity of 87.2% (95%CI: 81.0-91.6) and 84.4% (95%CI: 79.4-88.3) for CRC, respectively[9].

In this meta-analysis, the number needed to scope to detect a CRC as well as the number of missed CRC per 1000 patients according to expected CRC prevalence in primary care was calculated. The number of missed CRC per 1000 patients if a patient has a 'negative' FIT result in a population with a CRC prevalence of 2% is expected to increase from four to five when using the threshold of 20 μg Hb/g faeces instead of 10 μg Hb/g faeces. However, at the same CRC prevalence, the number needed to scope is expected to decrease from 10 to 4 if the 150 μg Hb/g faeces threshold is used instead of 10 μg Hb/g faeces[9].

Accuracy of FIT in detecting SCL and gastrointestinal cancer in patients with abdominal symptoms

As previously discussed, abdominal symptoms are non-specific and shared among benign and malignant diseases. Thus, the aim of a physician is not only to rule out CRC as long as other benign conditions may also present with the same symptoms and may benefit from diagnosis. In this sense, a previous meta-analysis revealed that FIT may not be sensitive enough to rule out all SCL[8]. Unfortunately, there is a high degree of heterogeneity among the studies evaluated due to the FIT brands used, the threshold selected, and especially, the differences in SCL definitions. The definitions used vary among inflammatory bowel disease, cancer, or high-risk adenoma; plus any type of colitis; plus any advanced adenoma, polyposis, complicated diverticular disease, colonic ulcer and bleeding angiodysplasia or even any colonic lesion detection regardless of its importance[8]. In a recently published meta-analysis, the overall pooled sensitivity and specificity of FITs for SCL for studies that used the limit of detection as a threshold (seven studies; n = 22624 patients) was 70.4% (95%CI: 68.4-72.3) and 78.4% (95%CI: 77.8-78.9), respectively. At the ≥ 10 μg Hb/g faeces threshold (seven studies; n = 20407 patients), the sensitivity and specificity were 69.1% (95%CI: 60.5-76.5) and 87.2% (95%CI: 83.4-90.2), respectively. Furthermore, three studies (n = 20528 patients) evaluated the diagnostic accuracy of FIT with a threshold of ≥ 150 μg Hb/g faeces showing sensitivity and specificity of 35.9% (95%CI: 33.8-38.1) and 97.5% (95%CI: 97.3-97.8), respectively[9].

Gastrointestinal (GI) symptoms are non-specific and could be related to different GI diseases. A relevant question is whether symptomatic patients with a completely normal colonoscopy after a positive FIT require further evaluation. In a recently published study, the risk of GI cancer detection (upper GI cancer and CRC) after a complete colonoscopy without CRC was evaluated according to the FIT result (threshold 10 μg Hb/g faeces). During a mean time of 45.5 ± 20.0 mo, GI cancer was detected in 57 (2.1%) patients: upper GI cancer in 35 (1.3%) and CRC in 14 (0.5%). FIT-positive subjects revealed a higher CRC risk (hazard ratio [HR] 3.8, 95%CI: 1.2-11.9] with no differences in GI (HR 1.5, 95%CI: 0.8-2.7) or upper GI cancer risk (HR 1.0, 95%CI: 0.5-2.2). Upper GI cancer was detected in 22 (0.8%) patients during the first year. Two variables were independently associated: anaemia (odds ratio [OR] 5.6, 95%CI: 2.2-13.9) and age ≥ 70 years (OR 2.7, 95%CI: 1.1-7.0)[23].

Does one sample with a cut-off point of 10 μg Hb/g of faeces fit everybody?

NICE recommends a single f-Hb cut-off of 10 μg Hb/g faeces to be used in the evaluation of symptomatic patients at all ages regardless of sex. However, using FIT with the same cut-off for asymptomatic populations has been associated with a higher accuracy for advanced adenoma detection in males[24]. However, a recently published meta-analysis on the diagnostic accuracy for CRC detection in asymptomatic patients did not show any differences between males and females[7]. These variations may be related to the differences in advanced adenoma prevalence and location as well as colonic transit.

One key question is what threshold is used to determine a positive result in the evaluation of symptomatic patients. Most studies have evaluated the 10 μg Hb/g of faeces threshold to triage symptomatic patients as this cut-off approximates to the quantitation limit documented by the manufacturers of most FIT analytical systems[8, 10]. Nonetheless, a recent study showed that using a cut-off point of 20 μg Hb/g of faeces could reduce the number of colonoscopy examinations without missing more than 1 CRC per 1000 patients evaluated belonging to the low-risk group defined by

NICE DG 30[9]. Moreover, other thresholds have also been proposed: limit of determination, and recently, 150 μg Hb/g of faeces[25]. Thus, while patients with a concentration below the limit of determination had a risk of detecting a CRC less than 0.2%; in patients with a Hb greater than 150 μg/g of faeces the risk of detecting a CRC was greater than 31.1%.

In fact, the choice of threshold is a trade-off among the number of patients required to be referred to colonoscopy, the number of missing CRC, and CRC prevalence. In our meta-analysis, we calculated the number needed to scope to detect a CRC as well as the number of missed CRC per 1000 patients according to expected CRC prevalence in primary care. The number of missed CRC per 1000 patients if a patient has a 'negative' FIT result in a population with a CRC prevalence of 2% is expected to increase from four to five when using the threshold of 20 μg Hb/g faeces instead of 10 μg Hb/g faeces. However, at the same CRC prevalence, the number needed to scope is expected to decrease from 10 to 4 if the 150 μg Hb/g faeces threshold is used instead of 10 μg Hb/g faeces[9]. With respect to colonoscopy, we must take into account not only the resources required to evaluate patients and waiting lists but also the risks associated with colonoscopy. In a recently published meta-analysis, they were estimated at 5.8 perforations per 10000 colonoscopies (95%CI: 5.7-6.0) and 2.4 cases of relevant bleeding per 1000 colonoscopies (95%CI: 2.4-2.5)[26]. In this sense, it also seems reasonable to use a higher cut-off than that recommended by NICE, if there is well-planned safety netting to evaluate symptomatic patients with a negative result if symptoms persist. Furthermore, results could also be closely monitored locally to enable the rapid adjustment of cut-offs to optimize each area's resources[27]. However, because f-Hb correlates directly with the severity of colorectal lesions, raising the f-Hb cut-off will lead to losing a higher number of SCLs. However, those are less urgent for diagnosis and could easily be subsequently rescued in an environment whose colonoscopy resources are preserved through the appropriate use of FIT as a first line triage test.

There is the option of using more than one FIT determination. Two studies included in a previous meta-analysis[8,10] examined the utility of one *vs* two faecal samples for detecting advanced neoplasia (CRC plus advanced adenoma). The diagnostic yield of the two samples using a cut-off of 20 μg Hb/g faeces was attained with only one sample using a cut-off of 10 μg Hb/g faeces. This highlights the need for further investigation to verify the efficiency of using different strategies to triage not only advanced adenoma but also any SCL, if the use of a higher cut-off than that recommended by NICE (10 μg Hb/g faeces) is conditioned to a "safety netting", which requires more than one FIT sample.

RISK PREDICTION MODELS FOR CRC INCORPORATING FIT IN SYMPTOMATIC PATIENTS

A number of predictive models have been developed to improve clinical judgement in patients with abdominal symptoms, and some have included quantitative FITs[28]. In recent years, two prediction models (COLONPREDICT and the FAST score) have been developed and were externally validated, not only to identify people at higher risk of CRC but also to define a subgroup whose CRC risk is so low that we can ensure that no further evaluation is required[29,30]. For that purpose, both prediction models defined two cut-offs. The first (COLONPREDICT< 3.50 and FAST score < 2.12 respectively) was evaluated to identify a low-risk population with a negative predictive value (NPV) of having CRC higher than 99%. In this subgroup, no further evaluation should be recommended as the risk of performing a colonoscopy is similar to the risk of severe complications associated with this exploration[26]. A second cut-off (COLONPREDICT ≥ 5.60, FAST score ≥ 4.50) was calculated to define a high-risk subgroup in which at least 90% of CRC would be detected.

Despite COLONPREDICT, the model has shown a high diagnostic performance, with an area under the curve (AUC) of 0.92 in both derivation and validation cohorts, it has been criticized as too complex for routine primary healthcare practice due to considering too many clinical (age, sex, acetylsalicylic acid treatment, previous colonoscopy, rectal mass, benign anorectal lesion, rectal bleeding and change in bowel habit) and laboratory (serum carcinoembryonic antigen, faecal and blood haemoglobin) variables[29]. Conversely, the FAST score combines only three variables (f-Hb, sex and age). In addition to being easier, this model has also shown high accuracy to predict the individual risk of CRC and SCL in symptomatic patients (AUC = 0.88)[30]. To date, none of these prediction models have been validated in primary

healthcare.

A similar risk score was developed by Rodríguez-Alonso *et al*[31] for advanced neoplasia detection. A score between 0 and 11 is calculated for each patient according to three variables (sex, age, and FIT), and a risk score ≥ 5 is considered the optimal cut-off point for colonoscopy referral. More recently, the COLONOFIT score aims to assess the risk of advanced neoplasia (CRC plus advanced adenoma) in symptomatic patients with indication of a fast-track colonoscopy[32]. This model is based on age, colonoscopy (in the previous 5 years), tobacco use, and variables related to FIT (maximum f-Hb value and number of samples with FIT > 4 μg Hb/g faeces). A COLONOFIT score > 10 points enables diagnosis of 98% of CRC (NPV = 99.7%) and 77% of advanced adenomas, and has been shown to classify patients 3% to 4% better than the FAST score in this study. However, COLONFIT needs the submission of three FIT samples, which could reduce adherence and compromise its successful implementation in primary healthcare.

COMBINATION OF FIT WITH OTHER NON-INVASIVE BIOMARKERS IN PATIENTS WITH ABDOMINAL SYMPTOMS

Although many biomarkers have been evaluated for the detection of CRC in a screening setting[33], they have shown little applicability in clinical practice. A few studies have explored the possibility of improving the diagnostic performance of FIT in combination with other non-invasive biomarkers, mainly faecal calprotectin, M2-piruvate kinase and volatile organic compounds, in symptomatic patients[19,34-36]. In general, adding a second biomarker either improves sensitivity reducing specificity and increasing the number of patients referred to colonoscopy, or by contrast, increases specificity reducing sensitivity and the number of patients with a positive result. Only one study has evaluated the concomitant analysis of FIT and faecal calprotectin. This has shown mixed results, presumably due to heterogeneity of both targets, FIT assays, and cut-offs used. Mowat *et al*[19] reported the diagnostic accuracy of FIT (OC-Sensor®) and faecal calprotectin to detect SCL (CRC plus higher risk adenoma plus inflammatory bowel disease) using different cut-offs. The sensitivity of one sample of the OC-Sensor to detect CRC and SCL was 89.3% and 68.6% respectively (cut-off 10 μg Hb/g faeces). Furthermore, when using the limit of haemoglobin detection as a cut-off, the sensitivity to detect CRC and SCL improved by 100% and 88.2%, respectively. Finally, when adding the measurement of faecal calprotectin, the sensitivity to detect SCL increased by 91.2% and 96.1% using the 200 μg Hb/g faeces and 50 μg Hb/g faeces cut-offs, respectively[19].

RECOMMENDATIONS ON USE OF FIT IN PRIMARY HEALTHCARE

Not many international clinical guidelines have opted for the potential advantages of introducing FIT in daily clinical practice. The available literature was reviewed by van Melle *et al*[37], which identified a limited number of countries with clinical guidelines that explicitly recommended use of FIT in symptomatic patients: Australia[38], Spain [5], United Kingdom[16,17] and Denmark (limited to specialized healthcare). We searched most guidelines mentioned by van Melle *et al*[37], in addition to others such as the Italian[39], Colombian[40] and English versions of the Chinese guideline[41]. However, only the 2019 update of the Scottish guideline[42] raised the possibility of including FIT, after completion of several pilot studies currently being developed in Scotland (Table 1).

As previously noted, the NICE recommended that FIT be performed in primary healthcare in symptomatic patients with a positive predictive value below 3%[16,17] after performing a systematic review[10]. Specifically, in the National Institute for Health and Care Excellence guideline, 12 FIT is recommended in patients without rectal bleeding aged under 60 with altered bowel habits or iron deficiency anaemia, patients without rectal bleeding aged over 50 with abdominal pain or weight loss (in which the combination of both symptoms would have been a fast-track criterion); and patients older than 60 with anaemia[17]. Patients with a positive FIT should be referred through the fast-track pathway for further evaluation. In the DG 30, FIT is recommended in any symptomatic patient that does not meet any of the fast-track criteria[18]. The Australian guideline[38] includes the indications of the NICE guidelines to recommend FIT in patients aged under 60 without overt bleeding with a

Table 1 Clinical guidelines and recommendations on colorectal cancer diagnosis that include the use of faecal immunochemical test in primary healthcare

Guideline	Year	Criteria to use FIT
NICE DG 30 United Kingdom [16]	2017	People without rectal bleeding who have unexplained symptoms but do not meet the criteria for a suspected cancer pathway referral outlined in NICE's guideline on suspected cancer
Australia[38]	2018	In people with symptoms other than overt rectal bleeding, FIT can be used as part of the diagnostic assessment in primary healthcare. It is of particular use in the following circumstances to support diagnostic assessment and notify the urgency of colonoscopy: people over 50 yr with either unexplained weight loss or abdominal pain; and people under 60 yr with either altered bowel habit or anaemia
Spain[5]	2018	Patients with lower gastrointestinal symptoms of recent onset who do not meet criteria for referral without delay to a specialist service due to high suspicion of CRC (rectal or abdominal mass, rectal bleeding or iron-deficiency anaemia) should undergo a FIT
NICE NG12 United Kingdom [17]	2021	Offer FIT to assess for CRC in adults without rectal bleeding who: Are aged 50 and over with unexplained abdominal pain or weight loss, or are aged under 60 with changes in their bowel habit, or iron-deficiency anaemia, or are aged 60 and over and have anaemia even in the absence of iron deficiency

CRC: Colorectal cancer; DG: Diagnostics guidance; FIT: Faecal immunochemical test; NG: National Institute for Health and Care Excellence guideline; NICE: National Institute for Health and Care Excellence.

change in bowel habit or anaemia; and in those over 50 with abdominal pain or weight loss. Based on the article published by Mowat *et al*[19], the recommendation justified performing FIT to rule out SCL.

The Spanish guideline on CRC diagnosis and prevention[5] is based on the NICE DG 30 (including the 10 μg Hb/g of faeces cut-off) and the systematic review performed by Westwood *et al*[10]. FIT is recommended for the evaluation of any patient with lower GI symptoms if they do not meet the criteria for urgent referral to colonoscopy (tumour or mass on examination or imaging tests, rectal bleeding associated with CRC or iron deficiency anaemia). Therefore, this guide includes non-suspicious rectal bleeding as a FIT application criterion. In the event of a positive result, the guide recommends requesting a colonoscopy and in case of negative result, to monitor symptoms and refer the patient to a specialized level if symptoms persist.

On the other hand, guidelines that do not recommend FIT in primary healthcare generally propose referral to specialized healthcare for all high-risk symptoms and only recommend observation in patients with low-risk symptoms such as loss of appetite, constipation or mucus in the faeces[37]. The specialist will probably finally request the colonoscopy and decide priority himself. The New Zealand guide, updated in 2014[43], explicitly advises against its use, due to the lack of evidence in its favour and compares it with carcinoembryonic antigen. The Ontario clinical guidelines[44] withdrew the recommendations that included cFOBTs in 2017, to avoid a possible conflict with its screening programme. In 2019, this exclusion was extended to FITs. Other clinical guidelines only contemplate cFOBTs[45].

EFFECT OF A FIT-BASED STRATEGY ON RESOURCES

As we have seen, the guidelines that recommend FIT in primary healthcare are intended to identify a subgroup of patients at high risk of CRC detection despite presenting mild symptoms. Otherwise, these patients would not have been included in the fast-track colonoscopy. However, a negative FIT result in these patients would reduce the number of colonoscopies performed with no relevant findings[10]. This strategy may avoid colonoscopy-related risks in subjects with a low CRC risk and facilitate better prioritization on colonoscopy waiting lists. In this sense, an estimation performed based on the results obtained in the Pin-Vieito study[8] with a 3% CRC estimated prevalence and 10 μg Hb/g and 20 μg Hb/g of faeces cut-off (cohorts 100% symptomatic) highlights that FIT will avoid approximately 2/3 of the colonoscopy with two missed CRC out of 1000 patients evaluated (Figure 1). If we take the SCL into consideration with 12% and 10 μg Hb/g of faeces cut-off, the positive predictive value would be 24.8%, and the number of undetected SCL would be 24 out of 1000 subjects, mainly advanced adenomas.

Due to the COVID-19 pandemic, the risk stratification of subjects with GI symptoms has become of the utmost relevance. A modelling study has recently been published.

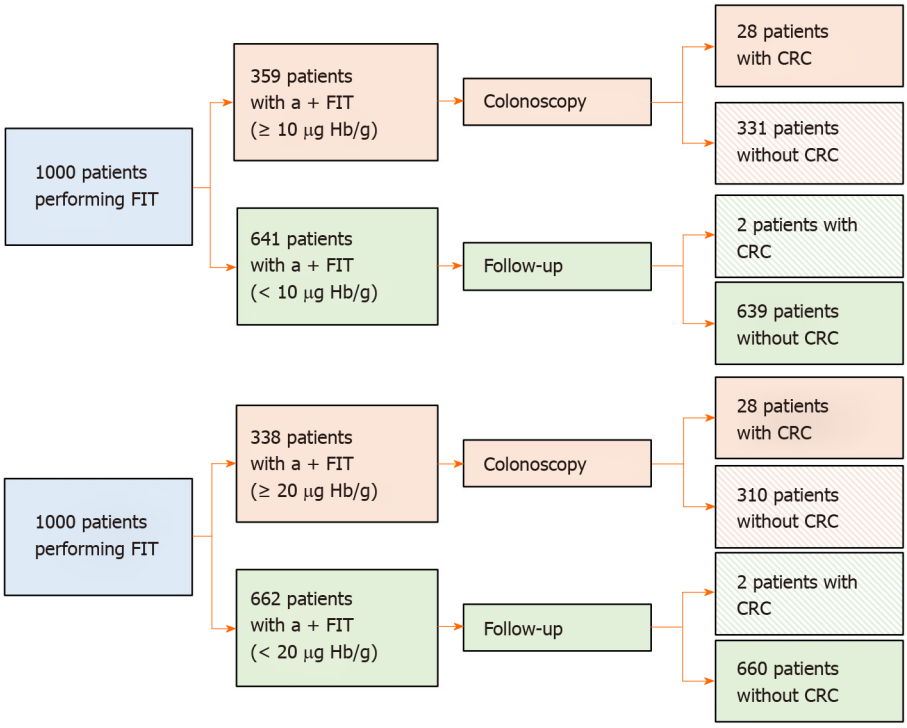

Figure 1 Estimation of the number of patients who need to be referred for colonoscopy, and the diagnostic yield for colorectal cancer in the 10 and 20 μg Hb/g of faeces thresholds[8]. CRC: Colorectal cancer; FIT: Faecal immunochemical test.

This model evaluated the effect of delays for colonoscopy on CRC mortality due to lack of endoscopic capacity and prioritization of colonoscopy in patients with a f-Hb ≥ 10 μg Hb/g of faeces. A delay higher than 6 mo would lead to 2250 attributable deaths and loss of 32799 life-years. In contrast, using FIT to stratify only 18% of symptomatic patients would be referred to colonoscopy; 89% of these deaths would be avoided and the requirements for colonoscopy would be reduced by > 80%[13]. This information has been confirmed in a recently published retrospective study in primary healthcare with a sample size of 14487 consecutive patients who underwent a FIT due to low-risk symptoms without fast-track criteria[46]. Using a ≥ 10 μg Hb/g faeces threshold, 10% of adults would be investigated to detect 91% of cancers with a number needed to scope of ten to detect one cancer and three to detect a SCL. Only 12 CRCs (< 2/1000 subjects) would be missed in the subjects evaluated. This proportion is similar to the colonoscopy-associated side effects[26] and CRC prevalence in asymptomatic adults aged 50-69[47].

A relevant topic is the strategy in subjects with a negative FIT result. Unfortunately, the information is limited. Hypothetically, symptoms in patients with CRC and a negative FIT will persist or even worsen, so they would require additional medical healthcare. In the Spanish guidelines, follow-up and request for colonoscopy are recommended if symptoms persist[5]. The results of the study performed by Nicholson *et al*[46] support this strategy because seven out of the 12 FIT-negative CRC were detected within 1 mo after FIT. A better understanding of the characteristics of patients with false negative results and their clinical course, is key to guide the most effective diagnostic tests to perform during follow-up.

Westwood *et al*[10] published a cost-effectiveness analysis to inform NICE about the use of FIT in symptomatic patients. For the effectiveness analysis, a meta-analysis was performed and for the cost analysis, a model based on quality-of-life-adjusted life years (QALYs) was devised. Researchers compared three strategies: triage with FIT, triage with cFOBT and no triage (direct referral to colonoscopy). The cost of a colonoscopy and the FIT was estimated at £372 and £4.53, respectively. The differences in QALYs between the three strategies were minimal. The triage strategy using FIT demonstrated a higher cost, but also higher efficacy and cost-effectiveness than cFOBT. It would only be surpassed in cost-effectiveness by placing the cut-off point at the detection threshold of 2 μg/g of Hb in faeces. The FIT also demonstrated greater cost-effectiveness compared to no triage, with an incremental cost of £258.09 per patient. Taking into account the lost QALYs due to FIT false-negatives, saving of £2578.543 per lost QALY was calculated using triage with FIT.

EFFECT OF FIT ON CRC PROGNOSIS

The main objective of any diagnostic strategy is to improve the prognosis of the disease detected. We have evidence that FIT-based CRC screening improves CRC prognosis through early detection[48]. However, the information regarding CRC prognosis detected after a positive FIT in symptomatic subjects is still limited. In this sense, a Polish retrospective analysis of 535 CRC detected in symptomatic subjects evaluated the effect of the pathway to CRC diagnosis on prognosis. CRC detected after a positive FIT (HemCheck-1) result revealed better prognosis than CRC detected on the basis of clinical evaluation (40 ± 47 mo *vs* 25 ± 38 mo; $P < 0.001$)[49].

In a recently published Spanish population-based study[14], a significantly longer 3-year survival was observed in patients with CRC diagnosed after a positive FIT in comparison with CRC detected after a negative result or without a FIT (HR 1.50; 95%CI: 1.22-1.84). These differences in prognosis were related to an earlier CRC stage at diagnosis in the positive FIT group. The reason for these findings is unclear; it is hypothesized that requesting a FIT could reduce diagnostic delay. However, there is a risk of bias, since in one-third of the positives the reason for the FIT request could not be confirmed and they could be related to opportunistic CRC screening.

Although these two retrospective studies have limitations and risk of bias (ignorance of comorbidities, circumstances of the CRC diagnosis, indications for FIT, single-centre, *etc.*), they support the hypothesis that evaluation of symptomatic patients with FIT could modify CRC prognosis. The causes of these differences are not clear and may include diagnostic delay, severity of the onset symptoms and characteristics of the general practitioner. Prospective studies need to be performed that evaluate the effect of a FIT-based diagnostic strategy on CRC prognosis to fully identify the strengths and weaknesses and thus find their ideal place in clinical practice guidelines.

FIT IN SURVEILLANCE AFTER ADENOMA RESECTION

Colorectal polyps are precursor lesions for CRC. Therefore, their removal during colonoscopy reduces CRC risk[5]. These patients have an increased risk of developing more polyps and eventually CRC over the years, so adopting surveillance strategies is recommended[5]. In recent years, mainly motivated by the implementation of the CRC populations screening programmes, there has been an increase in the number of patients with resected colorectal polyps that require surveillance. This has meant that colonoscopies have also undergone a major increase, which require large amounts of resources and highlights the currently overloaded endoscopy services[50,51]. However, we must keep in mind that colonoscopy is an invasive and expensive procedure, with a risk of adverse events, associated both with the procedure itself and with the sedation necessary for it to be performed correctly[26]. For these reasons, it seems reasonable that surveillance colonoscopy for patients after polyp removal should be targeted at those most likely to benefit[51].

The aim of surveillance after colorectal polyp resection is to reduce CRC incidence [51]. In order to make decisions on surveillance, there are two questions that need to be answered: what is the long term (10 years) risk of CRC without surveillance, and does endoscopic surveillance reduce CRC risk compared with no surveillance/participation in a CRC screening programme?

Until recently, the information available was limited and referred to the short-term risk of advanced adenoma detection, an intermediate lesion[52]. Several long-term studies with CRC incidence as the main endpoint have recently been published (Table 2)[53-58]. They reveal that subjects with low-risk lesions (mainly 1-2 non-advanced adenomas or serrated lesions) have a long-term CRC risk similar to the control group (general population or subjects with normal colonoscopy). In contrast, CRC risk is increased in subjects with high-risk lesions (mainly advanced adenomas/serrated lesions and/or multiple adenomas). Taking into account these results, the available practice guidelines recommend in the low-risk group a surveillance strategy equivalent to that recommended in the general population: participation in a CRC screening programme[2,5,59-61].

However, the evidence regarding the benefits of endoscopic surveillance in high-risk lesions is limited to cohort studies. In the study published by Cottet *et al*[62] in 2012, the standardized incidence ratio was 1.10 (95%CI: 0.62-1.82) and 4.26 (95%CI: 2.89-6.04) in those patients with and without colonoscopy follow-up, respectively. In a recently published study including 6239 patients with high-risk lesions, endoscopic

Table 2 Colorectal cancer risk in patients with high risk adenomas or low risk adenomas

Ref.	Patients	Follow-up in yr	Comparison group	High risk lesions	Low risk lesions
Løberg *et al*[53], 2014	40826	7.7	General population	SIR 1.62 (95%CI: 1.50-1.75)	SIR 0.98 (95%CI: 0.89-1.08)
Click *et al*[56], 2018	15935	12.9	No adenoma group	RR 2.7 (95%CI: 1.9-3.7)	RR 1.2 (95%CI: 0.8-1.7)
Lee *et al*[54], 2020	64422	8.1	No adenoma group	HR 2.61 (95%CI: 1.87-3.63)	HR 1.29 (95%CI: 0.89-1.88)
Wieszczy *et al*[55], 2020	236089	7.1	General population	SIR 0.65 (95%CI: 0.51-0.82)	SIR 0.35 (95%CI: 0.26-0.45)
He *et al*[58], 2020	122899	10	No adenoma group	HR 4.07 (95%CI: 2.89-5.72)	HR 1.21 (95%CI: 0.68-2.16)
Cross *et al*[57], 2021	21318	10.1	General population	SIR 1.30 (95%CI: 1.03-1.62)	SIR 0.75 (95%CI: 0.63- 0.88)

HR: Hazard ratio; RR: Rate ratio; SIR: Standardized incidence ratio.

surveillance was associated with a reduction in CRC risk (HR 0.71, 95%CI: 0.49-1.03 for 1 visit; 0.44, 0.28-0.70 for ≥ 2 visits)[57]. Atkin *et al*[63] also showed in a study including 12000 patients with high-risk lesions (1-2 adenomas ≥ 10 mm or 3-4 adenomas < 10 mm), that performing at least one endoscopic surveillance reduces the incidence of CRC (HR 0.57, 95%CI: 0.40-0.80). However, this risk reduction was limited to a subgroup of patients: low-quality colonoscopy, large (≥ 20 mm), high-grade dysplasia and proximal adenomas. In this respect, available guidelines recommend performing baseline colonoscopy with full exploration of the colonic mucosa and resection of all polyps detected[2,5,59-61]. CRC detection during surveillance depends not only on the characteristics of the polyps but also on the endoscopist's technical ability. A recently published Polish study revealed that long-term risk of CRC is increased (HR 2.69, 95%CI: 1.62-4.47) if baseline colonoscopy is performed by low-performing endoscopists (adenoma detection rate < 20%)[64].

One limitation of FIT is its limited diagnostic accuracy for adenomas at a single determination. In asymptomatic subjects, at a single determination FIT detects 31% and 21% of advanced adenomas at the 10 μg Hb/g and 20 μg Hb/g of faeces thresholds, respectively, with specificity higher than 90%[7]. There are several characteristics of the adenomas associated with a positive FIT: number, location, morphology and size[5]. However, the strength of a FIT-based CRC screening is that it is based on periodic (annual or biennial) determination. Furthermore, the threshold used can be tailored according to colonoscopy capacity and long-term objective. The evidence available on a FIT-based surveillance is limited. A prospective British study published in 2019 investigated whether faecal FIT could reduce the surveillance burden on patients and endoscopy services. The study population was patients with intermediate risk of CRC after polyp removal (1-2 adenomas ≥ 10 mm or 3-4 adenomas < 10 mm). Subjects were offered an annual FIT and all subjects underwent a 3-year scheduled colonoscopy. The number of patients that required work-up colonoscopy using the 10 μg Hb/g threshold was 28.8%. The 3-year programme sensitivity for CRC and advanced adenoma was 72.4% and 56.6% with a specificity of 71.1% and 73.7%, respectively. Incremental cost-effectiveness of colonoscopy *vs* FIT surveillance was £7354 per additional advanced adenoma detected and £180778 per additional CRC detected[11].

Similar results were obtained in a diagnostic accuracy study that evaluated FIT (2 μg Hb/g) in a cohort of high-risk patients who underwent endoscopic surveillance. A total of 593 patients were included, including 41 (6.9%) with advanced neoplasia (4 CRC, 37 higher-risk adenoma). Of the 238 patients (40.1%) who had detectable FIT, 31 (13.0%) had advanced neoplasia (2 CRC, 29 higher-risk adenoma) compared with 10 (2.8%) with undetectable FIT (2 CRC, 8 higher-risk adenoma). A detectable FIT gave NPV of 99.4% for CRC and 97.2% for CRC plus higher-risk adenoma. According to these results, a FIT determination can provide an objective estimate of the risk of advanced neoplasia, and could enable tailored scheduling of colonoscopy[65].

In the absence of results from randomized clinical trials evaluating a FIT-based surveillance strategy, we have information from a simulation study[66]. This study evaluated the additional benefit in terms of cost-effectiveness of adding colonoscopy surveillance to a CRC screening programme. Based on the information obtained from the Dutch CRC screening programme, FIT screening without colonoscopy surveillance after adenoma removal reduces CRC mortality by 50.4% compared with no screening or surveillance. Adding colonoscopy surveillance after adenoma resection to FIT screening would reduce mortality by an additional 1.7% to 52.1% but would increase

lifetime colonoscopy demand by 62% at an additional cost of €68000, for an increase of 0.9 life-year. Despite the reduction in mortality provided by endoscopic surveillance compared to FIT follow-up, this study concludes that it is not a cost-effective strategy based on the incremental cost-effectiveness ratios, which exceeds the Dutch willingness-to-pay threshold of €36602 per life-year gained and also substantially increases colonoscopy demand[66].

CONCLUSION

In conclusion, we have enough evidence to recommend use of FIT to triage symptomatic patients in primary healthcare. FIT improves use of available endoscopic resources, avoids unnecessary colonoscopies, accurately predicts the risk of CRC and may have an impact on CRC prognosis. On the other hand, although endoscopic surveillance after adenoma resection is widely extended, there are relevant doubts about its efficiency in the context of high-quality baseline colonoscopies. Moreover, in terms of evaluating the effect on CRC incidence, endoscopic surveillance should be compared with participation in a CRC screening programme. In this sense, we require a randomized controlled trial comparing endoscopic with FIT-based surveillance after high-risk polyp resection.

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ORIGINAL ARTICLE

Basic Study

Fecal metabolomic profiles: A comparative study of patients with colorectal cancer *vs* adenomatous polyps

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Abstract

BACKGROUND

Colorectal cancer (CRC), the third most common cause of death in both males and females worldwide, shows a positive response to therapy and usually a better prognosis when detected at an early stage. However, the survival rate declines when the diagnosis is late and the tumor spreads to other organs. Currently, the measures widely used in the clinic are fecal occult blood test and evaluation of serum tumor markers, but the lack of sensitivity and specificity of these markers restricts their use for CRC diagnosis. Due to its high sensitivity and precision, colonoscopy is currently the gold-standard screening technique for CRC, but it is a costly and invasive procedure. Therefore, the implementation of custom-made methodologies including those with minimal invasiveness, protection, and reproducibility is highly desirable. With regard to other screening methods, the screening of fecal samples has several benefits, and metabolomics is a successful method to classify the metabolite shift in living systems as a reaction to pathophysiological influences, genetic modifications, and environmental factors.

AIM

To characterize the variation groups and potentially recognize some diagnostic markers, we compared with healthy controls (HCs) the fecal nuclear magnetic resonance (NMR) metabolomic profiles of patients with CRC or adenomatous polyposis (AP).

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METHODS

Proton nuclear magnetic resonance spectroscopy was used in combination with multivariate and univariate statistical approaches, to define the fecal metabolic profiles of 32 CRC patients, 16 AP patients, and 38 HCs well matched in age, sex, and body mass index.

RESULTS

NMR metabolomic analyses revealed that fecal sample profiles differed among CRC patients, AP patients, and HCs, and some discriminatory metabolites including acetate, butyrate, propionate, 3-hydroxyphenylacetic acid, valine, tyrosine and leucine were identified.

CONCLUSION

In conclusion, we are confident that our data can be a forerunner for future studies on CRC management, especially the diagnosis and evaluation of the effectiveness of treatments.

Key Words: Colorectal cancer; Adenomatous polyps; Nuclear magnetic resonance metabolomics; Fecal samples; Fecal metabolomics

Core Tip: Colorectal cancer (CRC) is the third leading cause of cancer-related death worldwide. Fecal occult blood and serum tumor markers are indicators currently used in the clinic, but their lack of sensitivity and precision limit their use for CRC diagnosis. Colonoscopy is the gold-standard screening technique for CRC, but it is costly and invasive. Using readily accessible biological samples such as stool specimens, in conjunction with high-throughput molecular profiling techniques, could significantly contribute to diagnosing and understanding the patient's relationship with CRC. We compared with healthy subjects the fecal nuclear magnetic resonance metabolomic profiles of patients with CRC or adenomatous polyposis.

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INTRODUCTION

Colorectal cancer (CRC) is globally the third most common cause of cancer-related death in both men and women[1]. Many CRC cases are attributable to changeable (and therefore potentially preventable)[2] risk factors including diet, smoking, high alcohol consumption, physical inactivity, and being overweight. If diagnosed at an early stage, CRC shows a good response to therapy and usually a better prognosis, while survival decreases when the diagnosis is late, and the tumor spreads to other organs[3]. In addition, it is well documented that about 95% of CRCs are adenocarcinomas and begin as colonic adenomatous polyps (AP)[4] or adenomas. A series of molecular alterations and mutations induce CRC development. Tumors can be prevented with polyps excision and adequate treatment[5]. The fecal occult blood test and evaluation of serum tumor markers are commonly used in the clinic[6]; however, the lack of sensitivity and specificity of these markers limits their application in CRC diagnosis[6-8]. Currently, colonoscopy represents the gold-standard screening procedure for CRC due to its high sensitivity and specificity, but it is an expensive and especially invasive procedure[9,10]. Therefore, it is highly desirable to introduce custom-made methodologies combining minimal invasiveness, safety, and reproducibility. The screening of fecal samples has many advantages with respect to other screening techniques. In fact, stool screening is certainly non-invasive and primarily reflects the colorectal status. Moreover, the use of easily accessible and non-invasive biological samples, such as

stool specimens, combined with high-throughput molecular profiling techniques, can significantly contribute to the diagnosis of CRC and to the understanding of its interaction with the patient.

Metabolomics is an omics science that is an efficient approach to characterizing the change of metabolites in living systems as a response to pathophysiological stimuli, genetic modifications, and environmental factors. Metabolites are low molecular weight organic molecules that take part (as substrates or products) in the biochemical processes essential for sustaining life. Thus, the comprehensive evaluation of metabolites and their changes are fundamental to observe and measure the response of the organism to diverse conditions.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most useful high-throughput techniques to obtain metabolomics information from biological samples [11,12]. NMR-based metabolomics has been successfully applied for disease classification[13-18] and prognosis. Starting from these premises, we compared the fecal NMR metabolomic profiles of patients with CRC or AP with those of healthy controls (HCs) to characterize the differences among the groups and possibly identify diagnostic markers.

MATERIALS AND METHODS

Patients and biological samples

A total of 86 patients including 32 CRC patients, 16 AP patients, and 38 HCs were enrolled for different studies between January 2016 and February 2019 at the Careggi Hospital and University of Florence, Italy. The Ethics Committee Area Vasta Toscana Centro (Italy) approved the studies. All fecal samples were taken at diagnosis, before starting any treatment (*e.g.*, surgical resection, chemotherapy, probiotic intake). Moreover, patients with evidence of serious illness, immunodeficit, autoimmune or infectious diseases were excluded. CRC patients, AP patients, and HCs were well matched in age, sex and body mass index. Table 1 summarizes the clinical characteristics of the enrolled patients.

Stool samples were collected in pre-labeled collection cups. Fecal water was extracted to ratios of 1:2 (g/mL, weight of unthawed feces-to-buffer) in 0.75 M phosphate-buffered saline (PBS, pH 7.4)[19]. The buffered samples where homogenized by whirl mixing for 30 s and sonicated for 15 min. Then each sample was centrifuged at 10000 g for 10 min at 4 °C, and 700 μL supernatant was transferred to 1.5 mL Eppendorf tubes and centrifuged again at 14000 rpm for 5 min at 4 °C. The clear supernatant was used for NMR analyses.

NMR sample preparation and analyses

A total of 70 μL buffer solution (1.5 M KH_2PO_4/d_2O, pH 7.4; 2 mmol/L NaN_3; 0.1% TMSP) was added to 630 μL of each fresh fecal water sample, and a total of 600 μL of this mixture was transferred to a 5 mm NMR tube.

One-dimensional proton NMR (^{1}H-NMR) spectra for all samples were acquired using the Bruker 600 MHz spectrometer (Bruker BioSpin srl; Rheinstetten, Germany) operating at 600.13 MHz proton Larmor frequency and equipped with a 5 mm PATXI ^{1}H-^{13}C-^{15}N and ^{2}H-decoupling probe including a *z* axis gradient coil, an automatic tuning-matching, and an automatic and refrigerated sample changer (SampleJet, Bruker BioSpin srl; Rheinstetten).

The BTO 2000 thermocouple served for temperature stabilization at the level of approximately 0.1 K at the sample. Before measurement, samples were kept for at least 3 min inside the NMR probe head for temperature equilibration.

Two one-dimensional ^{1}H-NMR spectra, namely one-dimensional (1D) NOESY and Carr-Purcell-Meiboom-Gill (CPMG), were acquired at 310 K with different pulse sequences: a standard nuclear Overhauser effect spectroscopy pulse sequence 1D NOESY PRESAT (noesygppr1d.comp; Bruker BioSpin) pulse sequence, using 64 scans, 98304 data points, a spectral width of 18028 Hz, an acquisition time of 2.7 s, a relaxation delay of 4 s, and a mixing time of 0.1 s; and a standard spin echo CPMG[20] (cpmgpr1d.comp; Bruker BioSpin) pulse sequence applied to a standard 1D sequence, with 64 scans, 73728 data points, a spectral width of 12019 Hz, and a relaxation delay of 4 s.

Spectral processing and statistical analysis

Free induction decays were multiplied by an exponential function equivalent to 0.3 Hz line-broadening factor before applying Fourier transform. Transformed spectra were

Table 1 Clinical characteristics of colorectal cancer patients, adenomatous patients, and healthy controls

Code	Gender ratio M/F	Median age, yr	Range of age, yr	Tumor staging T0/T1/T2/T3/T4 (*n* of patients)	Diet	Race
CRC	22 M-10 F; M/F = 2, 2	72	36-85	2/6/10/6/8	Mediterranean	Caucasian
AP	9M-7F; M/F = 1, 3	59	41-79	-	Mediterranean	Caucasian
HS	28M-10F; M/F = 2,8	47	27-68	-	Mediterranean	Caucasian

AP: Adenomatous patients; CRC: Colorectal cancer; HCs: Healthy controls.

automatically corrected for phase and baseline distortions and fecal spectra calibrated to TMSP singlet at 0 ppm using the TopSpin version 4.1.0 (Bruker BioSpin GmbH).

NMR spectra were segmented into bins of 0.02 ppm in the spectral range between 0.2 and 10.00 ppm. Regions containing residual water signal (between 4.6 and 4.9 ppm) were removed from the binning. The spectral intensity within each bin was integrated using Assure NMR 2.2, and the corresponding area was calculated to obtain the variable used as input for the statistical methods.

Before analysis of the generated data matrix, probabilistic quotient normalization [21] normalization and mean centering of the variables were performed.

Statistical analyses of the data were performed using R[22]. On processed NMR spectra, multivariate data analyses were performed. Principal component analysis (PCA) was used as unsupervised technique for exploratory analyses to check the homogeneity of the acquired spectra and to visualize the presence of outliers. As a supervised technique, orthogonal projections to latent structures-discriminant analysis (OPLS-DA) was applied. The OPLS-DA method is a multivariate projection approach that is commonly used to model spectroscopic data. Compared to PCA or partial least squares projection to latent structures (PLS), OPLS is able to distinguish between "response-related" and "response-orthogonal" fluctuations in data, delivering benefits in terms of model interpretation[23]. All of the accuracies reported and the confusion matrix for different classifications were assessed by means of 100 cycles of the Monte Carlo cross-validation scheme (MCCV, R script developed in-house). In this case, 90% of the data were randomly chosen at each iteration as a training set to build the model. Then the remaining 10% was tested, and the sensitivity, specificity, and accuracy of the classification were assessed. Metabolite identification was performed manually based on previous literature[19,24], the human metabolome database public database, and a library of pure organic compounds (BBIOREFCODE; Bruker BioSpin). The relative metabolite concentrations (expressed in arbitrary units) were calculated integrating and calculating the peaks area[25].

To determine the discriminating molecules among all classes under study, the Wilcoxon test was chosen to infer differences between two groups of subjects[26]. False discovery rate (FDR) correction was applied using the Benjamini & Hochberg method, and adjusted $P < 0.05$ was considered statistically significant[27]. The effect size, using the Cliff's delta (Cd) formulation[28], was also calculated to aid in the identification of the meaningful signals giving an estimation of the magnitude of the separation between the different groups. The magnitude was assessed using the thresholds provided in Romano and Coll[29], *i.e.* $|Cd| < 0.147$ "negligible," $|Cd| < 0.33$ "small," $|Cd| < 0.474$ "medium,"and otherwise "large."

Changes in metabolite levels were calculated as the $\log_2$ (fold change) ratio of the normalized median intensities of the corresponding signals in the spectra of the two groups. MetaboAnalyst 4.0 free online software was used for pathway analysis[30].

RESULTS

Metabolic fingerprint of CRC and AP patients

The NMR spectra of 86 fecal extract samples (32 CRC, 16 AP, and 38 HC) were acquired. Because of the suboptimal shimming quality of 7 spectra, only 79 spectra (1D NOESY: 26 CRC, 15 AP and 38 HCs; CPMG: 27 CRC, 14 AP, and 38 HCs) were used in subsequent analyses.

PCA was initially carried out to generate an overview of the variation among the different groups of patients (CRC, AP, and HCs) using bucketed spectra of fecal extracts. Some trends could be detected in the score plots of the first two principal components as shown in Supplementary Figure 1. Indeed, both score plots reveal that

CRC and AP patient groups tend to spread in the plots more than HCs that are more grouped. However, no net clustering seems to appear in the metabolomic profiles of the groups of patients neither with respect to HCs using this unsupervised approach.

Comparative analyses among the groups have been performed to test the capability of ^{1}H-NMR fecal water spectra to classify the samples of the patients according to the diagnosis. Several models have been built using a MC cross-validated supervised OPLS-DA approach. First, all three groups (CRC, AP, and HCs) were used in the same model to test the accuracy of the approach in the prediction of the healthy or the patho-logical state using a single bucketed NMR fecal spectrum (Supplementary Figure 2). As is shown in the score plots of Supplementary Figure 2, the AP samples occupied the middle metabolomic space between HCs and CRC. Indeed, the resulting true AP-positive percentages of the OPLS-DA NOESY and CPMG models were 37.7% and 18%, respectively (Supplementary Figure 2), and most of the AP patients were misclassified within the metabolomic space of HCs or CRC.

Prognostic data about patients are not available therefore was impossible to assess whether the AP patients predicted within the metabolomic space of CRC were more predisposed to developing cancer.

The capability to correctly classify HCs provides another important challenge for clinical screening. Indeed, other OPLS-DA models have been attempted to distinguish the fecal water spectra of HCs compared to AP + CRC patients yielding an overall predictive accuracy of 85.3% using 1D NOESY binned spectra (Table 2, Supplementary Figure 3A).

Furthermore, separated models were established comparing separately the ^{1}HNMR binned spectra of HCs *vs* CRC, HCs *vs* AP, and CRC *vs* AP patients (Table 2, Supplementary Figure 3B-D). As reported in Table 2, all models built on 1D NOESY bucketed spectra are better performing than those built on CPMG spectra. Supplementary Figure 3 shows the score plots related to the higher predictive accuracy among the models listed in Table 2. The reported models suggest the existence of a metabolomic fingerprint in fecal extracts of CRC and AP compared with HCs, confirming what was previously suggested by PCA and the literature available[24,31-33].

In most biofluids, low mass metabolites coexist with high mass biomolecules such as lipids, proteins, and lipoproteins. Here, two NMR pulse sequences were used to selectively observe the different components: 1D NOESY pulse sequence yields a spectrum in which both signals of metabolites and high molecular weight molecules (*e.g.*, lipids, lipoproteins, and albumin) are visible; the CPMG pulse sequence enables the selective observation of small molecule components in solutions containing macromolecules (*via* T2 filtering). Representative one-dimensional ^{1}H-NMR spectra of fecal extracts obtained with the mentioned pulse sequences are shown in Supplementary Figure 4. Indeed, NOESY experiments, which are sensitive to both low and high molecular weight compounds, resulted more accurate classifiers of all of the cases considered in this study and are described in Table 2 (HCs *vs* AP and CRC: sensitivity 84.9%, specificity 85.7%, predictive accuracy 85.3%; HCs *vs* CRC: sensitivity 85.0%, specificity 88.6%, accuracy 86.8%; HCs *vs* AP: sensitivity 71.7.8%, specificity 83.8%, predictive accuracy 77.8%: AP *vs* CRC: sensitivity 87.4%, specificity 71.6%, predictive accuracy 79.5%).

Metabolic profiles of CRC and AP patients

With the aim of identifying metabolite-level variations characteristic for each group, univariate analyses were applied to the identified fecal metabolites. Marked changes were observed in the metabolic profiles of fecal samples between CRC patients and HCs. Indeed, the first were characterized by a significantly lower content of 3-hydroxyphenylacetate, methanol, galactose, acetate, xylose and isobutyrate and a higher content of glycerol and phenylalanine (Figure 1A). Compared to HCs, AP patients had significantly lower amounts of 3-hydroxyphelylacetate, butyrate, acetate, propionate, isobutyrate and lactate+threonine (considered together because of the overlapping doublets at 1.33 ppm) as reported in Figure 1C. In CRC fecal extract profiles, when compared to AP patients, only leucine, tyrosine, and valine remained statistically significant and were present in higher amounts (Figure 1B). The complete list of fecal extract metabolites identified is reported in Supplementary Table 1. The most relevant pathways identified are reported in Table 3 and Supplementary Figure 5.

The top six identified metabolomic pathways in CRC ($P < 0.05$) were aminoacyl-tRNA biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis, valine, leucine and isoleucine biosynthesis, phenylalanine and galactose metabolism and valine, leucine and isoleucine degradation. Among them, valine, leucine and isoleucine biosynthesis, aminoacyl-tRNA biosynthesis, valine, leucine and isoleucine

Table 2 Summary of orthogonal projections to latent structures-discriminant analysis models built fecal water spectra acquired using selective one-dimensional proton nuclear magnetic resonance pulse sequences, sensitivity (%), specificity (%) and predictive accuracy (%) assessed following 100 runs of Monte Carlo cross-validation are reported for each model

	^{1}H NMR fecal extract spectra OPLS-DA cv models			
Group (*n* of samples)	Pulse sequences	Sensitivity (%)	Specificity (%)	Predictive accuracy (%)
HS (38) *vs* AP&CRC (41)	1D noesy; CPMG	84.9	85.7	85.3
HS (38) *vs* AP&CRC (41)		77.5	83.8	80.5
HS (38) *vs* CRC (26)	1D noesy; CPMG	94.2	90.2	93.7
HS (38) *vs* CRC (27)		97.3	85.2	91.0
HS (38) *vs* AP (15)	1D noesy; CPMG	90.8	76.6	87.0
HS (38) *vs* AP (14)		69.0	67.6	81.1
AP (15) *vs* CRC (26)	1D noesy; CPMG	77.0	90.4	79.3
AP (14) *vs* CRC (27)		67.5	74.0	72.7

AP: Adenomatous patients; CPMG: Carr-Purcell-Meiboom-Gill; CRC: Colorectal cancer.
^{1}HNMR: One-dimensional proton nuclear magnetic resonance; HCs: Healthy controls; OPLS-DA: Orthogonal projections to latent structures-discriminant analysis.

Table 3 Identified pathways from fecal water metabolites

Pathway analyses fecal water samples						
Fecal metabolites		Metabolites	*P*	Holm *P*	FDR	Impact
AP	Valine, leucine and isoleucine biosynthesis	Leucine, valine	8.25×10^{-4}	0.069	0.069	0.0
	Aminoacyl-tRNA biosynthesis	Valine, leucine, tyrosine	0.002	0.17	0.08	0.0
	Valine, leucine and isoleucine degradation	Leucine, valine	0.02	1	0.48	0.0
	Phenylalanine, tyrosine and tryptophan biosynthesis	Tyrosine	0.02	1	0.48	0.5
CRC	Aminoacyl-tRNA biosynthesis	Phenylalanine, valine, leucine, tyrosine	2.3×10^{-4}	0.019	0.01	0
	Phenylalanine, tyrosine and tryptophan biosynthesis	Phenylalanine, tyrosine	2.7×10^{-4}	0.022	0.035	1
	Valine, leucine and isoleucine biosynthesis	Leucine, valine	0.0012	0.1	0.035	0.0
	Phenylalanine metabolism	Phenylalanine, tyrosine	0.0019	0.16	0.041	0.36
	Galactose metabolism	Galactose, glycerol	0.014	1.0	0.2	0.05
	Valine, leucine and isoleucine degradation	Valine, leucine	0.03	1.0	0.43	0.0

An integrated analysis based on MetaboAnalyst 4.0 software built on significantly altered metabolites in colorectal cancer (CRC); and adenomatous patients (AP): View of most contributing pathways; *P* is the original *P* value calculated from the enrichment analysis; Holm *P* is the *P* value adjusted by Holm-Bonferroni method; the false discovery rate is the *P* value adjusted using false discovery rate (FDR); Impact is the pathway impact value calculated from pathway topology analysis.

degradation and phenylalanine, tyrosine and tryptophan biosynthesis pathways were also altered in AP patients (Table 3).

DISCUSSION

In this study, we evaluated the fecal metabolomic profiles of CRC and AP patients

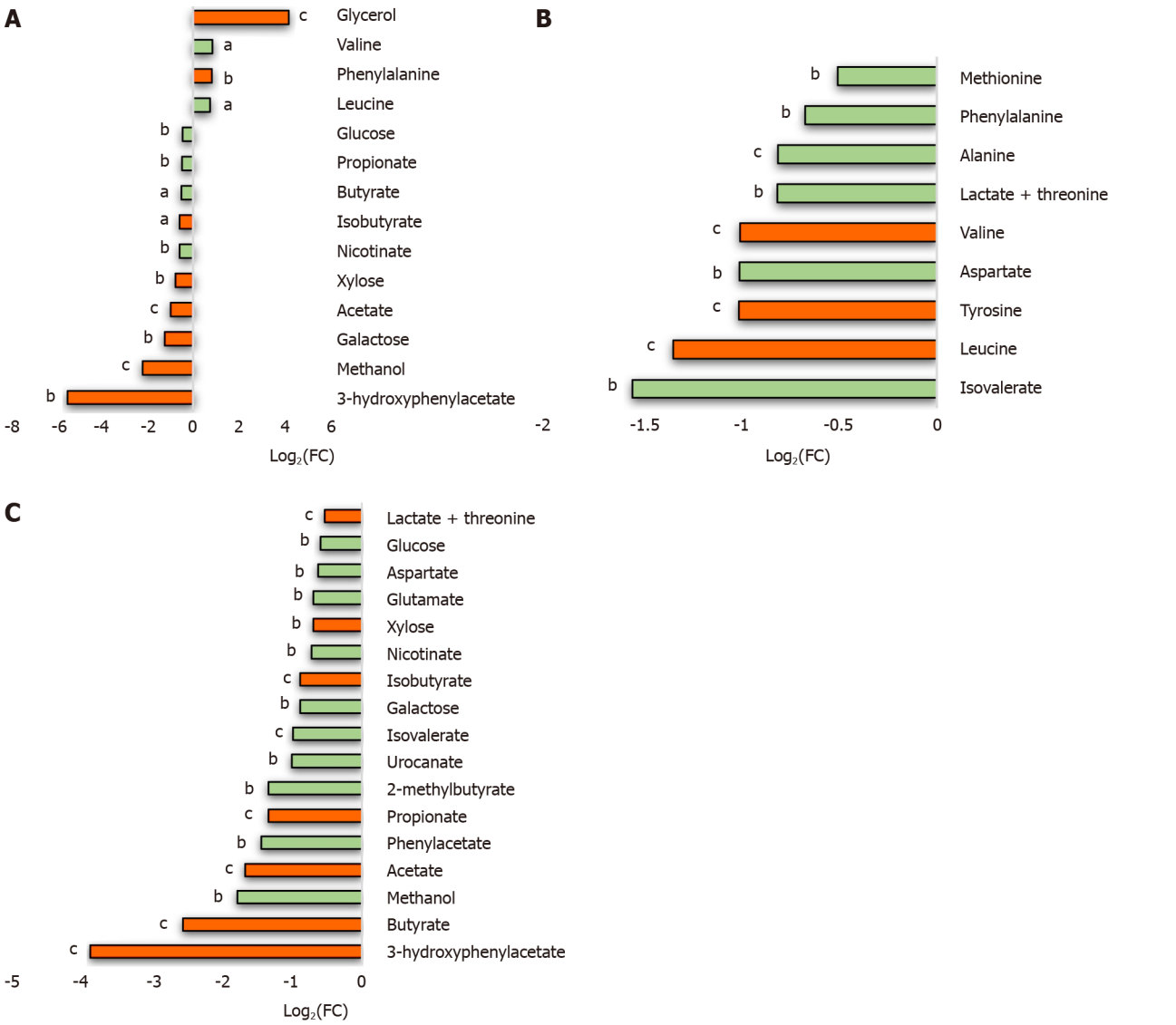

Figure 1 Fecal water metabolite levels of colorectal cancer, polyps' patients and healthy controls. Boxplots of fold-change (FC) values for the significantly altered metabolites. Red bars represent metabolites levels that remain significant after the false discovery rate (FDR) correction (FDR $P < 0.05$), green bars are the metabolites that are no more significant after the FDR correction ($P < 0.05$). Cliff's delta effect size is also reported for each metabolites in the comparisons (a: Small effect, b: Medium effect, c: Large effect). A: Comparison between healthy controls (HCs) and colorectal cancer (CRC) patients, negative $\log_2$ (FC) values mean lower metabolite levels in CRC fecal samples, positive $\log_2$(FC) values report higher content in CRC compared to HCs; B: Negative $\log_2$(FC) represent higher metabolite levels in CRC patients compared to polyp patients; C: Comparison between HCs and polyp patient-negative $\log_2$(FC) values mean lower metabolite levels in polyp patients fecal water.

with respect to HCs using NMR spectroscopy. The metabolome is a quantitative collection of low molecular weight compounds generated by metabolism[34]. Metabolomics is an emerging field of research downstream from genomics, proteomics, and transcriptomics. The metabolic screening of stool samples, which reflects the colorectal status, might contribute to the development of non-invasive screening tests[35,36]. The power of metabolomics consists in the capacity to detect and characterize tumors because cellular metabolism alterations represent a key hallmark of cancer[37]. To date, studies evaluating fecal metabolic changes associated with CRC are still lacking. Notably, no study has described fecal metabolomic changes associated with adenoma. Despite the high variability in fecal water profiles, reliable metabolic differences between patients and HCs were observed by preliminary analyses of the spectra. First, the PCA of the ^{1}H-NMR spectral data was carried out to identify some trends and outliers, showing some separation among cancer, adenoma, and HC groups. However, this unsupervised approach does not seem to clearly characterize the groups. These results were not surprising in light of the inter-individual variability introduced with diet, lifestyle, sex *etc.*

To optimize the separation among groups, we used OPLS-DA MCCV models, which were effective in discriminating the fecal metabolomic fingerprints of CRC patients and HCs (overall predictive accuracy of 93.7%). From the above analyses we can conclude that both low and high molecular weight molecules, visible using the 1D NOESY pulse sequence, are important to characterize at metabolic level the two pathological profiles. Moreover, the capability to accurately predict HCs when compared to patients with colonic AP or adenomas and CRC patients (showed predictive accuracy of 85.3%) using ^{1}H-NMR fecal water spectra, could be tested on a larger number of subjects to develop fast screening following a positive fecal occult blood test in order to spare colonoscopy in some patients who have bleeding due to other reasons[38,39].

In detail, the short-chain fatty acids (SCFAs) were found to be significantly decreased in CRC and AP patients, and in particular, lower levels of acetate were observed with respect to the HCs. SCFAs are microbial-derived metabolites, produced through gut bacteria fermentation of complex carbohydrates. SCFAs can be absorbed by the colonic epithelium, suppling energy and playing a crucial role in the regulation of fatty acids, glucose, and cholesterol metabolism[40-43]. A decrease in SCFA abundance is strictly linked to an unhealthy gut microbiota status, and alterations in the fecal SCFA profile may be the result of gut microbiota dysbiosis, inflammatory changes, or both[44]. Our data confirmed what has been previously reported by other studies. In particular, the analyses by Lin *et al*[45] demonstrated a high diagnostic performance of fecal acetate in CRC patients with respect to HCs.

In contrast with previous data, we did not find decreased levels of butyric acid in CRC patients with respect to HCs; however, we observed significantly decreased levels in AP. Butyric acid has an important homeostatic role in the human colon, and *in vitro* and *in vivo* studies have demonstrated its ability in preventing CRC[46-48]. However, other studies have showed contrasting results, suggesting a pro-cancer role of butyric acid, and low levels of butyrate usually seem to be linked with CRC development[31,35]. This double-edged role is named "butyrate paradox"[49,50]. Our results could be very interesting in this respect, suggesting that low levels of butyric acid in adenoma could lead to CRC development, while "normal" levels of butyrate in CRC could support cancer progression and promote the differentiation of regulatory T cells, which show a pro- tumorigenic role[51], especially in advanced CRC.

In addition, we found significantly low levels of 3-hydroxyphenylacetic (3-HPAA) acid in both CRC and AP patients. 3-HPAA acid is a weak acid and one of the most abundant products of polyphenol degradation in the large intestine[52,53]. Food polyphenols are broken down into other phenolic compounds by colonic bacteria action. In this way, poorly absorbable large-size polyphenols are converted to small-sized bioavailable metabolites, including 3-HPAA, which could be more biologically active[54,53]. Polyphenolic colon metabolites could be important endogenous antioxidants able to scavenge excess of free radicals, suppressing their effects on protein, lipid, and DNA damage[55]. A recent study demonstrated that polyphenol metabolites produced by colonic microbiota reduce some enzymatic activities involved in human tumorigenesis[56]. 3-HPAA can act as a CRC preventive agent by inhibition of cyclooxygenase-2 (COX-2)[57], a mediator of inflammation that is significantly overexpressed in a variety of human malignancies. Moreover, some studies have reported that COX-2 inhibitors not only prevent tumor formation but also decrease the number of already established polyps in patients with familial AP[58]. Accordingly, our results suggest that low levels of 3-HPAA are insufficient to inhibit COX-2, and consequently COX-2 expression can promote the development, tumor growth, immune suppression, angiogenesis, and metastasis of cancer cells.

Furthermore, higher amounts of amino acids such as leucine, tyrosine and valine, were present in the stool of CRC patients (compared to AP), probably resulting from malabsorption due to large epithelial inflammation and damage associated with CRC [59]. Previous metabolomic studies on fecal water have suggested that amino acid concentrations mirror the malabsorption of nutrients caused by the malfunction of epithelium barrier protection[60]. In agreement with previous studies, we documented higher levels of amino acids in the fecal water of CRC patients[31,35,61]. Alterations of amino acid levels can be associated with altered cancer cell activities, including the synthesis of proteins or catabolism to provide energy and/or other metabolite substrates.

CONCLUSION

In summary, our NMR metabolomics investigation revealed for the first time that fecal sample profiles can discriminate among CRC AP, AP patients and HCs, and some discriminatory metabolites were identified including acetate, butyrate, propionate, 3-HPAA acid, valine, tyrosine, and leucine. These altered metabolites suggest that changes in CRC and adenoma are associated with different pathway perturbations including valine, leucine and isoleucine biosynthesis, aminoacyl-tRNA biosynthesis, valine, leucine and isoleucine degradation phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism and galactose metabolism. In conclusion, we are confident that our data can be a forerunner for future studies on CRC management, especially the diagnostics and evaluation of the effectiveness of the treatments.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer (CRC) is globally the third most common cause of death. If diagnosed at an early stage, CRC shows a good response to therapy and usually a better prognosis. Unfortunately, despite its invasiveness, colonoscopy represents the gold-standard screening procedure for CRC.

Research motivation

Considering that colonoscopy is an expensive and invasive procedure, it seems to be essential to introduce custom-made methodologies combining minimal invasiveness, safety, and reproducibility. Fecal sample screening has many advantages with respect to other screening techniques.

Research objectives

The main objective of our study was to compare with HCs (HCs) the fecal nuclear magnetic resonance (NMR) metabolomic profiles of patients with CRC or adenomatous polyposis (AP) to characterize the variations among the groups and potentially identify some diagnostic markers.

Research methods

In order to define the fecal metabolic profile of 32 CRC, 16 AP patients and 38 HCs we used proton nuclear magnetic resonance spectroscopy in combination with multivariate and univariate statistical approaches.

Research results

The NMR spectra of 86 fecal extract samples have been acquired. With the aim of identifying metabolite level variations characteristic for each group, univariate analyses were applied to the identified fecal metabolites. The most marked changes were observed in the metabolic profiles of fecal samples of CRC patient *vs* HCs. AP patients, compared to HCs show significant lower amount of 3-hydroxyphelylacetate, butyrate, acetate, propionate, isobutyrate and lactate+threonine. In CRC fecal extract profiles, when compared to AP patients, only leucine, tyrosine, and valine remained statistically significant and present in higher amounts.

Research conclusions

The metabolic screening of stool samples might contribute to the development of non-invasive screening tests. To date, studies evaluating fecal metabolic changes associated with CRC are still lacking. Furthermore, no study has described fecal metabolomic changes associated with adenoma. The short-chain fatty acids were found to be significantly decreased in CRC and AP patients, and in particular, lower levels of acetate were observed with respect to HCs. In contrast with previous data, we did not find decreased levels of butyric acid in CRC patients compared to HCs; however, we observed significantly decreased levels in AP patients. We showed significantly low levels of 3-hydroxyphenylacetic (3-HPAA) acid in both CRC and AP patients. Finally, higher amounts of amino acids such as leucine, tyrosine, and valine were present in the stool of CRC patients (compared to AP), probably resulting from malabsorption due to large epithelial inflammation and damage.

Research perspectives

For the first time, we showed that fecal sample profiles can discriminate among CRC patients, AP patients and HCs, and some discriminatory metabolites were identified including acetate, butyrate, propionate, 3-HPAA acid, valine, tyrosine, and leucine. We believe that our data will be a starting point for future studies on CRC management, especially the diagnostics and evaluation of the effective of the treatments.

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ORIGINAL ARTICLE

Retrospective Cohort Study

High total Joule heat increases the risk of post-endoscopic submucosal dissection electrocoagulation syndrome after colorectal endoscopic submucosal dissection

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Abstract

BACKGROUND

We hypothesized that thermal damage accumulation during endoscopic submucosal dissection (ESD) causes the pathogenesis of post-ESD electrocoagulation syndrome (PECS).

AIM

To determine the association between Joule heat and the onset of PECS.

METHODS

We performed a retrospective cohort study in patients who underwent colorectal ESD from May 2013 to March 2021 in Japan. We developed a novel device that measures swift coagulation time with a sensor adjacent to the electrosurgical coagulation unit foot switch, which enabled us to calculate total Joule heat. PECS was defined as localized abdominal pain (visual analogue scale ≥ 30 mm during hospitalization or increased by ≥ 20 mm from the baseline) and fever (temperature ≥ 37.5 degrees or white blood cell count ≥ 10000 μ/L). Patients exposed to more or less than the median Joule heat value were assigned to the high and low Joule heat groups, respectively. Statistical analyses included Mann-Whitney U and chi-square tests and logistic regression and receiver operating characteristic curve (ROC) analyses.

RESULTS

We evaluated 151 patients. The PECS incidence was 10.6% (16/151 cases), and all

patients were followed conservatively and discharged without severe complications. In multivariate analysis, high Joule heat was an independent PECS risk factor. The area under the ROC curve showing the correlation between PECS and total Joule heat was high [0.788 (95% confidence interval: 0.666-0.909)].

CONCLUSION

Joule heat accumulation in the gastrointestinal wall is involved in the onset of PECS. ESD-related thermal damage to the peeled mucosal surface is probably a major component of the mechanism underlying PECS.

Key Words: Post-endoscopic submucosal dissection electrocoagulation syndrome; Joule heat; Colorectal endoscopic submucosal dissection; Colorectal neoplasms; Electrocoagulation; Gastrointestinal tract

Core Tip: We investigated the association between Joule heat and the onset of post-endoscopic submucosal dissection electrocoagulation syndrome (PECS), using originally developed a device to measure swift coagulation time with a sensor adjacent to the electrosurgical coagulation unit foot switch which enabled us to calculate total Joule heat. High Joule heat was an independent PECS risk factor. Moreover, the area under the operating characteristic curve showing the correlation between PECS and total Joule heat was high. Joule heat accumulation is involved in the onset of PECS. endoscopic submucosal dissection-related thermal damage of the peeled mucosal surface is probably a major component of the mechanism underlying PECS.

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INTRODUCTION

Colorectal endoscopic submucosal dissection (ESD), a minimally invasive operation, is the best endoscopic procedure for *en bloc* resection of superficial colorectal tumors[1-4]. However, ESD is associated with severe complications, with rates of perforation and bleeding of 2% to 14% and 0.7% to 3.1%, respectively[4-10]. Post-ESD electrocoagulation syndrome (PECS) is another notable adverse event that can occur after colorectal ESD. The incidence of PECS is 9% to 40%, although most cases improve with conservative therapy[11-15]. In addition, PECS sometimes manifests as a delayed perforation. Therefore, physicians require clinically useful PECS predictors to identify patients who are at risk[12,16].

Currently, two main hypotheses explain the mechanism underlying PECS. One suggests that ESD exposes the mucosa, which is then infected by intestinal bacteria, resulting in inflammation[13,17]. The other proposes that the peeled mucosal surface is inflamed by thermal damage during ESD[13,18]. Interestingly, a clipping closure method performed to prevent intestinal bacteria from infecting the exposed mucosa did not reduce the onset of PECS[19]. In contrast, few studies have examined the impact of thermal damage during ESD on the onset of PECS.

We have often encountered patients with PECS who underwent a lengthy colorectal ESD, and previous studies of PECS risk factors implicated prolonged ESD procedures [20,21]. We hypothesized that electrocoagulation is associated with Joule heat capable of causing thermal damage to the gastrointestinal wall during long ESD operations. Therefore, we aimed to determine the association between Joule heat and the onset of PECS.

statement: The Hitachi General Hospital Institutional Review Board approved this study (2019-97, 2020-1), and it was performed according to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments.

Informed consent statement: Informed consent was obtained using an opt-out option on the Hitachi General Hospital's website (see institution website uniform resource locators: http://www.hitachi.co.jp/hospital/hitachi/infor/opto-out/index.html).

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MATERIALS AND METHODS

Study design and patient selection

We performed a retrospective study in patients who underwent colorectal ESD at Hitachi General Hospital in Japan from May 2013 to March 2021. Case selection was according to the indications for colorectal ESD established by the Japan Gastroenterological Endoscopy Society guidelines[22]. Therefore, our inclusion criteria were as follows: (1) Laterally spreading tumors of the non-granular type with the Vi-type pit pattern, carcinomas with shallow T1 submucosal (SM) invasion, large depressed-type tumors, and large protruded-type tumors that are difficult to remove en bloc by endoscopic mucosal resection; and (2) Mucosal tumors with SM fibrosis. Patients with multiple colorectal neoplasms or apparent deeply invasive T1 SM carcinoma were excluded. R0 resection was defined as no cancer cells seen microscopically at the primary tumor site. We converted the swift-coagulation mode time, measured by an electrosurgical-coagulation unit with a high-frequency generator (VIO 300D, ERBE Co. Ltd., Tubingen, Germany), to Joule heat.

The Hitachi General Hospital Institutional Review Board approved the study (No. 2019-97, 2020-1), and our research was performed according to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments. The study is registered on the University Hospital Medical Information Network (ID: UMIN000038704, UMIN 000041580). Although our ethics committee waived the requirement for informed consent from each patient because we used anonymized data, we obtained informed consent using an opt-out option on our facility's website (uniform resource locator below).

PECS definition

PECS was defined as localized abdominal pain and fever without apparent perforation after colorectal ESD. We used a visual analog scale (VAS) to evaluate localized abdominal pain. Nurses who were not research participants administered the VAS. The pain criteria were defined as a score ≥ 30 mm from the postoperative day (POD) 1 to discharge or increased by ≥ 20 mm from the hospital admission VAS score. Our fever criteria were body temperature ≥ 37.5 degrees or white blood cell count ≥ 10000 μ/L from POD 1 to discharge.

Colorectal ESD procedures

All patients underwent bowel preparation (polyethylene glycol, 2 L) before colorectal ESD. Then, the patients were sedated in the endoscopy room using intravenous injections of pentazocine (15-30 mg/kg body weight). We added midazolam (1-3 mg/session) if pentazocine was insufficient for sedation. Electrocardiogram monitoring was performed in each case. The procedures were performed by 10 endoscopists (one expert colorectal endoscopic surgeon and 9 less experienced colorectal endoscopic surgeons). An expert was defined as an endoscopist who performed more than 40 colorectal ESDs[23]. We used a water-jet system colonoscope (PCF-Q260JI, Olympus Corporation, Tokyo, Japan) with a transparent hood (DH-29CR for scope tip, Fujifilm, Tokyo, Japan) attached to the scope tip. Marking, mucosal incision, and mucosal detachment were performed with a dual knife (KD-655Q, Olympus Corporation). We injected 0.4% sodium hyaluronate acid diluted in physiological saline into the SM layer directly below the lesion before mucosal detachment. A coagrasper (FD-411QR, Olympus Corporation) and an endoscopic clip (HX-610-090, Olympus Corporation) were used to obtain hemostasis.

Calculating Joule heat

We used the electrosurgical-coagulation unit to detach the lesion's colorectal mucosa. We changed the setting mode according to the procedure step and used dry-cut, swift-coagulation, and soft-coagulation modes. We calculated the power applied using the diagram in the manufacturer's technical manual that shows the relationship between resistance (Ω) and power (W) in the swift-coagulation mode (Supplementary material). As human internal resistance is 1000-1600 Ω[24], the lesion resistance was defined as 1300 Ω. Thus, lesions are detached while the electrosurgical-coagulation unit automatically adjusts the power output. However, this automatic power adjustment is difficult to monitor.

The curve describing the relationship between power (vertical axis) and resistance (horizontal axis) in the manufacturer's technical diagram (we used effect 4 in our study) showed that the power corresponding to 1300 Ω is 50 W (Supplementary material). Therefore, we obtained the formula: power consumption (J) = power (W) ×

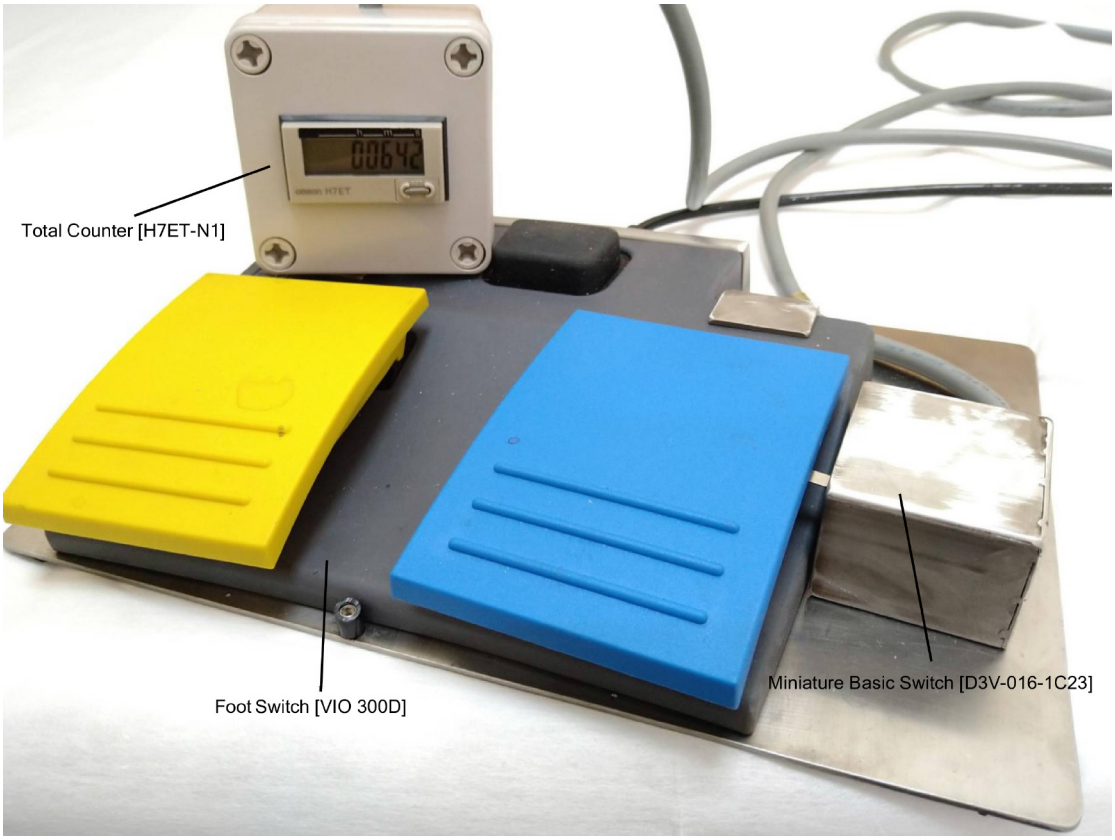

Figure 1 A novel device for measuring the swift-coagulation mode time with a sensor adjacent to the foot switch of the electrosurgical coagulation unit.

time (s) from the relationship between power and power consumption. We used this formula to calculate the total Joule heat applied to the lesion.

We developed a novel device to measure the swift coagulation time with a sensor [Miniature Basic Switch (D3V-016-1C23), OMRON Corporation, Kyoto, Japan] adjacent to the foot switch of the electrosurgical coagulation unit (Figure 1). This sensor was activated when the operator stepped down on the foot switch, and the swift-coagulation mode time was recorded by a counter [Total Counter (H7ET-N1), OMRON Corporation]. In cases where the swift-coagulation time was not measured, we retrospectively calculated the time using the data (swift-coagulation mode time/procedure time: Mean 3%) obtained from the measured cases. Patients exposed to more or less than the median Joule heat value (8460 J) were assigned to the high and low Joule heat groups, respectively.

Post-ESD hospitalization

In all patients, on POD 1, the vital signs and a blood sample were examined to check for bleeding, the pain VAS was administered, and radiography was performed to check for free air. We considered a fever, localized abdominal pain, or increased C-reactive protein suspicious for delayed perforation associated with PECS. If the pain or fever criteria were satisfied, we performed computed tomography. In the absence of adverse findings, patients resumed eating, starting with dinner on POD 1. If no fever, localized abdominal pain, or bleeding occurred after eating resumed, patients were discharged on POD 6.

Statistical analysis

We analyzed continuous and categorical variables using Mann-Whitney U and chi-square tests, respectively. In our multivariate analysis, we performed logistic regression analyses using important factors identified by univariate analysis. We determined the cutoff of the total Joule heat value that predicted the onset of PECS using receiver operating characteristic (ROC) curve analysis. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (version 26, IBM Corp., Armonk, NY, United States). The statistical methods of this study were reviewed by Masanori Ochi from the Department of

Gastroenterology, Hitachi General Hospital, Japan.

RESULTS

Baseline characteristics

Of 170 patients admitted for colorectal ESD, 19 met the exclusion criteria (multiple lesions, *n* = 6; post-ESD infection, *n* = 2; perforation during ESD, *n* = 7; ESD discontinuation, *n* = 4) (Figure 2). We analyzed the remaining 151 patients [mean age, 67.3 years (range 39-92); male, 62.3%]. Tumors occurred in the right colon (*n* = 59, 39.1%), left colon (*n* = 41, 27.2%), and rectum (*n* = 51, 33.8%); R0 and *en bloc* resections were achieved in 115 patients (76.2%) and 135 patients (89.4%), respectively; and PECS occurred in 16 patients (10.6 %) (Table 1).

Primary outcome

The high (*n* = 76) and low (*n* = 75) Joule heat groups showed no statistically significant difference in sex; tumor morphology, location, or depth of pathological invasion; R0 resection rate; or incidence of SM fibrosis (Table 2). Univariate analyses revealed that patient age was significantly greater, and the PECS incidence was significantly higher in the high Joule group than in the low Joule group; the specimen size was also significantly larger in the high Joule group. Compared to the low Joule heat group, the high Joule heat group included more patients who had undergone ESD performed by a trainee, and the difference was significant. In addition, the R0 resection rate was significantly higher in the low Joule group. Multivariate analysis showed that the R0 resection rate [odds ratio (OR), 3.27; 95% confidence interval (CI): 1.26-8.45; $P = 0.01$] and PECS incidence (OR, 4.83; 95%CI: 1.08-21.50; $P = 0.03$) were significantly higher and the specimen size (OR, 1.07; 95%CI: 1.03-1.11; $P < 0.01$) and number of ESD procedures performed by a trainee (OR, 5.30; 95%CI: 2.32-12.10; $P < 0.01$) were significantly larger in the high Joule heat group than in the low Joule heat group (Table 3).

PECS and Joule heat correlation analysis

The area under the ROC curve showing the correlation between PECS and total Joule heat was 0.788 (95%CI: 0.666-0.909), and the PECS onset cutoff value was 15390 J (sensitivity, 0.625; specificity, 0.822) (Figure 3).

DISCUSSION

To our knowledge, this study, performed using our novel device, is the first to indicate high total Joule heat exposure during colorectal ESD as a PECS risk factor. Additionally, PECS and Joule heat were highly correlated, and the Joule heat cutoff value where PECS occurred was 15390 J.

PECS is a state of temporary inflammation resulting from transmission of electrocoagulation heat to the resection site muscle layer and serous membrane during endoscopic therapy[18,25,26]. Previously reported PECS risk factors include tumor location[11,13,20,27], SM fibrosis[27], long procedure time[20,21], and specimen diameter[11,13]. Long procedures lead to the occurrence of PECS due to increased electrical current load on the gastrointestinal wall[11]. Additionally, ischemic changes occur in the gastrointestinal wall due to excessive current during incision, dissection, and hemostasis[28]. These reports suggest that Joule heat of the peeled mucosal surface is associated with the occurrence of PECS.

A study of lengthy procedures (mean, 90 min) demonstrated that long procedure time is a PECS risk factor[20]. However, several studies that did not include long procedure time as a PECS risk factor showed that the procedures were short (range, 52-67 min)[11,28,29]. Our study revealed that ESD procedures performed by trainees are associated with the onset of PECS. A previous study showed that ESD procedures performed by trainees are longer than those performed by experienced endoscopists [23]. Because the swift-coagulation mode time probably increases as the procedure time lengthens, high Joule heat accumulation is likely to occur during long ESDs, causing severe damage to the peeled mucosal surface, leading to PECS. Previous studies were limited in that they did not consider the impact of the ESD procedure time on the Joule heat delivered to the lesion. In this study, measurement of swift-coagulation mode time allowed us to investigate the association between PECS and

Table 1 Characteristics of patients who underwent endoscopic submucosal dissection, *n* (%)

Patient features	*n* (%)
Number of patients	151
Male	94 (62.3)
Age (yr), mean ± SD	67.3 ± 10.9
BMI (kg/m^2), mean ± SD	22.9 ± 3.0
Specimen size	
< 40 mm	132 (87.4)
≥ 40 mm	19 (12.6)
Total Joule heat	
< 8460 J[1]	75 (49.7)
≥ 8460 J	76 (50.3)
Tumor location	
Right colon	59 (39.1)
Left colon	41 (27.1)
Rectum	51 (33.8)
Tumor morphology	
0-Is/Ip	29 (19.2)
LST-G	75 (49.7)
LST-NG (0-IIc)	47 (31.1)
Depth of pathological invasion	
Tis (M)	130 (86.1)
T1 (SM)	21 (13.9)
Histological diagnosis	
Adenoma	67 (44.4)
Adenocarcinoma	80 (53.0)
Other	4 (2.6)
ECOG performance status after ESD	
0	144 (95.4)
≥1	7 (4.6)
R0 resection	115 (76.2)
ESD procedure performed by trainees	52 (34.4)
Additional endoscopic therapy after ESD	2 (1.3)
Additional surgery after ESD	12 (7.9)
En bloc resection	135 (89.4)
Intraoperative perforation or penetration	7 (4.6)
Submucosal fibrosis	45 (29.8)
PECS	16 (10.6)

[1]Patients exposed to more or less than the median Joule heat value (8460 J) were assigned to the high and low Joule heat groups, respectively.
BMI: Body mass index; Is: Sessile type; Ip: Pedunculated type; LST-G: Lateral spreading tumor granular type; LST-NG: Lateral spreading tumor non-granular type; IIc: Superficial depressed type; Tis (M): Carcinoma in situ or intramucosal carcinoma; T1 (SM): Tumor invades submucosa; R0: Microscopically margin-negative; ESD: Endoscopic submucosal dissection; PECS: Post-endoscopic submucosal dissection electrocoagulation syndrome; SD: Standard deviation; ECOG performance status: Eastern Cooperative Oncology Group performance status.

total Joule heat. Our study revealed that the total Joule heat applied to the peeled

Table 2 Clinicopathological factors in the high and low Joule heat groups, *n* (%)

	High Joule heat	Low Joule heat	*P* value
	n = 76	*n* = 75	
Sex			0.54
Male	45 (59.2)	49 (65.3)	
Female	31 (40.8)	26 (34.7)	
Age (yr), mean ± SD	69 ± 11.5	66 ± 10.2	< 0.01
BMI (kg/m^2), mean ± SD	22.8 ± 2.3	23.0 ± 3.2	0.77
Specimen size			< 0.01
< 40 mm	60 (78.9)	73 (97.3)	
≥ 40 mm	16 (21.1)	2 (2.7)	
Tumor location			0.84
Right colon	28 (36.9)	31 (41.3)	
Left colon	21 (27.6)	20 (26.7)	
Rectum	27 (35.5)	24 (32.0)	
Tumor morphology			0.56
0-Is/Ip	18 (23.7)	21 (28.0)	
LST-G	36 (47.4)	29 (38.7)	
LST-NG (0-IIc)	22 (28.9)	25 (33.3)	
Depth of pathological invasion			0.66
Tis (M)	64 (84.2)	66 (88.0)	
T1 (SM)	12 (15.8)	9 (12.0)	
Histological diagnosis			0.12
Adenoma	34 (44.7)	33 (44.0)	
Adenocarcinoma	42 (55.3)	38 (50.7)	
Other	0 (0.0)	4 (5.3)	
Performance status after ESD			0.45
0	71 (93.4)	73 (97.3)	
≥ 1	5 (6.6)	2 (2.7)	
R0 resection	50 (65.8)	65 (86.7)	< 0.01
ESD procedure performed by trainees	37 (55.3)	15 (13.3)	< 0.01
Additional endoscopic therapy after ESD	1 (1.3)	1 (1.3)	0.999
Additional surgery after ESD	8 (10.5)	4 (5.3)	0.38
En bloc resection	69 (90.8)	73 (97.3)	0.18
Submucosal fibrosis	27 (35.5)	18 (24.0)	0.17
PECS	13 (17.1)	3 (4.0)	0.02

BMI: Body mass index; Is: Sessile type; Ip: Pedunculated type; LST-G: Lateral spreading tumor granular type; LST-NG: Lateral spreading tumor non-granular type; IIc: Superficial depressed type; Tis (M): Carcinoma in situ or intramucosal carcinoma; T1 (SM): Tumor invades submucosa; R0: Microscopically margin-negative; ESD: Endoscopic submucosal dissection; PECS: Post-endoscopic submucosal dissection electrocoagulation syndrome; SD: Standard deviation.

surface is involved in the development of PECS. Furthermore, using the total Joule heat cutoff value of PECS occurrence could allow the introduction of PECS prevention measures, including online measurement of swift-coagulation mode time during colorectal ESD. For example, an online PECS alert system is promising.

Table 3 Multivariate analysis of risk factors associated with high Joule heat accumulation

	Odds ratio	95%CI	*P* value
Age	1.03	0.99-1.06	0.17
R0 resection	3.27	1.26-8.45	0.01
Specimen size	1.07	1.03-1.11	< 0.01
ESD procedure performed by trainees	5.30	2.32-12.10	< 0.01
PECS	4.83	1.08-21.50	0.03

PECS: Post-endoscopic submucosal dissection electrocoagulation syndrome; R0: Microscopically margin-negative; 95% CI: 95% confidence interval.

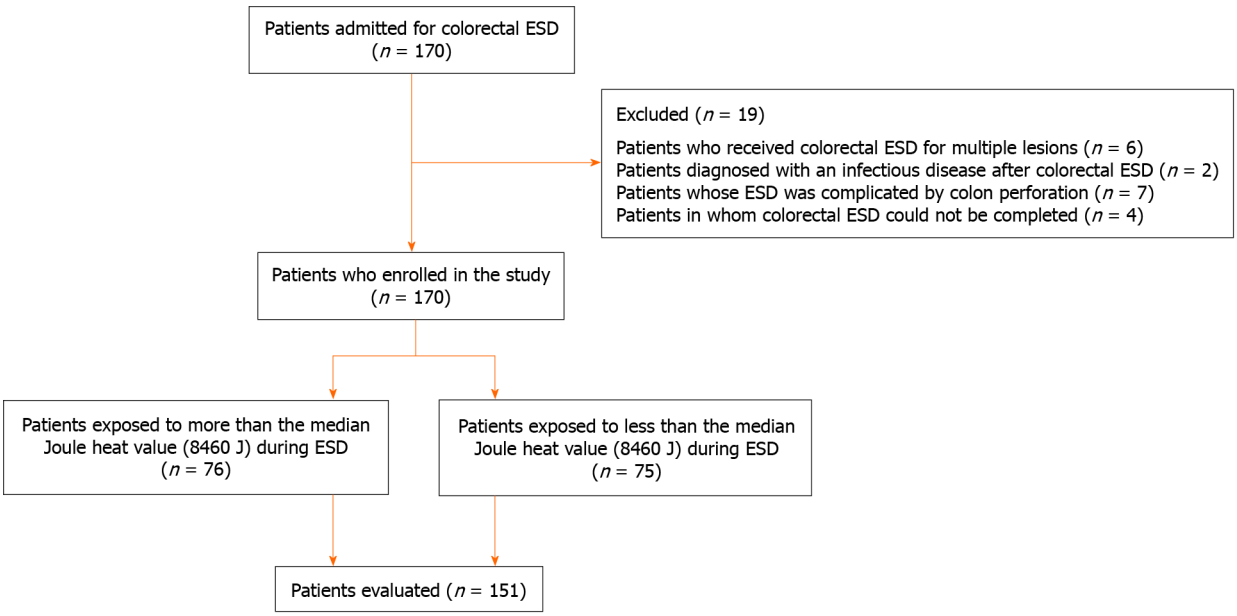

Figure 2 Study flowchart. ESD: Endoscopic submucosal dissection.

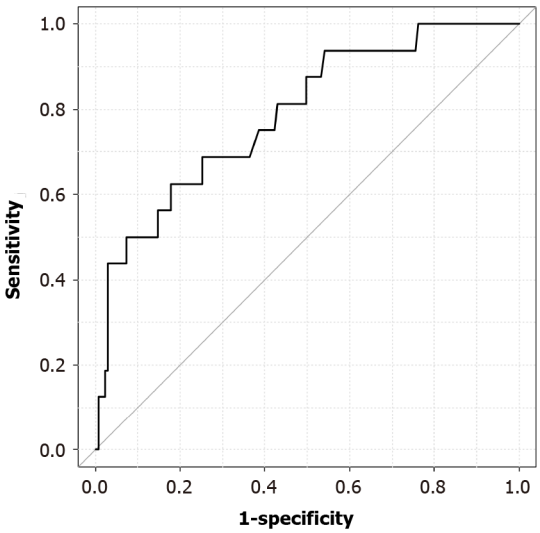

Figure 3 Receiver-operating characteristic curve showing the correlation between post-endoscopic submucosal dissection electrocoagulation syndrome and total Joule heat [area under the curve = 0.788 (95% confidence interval: 0.666-0.909)].

Limits of the study

First, because this was a retrospective cohort study, we could not rule out poor endoscope operability as a confounding factor. Second, as this was a single-center study, selection bias cannot be excluded. Nevertheless, we imposed strict inclusion and exclusion criteria to mitigate selection bias to the greatest extent possible. Last, the sample size was small. In the future, a prospective study should be performed to establish the validity of our results.

CONCLUSION

We found that Joule heat accumulation within the gastrointestinal wall is involved in the onset of PECS. ESD-related thermal damage to the mucosal peeled surface is probably a major component of the mechanism underlying PECS.

ARTICLE HIGHLIGHTS

Research background

Few studies have examined the impact of endoscopic submucosal dissection (ESD)-related thermal damage on the onset of post-ESD electrocoagulation syndrome (PECS).

Research motivation

We hypothesized that electrocoagulation is associated with Joule heat capable of causing thermal damage to the gastrointestinal wall during long ESD operations.

Research objectives

We aimed to determine the association between high Joule heat and the onset of PECS.

Research methods

We developed a novel device to measure the swift coagulation time with a sensor adjacent to the electrosurgical coagulation unit foot switch, which enabled us to calculate total Joule heat. PECS was defined as localized abdominal pain (visual analogue scale ≥ 30 mm during hospitalization or increased by ≥ 20 mm from the baseline) and fever (temperature ≥ 37.5 degrees or white blood cell count ≥ 10000 μ/L). Patients exposed to more or less than the median Joule heat value were assigned to the high and low Joule heat groups, respectively.

Research results

We evaluated 151 patients. The PECS incidence was 10.6% (16/151 cases), and all patients were followed conservatively and discharged without severe complications. In multivariate analysis, high Joule heat was an independent PECS risk factor. The area under the ROC curve showing the correlation between PECS and total Joule heat was high [0.788 (95% confidence interval: 0.666-0.909)].

Research conclusions

Joule heat accumulation in the gastrointestinal wall is involved in the onset of PECS. ESD-related thermal damage of the peeled mucosal surface is probably a major component of the mechanism underlying PECS.

Research perspectives

Future prospective studies should be performed to establish the validity of our results.

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ORIGINAL ARTICLE

Retrospective Study

Effects of acute kidney injury on acute pancreatitis patients' survival rate in intensive care unit: A retrospective study

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Abstract

BACKGROUND

Acute kidney injury (AKI) is one of the most common acute pancreatitis (AP)-associated complications that has a significant effect on AP, but the factors affecting the AP patients' survival rate remains unclear.

AIM

To assess the influences of AKI on the survival rate in AP patients.

METHODS

A total of 139 AP patients were included in this retrospective study. Patients were divided into AKI group (n = 72) and non-AKI group (n = 67) according to the occurrence of AKI. Data were collected from medical records of hospitalized patients. Then, these data were compared between the two groups and further

GD, and Gao Y performed manuscript review; All authors have read and approved the content of the manuscript; and Shi N and Sun GD contributed equally to this work.

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Institutional review board statement: This study was approved by the Ethics Committee of The Second Affiliated Hospital of Harbin Medical University.

Informed consent statement: Due to the nature of retrospective study, the written informed consent of this study was waived.

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Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0

analysis was performed.

RESULTS

AKI is more likely to occur in male AP patients (P = 0.009). AP patients in AKI group exhibited a significantly higher acute physiologic assessment and chronic health evaluation II score, higher Sequential Organ Failure Assessment score, lower Glasgow Coma Scale score, and higher demand for mechanical ventilation, infusion of vasopressors, and renal replacement therapy than AP patients in non-AKI group (P < 0.01, P < 0.01, P = 0.01, P = 0.001, P < 0.01, P < 0.01, respectively). Significant differences were noted in dose of norepinephrine and adrenaline, duration of mechanical ventilation, maximum and mean values of intra-peritoneal pressure (IPP), maximum and mean values of procalcitonin, maximum and mean serum levels of creatinine, minimum platelet count, and length of hospitalization. Among AP patients with AKI, the survival rate of surgical intensive care unit and in-hospital were only 23% and 21% of the corresponding rates in AP patients without AKI, respectively. The factors that influenced the AP patients' survival rate included body mass index (BMI), mean values of IPP, minimum platelet count, and hospital day, of which mean values of IPP showed the greatest impact.

CONCLUSION

AP patients with AKI had a lower survival rate and worse relevant clinical outcomes than AP patients without AKI, which necessitates further attention to AP patients with AKI in surgical intensive care unit.

Key Words: Acute kidney injury; Acute pancreatitis; Surgical intensive care unit; Survival rate; Risk factors; Intra-peritoneal pressure

Core Tip: Acute pancreatitis (AP) has become a common gastrointestinal disorder in surgical intensive care unit, and excessive secretion and/or poor drainage of pancreatic juice are the essence of AP onset. Acute kidney injury (AKI) is a common complication of AP, which is ordinarily associated with adverse outcomes. Among AP patients with AKI, the survival rate of surgical intensive care unit and in-hospital were only 23% and 21% of the corresponding rates in AP patients without AKI, respectively. The factors that influenced the AP patients' survival rate included body mass index (BMI), mean values of intra-peritoneal pressure, minimum platelet count, and hospital day, of which mean values of intra-peritoneal pressure showed the greatest impact.

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INTRODUCTION

Acute pancreatitis (AP) is an excessive inflammatory response caused by digestion of the pancreas itself, which can further lead to local and distant organ damage, or even single or multiple organ failure. In the wake of continuous in-depth understanding of pathophysiological mechanism of AP and improvement of treatment measures, AP-related mortality rate is annually declining, whereas AP-associated hospitalization rises year-by-year worldwide[1,2]. In clinical practice, about 80% of the common etiologies are attributed to gallstones and alcohol consumption[3], however, the proportion of different etiologies significantly varies among different countries. In addition, hypertriglyceridemia is a high-risk factor for AP, and may lead to direct damage to pancreas and pancreatic exocrine function[4,5]. Thus, identification of

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etiologies is highly essential to manage better AP patients. AP can be categorized into mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP), and severe acute pancreatitis (SAP) according to the presence and duration of organ failure presented in the revised Atlanta classification (2013)[6]. The mortality rate is high among SAP patients, reaching 15%-30% or even higher[7], due to persistent organ failure. Therefore, earlier identification and appropriate use of intensive care support can be conducive to prevent further disease progression, thereby improving AP patients' prognosis[8].

Acute kidney injury (AKI) is one of the most common AP-associated complications, resulting from uncontrolled inflammatory response, release of pancreatic amylase, hypovolemia, insufficient renal perfusion, micro-circulatory disturbance, intra-abdominal hypertension, and reactive oxygen species[9]. At present, AKI is mainly diagnosed based on the criteria presented by the Kidney Disease Improving Global Outcomes (KIDGO) guidelines[10,11]. The incidence of AKI has gradually, while steadily, increased year-by-year worldwide[12]. AKI can deteriorate AP patient's medical status and is an independent risk factor for increased mortality and development of chronic kidney disease (CKD). When AKI and AP occur simultaneously, a worse clinical prognosis is expected[13,14], involving longer period of hospitalization and higher mortality rate. However, in clinical practice, there is no an effective therapeutic approach for AKI except for renal replacement therapy (RRT)[15]. AKI has imposed a huge medical burden in China as well as in the world. Therefore, development of early detection and prevention measures is highly significant to avoid adverse outcomes associated with AKI[16].

Although previous studies have confirmed that concurrent AKI is associated with a poor prognosis[17], there is no reliable research assessing the influences of AKI on Chinese AP patients' survival rate who were hospitalized at surgical intensive care unit (SICU). Hence, to address this scientific gap, the current retrospective study was conducted.

MATERIALS AND METHODS

Study design

This retrospective study enrolled 139 AP patients who were admitted to the SICU of The Second Affiliated Hospital of Harbin Medical University (Harbin, Heilongjiang Province, China) between January 2014 and March 2019. Baseline and clinical data were collected during hospitalization. The enrolled AP patients were divided into AKI group (n = 72) and non-AKI group (n = 67) according to occurrence of AKI. This study was approved by the Ethics Committee of The Second Affiliated Hospital of Harbin Medical University.

Study population

The inclusion criteria for this retrospective study were as follows: Patients who were admitted to the SICU of The Second Affiliated Hospital of Harbin Medical University; patients who were diagnosed with AP; patients' age > 18-years-old. The exclusion criteria were as follows: Pregnant or breastfeeding women; patients with CKD; patients with recurrent pancreatitis; patients who received renal transplantation; incomplete medical data.

Diagnosis of AP

A combination of medical history, symptoms and physical examinations, laboratory tests, and radiographic examinations (*e.g.*, abdominal ultrasound, contrast-enhanced computed tomography, or magnetic resonance imaging) was applied to confirm the diagnosis of AP. A limited number of diagnosed AP patients were eventually confirmed by undergoing exploratory laparotomy.

Diagnosis and classification of AKI

Diagnosis and classification of AKI were conducted based on the criteria presented by the KIDGO guidelines (2012)[18], in which serum creatinine criteria was defined as an increased absolute value in serum creatinine level of ≥ 0.3 mg/dL (≥ 26.4 μmol/L) or a percentage increase in serum creatinine level of ≥ 50% within 48 h. Baseline serum creatinine level was defined as the lowest serum creatinine level measured within 2 d prior to admission to SICU. If no serum creatinine level was measured, the serum creatinine level recorded in the first measurement within 2 d after admission to SICU

was considered as baseline serum creatinine level.

RRT

In the present study, all AP patients who needed to undergo RRT were managed with continuous veno-venous hemofiltration (CVVH), anti-coagulation with heparin, and fixed pre- and post-dilution strategies. Blood flow rates, amount of substitute fluid, and dehydration volume were individually adjusted according to each patient's medical status.

Measurement of serum procalcitonin level and intra-peritoneal pressure

Serum procalcitonin (PCT) level was intermittently measured after AP patients' admission to SICU through Mini-VIDAS (Hain Lifescience GmbH; Nehren, Germany). Intra-peritoneal pressure (IPP) was indirectly reflected *via* measuring intravesical pressure with Freund's catheter.

Data collection

Baseline and clinical data, including patients' age, gender, body mass index (BMI), Acute Physiologic Assessment and Chronic Health Evaluation II (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score, Glasgow Coma Scale (GCS) score, duration of mechanical ventilation (MV), abdominal puncture drainage, gallbladder puncture drainage, infusion of vasopressors, demand of RRT, IPP, body temperature, PCT, creatinine, platelet count, hospital day, and prognosis were collected from medical records of hospitalized patients. APACHE II score and SOFA score were calculated by the first 24 h clinical data after SICU admission.

Statistical analysis

Variables conforming to normal distribution were described as mean ± SD, while those abnormally distributed variables were expressed as median (range). Normality analysis was applied to continuous data. SPSS 22.0 software (IBM, Armonk, NY, United Sates) was used to carry out statistical analyses. Independent-samples *t*-test was used to perform inter-group comparison for normally distributed data, while Mann-Whitney *U* test was employed for inter-group comparison for abnormally distributed data. Classification data were expressed by the number of samples, and χ^2 test was adopted. The prognosis of AP patients who were admitted to SICU was analyzed by binary logistic regression analysis, while the remaining indicators were analyzed by the *t*-test or Mann-Whitney *U* test. P value < 0.05 was considered statistically significant.

RESULTS

Patients' baseline and clinical data

A total of 139 AP patients were enrolled in this retrospective study. The enrolled AP patients were divided into AKI group (n = 72) and non-AKI group (n = 67) according to occurrence of AKI. As shown in Table 1, AKI was more likely to occur in male AP patients (P = 0.009). AP patients with AKI exhibited a significantly higher APACHE II score, higher SOFA score, lower GCS score, and higher demand for MV, infusion of vasopressors, and RRT than non-AKI AP patients (P < 0.01, P < 0.01, P = 0.01, P = 0.001, P < 0.01, P < 0.01, respectively). No significant difference was observed in the remaining baseline and clinical data, including patients' age, BMI, proportion of abdominal puncture drainage, and gallbladder puncture drainage.

Vasopressor infusion

There were significant differences in types of vasopressors and proportion of norepinephrine, adrenaline, and vasopressor infusion between the two groups (Table 2).

Analysis of prognostic indicators

Significant differences were noted in dose of norepinephrine and adrenaline, duration of MV, maximum and mean values of IPP, maximum and mean values of PCT, maximum and mean serum levels of creatinine, minimum platelet count, prognosis of AP patients admitted to SICU, and hospital day between the two groups (Table 3). Among AP patients with AKI, the survival rates of SICU and in-hospital were only 23% and 21% of the corresponding rates in AP patients without AKI, respectively.

Table 1 Baseline and clinical data of patients

	AKI (*n* = 72)	Non-AKI (*n* = 67)	Z/χ^2	*P* value
Age	45.00 (17.50)	44.00 (21.00)	-0.07	0.94
Gender			6.745	0.009
Male	50	32		
Female	22	35		
BMI	24.45 (2.38)	23.80 (2.60)	-1.20	0.23
APACHE II Score	18.00 (7.75)	9.00 (7.00)	-7.47	< 0.01
SOFA Score	9.00 (6.00)	4.00 (4.00)	-6.50	< 0.01
GCS Score	15.00 (3.00)	15.00 (0.00)	-2.71	0.01
MV			10.94	0.001
Yes	46	24		
No	26	43		
Abdominal puncture drainage			0.19	0.66
Yes	36	36		
No	36	31		
Gallbladder puncture drainage			2.36	0.12
Yes	17	9		
No	55	58		
Vasopressor infusion			26.84	< 0.01
Yes	21	18		
No	51	49		
RRT				
Yes	44	20	13.651	< 0.01
No	28	47		

AKI: Acute kidney injury; APACHE II: Acute Physiology and Chronic Health Evaluation II; BMI: Body mass index; GCS: Glasgow Coma Scale; MV: Mechanical ventilation; RRT: Renal replacement therapy; SOFA: Sequential Organ Failure Assessment.

Analysis of the related factors influencing AP patients' survival rate

The factors influencing the AP patients' survival rate who were hospitalized at SICU included BMI, mean values of IPP, minimum platelet count, and hospital day. Among these factors, mean values of IPP showed the greatest effect (Table 4). The values of area under the receiver operating characteristic (ROC) curve for the four factors related to patients with or without AKI were calculated to predict the AP patients' survival rate, and were 0.896 and 0.891, respectively (Figure 1 and Table 5; $P > 0.5$). However, there was no significant different in values of the area under the two ROC curves. The sensitivity and specificity of the two ROC curves were approximately the same [sensitivity (80.6%) *vs* specificity (88.0%)].

Risk thresholds of the related factors

Risk thresholds of BMI, mean values of IPP, minimum platelet count, and hospital day were ≥ 24, ≥ 23, ≤ 77, and ≤ 4, respectively, which indicated that BMI ≥ 24, mean values of IPP ≥ 23, minimum platelet count ≤ 77, and hospital day ≤ 4 were risk factors for AP patients' survival rate who were hospitalized at SICU.

DISCUSSION

Excessive secretion and/or poor drainage of pancreatic juice are the essence of AP onset. With the increasing incidence of gallstones and alcohol abuse, AP had become a

Table 2 Vasopressor infusion

	AKI, *n* = 72	Non-AKI, *n* = 67	χ^2	*P* value
Types of vasopressors			27.44	< 0.01
No	21	49		
1	34	14		
≥ 2	17	4		
Norepinephrine infusion			35.35	< 0.01
Yes	49	17		
No	23	50		
Adrenaline infusion			12.75	< 0.01
Yes	15	1		
No	57	66		
Vasopressor infusion			26.84	< 0.01
Yes	51	18		
No	21	49		

AKI: Acute kidney injury.

common gastrointestinal disorder. The majority of cases with AP are MAP characterized by no new onset of organ dysfunction, whereas SAP not only leads to new onset of organ dysfunction, but also lasts for more than 48 h[6]. With aggravation of the condition from MAP to SAP, the length of stay at SICU and mortality rate significantly increased. Hence, SAP patients should be managed in SICU for multiple organ support therapy and surgical interventions to avoid further disease progression [19]. When AP happens, hemodynamic status should be assessed immediately to avoid hypovolemia or volume overload caused by excessive fluid resuscitation[20], which might cause detrimental influences[21]. Routine use of prophylactic antibiotics in SAP or necrotizing pancreatitis was not clinically recommended in the guidelines [22], because of no mortality benefit or reduction in the incidence of infected necrosis [23]. The ability to penetrate pancreatic necrotic tissue is of great significance in the selection of antibiotics. Less than 50% of necrotizing pancreatitis patients need surgical interventions, in which step-up approach and minimally invasive strategies were advocated[24-27], involving endoscopic nasobiliary drainage, percutaneous transhepatic gallbladder drainage, percutaneous transhepatic biliary drainage, endoscopic retrograde cholangiopancreatography, endoscopic transgastric/transduodenal drainage, and video-assisted retroperitoneal debridement.

During AP, kidney is the most vulnerable organ and is typically sacrificed to protect other important organs, such as heart, lung, brain, and liver. As a consequence, AKI is a common complication of AP, which is ordinarily associated with adverse outcomes, including risk of subsequent CKD, end-stage renal disease, RRT dependence, in-hospital and post-discharge mortality, and even healthcare cost-containment concerns [28-30]. It was generally believed that the incidence of AKI was about 20% in critically ill adult patients[31]. More than 50% of SAP patients would develop into AKI according to previous literature[3,17,32]. Different organs interact with each other in the whole body, and the deteriorated kidney function can inevitably cause or aggravate damage to other organs without early intervention. Diuretics had been demonstrated to be an independent risk factor for AKI[33], and thus, were no longer recommended for routine use in clinical practice. When RRT is required, the mortality rate may even exceed 75%[34]. Compared with developed countries, AKI is typically substantial underdiagnoses and undertreatment in developing countries[35], especially in African countries, which may be partially explained as seriously inadequate repeated serum creatinine assay[36].

At present, about 20% of AKI patients require to undergo RRT[37], and this rate continues to increase worldwide. Some eligible patients for RRT may decline to undergo this intervention because of resource constraints, high costs of therapy, or severe comorbidities[38], which significantly increase mortality rate compared with those cases who received it[39]. RRT involves different treatment modalities, such as

Table 3 Analysis of prognostic indicators

	AKI, *n* = 72	Non-AKI, *n* = 67	*Z/t/Wald*	*P* value	OR
Prognosis of SICU			9.54	0.002	0.23
cure	48	60			
other	24	7			
Prognosis of in-hospital			11.33	0.001	0.21
cure	46	60			
other	26	7			
Duration of MV	34.00 (87.75)	0.00 (18.00)	-3.92	< 0.01	
Maximum values of IPP	24.50 (10.00)	18.00 (10.00)	-5.39	< 0.01	
Mean values of IPP	21.40 (6.82)	15.29 (4.73)	6.175	< 0.01	
Maximum values of body temperature	37.80 (1.20)	37.50 (1.00)	-2.00	0.05	
Mean values of body temperature	37.00 (0.50)	37.00 (0.30)	-0.59	0.56	
Maximum values of PCT	16.28 (21.12)	2.87 (9.28)	-5.96	< 0.01	
Mean values of PCT	8.52 (11.52)	1.58 (4.20)	-6.42	< 0.01	
Maximum serum levels of creatinine	284.35 (215.73)	87.60 (47.80)	-9.94	< 0.01	
Mean serum levels of creatinine	197.45 (172.10)	65.60 (29.60)	-9.48	< 0.01	
Minimum platelet count	93.00 (87.75)	137.00 (73.00)	-3.18	< 0.01	
Mean platelet count	174.40 (112.15)	198.00 (103.60)	-1.64	0.10	
Hospital day	9.00 (12.75)	8.00 (11.00)	-0.07	0.94	
Dose of norepinephrine	16.00 (52.00)	0.00 (4.00)	-5.11	< 0.01	
Dose of adrenaline	0.00 (0.00)	0.00 (0.00)	-3.58	< 0.01	

AKI: Acute kidney injury; MV: Mechanical ventilation; IPP: Intra-peritoneal pressure; OR: Odds ratio; PCT: Procalcitonin; SICU: Surgical intensive care unit.

Table 4 Analysis of the related factors influencing acute pancreatitis patients' survival rate

	B	SE	Wald	*df*	*P* value	Exp (B)
BMI	1.4904	0.6467	5.3105	1.0000	0.0212	4.4387
Mean values of IPP	2.6477	0.6217	18.1355	1.0000	0.0000	14.1211
Minimum platelet count	-1.2285	0.6125	4.0237	1.0000	0.0449	0.2927
Hospital day	-1.8571	0.5965	9.6913	1.0000	0.0019	0.1561
Constant	-0.9863	0.8689	1.2883	1.0000	0.2564	0.3730

BMI: Body mass index; IPP: Intra-peritoneal pressure; SE: Standard error.

hemodialysis, hemofiltration, hemodiafiltration, and peritoneal dialysis. It has been suggested that continuous RRT has several advantages over intermittent RRT, including better hemodynamic stability (blood pressure control and blood circulation), improved survival, and greater likelihood of renal recovery[38,40,41]. Thus, CVVH had occupied the mainstream of RRT, particularly for AP patients who were hospitalized at SICU, because of its outstanding effectiveness and safety. Although it has been suggested that early application of RRT in patients with severe sepsis, irrespective of the presence of renal failure, might be beneficial, CVVH did not limit further organ damage and even prolonged the need for organ support. RRT is a non-selective method, which can simultaneously remove detrimental and beneficial substances. In clinical practice, in spite of great advances in RRT development, there is still a lack of uniform standards for RRT-associated strategies, such as optimal timing

Table 5 Area under the receiver operating characteristic curve

Variables	Area	SE	Asymptotic, *P* value	Asymptotic 95%CI	
				Lower bound	Upper bound
With AKI	0.896	0.033	0	0.831	0.961
Without AKI	0.891	0.037	0	0.819	0.963

AKI: Acute kidney injury; CI: Confidence interval; SE: Standard error.

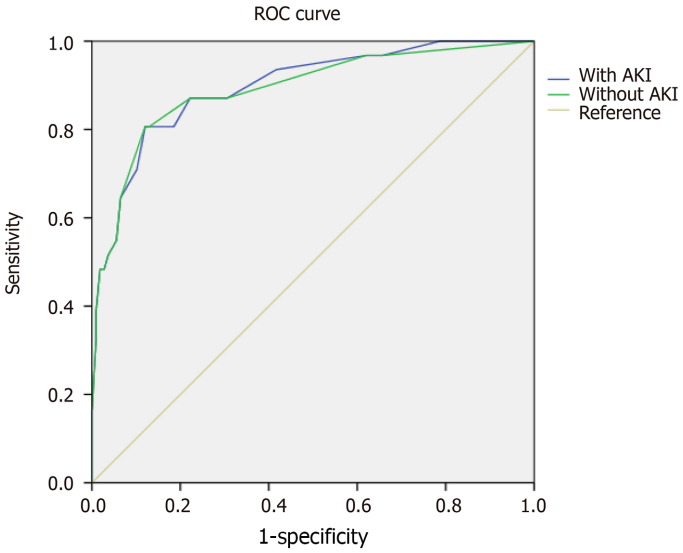

Figure 1 The receiver operating characteristic curve of these four factors related to acute pancreatitis patients without or with acute kidney injury. AKI: Acute kidney injury; ROC: Receiver operating characteristic.

for initiation of RRT, anti-coagulation, and optimal dosage[42,43].

Among several scoring systems, APACHE II score and SOFA score are commonly used to reflect timely and accurately illness severity and predict prognosis of AP patients[44]. It is well known that GCS score is a simple and valuable tool for indication of nervous system function and prediction of short- and long-term mortality [45], in which its discriminative power is similar to other more complex scoring systems[46]. Therefore, these three scoring systems were selected to assess the severity of AP in the present research. A number of scholars pointed out that demand for MV, infusion of vasopressors, and RRT could be risk factors for increased mortality in AP patients[47]. PCT level may contribute to earlier and better stratification of septic patients who are at risk of death and admitted to SICU[48]. To our knowledge, the increased IPP and decreased platelet count are closely associated with the severity and prognosis of AP in clinical practice[49]. Hence, the above-mentioned indicators were employed in the current study.

The present retrospective study provided a comprehensive description about the influences of AKI on AP patients' survival rate who were hospitalized at SICU in accordance with the criteria presented by the KIDGO guidelines. Our findings revealed that AP patients with AKI had more severe degree of illness than AP patients without AKI, as evidenced by higher APACHE II score, higher SOFA score, and lower GCS score, and the survival rates of SICU and in-hospital were only 23% and 21% of the corresponding rates in AP patients without AKI, respectively, which were roughly consistent with previously reported rates[10,31]. These gaps can be partially explained by the increased emphasis placed on AP and improvement of diagnostic methods and standardized therapeutic bundles. For AP patients with AKI, other influences were found to be associated with demand for MV, infusion of vasopressors and RRT, dosages of norepinephrine and adrenaline, duration of MV, maximum and mean values of IPP, maximum and mean values of PCT, maximum and mean serum levels of creatinine, and minimum platelet count. It was revealed that AKI negatively and seriously affected AP patients who were hospitalized at SICU.

The factors that influenced AP patients' survival rate who were hospitalized at SICU included BMI, mean values of IPP, minimum platelet count, and hospital day. With every unit change in BMI, mean values of IPP, minimum platelet count, and hospital day, the AP patients' survival rate was 4.4387, 14.1211, 0.1561, and 0.3730 of the original rates, respectively. Therefore, mean values of IPP had the greatest influence on the AP patients' survival rate who were hospitalized at SICU. Risk thresholds of BMI, mean values of IPP, minimum platelet count, and hospital day were ≥ 24, ≥ 23, ≤ 77, and ≤ 4, respectively, which indicated that BMI ≥ 24, mean values of IPP ≥ 23, minimum platelet count ≤ 77, and hospital day ≤ 4 were risk factors for the AP patients' survival rate who were hospitalized at SICU.

The present study contains a number of limitations. First, although significant differences were detected, this was only a small sample-size, single-center, retrospective study, which might reduce the reliability of our conclusion and its application to AP patients in clinical practice. Second, clinical data were obtained only on the basis of the medical records during hospitalization, which might lead to research bias. Last but not least, the etiology of AP had not been further differentiated. It is noteworthy that different etiologies may induce different clinical manifestations and prognosis, necessitating conducting further studies in the future.

CONCLUSION

In conclusion, this study attempted to clarify the influence of AKI on AP patients' survival rate who were hospitalized at SICU. Results showed that AP patients with AKI exhibited lower survival rate and worse relevant clinical outcomes than AP patients without AKI. Besides, BMI, mean values of IPP, minimum platelet count, and hospital day may play significant roles in predicting the AP patients' survival rate who were hospitalized at SICU. Our findings suggest that prevention of AKI is clinically important.

ARTICLE HIGHLIGHTS

Research background

Acute kidney injury (AKI) is common in acute pancreatitis (AP) patients, but the risk factors are not clear.

Research motivation

This study tried to explore the associated risk factors of AKI in AP patients.

Research objectives

This study aimed to assess the influences of AKI on the survival rate in AP patients.

Research methods

Patients were divided into two groups based on AKI status, and comparisons between the groups were calculated for diverse variables.

Research results

AKI is more likely to occur in male AP patients.

Research conclusions

AP patients with AKI exhibited lower survival rate and worse relevant clinical outcomes than AP patients without AKI in SICU.

Research perspectives

This study provided clinical evidence for prevention of AKI in AP patients.

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ORIGINAL ARTICLE

Retrospective Study

Magnetic resonance imaging-radiomics evaluation of response to chemotherapy for synchronous liver metastasis of colorectal cancer

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Abstract

BACKGROUND

Synchronous liver metastasis (SLM) is an indicator of poor prognosis for colorectal cancer (CRC). Nearly 50% of CRC patients develop hepatic metastasis, with 15%-25% of them presenting with SLM. The evaluation of SLM in CRC is crucial for precise and personalized treatment. It is beneficial to detect its response to chemotherapy and choose an optimal treatment method.

AIM

To construct prediction models based on magnetic resonance imaging (MRI)-radiomics and clinical parameters to evaluate the chemotherapy response in SLM of CRC.

METHODS

A total of 102 CRC patients with 223 SLM lesions were identified and divided into disease response (DR) and disease non-response (non-DR) to chemotherapy. After standardizing the MRI images, the volume of interest was delineated and radiomics features were calculated. The MRI-radiomics logistic model was constructed after methods of variance/Mann-Whitney *U* test, correlation analysis, and least absolute shrinkage and selection operator in feature selecting. The radiomics score was calculated. The receiver operating characteristics curves by the DeLong test were analyzed with MedCalc software to compare the validity of all models. Additionally, the area under curves (AUCs) of DWI, T2WI, and portal phase of contrast-enhanced sequences radiomics model (Ra-DWI, Ra-T2WI, and

appendix, statistical code, and dataset available from the corresponding author at email address. Participants informed consent was not obtained but the presented data are anonymized and risk of identification is low.

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Ra-portal phase of contrast-enhanced sequences) were calculated. The radiomics-clinical nomogram was generated by combining radiomics features and clinical characteristics of CA19-9 and clinical N staging.

RESULTS

The AUCs of the MRI-radiomics model were 0.733 and 0.753 for the training (156 lesions with 68 non-DR and 88 DR) and the validation (67 lesions with 29 non-DR and 38 DR) set, respectively. Additionally, the AUCs of the training and the validation set of Ra-DWI were higher than those of Ra-T2WI and Ra-portal phase of contrast-enhanced sequences (training set: 0.652 *vs* 0.628 and 0.633, validation set: 0.661 *vs* 0.575 and 0.543). After chemotherapy, the top four of twelve delta-radiomics features of Ra-DWI in the DR group belonged to gray-level run-length matrices radiomics parameters. The radiomics-clinical nomogram containing radiomics score, CA19-9, and clinical N staging was built. This radiomics-clinical nomogram can effectively discriminate the patients with DR from non-DR with a higher AUC of 0.809 (95% confidence interval: 0.751-0.858).

CONCLUSION

MRI-radiomics is conducive to predict chemotherapeutic response in SLM patients of CRC. The radiomics-clinical nomogram, involving radiomics score, CA19-9, and clinical N staging is more effective in predicting chemotherapeutic response.

Key Words: Radiomics; Synchronous liver metastasis; Colorectal cancer; Chemotherapy; Magnetic resonance; Nomogram

Core Tip: Synchronous liver metastasis (SLM) indicates poor prognosis for colorectal cancer. Nearly 50% of colorectal cancer patients develop hepatic metastasis, with 15%-25% of them presenting with SLM. It is beneficial to detect the response of SLM to chemotherapy. Magnetic resonance imaging-radiomics could provide a non-invasive approach to predict the risk of SLM. The logistic model of DWI sequence behaved the best in evaluating the chemotherapeutic response in SLM compared with T2WI, DWI, and portal phase of contrast-enhanced sequences. Moreover, the radiomics-clinical nomogram containing radiomics score, CA19-9, and clinical N staging is more effective in predicting the chemotherapeutic response of SLM patients.

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INTRODUCTION

Colorectal cancer (CRC) is the fourth most common malignancy worldwide[1], accounting for approximately one-third of cancer related deaths in western countries [2]. Nearly 50% of CRC patients developed hepatic metastasis throughout the course of disease, and 15%-25% of them were associated synchronous liver metastasis (SLM) [3]. SLM is confirmed as an indicator of poor prognosis for CRC, which was defined as a lesion identified within 90 d after the diagnosis of the primary tumor[4]. Currently, the standard guideline for the treatment of CRC patients with SLM remains undetermined. Conventional treatment for this condition is colectomy, followed by chemotherapy and liver resection[5]. Preoperative chemotherapy has superiority on early treatment of metastatic disease, which may help to achieve a negative resection margin[6] and reduce the risk of local recurrence[1]. However, liver injuries can be induced by chemotherapy, such as vascular changes and chemotherapy-associated steatohepatitis[7]. Previous studies have reported that administration of more than 12

cycles of preoperative chemotherapy increased the risk of re-operation and prolonged hospital stay[8]. Excessive cycles of preoperative chemotherapy may result in increased damage to the liver and lost potential opportunity to receive surgery[7] since progression of chemotherapy is irreversible. Therefore, precise and non-invasive assessment of the response of SLM patients to preoperative chemotherapy is a critical step in individualized treatment. In addition, SLM patients who were predicted as non-responders could benefit from alternative therapies to avoid dispensable chemotherapy.

Radiomics is a promising and non-invasive method to analyze conventional imaging features and incorporate them into predictive models to evaluate tumor behaviors[9]. Previous work has concluded that the nomogram combining radiomics and clinical factors exhibited favorable ability and accuracy in evaluating metastatic pulmonary nodules in CRC patients[10]. Analysis of liver texture is potentially a supplement to routine computed tomography examination and may provide prognostic markers for CRC patients[11]. A radiomics signature was validated to be a complementary predictor for preoperative staging of CRC patients[12]. It has been suggested that magnetic resonance imaging (MRI)-radiomics of CRC patients could provide a non-invasive approach to predict the risk of SLM[13].

To the best of our knowledge, little attention has been paid to predict the response of chemotherapy in SLM patients. This retrospective study examined the emerging role of MRI-radiomics signature in order to detect the prediction efficiency of models in chemotherapeutic response of SLM patients and avoid ineffective chemotherapy.

MATERIALS AND METHODS

Patient selection

This retrospective study was approved by the institutional review board of our hospital. For the characteristics of retrospective study, formal written consent is not applicable. Research methods were carried out in accordance with the Declaration of Helsinki.

A total of 102 CRC patients with 223 SLM lesions were identified from 2017 to 2020 in our hospital. SLM was a histopathologically confirmed intrahepatic lesion within 90 d of the diagnosis of CRC[4]. Inclusion criteria included: (1) Patients were histopathologically diagnosed as classical adenocarcinoma in CRC, excluding mucinous and signet ring adenocarcinoma[14]; (2) Patients have at least one SLM lesion; (3) For patients with multiple SLM lesions, the top three largest ones were selected to analyze; (4) Patients underwent baseline and 3 mo follow-up MRI examination after the start of chemotherapy; and (5) Patients underwent mFOLFOX7 chemotherapy regimen. Exclusion criteria included: (1) Patients underwent anti-tumor treatments such as chemotherapy, radiotherapy, or transarterial chemoembolization before baseline MRI examinations; (2) Patients were diagnosed with CRC with biopsy but not with surgery; (3) History of other malignancies; and (4) Patients were diagnosed as mucinous or signet ring adenocarcinoma. The general characteristics involved gender, age, tumor markers, and clinical T/N staging were recorded. The tumor markers encompassed alpha-fetoprotein (normal range: 0.0-20.0 μg/L), carcinoembryonic antigen (normal range: 0.0-5.0 μg/L), and CA19-9 (normal range: 0.0-37.0 U/mL) that all were divided into normal and abnormal subgroups.

The response to chemotherapy was assessed after 3 mo from the start of chemotherapy by MRI examinations. The response of lesions was categorized into four subgroups according to the Response Evaluation Criteria in Solid Tumors (version 1.1) criterion[15]: (1) Complete response refers that all target lesions disappeared; (2) Partial response is defined as lesions having at least a 30% decrease in the sum diameters of lesions; (3) Progressive disease is defined as lesions having at least a 20% increase in the sum diameters of lesions; and (4) Stable disease is defined as tumors with neither sufficient shrinkage nor sufficient increase in the lesions. None of the patients in this study belonged to complete response. Patients with partial response were classified as disease response (DR) group (Figure 1), while patients with progressive or stable disease were merged into disease non-response (non-DR) group.

MRI examination and image processing

All examinations were performed using 3.0-T MRI (Discovery 750, GE Healthcare, Waukesha, WI, United States). The axial T2WI, DWI, and portal phase of contrast-enhanced sequences (CE_{PP}) were taken. CE-MRI was performed with gadobenate dimeglumine being injected *via* a dual head pressure injector at a rate of 2 mL/s and

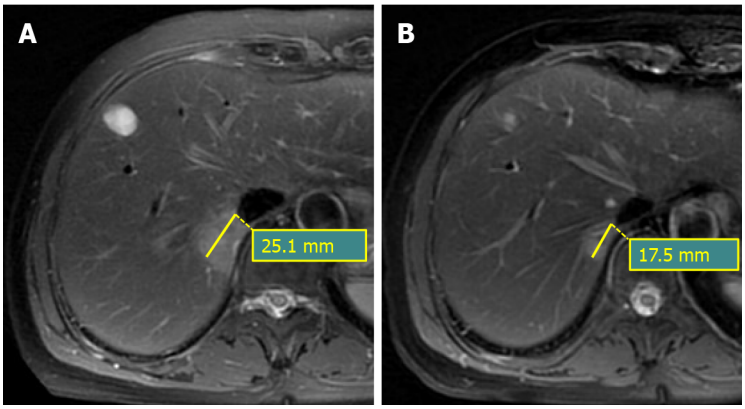

Figure 1 Magnetic resonance imaging examinations. A: A 48-year-old female had a synchronous liver metastasis with baseline long diameter of 25.1 mm; B: After chemotherapy, the lesion a had 30.3% decrease in long diameter of 17.5 mm, which belonged to the disease response group for analysis.

followed by 20 mL saline flush at the same rate. Post-contrast image acquisition was done in the axial plane in arterial phase (AP), PP, and equilibrium phases (EP). The imaging parameters were as follows: T2WI (TR 10000-12000 ms, TE 85 ms; FOV 36 cm × 40 cm, matix 320 × 320, thickness 5.0 mm, interval 1.0 mm), CE (TR 3.7 ms, TE 2.2 ms; FA 12°, matix 260 × 260, thickness 5.0 mm, interval 1.0 mm, 0.2 mL/Kg), and DWI (TR 3500 ms, TE 75 ms; FOV 32 cm × 32 cm, matix 128 × 128, thickness 3.0 mm, interval 0.6 mm, *b* value 0 and 800 s/mm^2).

The process of image standardization included resampling images into a 1.0 mm × 1.0 mm × 1.0 mm voxel size of X/Y/Z-spacing, denoising images by Gaussian, and normalizing the gray level of images to a scale from 1 to 32, which is automatically performed with the software of AK (Artificial Intelligence Kit, version 3.0.0, GE Healthcare).

Then, the three-dimensional volume of interest (VOI) was manually delineated in all the images by software of "ITK-SNAP" (version 3.4.0, http://www.itksnap.org/) by two radiologists with 9 and 12 years of experience in MRI diagnosis, respectively. Finally, the radiomics features of two radiologists were automatically calculated in AK software.

MRI-radiomics signature construction

A total of 396 radiomics features were automatically calculated by AK software, including 42 histogram parameters, 54 texture parameters, 9 form factor parameters, 100 gray-level co-occurrence matrices parameters, 180 gray-level run-length matrices parameters (RLM), and 11 gray-level size zone matrices parameters. The specific description of radiomics features is presented in the Supplemental Material. These radiomics features have underlying relationships with pathophysiological characteristics[16], intracellular heterogeneity[17], as well as genotypes[18], and so on.

Five steps were carried out to select radiomics features. First, the intra-class correlation coefficient (ICC) for all features by two radiologists was analyzed. Features with ICC greater than 0.80 were selected[19], and the mean values of radiomics features from two radiologists were calculated as robust features for further analysis. Second, we normalized the selected radiomics features by replacing the abnormal values with mean and converting the features into non-dimensional values *via* subtracting by mean and dividing by standard deviation value to eliminate discrepancies. Third, we randomly grouped the cohort into a training set and a validation set with a proportion of 7:3 (156 lesions in the training set with 68 non-DR and 88 DR lesions, 67 lesions in the validation set with 29 non-DR and 38 DR lesions). Fourth, we applied analysis of variance/Mann-Whitney *U* test, correlation analysis, and least absolute shrinkage and selection operator to select optimal features. The specific explanation of the methods to select radiomics features is summarized in the Supplemental Material. Last, the MRI-radiomics logistic model to differentiate DR and non-DR patients was constructed in the training set and verified by the validation set. The workflow of the radiomics signature in differentiating DR and non-DR patients was illustrated in Figure 2.

The calibration curves were depicted to compare the consistency between predicted and actual ability to evaluate response to chemotherapy, accompanied by Hosmer-Lemeshow test. The receiver operating characteristic curve was constructed by DeLong test, and the area under curve (AUC) was calculated to evaluate the validity of

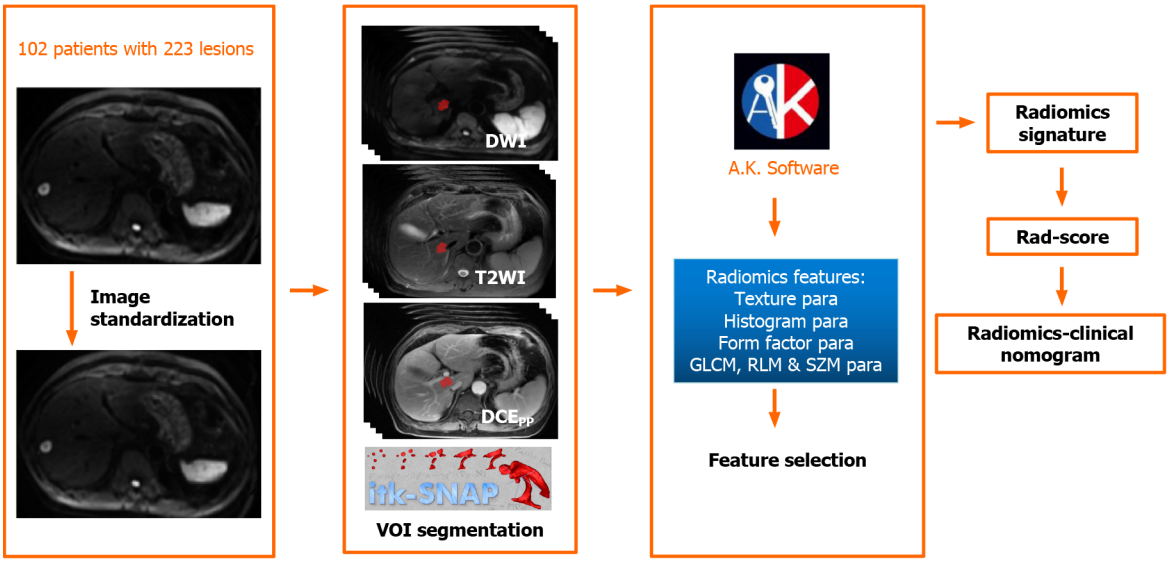

Figure 2 The workflow of radiomics signature in differentiating the responses of synchronous liver metastasis patients to chemotherapy.
VOI: Volume of interest; GLCM: Gray-level co-occurrence matrices; RLM: Run-length matrices; SZM: Size zone matrices; Rad-score: Radiomics score.

MRI-radiomics logistic models.

Statistical analysis

Analysis of variance/Mann-Whitney *U* test /MW, correlation analysis, least absolute shrinkage and selection operator, the logistic model construction, the calibration curve establishment, and radiomics-clinical nomogram development were performed with R software V4.0.1 to select features that potentially predict chemotherapeutic response. The calibration curves were depicted with Hosmer-Lemeshow test to compare the consistency between predicted and actual ability of evaluating response of chemotherapy. The receiver operating characteristic curves were constructed to calculate the AUC with a 95% confidence interval (CI), and the DeLong test was made to evaluate the validity of models in MedCalc V18.2.1. The general information, such as gender, age, tumor index, and T/N stage, was analyzed with IBM SPSS V22.0, using χ^2 or independent samples *t*-test. A two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Patients' general information

General information of patients were listed in Table 1. A total of 102 patients with 223 lesions were enrolled. There were 53 patients with 97 lesions in the non-DR group and 49 patients with 126 lesions in the DR group. The mean age of non-DR was 63.2 ± 9.5-years-old, and that of DR was 59.9 ± 11.6-years-old. In general, baseline demographics and tumor characteristics were balanced in the DR and non-DR groups, with exceptions of CA19-9 ($P = 0.045$) and clinical N staging ($P = 0.030$). Higher ratios of patients with normal CA19-9 levels were enrolled in the non-DR group (non-DR was 56.6% *vs* DR was 36.7%). In regard to clinical N staging, patients in the non-DR group primarily were staged to be N1 (52.8%), while stage N2 (73.5%) ranked the top in the DR group.

MRI-radiomics logistic model construction

Among the total 1188 radiomics features from T2WI, DWI, and CE_{PP} sequences of MRI examination, 893 features with ICC greater than 0.80 between two radiologists remained for the following analysis. After decreasing redundant features with the methods of analysis of variance/Mann-Whitney *U* test, correlation analysis, and least absolute shrinkage and selection operator, 12 features were selected to construct the MRI-radiomics logistic model for predicting responses of chemotherapy (Figure 3) and the radiomics score (rad-score) was calculated accordingly. The 12 optimal features included four DWI features , six T2WI features , and two CE_{PP} features. The AUC of

Table 1 Baseline patient characteristics

General characteristics	non-DR, *n* = 53	DR, *n* = 49	*P* value
Demographics			
Gender (female/male)	28/25	17/32	0.065
Age (mean ± SD)	63.3 ± 11.1/63.2 ± 7.5	58.6 ± 11.2/59.7 ± 14.1	0.117
Tumor markers			
AFP (normal/abnormal)	51/2	47/2	0.936
CEA (normal/abnormal)	14/39	14/35	0.807
CA19-9 (normal/abnormal)	30/23	18/31	0.045
Clinical T/N staging			
T1/T2/T3/T4	0/1/48/4	0/10/33/6	0.136
N0/N1/N2	3/28/22	5/8/36	0.030

The characteristics of age and clinical T/N staging were analyzed by independent-samples *t*-test. The data of gender and tumor markers of AFP/CEA/CA19-9 were analyzed by Pearson χ^2. A $P < 0.05$ was viewed as statistically significant. Non-DR: Disease non-response group; DR: Disease response group; SD: Standard deviation; AFP: Alpha-fetoprotein; CEA: Carcinoembryonic antigen.

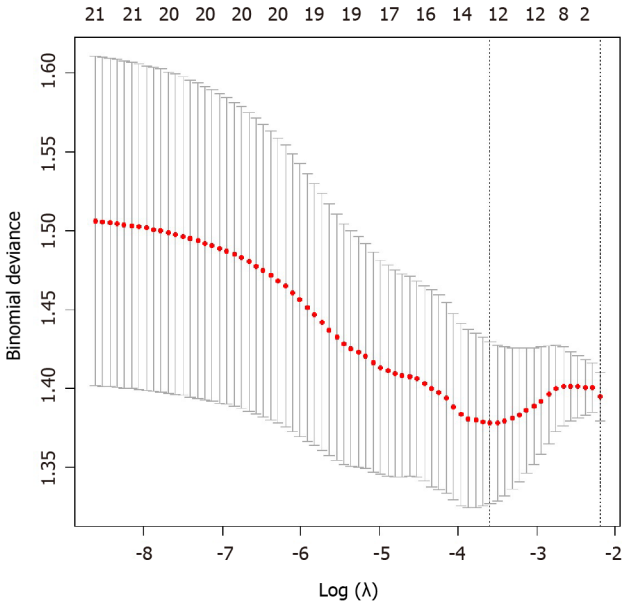

Figure 3 The feature selection method of least absolute shrinkage and selection operator. A total of 12 optimal features were selected.

this model was 0.733 (95%CI, 0.656-0.800) in the training set and was 0.753 (95%CI, 0.633-0.849) in the validation set. The calibration curves showed good consistency in the predicted and the actual ability to evaluate chemotherapy responses in both training and validation sets. The non-significant Hosmer-Lemeshow test suggested goodness-of-fit for the MRI-radiomics logistic models in the training ($P = 0.858$) and validation ($P = 0.374$) set.

Furthermore, we also compared prediction efficiency in chemotherapeutic response between different radiomics models of single DWI, T2WI, or CE_{PP} sequence (Ra-DWI, Ra-T2WI, Ra-CE_{PP}). The AUCs of these models were listed in Table 2. The AUCs of the training set and validation set of Ra-DWI were 0.652 (95%CI, 0.571-0.726) and 0.661 (95%CI, 0.536-0.772), which were higher than those of Ra-T2WI and Ra-CE_{PP} (Table 2). Then we compared the radiomics features of DWI between the baseline and after chemotherapy in the DR group. There were 105 lesions enrolled, as the post-chemotherapy images of another 21 lesions were not satisfactory. By features selection, 11 significant delta-radiomics features were left. The remaining features included histogram (Quantile 0.025), texture (ClusterShade_angle90_offset1), gray-level co-occurrence matrices Entropy (GLCMEntropy_angle135_offset7/_angle90_offset1, and

Table 2 The area under the curve of radiomics model of DWI sequence, radiomics model of T2WI sequence, and radiomics model of portal phase of contrast-enhanced sequences models in the training set and the validation set

Sequence	Training set	*P* value	Validation set	*P* value
Ra-DWI	0.652 (95%CI, 0.571-0.726)	0.001	0.661 (95%CI, 0.536-0.772)	0.018
Ra-T2WI	0.628 (95%CI, 0.547-0.704)	0.005	0.575 (95%CI, 0.450-0.695)	0.291
Ra-CE_{PP}	0.633 (95%CI, 0.552-0.709)	0.003	0.543 (95%CI, 0.418-0.664)	0.544

A $P < 0.05$ of the DeLong test was considered statistically significant. The DeLong test of validation set in radiomics model of T2WI sequence and radiomics model of portal phase of contrast-enhanced sequences had no statistical significance. Ra-DWI: Radiomics model of DWI sequence; Ra-T2WI: Radiomics model of T2WI sequence; Ra-CE_{PP}: Radiomics model of portal phase of contrast-enhanced sequences; CI: Confidence interval.

differenceEntropy), RLM (LongRunHighGreyLevelEmphasis_AllDirection_offset1_SD/_angle45_offset7, ShortRun Emphasis_angle45/90_offset1/HighGreyLevelEmphasis_angle90_offset7), and size zone variability. The top 4 of the 11 delta-radiomics features belonged to RLM parameters.

Radiomics-clinical nomogram analysis

For clinical characteristics, tumor marker CA19-9 ($P = 0.045$) and clinical N staging ($P = 0.030$) were demonstrated to be statistically different between DR and non-DR groups. Thus, CA19-9 and clinical N staging, together with rad-score were integrated into the logistic model to construct the radiomics-clinical nomogram (Figure 4). The formula of the logistic model was: Y = -2.141 + 1.018 × [rad-score] + 0.893 × [CA19-9] + 1.042 × [N staging]. The AUC of the radiomics-clinical nomogram was 0.809 (95%CI, 0.751-0.858).

DISCUSSION

The standardized treatment for SLM patients is unascertained, and early detection of patients with DR or non-DR is crucial for personalized treatment planning. In order to predict patients' responses to chemotherapy, we generated a MRI-radiomics based model in this study. The AUC of this model was 0.733 in the training set and was 0.753 in the validation set. Non-significant Hosmer-Lemeshow test and the calibration curve of the MRI-radiomics model showed good consistency between the predicted and actual probability. Although the AUC value was not ideal enough, the non-invasive MRI examination is still beneficial to differentiate non-DR and DR in clinical practice. There were 156 lesions with 68 non-DR and 88 DR lesions in the training set and 12 radiomics features to construct MR-radiomics logistic model. The sample size of the logistic model often relies on an events per predictor variable[20]. Vittinghoff *et al*[21] conducted a large simulation study of other influences on relative bias, confidence interval coverage, and type I error and found that the events per predictor variable between 5 to 9 could achieve acceptable results. In our study, the events per predictor variable were 5.7 in non-DR group and were 7.3 in the DR group, which were both in the range of 5 to 9. Therefore we believed that the MRI-radiomics model based on 156 lesions in the training set was valid.

As for the selection of MRI sequences, a recent investigation in reproducibility and robustness of MRI radiomics has suggested that caution should be taken in the interpretation of clinical studies using T1WI features to delineate VOI[22]. Meanwhile, after making some attempt in the exploration stage, we realized that it was difficult and inaccurate to depict VOI in AP and EP of CE sequence. Thus, we selected DWI, T2WI, and PP of CE sequences to do future research. Features with ICC more than 0.80 identified by two radiologists were selected, and the mean values of selected features were calculated as robust features for further analysis. After comparing the predictive efficiency between Ra-DWI, Ra-T2WI, and Ra-CE_{PP}, Ra-DWI demonstrated outstanding predictive value compared with Ra-T2WI and Ra-CE_{PP} (AUCs in the training set: 0.652 *vs* 0.628, 0.633; AUCs in the validation set: 0.661 *vs* 0.575, 0.543). We should think highly of DWI due to its potential for evaluating DR and non-DR in clinical practice. As has been investigated that delta-radiomics analysis explored the change of radiomics features between baseline and follow-up computed tomography images can improve the differentiation of pre-invasive ground glass nodules from invasive ground glass nodules[23].

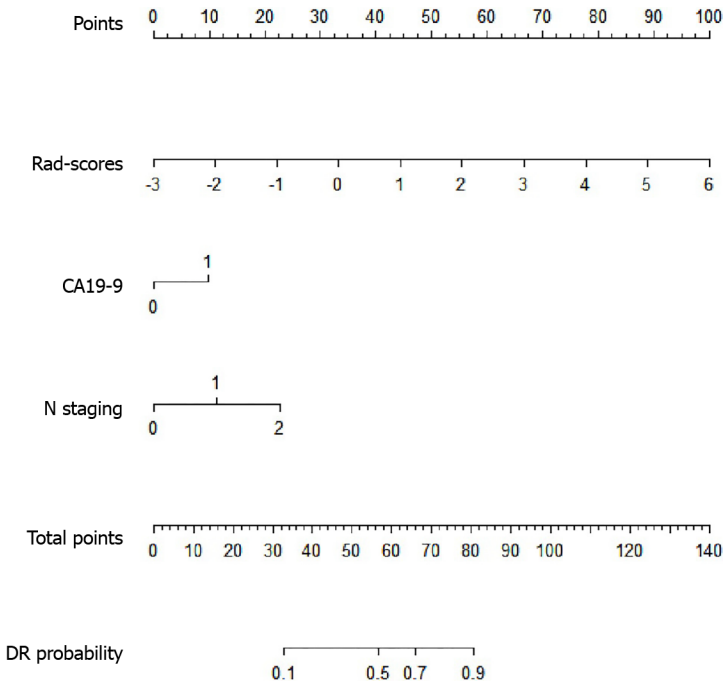

Figure 4 The nomogram of radiomics-clinical analysis to differentiate patients with disease non-response group from disease response group. The zero in CA19-9 represented normal and one referred to abnormal. DR: Disease response group; Rad-score: Radiomics score.

After comparing the delta-radiomics of DR between baseline and post-chemotherapy MRI examination, we obtained the top 4 of 11 delta-radiomics features of post-chemotherapy belonging to RLM parameters. Kim *et al*[24] analyzed the association between pathological characteristics and gray-level run-length matrices features of pancreatic cancer and revealed that gray-level non-uniformity values of RLM were powerful indicators for prognosis. RLM is more sensitive to reflect changes of regional heterogeneity since it analyzes radiomics changes through the whole length of the run [25]. These results suggested that DWI helped to discriminate patients with DR from non-DR in clinical practice.

The nomogram of incorporated independent risk factors for clinical events, such as differentiation[26], survival[27], and recurrence[28], has been widely applied in the field of oncology. The radiomics-clinical nomogram contained rad-score, CA19-9, and clinical N staging demonstrated better predictive accuracy compared with MRI-radiomics signature (AUC: 0.809 *vs* 0.733 in the training set, and 0.753 in the validation set). In patients with SLM, elevation of CA19-9 and carcinoembryonic antigen is a prognostic indicator and can predict response to treatment[29]. A previous study identified that CA19-9 was the best prognostic indicator of metastatic CRC[30] and also was a significant prognostic indicator for CRC patients treated with neoadjuvant chemoradiotherapy[29]. Similar to a previous study[31], we demonstrated that more patients (63.3%) with DR had elevated levels of CA19-9 than those with non-DR (43.4%), suggesting that CA19-9 was a promising indicator for predicting response to chemotherapy ($P < 0.05$). A study by Märkl *et al*[32] illustrated that lymph node staging played a significant role in prognosis evaluation and treatment stratification for CRC. In the current study, we confirmed that clinical N staging had a correlation to chemotherapeutic response. Taken together, the proposed radiomics-clinical nomogram is beneficial in estimating the chemotherapeutic response and in selecting appropriate patients to receive chemotherapy.

Our study had several limitations. First, we only took PP of CE for analysis since the lesions in AP and EP of CE sequence were not visible enough for VOI segmentation. An automatic segmentation method to deal with the AP/EP images remains to be developed. Second, this is a single center study, and the prediction models should be further verified in other centers and in a larger cohort. Third, the inevitable flaw may occur in this retrospective study since the histopathological grade and clinical characteristics of selected patients may be unbalanced. Fourth, as for the criteria for evaluating the response of chemotherapy, we chose the Response Evaluation Criteria in Solid Tumors criterion instead of considering histopathological evidence, which may be complementary to the results of the radiomics. Therefore, our results should be further validated in future multiangle and multiclassification sample studies.

CONCLUSION

In conclusion, our study indicated that the MRI-radiomics logistic model was a helpful and non-invasive predictor for differentiating patients with non-DR from DR. Ra-DWI was more efficient in distinguishing patients with non-DR from DR than that of Ra-T2WI and Ra-CE_{PP}, and the RLM parameter of Ra-DWI was superior in reflecting the delta-radiomics after chemotherapy. Furthermore, the radiomics-clinical nomogram based on the MRI-radiomics signature and clinical factors of CA19-9 and clinical N staging is conducive to better predict non-DR and DR of SLM patients and provides a theoretical and practical basis for the choice of treatment strategies.

ARTICLE HIGHLIGHTS

Research background

Synchronous liver metastasis (SLM) frequently occurs in colorectal cancer (CRC). Nearly 50% of CRC patients develop hepatic metastasis, with 15%-25% of them presenting with SLM. The evaluation of SLM in CRC is crucial for a precise and personalized treatment.

Research motivation

To construct prediction models based on magnetic resonance imaging (MRI)-radiomics and clinical parameters to evaluate the chemotherapy response in SLM patients in the context of CRC.

Research objectives

A total of 102 patients with 223 SLM lesions were identified and divided into disease response (DR) and disease non-response (non-DR) to chemotherapy.

Research methods

The MRI-radiomics logistic models containing T2WI, DWI, and portal phase of dynamic contrast-enhanced sequences radiomics models (Ra-T2WI, Ra-DWI and Ra-portal phase of dynamic contrast-enhanced sequences) were constructed after methods of feature dimension, and the respective radiomics score was calculated. Then radiomics-clinical nomogram was generated by combining radiomics score, CA19-9, and clinical N staging.

Research results

The AUCs of the training and validation set of Ra-DWI were 0.652 and 0.661, which were higher than those of Ra-T2WI and Ra-portal phase of dynamic contrast-enhanced sequences. After chemotherapy, the top four delta-radiomics features of Ra-DWI in DR group belonged to gray-level run-length matrices parameters. The radiomics-clinical nomogram was built with an AUC of 0.809 and can effectively discriminate the patients with DR from non-DR.

Research conclusions

MRI-radiomics is conducive to predict chemotherapeutic response in SLM patients. The Ra-DWI logistic model behaved the best in differentiating DR and non-DR. Run-length matrices parameters of Ra-DWI were more sensitive to reflect the delta-radiomics after chemotherapy. The radiomics-clinical nomogram is more effective in predicting chemotherapeutic response.

Research perspectives

This study provides new insights into the potential ability of MRI-radiomics in evaluating chemotherapeutic response in SLM patents. The MRI-radiomics features combined with clinical characteristics is more effective in evaluation.

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ORIGINAL ARTICLE

Observational Study

Deep learning *vs* conventional learning algorithms for clinical prediction in Crohn's disease: A proof-of-concept study

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Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained retrospectively.

Conflict-of-interest statement: Con D has no relevant conflicts of interest to declare. AV has received

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Abstract

BACKGROUND

Traditional methods of developing predictive models in inflammatory bowel diseases (IBD) rely on using statistical regression approaches to deriving clinical scores such as the Crohn's disease (CD) activity index. However, traditional approaches are unable to take advantage of more complex data structures such as repeated measurements. Deep learning methods have the potential ability to automatically find and learn complex, hidden relationships between predictive markers and outcomes, but their application to clinical prediction in CD and IBD has not been explored previously.

AIM

To determine and compare the utility of deep learning with conventional algorithms in predicting response to anti-tumor necrosis factor (anti-TNF) therapy in CD.

METHODS

This was a retrospective single-center cohort study of all CD patients who commenced anti-TNF therapy (either adalimumab or infliximab) from January 1, 2010 to December 31, 2015. Remission was defined as a C-reactive protein (CRP) < 5 mg/L at 12 mo after anti-TNF commencement. Three supervised learning algorithms were compared: (1) A conventional statistical learning algorithm using multivariable logistic regression on baseline data only; (2) A deep learning algorithm using a feed-forward artificial neural network on baseline data only; and (3) A deep learning algorithm using a recurrent neural network on repeated data. Predictive performance was assessed using area under the receiver operator characteristic curve (AUC) after 10× repeated 5-fold cross-validation.

financial support to attend educational meetings from Ferring. van Langenberg DR has served as a speaker and/or received travel support from Takeda, Ferring and Shire. He has consultancy agreements with Abbvie, Janssen and Pfizer. He received research funding grants for investigator-driven studies from Ferring, Shire and AbbVie.

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RESULTS

A total of 146 patients were included (median age 36 years, 48% male). Concomitant therapy at anti-TNF commencement included thiopurines (68%), methotrexate (18%), corticosteroids (44%) and aminosalicylates (33%). After 12 mo, 64% had CRP < 5 mg/L. The conventional learning algorithm selected the following baseline variables for the predictive model: Complex disease behavior, albumin, monocytes, lymphocytes, mean corpuscular hemoglobin concentration and gamma-glutamyl transferase, and had a cross-validated AUC of 0.659, 95% confidence interval (CI): 0.562-0.756. A feed-forward artificial neural network using only baseline data demonstrated an AUC of 0.710 (95%CI: 0.622-0.799; P = 0.25 *vs* conventional). A recurrent neural network using repeated biomarker measurements demonstrated significantly higher AUC compared to the conventional algorithm (0.754, 95%CI: 0.674-0.834; P = 0.036).

CONCLUSION

Deep learning methods are feasible and have the potential for stronger predictive performance compared to conventional model building methods when applied to predicting remission after anti-TNF therapy in CD.

Key Words: Machine learning; Artificial intelligence; Precision medicine; Personalized medicine; Deep learning

Core Tip: Deep learning has vast potential, but its clinical utility in predicting outcomes in Crohn's disease (CD) has not been explored. This study showed that deep learning algorithms (a recurrent neural network) using a more complex information structure including repeated biomarker measurements had a better predictive performance compared to a conventional statistical algorithm using only baseline data. This proof-of-concept study therefore paves the way for further research in the use of deep learning methods in clinical prediction in CD.

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INTRODUCTION

Crohn's disease (CD) is a heterogeneous chronic inflammatory bowel disease (IBD) that is characterized by intermittent flares, medication changes, the potential need for surgery and substantial psychological morbidity[1,2]. As with many chronic conditions, predicting disease trajectory, outcomes and response to therapies in CD are key components of clinical practice where management is tailored to the individual [3]. Precision medicine has been in part driven by the vast expansion of available electronic health data, genomic data and novel disease biomarkers[3]. However, deciphering the complex relationships between large amounts of information and multiple data types presents new analytical challenges.

Traditional approaches to constructing prediction models rely on multivariable regression approaches, typically logistic regression for classification or proportional hazards regression for longitudinal prediction[4]. The resulting predictive models are thus typically only linear combinations of the included predictors and may have limited ability to learn more complex relationships within the data. The advantage of machine learning and artificial intelligence over traditional predictive tools is the potential ability for computational algorithms to automatically find and learn complex, hidden relationships between predictive markers and outcomes[5,6]. This is especially true for deep learning or artificial neural network (ANN) methods, although their 'black box' approach has been criticized for an inability to produce a causal

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explanation between predictors and outcomes[6].

Despite some limitations, there is much interest in developing and testing machine learning and deep learning tools to aid decision making[5,7]. In luminal gastroenterology, machine learning is gaining traction but its use has been relatively limited to automatic image recognition in endoscopy[8-11] as well as feature selection in genomic and microbiomics data[12,13]. Although there has been great interest in predicting clinical outcomes in CD such as response to therapeutics including biologics[14-18] and immunomodulators[19,20], studies investigating the utility of machine learning models for such predictive tasks have been more limited[21-23]. In particular, the utility of deep learning or ANNs specifically in clinical prediction of CD remains unknown[7].

We aimed to evaluate the utility of deep learning algorithms compared with conventional statistical learning algorithms for clinical prediction in this proof-of-concept study. In particular, we aimed to compare these algorithms as methods of learning and prediction in a general sense, rather than to develop any specific predictive model or score.

MATERIALS AND METHODS

Study design

This proof-of-concept study utilized a retrospective longitudinal cohort at a tertiary health network comprising three acute hospitals in Melbourne, Australia. The focus of the study was to compare the ability of two supervised learning algorithms (conventional statistical learning *vs* deep learning) to predict remission after 12 mo of treatment using clinical variables and biomarkers available at baseline. The performance of each algorithm was evaluated using cross-validation. The emphasis of the study was to compare the predictive performance of the two methods of learning rather than any specific model itself. This study was approved by the Eastern Health Office of Research & Ethics (approval number: LR 61/2015).

Study cohort

All adult patients > 18 years with confirmed CD according to standard criteria[24] were included if they were commenced on treatment with an anti-tumor necrosis factor (anti-TNF) agent (adalimumab or infliximab) for luminal CD and received at least one dose of the drug between January 2010 and December 2015. Patients receiving anti-TNF for perianal disease without luminal disease were excluded. Patients were followed up for 12 mo to determine rates of biochemical remission.

Outcomes

Response to anti-TNF was defined as having achieved biochemical remission as per serum C-reactive protein (CRP) < 5 mg/L at 12 mo. This endpoint was chosen because CRP is an accepted biomarker to reflect disease activity and predict outcomes in CD [25,26]. Additionally, normalization of CRP predicts better outcomes in CD patients in remission[27,28]. The first CRP measurement after 12 mo and before 18 mo was used. Patients who did not have a CRP measurement in this time period were excluded.

Data collection and pre-processing

Baseline characteristics were collected *via* hospital and clinic records, including Montreal classification, concomitant baseline therapies, prior anti-TNF exposure and prior surgeries. Biomarker data were collected at two time points: (1) A baseline measurement defined as the most proximate measurement prior to commencing anti-TNF, up to 3 mo before commencement; and (2) A prior measurement defined as the second most proximate measurement, up to 12 mo before commencement. Only patients with complete baseline data were included, while missing prior values were imputed with the respective baseline value. The following variables were log-transformed to correct skewness: serum bilirubin, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transferase (GGT). The data underlying this article cannot be shared publicly due to privacy and ethical concerns. The data will be shared upon reasonable request to the corresponding author.

Statistical learning algorithm (conventional approach)

The conventional approach to developing a predictive clinical model is to run univariable and multivariable regression analysis to find useful and preferably

independent predictors of the outcome of interest (see Figure 1). Criteria for variable selection usually involves significance testing (*P* values) or likelihood-based information criterion (such as the Akaike information criterion). In this study, logistic regression was used given the dichotomous nature of the outcome (CRP < 5 mg/L *vs* CRP ≥ 5 mg/L). The conventional approach typically only uses data from a single time-point, therefore we used baseline data only (the most proximate measurement for all biomarkers). For this conventional approach, we employed the following modelling algorithm: (1) Perform univariable logistic regression on each variable and retain all variables with $P < 0.5$; (2) Run backwards stepwise selection on all retained variables with removal criterion $P > 0.2$; and (3) Use the regression coefficients in the remaining multivariable model to derive the predictive score.

Deep learning algorithms (experimental approach)

A basic deep learning algorithm is a feed-forward ANN[6]. An ANN is composed of layers: an input layer (consisting of all the input predictor variables), an output layer (the prediction), and a number of 'hidden' layers (see Figure 1). Nodes within a hidden layer are called 'neurons'. The hidden layers allow an ANN to learn complex, non-linear relationships between input variables and the outcome of interest. The influence of nodes in a layer on other nodes in subsequent layers is 'trained' or fitted using a mathematical function and ultimately determines how information is propagated through the ANN — this is analogous to fitting a regression line on data in conventional statistics. An ANN with only an input and output layer, without hidden layers, can be analogous to simple logistic regression, although they are not equivalent.

However, like the conventional statistical algorithm, a basic feed-forward ANN is still only able to model relationships between predictors at a single time-point. A recurrent neural network (RNN) is a more advanced deep learning algorithm that is able to model repeated measurements over time. Like a feed-forward ANN, information is propagated from the input layer to the output layer. However, instead of only allowing the information to pass through once, information is fed to the RNN sequentially, or 'recurrently' — that is, each set of repeated measurements is inputted once at a time allowing the RNN to update its knowledge of the relationship between the predictors and the outcome. Therefore, the algorithm is additionally able to learn and utilize the dynamics of biomarkers over time, in a way that cannot be achieved by conventional statistical learning methods.

We tested the feed-forward ANN and the RNN in three separate experiments: (1) Using all baseline clinical data in a feed-forward ANN; (2) Using only baseline biomarker data in a feed-forward ANN; and (3) Using repeated biomarker data in an RNN. In this study after hyper-parameter tuning, we used a feed-forward ANN architecture of 3 hidden layers, each with 64 neurons, and an RNN architecture of 1 hidden layer with 64 neurons.

Comparison of algorithms

The predictive performances of the conventional statistical algorithm and the experimental deep learning algorithm (ANN) was defined as their ability to correctly classify 12-mo CRP < 5 mg/L measured using the area under the receiver operator characteristic curve (AUC). Because the learning ability of an ANN can be arbitrarily increased, an overly powerful ANN that is trained such that it has near-perfect prediction on the original training cohort, would suffer from poor predictive ability in an external cohort (this is called 'over-fitting', a well-known phenomenon). Similarly, the same conventional statistical learning algorithm might result in models with different variables when applied to different cohorts. Therefore, it is important to evaluate the ability of a learning algorithm to predict outcomes in patients that are not included in the original training cohort (external validity).

In the absence of external testing cohorts to assess external validity, cross-validation is an internal validation procedure that is suited to this purpose[4]. During cross-validation, the cohort is randomly divided into *k* equally sized sub-cohorts, known as 'folds' (where *k* is often 5 or 10 by convention). Then, one fold is set aside to be used to test the algorithm, after the algorithm is first trained on the remaining *k*-1 folds (see Figure 2). This allows the algorithms to be tested on patients that were not used during training. The process is then repeated for each fold (where each fold takes turns in being the test fold). The average AUC after repeating *k* times gives the cross-validated AUC. However, this procedure is not free from error, because the partitioning process may have randomly resulted in a better (or worse) than usual performance. Thus it is important to repeat the whole process a number of times, to reduce this error[29].

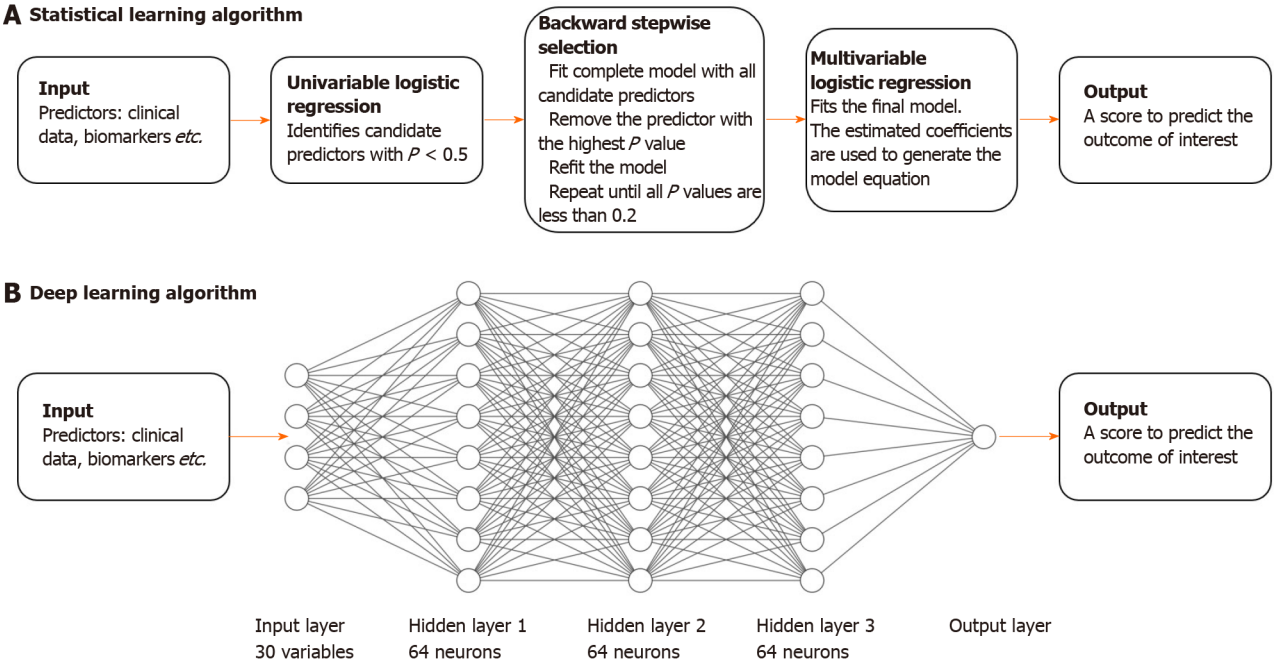

Figure 1 Comparison of the predictive modelling process using two supervised learning algorithms. A: Conventional statistical learning; B: Deep learning.

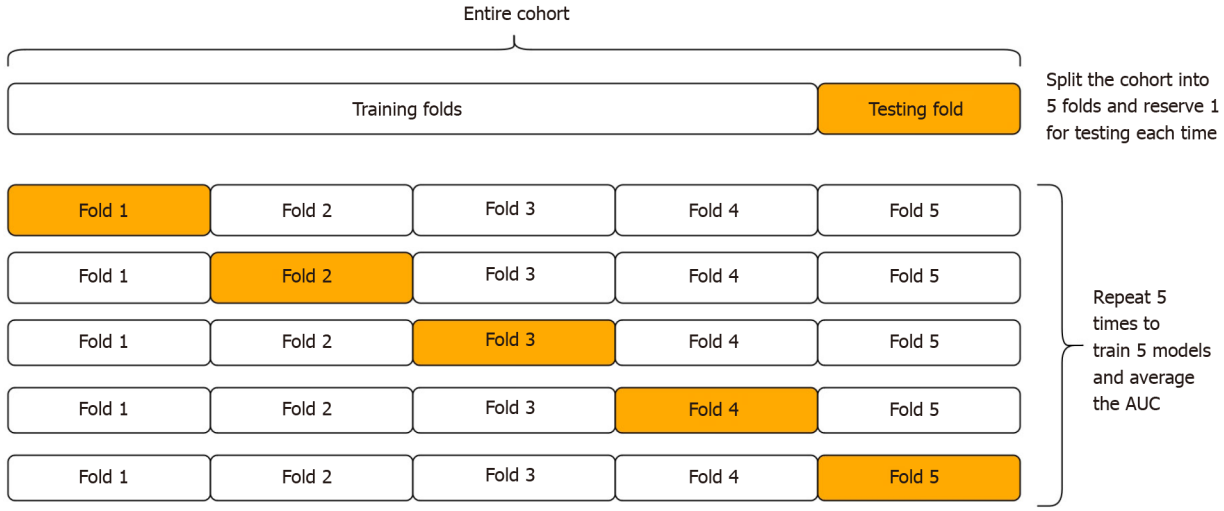

Figure 2 Schematic diagram of *k*-fold cross validation procedure for *k* = 5. This method is considered more reliable than a random train-test split, which would be analogous to training only one model, instead of the average of *k* models. AUC: Area under the receiver operator characteristic curve.

For this study, we used 5-fold cross-validation repeated 10 times to estimate the generalizability of each algorithm on unseen data. Statistical comparison of the cross-validated AUCs of each learning algorithm was made using the variance-corrected repeated *k*-fold *t* test instead of a conventional paired *t* test because of the independency violation from repeated partitioning of the same dataset[29]. For comparison, the naïve or apparent AUC of each model after training and testing on the same entire cohort was given, however this is non-informative. Sample size calculations were conducted only as a guide given the exploratory nature of the study and without prior similar studies on which to base AUC assumptions. The target sample size to detect a 10% difference in AUC with 80% power and 95% significance assuming an AUC variance of 10% was n = 157[30]. To instead detect a 15% difference in AUC under the same conditions, a sample size of n = 70 was required. The Python 3.8.4 programming language with the open-source module PyTorch was used to create the deep learning algorithm. Stata/IC 16 (Texas, United States, 2020) was used to create the statistical learning algorithm.

RESULTS

Baseline characteristics

A total of 146 CD patients were included (see Table 1). Their median age was 36 years [inter-quartile range (IQR) 25-50], 48% were male and median disease duration since diagnosis was 5 years (IQR 1-12). The anti-TNF commenced was infliximab in 58% and adalimumab in 42%. Concomitant therapy at anti-TNF commencement included thiopurines (68%), methotrexate (18%), corticosteroids (44%) and aminosalicylates (33%). Over a quarter of patients (28%) had prior intestinal surgery, while 15% had prior exposure to anti-TNF. After 12 mo, 94 (64%) patients were in biochemical remission (CRP < 5 mg/L).

Statistical learning algorithm

Univariable analysis: Baseline factors associated with biochemical remission at 12 mo on univariable testing included non-complex disease behavior (B1), higher albumin and mean corpuscular hemoglobin concentration (MCHC), and lower platelets, lymphocytes and monocytes (each $P < 0.05$; see Table 2), while lower neutrophil count was nearly significant ($P = 0.06$). There was no significant association with age, sex, disease location or baseline medical therapies (see Table 2).

Multivariable analysis: After backward stepwise selection, the following variables remained in the final multivariable model: Complex disease, baseline albumin, monocytes, lymphocytes, MCHC and GGT (see Table 2). The resulting prediction model was given by the following equation (coefficients correct to two significant figures): Score = 0.079 × (albumin, g/L) + 0.050 × (MCHC, mg/L) - 1.1 × (monocytes, 10^9/L) - 0.43 × (lymphocytes, 10^9/L) - 1.0 × (complex disease, y=1 | n=0) - 0.69 × $\log_e$ (GGT, IU/L).

Outcome prediction: After 10× 5-fold cross validation, the average AUC of the statistical learning algorithm was 0.659 [95% confidence interval (CI): 0.562-0.756]. This suggests the statistical learning algorithm is expected to accurately classify 65.9% of patients in external cohorts who have similar characteristics to the study cohort (see Table 3). The algorithm performed better than chance (AUC > 0.5) 94% of the time and had an AUC > 0.7 in 38% of occasions (see Figure 3). The apparent naïve AUC (when trained and tested on the same data) of the model was 0.771.

Deep learning algorithms

Feed-forward ANN with complete baseline data: The feed-forward ANN with complete baseline data had a cross-validated AUC of 0.710 (95%CI: 0.622-0.799) (see Figure 3 and Table 3). This difference was not statistically significant using the variance corrected t test ($P = 0.25$). The algorithm performed better than chance 100% of the time and had good performance (AUC > 0.7) 54% of the time (see Figure 3). For comparison, the naïve AUC of the model was 0.857.

Feed-forward ANN with baseline biomarker data only: The same feed-forward ANN using only baseline biomarker data had a similar cross-validated AUC of 0.706 (95%CI: 0.621-0.791), which was again not significantly different compared to the conventional algorithm ($P = 0.33$) (see Table 3). The algorithm performed better than chance 100% of the time and had good performance (AUC > 0.7) 58% of the time (see Figure 3). The naïve AUC of the model was 0.776.

RNN with repeated biomarker data: The same feed-forward ANN using only baseline biomarker data had a similar cross-validated AUC of 0.754 (95%CI: 0.674-0.834), which was significantly higher than the AUC of the conventional algorithm ($P = 0.036$) (see Table 3). This suggests the RNN is expected to accurately classify 75.4% of patients in external cohorts who have similar characteristics to the study cohort. The RNN algorithm performed better than chance 100% of the time and had good performance (AUC > 0.7) 72% of the time (see Figure 3). For comparison, the naïve AUC of the model was 0.892.

DISCUSSION

The rapid expansion of available health data has motivated the development of machine learning and deep learning tools to predict useful outcomes in clinical medicine[5,6]. The advent of machine learning and data science techniques is

Table 1 Baseline characteristics of study cohort (*n* = 146)

Characteristic	*n* (%)
Age, years, median (IQR)	36 (25-50)
Sex	
Female	76 (52)
Male	70 (48)
Smoker (active)	33 (23)
CD behavior	
B1: Non-stricturing, non-penetrating	75 (51)
B2: Stricturing	56 (38)
B3: Penetrating/fistulizing	15 (10)
CD location	
L1: Ileal	41 (28)
L2: Colonic	43 (29)
L3: Ileocolonic	62 (42)
L4: Isolated UGI	0 (0)
Perianal involvement	20 (21)
Initial anti-TNF commenced	
Infliximab	84 (58)
Adalimumab	62 (42)
Baseline thiopurine	99 (68)
Baseline methotrexate	27 (18)
Baseline corticosteroids	64 (44)
Baseline aminosalicylates	48 (33)
Prior anti-TNF	22 (15)
Prior intestinal surgery	41 (28)
Disease duration, yr, median (IQR)	5 (1-12)
Baseline investigations	
CRP, mg/L, median (IQR)	3 (2-8)
Albumin, g/L, median (IQR)	37 (36-41)

IQR: Inter-quartile range; CD: Crohn's disease; CRP: C-reactive protein; TNF: Tumor necrosis factor; UGI: Upper gastrointestinal.

especially applicable to IBD due to the heterogeneity and chronic nature of such conditions and the repeated measures of disease activity over time which provides data that may be more suitable for complex modelling techniques. For instance, those with CD typically present with a wide array of disparate disease phenotypes and underlying pathogeneses, and their response to treatment and the trajectory of their disease course varies substantially and changes based on their response[31]. This study has exhibited the potential of deep learning algorithms in predicting response to anti-TNF therapy in patients with CD. The ability to predict the likelihood of response to a given treatment is crucial for risk-benefit assessment, which in turn is crucial to facilitate shared decision making between clinicians and patients[32]. Further, although biologic therapies have revolutionized management in IBD[31], medical therapy is now the principal driver of healthcare costs[33,34] and health economic considerations will inevitably affect treatment choice. Ideally, patients should receive therapies that are both likely to work and cost-effective. Therefore, there can be no 'one-size-fits-all' strategy to management, and precision and personalized medicine are key objectives.

Table 2 Estimated odds ratios with 95% confidence intervals on univariable and multivariable logistic regression analysis

Predictor	Univariable		Multivariable	
	OR (95%CI)	*P* value	Adj. OR (95%CI)	*P* value
Age, per year	0.98 (0.96-1.00)	0.10	-	-
Male (*vs* female)	1.42 (0.72-2.82)	0.31	-	-
CD behavior				
B1	1.0		Not included	
B2	0.45 (0.22-0.94)	0.034	Not included	
B3	0.42 (0.13-1.29)	0.13	Not included	
CD location				
L1: ileal	1.0		Not included	
L2: colonic	1.33 (0.54-3.31)	0.54	Not included	
L3: ileocolonic	0.91 (0.40-2.06)	0.83	Not included	
Ileal location (L1)	0.94 (0.45-2.00)	0.88	Not included	
Complex disease (B2/B3)	0.44 (0.22-0.89)	0.021	0.36 (0.16-0.80)	0.012
Active smoker	0.76 (0.40-1.47)	0.42	-	-
Perianal involvement	1.14 (0.49-2.65)	0.77	Not included	
Anti-TNF type: infliximab (*vs* adalimumab)	1.12 (0.56-2.22)	0.75	Not included	
Baseline immunomodulator	1.24 (0.47-3.27)	0.66	Not included	
Baseline corticosteroids	1.10 (0.56-2.18)	0.78	Not included	
Baseline aminosalicylates	1.16 (0.56-2.40)	0.69	Not included	
Prior anti-TNF	0.96 (0.37-2.47)	0.94	Not included	
Prior intestinal surgery	0.71 (0.34-1.48)	0.36	-	-
Disease duration, per $\log_e$ year	0.83 (0.65-1.06)	0.14	-	-
Albumin, per g/L	1.12 (1.03-1.22)	0.006	1.08 (0.98-1.20)	0.12
Hemoglobin, per g/L	1.01 (0.99-1.04)	0.32	-	-
HCT, per %	0.91 (0.71-1.16)	0.44	-	-
RCC, per 10^9/L	1.07 (0.84-1.36)	0.60	Not included	
MCV, per fL	1.01 (0.96-1.07)	0.64	Not included	
MCH, per pg/cell	1.15 (0.99-1.32)	0.06	-	-
MCHC, per mg/L	1.05 (1.02-1.08)	0.002	1.05 (1.02-1.09)	0.004
Platelets, per 100 × 10^9/L	0.63 (0.43-0.93)	0.020	-	-
Neutrophils, per 10^9/L	0.91 (0.82-1.00)	0.06	-	-
Lymphocytes, per 10^9/L	0.66 (0.46-0.93)	0.019	0.65 (0.41-1.02)	0.06
Monocytes, per 10^9/L	0.23 (0.08-0.63)	0.004	0.34 (0.10-1.16)	0.09
Eosinophils, per 10^9/L	0.61 (0.08-4.77)	0.64	Not included	
Basophils, per 0.01 × 10^9/L	0.92 (0.80-1.06)	0.24	-	-
Bilirubin, per $\log_e$ μmol/L	1.38 (0.70-2.72)	0.36	-	-
ALT, per $\log_e$ IU/L	1.04 (0.60-1.80)	0.90	Not included	
ALP, per $\log_e$ IU/L	0.55 (0.18-1.64)	0.28	-	-
GGT, per $\log_e$ IU/L	0.71 (0.46-1.09)	0.12	0.69 (0.43-1.11)	0.13

Variables excluded after univariable regression are in grey; variables excluded after stepwise selection are marked with a dash. CI: Confidence interval; OR: Odds ratio; CD: Crohn's disease; TNF: tumor necrosis factor; HCT: Hematocrit; RCC: Red cell count; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase.

Table 3 Comparison of learning algorithms during cross-validation experiments

Algorithm	Dataset[1]	AUC (%)		*P* value[2]
		Mean	SD	
Conventional statistics	Baseline clinical + biomarker data	65.9	9.5	-
Feed-forward ANN	Baseline clinical + biomarker data	71.0	8.7	0.25
Feed-forward ANN	Baseline biomarker data only	70.6	8.3	0.33
Recurrent neural network	Baseline and prior biomarker data	75.4	7.8	0.036

[1]Clinical data refers to non-biochemical data such as age, sex, disease characteristics and concurrent treatments. Biomarker data refers to complete blood count, liver function tests and albumin.
[2]*P* value for comparison against conventional statistical algorithm, using the variance-corrected repeated *k*-fold *t* test. AUC: Area under the receiver operator characteristic curve; ANN: Artificial neural network.

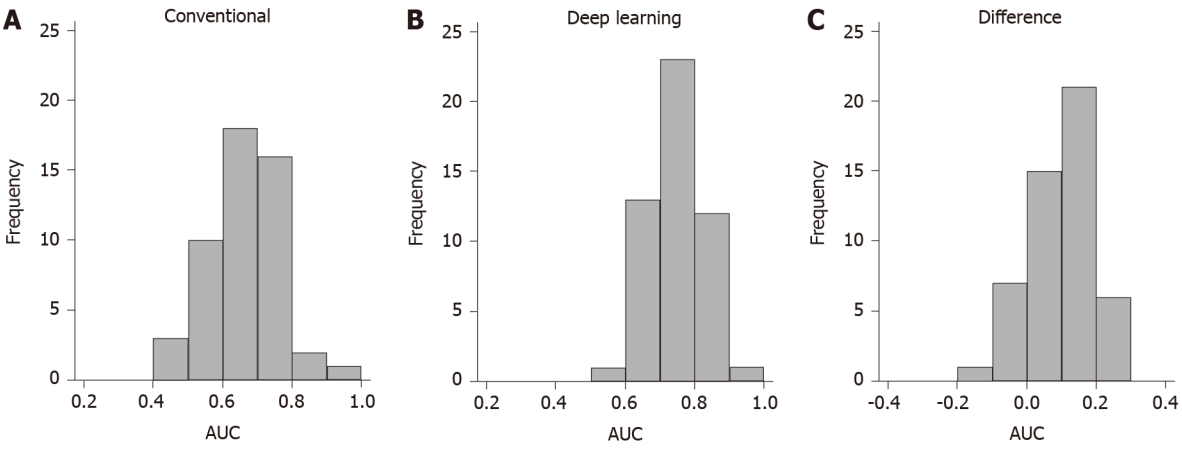

Figure 3 Distribution of area under the receiver operator characteristic curve after 10 × 5 fold cross validation. A: Conventional statistical learning algorithm (mean 0.659, SD 0.095); B: Recurrent neural network (mean 0.754, SD 0.078); C: Head-to-head comparison, matched at each fold and repetition (mean difference, + 0.095, *P* = 0.036). AUC: Area under the receiver operator characteristic curve.

Conventional statistical learning algorithms have generated many useful clinical scores, including the CD activity index[35], the simple endoscopic score for CD[36], scores to predict response to biologic therapies[16], and scores to differentiate CD from intestinal tuberculosis[37]. The advantage of conventional scores is often their simplicity and interpretability. A simple score can be memorized and calculated at the bed side and are intuitive as they utilize important risk factors of the outcome of interest. Yet clinical scores can only apply to a rather generic subgroup of patients and are never specific to any individual, as they utilize relatively few variables. Further, conventional methods are not readily able to model more complex, non-linear or time-dependent health states. With new genomic and microbiomic profiling, as well as the rapid uptake of comprehensive electronic medical records with mass data linkage, the ability of conventional learning algorithms to select useful predictive factors may become redundant[38].

Although the advantages of deep learning for the analysis of non-numerical data types is obvious, such as image data in endoscopy[39-41] and text or speech data in natural language processing[42], the utility of deep learning for the analysis of numerical data is less clear but remains promising. A recent study has demonstrated the utility of machine learning in predicting anti-TNF response in rheumatoid arthritis, but relied on genetic markers in addition to clinical data[43]. Another recent study

used machine learning to predict whether patients with ankylosing spondylitis required anti-TNF therapy, but did not evaluate whether response to therapy could be predicted[44]. It is anticipated that new data science and machine learning techniques are required to handle large amounts of data for use in clinical practice, although the optimal algorithms for this task remain unknown. Nevertheless, with the provision of comprehensive training data, machine learning tools have the potential to aid in individualized risk prediction, although no such model exists in IBD currently. In our cohort, the RNN deep learning algorithm was able to outperform the conventional algorithm after incorporating repeated biomarker measurements and thus additionally learn the non-linear temporal dynamics of the respective biomarkers — a feat that is not possible with conventional prediction models. It is expected that with enough training data, deep learning methods such as the RNN will be able to incorporate the time series data from multiple repeated health states of an individual patient over time. The clear trade-off with deep learning methods is the need for more data coordination and software to execute. However, the continued uptake of automated medical records in routine clinical practice may mitigate this limitation in future. Further, with the ever increasing breadth and volume of information from sources including comprehensive previous medical history, serum and fecal biomarkers, imaging and endoscopic data as well as genetics, the role of machine learning in prediction in chronic diseases including IBD is likely to expand.

This study has also demonstrated the importance of applying model validation techniques during model development[29]. ANNs and other powerful algorithms have the ability to learn intricate differences in data, yet poorly specified models that focus only on learning power have the propensity to learn the random variations or artefacts in the data, which are present only due to chance. This is evidenced by the RNN in this study achieving excellent AUC during training, but a reduced AUC when tested on unseen data (naïve AUC 0.892; cross-validated AUC 0.754). The same phenomenon occurred with the statistical learning algorithm but to a somewhat lesser extent (naïve AUC 0.771; cross-validated AUC 0.659). Therefore, studies developing predictive models should take care to avoid naïvely assessing predictive performance and ensure that effective cross-validation or bootstrapping methods are used for appropriate interval validation[4]. If available, external validation of predictive models in entirely new and different cohorts is the gold standard for model validation[4].

The dataset used in this study was retrospective and from a single center which subjects the results to information bias and limits their external validity. The outcome used was biochemical remission as this is a readily available as a repeated measure which allowed demonstration of more conventional and machine learning models, however it is acknowledged that clinical symptoms and/or mucosal healing are more clinically relevant end-points. Nevertheless, the goal of this study was to demonstrate the feasibility of deep learning methods in clinical prediction in this proof-of-concept study, rather than to develop a specific predictive model. Further, in practice, much larger cohorts will be required to properly train and calibrate deep learning models to maximize their utility in the real world. In future, all studies investigating specific predictive models should be subject to prospective controlled validation prior their application in clinical practice, specifically having shown that outcomes are improved after using predictive models to guide management.

CONCLUSION

In conclusion, we have demonstrated the feasibility of deep learning algorithms for clinical prediction in CD, which demonstrated an improved predictive performance compared to conventional methods. However, conventional statistical methods retain the advantage of simplicity and intuitiveness, allowing their use at the bedside. Yet with the rapid expansion of available health data, machine learning models have the potential to supersede currently conventional methods and greatly improve the development of tools for the clinical prediction of patient outcomes.

ARTICLE HIGHLIGHTS

Research background

Machine learning and artificial intelligence have the potential to revolutionize precision care in inflammatory bowel diseases. The greatest area of interest has been

the application of deep learning methods in automatic tumor detection during endoscopy, yet the application of such techniques in clinical outcome prediction has been lacking.

Research motivation

Traditional approaches to clinical prediction rely on conventional statistical algorithms such as regression, which are not suitable for more complex data such as repeated biomarker measurements.

Research objectives

To determine and compare the utility of deep learning with conventional algorithms in predicting response to anti-tumor necrosis factor (anti-TNF) therapy in Crohn's disease (CD).

Research methods

A retrospective cohort of CD patients commenced on anti-TNF therapy was used to experimentally develop and cross-validate three supervised learning algorithms: (1) Statistical learning algorithm; (2) Feed-forward artificial neural network; and (3) Recurrent neural network with repeated data. Predictive utility was quantified using the area under the receiver operator characteristic curve (AUC).

Research results

Within our cohort of 146 patients, the conventional statistical learning algorithm had the weakest performance [AUC 0.659, 95% confidence interval (CI): 0.562-0.756], compared to the feed-forward artificial neural network (AUC 0.710, 95%CI: 0.622-0.799; P = 0.25 *vs* conventional) and the recurrent neural network using repeated biomarker measurements (AUC 0.754, 95%CI: 0.674-0.834; P = 0.036 *vs* conventional).

Research conclusions

Deep learning methods are feasible and have the potential for stronger predictive performance compared to conventional model building methods when applied to predicting remission after anti-TNF therapy in CD.

Research perspectives

This has been the first study to investigate the utility of deep neural networks in predicting clinical outcomes using repeated clinical data in inflammatory bowel disease. Future studies should incorporate additional data types such as genetic, imaging and endoscopic factors.

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ORIGINAL ARTICLE

Observational Study

Serum soluble suppression of tumorigenicity 2 as a novel inflammatory marker predicts the severity of acute pancreatitis

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Abstract

BACKGROUND

Acute pancreatitis (AP) is an inflammatory disease in which the regulatory pathway is complex and not well understood. Soluble suppression of tumorigenicity 2 (sST2) protein receptor functions as a decoy receptor for interleukin (IL)-33 to prevent IL-33/suppression of tumorigenicity 2L (ST2L)-pathway-mediated T helper (Th)2 immune responses.

AIM

To investigate the role of sST2 in AP.

METHODS

We assessed the association between sST2 and severity of AP in 123 patients enrolled in this study. The serum levels of sST2, C-reactive protein (CRP) and Th1- and Th2-related cytokines, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, IL-2, IL-4, IL-5 and IL-13, were measured by highly sensitive ELISA, and the severity of AP in patients was evaluated by the 2012 Atlanta Classification Criteria.

RESULTS

Serum sST2 levels were significantly increased in AP patients, and further, these levels were significantly elevated in severe AP (SAP) patients compared to moderately severe AP (MSAP) and mild AP (MAP) patients. Logistic regression showed sST2 was a predictor of SAP [odds ratio (OR): 1.003 (1.001–1.006), P = 0.000]. sST2 cutoff point was 1190 pg/mL, and sST2 above this cutoff was associated with SAP. sST2 was also a predictor of any organ failure and mortality during AP [OR: 1.006 (1.003–1.009), P = 0.000, OR: 1.002 (1.001–1.004), P = 0.012,

respectively]. Additionally, the Th1-related cytokines IFN-γ and TNF-α in the SAP group were higher and the Th2-related cytokine IL-4 in the SAP group was significantly lower than those in MSAP and MAP groups.

CONCLUSION

sST2 may be used as a novel inflammatory marker in predicting AP severity and may regulate the function and differentiation of IL-33/ST2-mediated Th1 and Th2 Lymphocytes in AP homeostasis.

Key Words: Acute pancreatitis; Soluble suppression of tumorigenicity 2; T-helper 1 cells; T-helper 2 cells; Interleukin-33; Biomarker

Core Tip: Acute pancreatitis (AP) is an inflammatory disease in which the regulatory pathway is complex and not well understood. The interleukin (IL)-33/ suppression of tumorigenicity 2L (ST2L) functional pathway is involved in the pathological process of AP. Soluble suppression of tumorigenicity 2 protein (sST2) is a soluble receptor, which is released in the circulation acts as a decoy receptor by binding IL-33. However, sST2 as one of the most promising disease biomarker, has not been studied in the development of AP. In this study we studied the role of sST2 as an inflammatory marker for predicting the severity of acute pancreatitis.

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INTRODUCTION

Acute pancreatitis (AP) is caused by activation of pancreatic enzymes induced by a variety of etiologies, resulting in autodigestion, hemorrhage, edema, and even necrosis of pancreatic tissue, with or without other organ function changes. The clinical severity of AP is related to its prognosis. Severe AP (SAP) has a poor prognosis, and can cause severe disorders of multiple organ functions, and the mortality can reach 30%[1]. Excessive activation of inflammatory cells and their cytokines is one of the main mechanisms of pathogenesis of AP. The development and outcome of AP are closely related to immune function[2]. Research on changes in inflammatory mediators in patients with different clinical severity of AP is important clinically. A better understanding of the regulatory inflammatory pathways in AP is beneficial for discovery of new therapeutic targets.

Suppression of tumorigenicity 2 protein (ST2) is a member of the interleukin (IL)-1 receptor family with transmembrane (ST2L) and soluble (sST2) isoforms. The ligand of ST2 is IL-33, which can produce nuclear signal transduction and immunomodulatory functions in various cells when combined with ST2L. IL-33 is mainly produced by epithelial and endothelial cells. After exposure to pathogens, stress or necrosis caused by injury, IL-33 can signal the presence of tissue damage to local immune cells, thereby acting as a danger signal or alarm protein[3]. ST2L can form a heterodimer with IL-1R-related protein, which is widely present in the membranes of mast cells, T helper (Th)2 cells, dendritic cells, basophils, and macrophages[4,5]. The importance and role of the IL-33/ST2L axis have been evaluated and confirmed in several inflammatory and cardiac diseases and cancer. sST2, as a soluble receptor that is released in the circulation, acts as a decoy receptor by binding IL-33, and, thus, sST2 is involved as the counterbalance/response on IL-33/ST2L axis activation by inhibiting its signal transduction through ST2L.

The IL-33/ST2L functional pathway is involved in coxsackievirus B5 (CVB5)-induced pancreatitis[6]. Mice deficient in ST2L develop significantly more severe pancreatitis. Conversely, wild-type mice treated with recombinant IL-33 develop

study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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significantly lower viral titers, and pancreatitis is attenuated, indicating that IL-33/ST2L is involved in the pathological process of AP. SAP can cause severe disorders of multiple organ, including AP-related myocardial, kidney, or lung injury. In clinical studies, sST2 is recognized as an important marker for monitoring treatment in heart failure patients and higher sST2 is associated with worse right ventricular dysfunction and higher mean pulmonary and right atrial pressures[7-9]. sST2 is also recognized as an important prognostic marker in patients with kidney injury, where specific characteristics of sST2 enable better assessment of the risk of end-stage renal disease patients on dialysis[10]. It has been also found to be relevant in pulmonary diseases, sepsis, trauma, and gastrointestinal diseases[11-14]. sST2 can be used as a biomarker in heart, lung and kidney injury, and may also be served as biomarker in the severity of AP. However, sST2, as one of the most promising disease biomarkers, has not been studied in the development of AP.

The IL-33/ST2L pathway promotes $CD4^+$ T-cell differentiation to an atypical Th2 phenotype[15]. $CD4^+$ T cells play a major role in the pathogenesis of pancreatitis[16]. In this study, we investigated whether and how the IL-33/ST2L pathway was involved in AP in patients with different clinical severity of AP.

MATERIALS AND METHODS

Patients

A total of 123 hospitalized AP patients were recruited between January 2018 and August 2020 in the Emergency Surgery Department, Emergency Internal Medicine Department, and Comprehensive Intensive Care Unit (ICU) in the First Affiliated Hospital of Zhengzhou University. Inclusion criteria for subject enrollment included: (1) age 18–90 years; (2) clinically and radiographically confirmed AP; and (3) syndrome onset < 24 h prior to study enrollment. Exclusion criteria were: (1) Pregnancy or patients with immunodeficiency, acute and chronic hepatitis, end-stage liver and kidney disease, malignant tumors, or trauma; (2) Patients who have used hormones or immunosuppressive agents within the past 3 mo; and (3) Patients with chronic pancreatitis. This study complied with medical ethics standards and was approved by the hospital Ethics Committee. All participants provided written informed consent.

Demographic and clinical data

Demographic and clinical data were collected, including age, sex, ethnicity, body mass index (BMI), etiology, and comorbidities. According to the 2012 Atlanta Classification Criteria, patients were divided into: (1) Mild AP (MAP): No organ failure and local and systemic complications; (2) Moderately severe AP (MSAP): Local and/or systemic complications and/or transient organ failure (< 48 h); and (3) SAP: Persistent organ failure (> 48 h), with or without local complications.

Measurement of serum sST2, interferon-γ, tumor necrosis factor-α, IL-2, IL-4, IL-5, IL-13 and C-reactive protein

For serum preparation, peripheral venous blood samples (4 mL) were collected from each patient within 24 h after symptom onset and allowed to clot for 2 h at room temperature prior to centrifugation at 1500 rpm for 20 min. Serum was aliquoted and stored at -80 °C until further testing. The laboratory technicians were blinded to the baseline data and AP severity of the patients. Serum sST2 Levels were measured using a human sST2 ELISA kit (Elabscience, Wuhan, China). Serum C-reactive protein (CRP) and levels of Th1-cell-related inflammatory factors interferon (IFN)-γ, tumor necrosis factor (TNF)-α and IL-2 and Th2-cell-related inflammatory factors IL-4, IL-5 and IL-13 were measured in duplicate using an ELISA kit from Elabscience.

Statistical analysis

The Shapiro–Wilk test was used to test the normality of the data, and the Levene test was used to analyze the homogeneity of the data. Normally distributed measurement data are expressed as mean ± SE, and data were compared between groups by Student's *t* test and analysis of variance. Non-normally distributed measurement data are represented by median and interquartile range [M (IQR)], and comparison of data between groups used the Kruskal–Wallis test, and the nonparametric Mann–Whitney *U* test was used for comparison between the two groups. The *Z* value was used for the statistic. Categorical variables were described as frequencies with percentages, and

chi-square test was used to examine significant differences between categorical variables. The influence of serum sST2 levels on severity was assessed using univariate and multivariate binary logistic regression analysis, with significant confounding factors tested in the adjusted univariate analysis. Results were expressed as adjusted odds ratios (ORs) with the corresponding 95% confidence intervals (CIs). Optimal sST2 cutoff points were obtained using receiver operating characteristic (ROC) curve analysis. Additionally, IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-13 data were logarithmically transformed for analysis. The relationships between sST2, IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-13 and total length of hospital stay and ICU hospital stay were analyzed using Spearman's rank correlation. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 21.0.

RESULTS

Patient characteristics

We enrolled 123 patients: 55 (45%) with MAP, 37 (30%) with MSAP, and 31 (25%) with SAP. The etiology of AP was biliary stones (74.8%), alcohol (8.9%), laparoscopic retrograde cholangiopancreatography (1.6%), alcohol and biliary stones (8.1%) and metabolic factors (2.5%), drug-induced (0.8%), and idiopathic pancreatitis (3.3%). The etiology did not differ significantly among the groups. The incidence of acute pancreatitis with acute cholecystitis is 4.8%. Three patients had accompanying cholecystitis in the MAP group, one patient had cholecystitis in MSAP group, and two patients in the SAP group. There was no significant difference between the groups for cholecystitis.

The BMI of the healthy control group was lower than that of the MAP, MSAP, and SAP groups, and the difference was significant ($Z = -3.15$, $P = 0.002$, $Z = -3.16$, $P = 0.002$, $Z = -5.046$, $P = 0.000$). There was no significant difference in BMI between the MAP and MSAP groups ($Z = -2.27$, $P = 0.820$). The BMI of the MAP and MSAP groups was lower than that of the SAP group ($Z = -2.767$, $P = 0.006$, $Z = -2.452$, $P = 0.014$, respectively).

In the MAP group, three patients had accompanying hypertension, three were complicated by diabetes, and three were complicated by coronary heart disease. In the MSAP group, two patients had hypertension, three were complicated by diabetes, and one was complicated by coronary heart disease. Two patients in the SAP group had hypertension, five were complicated with diabetes, and one with coronary heart disease. There was no significant difference between the groups for co-morbidity, hypertension, diabetes, and coronary heart disease. None of the 123 patients were taking immunosuppressive agents. After statistical analysis, there was no significant difference in age, gender, or ethnicity between the AP groups. Patients in the AP groups had no significant difference in age, gender, or ethnicity compared with the healthy control group (Table 1).

sST2 Levels elevated in SAP patients

To identify the involvement of the IL-33/ST2L pathway in AP, we examined expression of IL-33 and sST2 in the serum after AP. The level of IL-33 was extremely low in the blood of all AP patients. Almost all samples were under the detection limit of the IL-33 ELISA, and only seven patients showed higher values. In addition, the low, almost undetectable levels of IL-33 were unlikely to be associated with worsening clinical outcomes. Nevertheless, we tested the levels of sST2 at the same time.

The sST2 content of the AP group and the healthy control group conformed to a normal distribution and uniformity of variance. We observed increased levels of sST2 in AP patients compared to healthy controls (1115.4 *vs* 211.1 pg/mL, $t = 9.355$, $P = 0.000$) (Figure 1). The sST2 value of the three AP groups did not conform to a normal distribution, and the nonparametric Kruskal–Wallis *H* test was used for comparison among multiple groups. The level of sST2 was significantly higher in the SAP group compared with MSAP and MAP groups ($Z = -3.510$, $P = 0.000$, $Z = -7.305$, $P = 0.000$, respectively). The level of sST2 in the MSAP group was significantly higher than in the MAP group ($Z = -6.489$, $P = 0.000$). The data are shown in Figure 2 and Table 2. The level of CRP in the SAP group was significantly higher than in the MSAP and MAP groups ($Z = -5.634$, $P = 0.000$, $Z = -7.150$, $P = 0.000$, respectively). The level of CRP in the MSAP group was higher than in the MAP group ($Z = -2.891$, $P = 0.000$) (Figure 3). This suggested that sST2 expression was increased in the SAP group, and that IL-33 signaling played a role in regulating the inflammatory response during AP.

Table 1 Demographic and clinical data of acute pancreatitis patients and health controls

	MAP (*n* = 55)	MSAP (*n* = 37)	SAP (*n* = 31)	Control (*n* = 42)	χ^2/t	*P* value
Age, yr	45.16 ± 1.74	50.35 ± 2.54	51.94 ± 2.42	45.60 ± 1.75	2.173	0.093
Gender					4.418	0.220
Male	34 (61.8)	21 (56.8)	14 (45.2)	18 (42.9)		
Female	21 (38.2)	16 (43.2)	17 (54.8)	24 (57.1)		
BMI, kg/m^2	25.63 (2.15)	25.81 (1.97)	26.54 (1.22)	24.61 (2.09)	28.518	0.000
Ethnicity					1.7	0.721
Han nationality	52 (94.5)	36 (97.3)	31 (100.0)	41 (97.6)		
Minority	3 (5.5)	1 (2.7)	0 (0.0)	1 (2.4)		
Etiology					9.374	0.677
Biliary stones	45 (81.8)	27 (73.0)	20 (64.5)			
Alcoholic	4 (7.3)	4 (10.8)	3 (9.7)			
ERCP	1 (1.8)	0 (0.0)	1 (3.2)			
Metabolic	1 (1.8)	1 (2.7)	1 (3.2)			
Mixed (alcohol + biliary stones)	2 (3.7)	3 (8.1)	5 (16.2)			
Drug-induced idiopathic	1 (1.8)	0 (0.0)	0 (0.0)			
Co-morbidity	1 (1.8)	2 (5.4)	1 (3.2)			
Hypertension	3 (5.5)	2 (5.4)	2 (6.5)		0.044	0.978
Diabetes	3 (5.5)	3 (8.1)	5 (16.1)		2.691	0.283
Coronary heart disease	3 (5.5)	1 (2.7)	1 (3.2)		0.515	0.860
Cholecystitis	3 (5.5)	1 (2.7)	2 (6.5)		0.582	0.747

Normally distributed continuous variables are represented by the mean ± SE, non-normally distributed continuous variables are represented by the median (interquartile range), and categorical variables are represented by the number of cases and percentages. AP: Acute pancreatitis; BMI: Body mass index; ERCP: Endoscopic retrograde cholangiopancreatography; MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis.

Table 2 Levels of Serum soluble suppression of tumorigenicity 2 and C-reactive protein in different acute pancreatitis groups

	MAP (*n* = 55)	MSAP (*n* = 37)	SAP (*n* = 31)	χ^2	*P* value
	Median (IQR)	Median (IQR)	Median (IQR)		
sST2 (pg/mL)	540 (385)	1250 (700)[b]	1890 (812)[b,d]	74.899	0.000
CRP 48 h (mg/L)	59.37 (32.92)	76.54 (31.79)[b]	120.78 (56.66)[b,d]	59.403	0.000

[b]$P < 0.001$ *vs* mild acute pancreatitis group.
[d]$P < 0.001$ *vs* moderately severe acute pancreatitis group. AP: Acute pancreatitis; MAP: Mild AP; MSAP: Moderately severe AP; SAP: Severe AP; sST2: Soluble suppression of tumorigenicity 2; CRP: C- reactive protein.

sST2 has predictive value for severity of AP

We conducted a multivariate analysis to evaluate whether the relationship of sST2 with SAP was really independent. Logistic regression showed that sST2 levels at admission [OR: 1.003 (1.001–1.006), $P = 0.000$] was a predictor of SAP. With the ROC curve, the optimal cut-off value of serum sST2 levels as an indicator for prediction of SAP was projected to be 1190 pg/mL, which yielded a sensitivity of 90.3% and specificity of 76.1%, with the area under the curve 0.889 (95%CI: 0.829–0.949; $P = 0.000$) (Figure 4).

sST2 was also a predictor of any organ failure and mortality during AP [OR: 1.006 (1.003–1.009), $P = 0.000$, OR: 1.002 (1.000–1.004), $P = 0.012$, respectively]. BMI and CRP

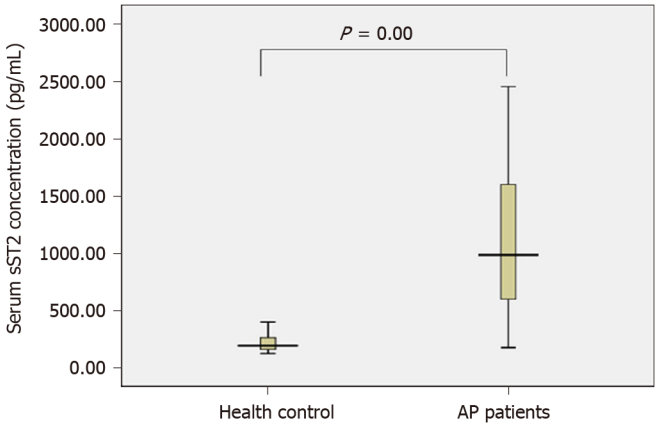

Figure 1 Serum soluble suppression of tumorigenicity 2 level in acute pancreatitis patients and health control. AP: Acute pancreatitis; sST2: Soluble suppression of tumorigenicity 2.

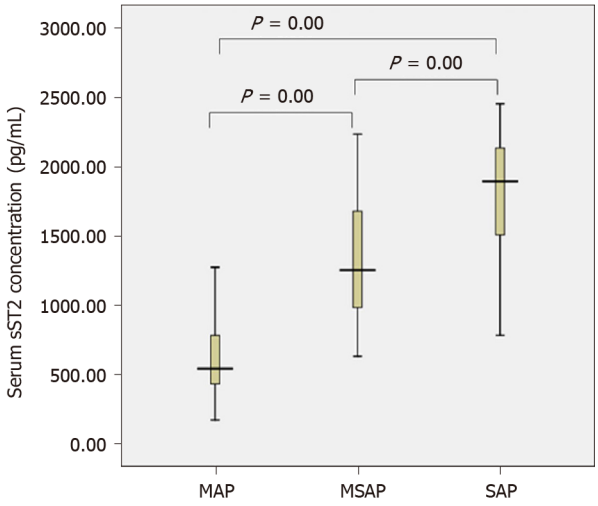

Figure 2 Serum soluble suppression of tumorigenicity 2 level in acute pancreatitis patients. AP: Acute pancreatitis; MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis; sST2: Soluble suppression of tumorigenicity 2.

were also predictors of SAP at admission [OR: 2.629 (1.075–6.429), P = 0.034, OR: 1.066 (1.031–1.101), P =0.002, respectively]. However, there was no value of BMI and CRP for predicting organ failure and mortality. sST2, CRP, age, BMI, and gender were not predictive of necrosis. The data are shown in Table 3. Furthermore, there was a significant correlation between the level of sST2 and total hospital stay and length of stay in ICU (r = 0.463, P = 0.000, r = 0.673, P = 0.000) (Table 4).

Relationship between levels of serum inflammatory factors of Th1 and Th2 cells and severity of AP

To further explore the biological mechanism of AP, we examined the production of Th1-related cytokines IFN-γ, TNF-α and IL-2 and Th2-related cytokines IL-4, IL-5 and IL-13 in the serum to clarify the role of the inflammatory process in the pathogenesis of AP. The concentration of inflammatory factors measured after logarithmic conversion was in accordance with the normal distribution. Statistical analysis showed that the serum levels of IFN-γ and TNF-α in the SAP and MSAP groups were higher than those in the MAP group, and the difference was significant. There was no significant difference in IFN-γ and TNF-α between the SAP and MSAP groups. IL-4 expression in the SAP group was significantly lower than that in the MAP and MSAP groups, but there was no significant difference between the MAP and MSAP groups. There was no significant difference in the expression of IL-2, IL-5 and IL-13 between the MAP, MSAP and SAP groups (Table 5).

Expression of Th1-cell-associated inflammatory factor IFN-γ and TNF-α was positively correlated with the length of total hospital stay and total ICU stay, while

Table 3 Multivariate binary regressions in acute pancreatitis patients showing association between soluble suppression of tumorigenicity 2 levels on admission and severity, necrosis, organ failure and mortality

Variables	OR (95%CI)	*P* value	Variables	OR (95%CI)	*P* value
MAP/MSAP and SAP			Necrosis		
Age	1.031 (0.969-1.097)	0.330	Age	1.019 (0.990-1.049)	0.209
BMI	2.629 (1.075-6.429)	0.034	BMI	0.973 (0.695-1.362)	0.847
Gender	1.096 (1.001-1.006)	0.910	Gender	0.529 (0.234-1.198)	0.127
CRP	1.066 (1.031-1.101)	0.002	CRP	1.009 (0.995-1.023)	0.209
sST2	1.003 (1.001-1.006)	0.000	sST2	1.000 (0.999-1.001)	0.837
Any organ failure			Mortality		
Age	1.017 (0.963-1.074)	0.546	Age	1.024 (0.967-1.085)	0.411
BMI	1.407 (0.788-2.511)	0.248	BMI	1.685 (0.731-3.883)	0.220
Gender	0.246 (0.050-1.202)	0.083	Gender	0.249 (0.040-1.563)	0.138
CRP	1.030 (0.991-1.071)	0.128	CRP	1.009 (0.987-1.032)	0.415
sST2	1.006 (1.003-1.009)	0.000	sST2	1.002 (1.000-1.004)	0.012

AP: Acute pancreatitis; MAP: Mild AP; MSAP: Moderately severe AP; SAP: Severe AP; BMI: Body mass index; CRP: C-reactive protein; sST2: Soluble suppression of tumorigenicity 2.

Table 4 Correlation between serum inflammatory factor and total hospital stay and intensive care unit stay

sST2/LN (Inflammatory factors, pg/mL)	Total hospital stay		Total ICU stay	
	r	*P* value	*r*	*P* value
sST2	0.463[b]	0.000	0.673[b]	0.000
LN (IFN-γ)	0.430[b]	0.000	0.700[b]	0.000
LN (TNF-α)	0.341[b]	0.000	0.652[b]	0.000
LN (IL-2)	-0.043	0.638	-0.082	0.366
LN (IL-4)	-0.483[b]	0.000	-0.440[b]	0.000
LN (IL-5)	-0.123	0.174	0.062	0.494
LN (IL-13)	-0.006	0.946	-0.139	0.126

[b]$P < 0.001$. sST2: Soluble suppression of tumorigenicity 2; ICU: Intensive care unit; IFN-γ: Interferon-γ; TNF-α: Tumor necrosis factor-α; IL: Interleukin.

expression of Th2-cell-associated inflammatory factor IL-4 was negatively correlated with the length of total hospital stay and total ICU stay (Table 4).

DISCUSSION

The results of our study showed that the levels of sST2 were significantly increased in SAP patients, and there were significant increases in Th1-related cytokines IFN-γ and TNF-α, and a decrease in Th2-related cytokine IL-4. This suggested an insufficient IL-33-driven Th2-type inflammatory response was involved in the pathophysiological process of AP. sST2 is a secreted form of the ST2 receptor and acts as a decoy receptor for IL-33, thus inactivating the functions of IL-33. sST2 expression increases in response to proinflammatory cytokines[17]. Our data were in line with a previous study showing elevated levels of sST2 in AP[18]. Importantly, in our study the levels of sST2 predicted the total hospital stay and length of stay in the ICU, as well as predicted organ failure and mortality during AP. These data support the findings of our study showing that SAP patients more frequently had higher serum levels of sST2 compared with MAP and MSAP patients, and that the early elevated levels of sST2

Table 5 Serum inflammatory factor levels in patients with acute pancreatitis

LN (inflammatory factors pg/mL)	MAP, mean ± SE	MSAP, mean ± SE	SAP, mean ± SE	*F*	*P* value
LN (IFN-γ)	1.98 ± 0.07	2.57 ± 0.08[b]	3.08 ± 0.07[b]	53.393	0.000
LN (TNF-α)	1.63 ± 0.04	2.07 ± 0.05[b]	2.33 ± 0.05[b,d]	45.369	0.000
LN (IL-2)	1.06 ± 0.05	1.05 ± 0.06	0.99 ± 0.05	0.362	0.697
LN (IL-4)	1.02 ± 0.03	0.99 ± 0.04	0.64 ± 0.03[b,d]	23.195	0.000
LN (IL-5)	0.10 ± 0.05	0.10 ± 0.07	0.13 ± 0.08	0.043	0.958
LN (IL-13)	0.76 ± 0.03	0.68 ± 0.06	0.63 ± 0.07	1.703	0.187

Due to the logarithmic transformation of the data, some data are displayed as negative values. The data are expressed as mean ± SE.
[b]$P < 0.001$ *vs* mild acute pancreatitis group.
[d]$P < 0.001$ *vs* moderately severe acute pancreatitis group. AP: Acute pancreatitis; MAP: Mild AP; MSAP: Moderately severe AP; SAP: Severe AP; IFN-γ: Interferon-γ; TNF-α: Tumor necrosis factor-α; IL: Interleukin.

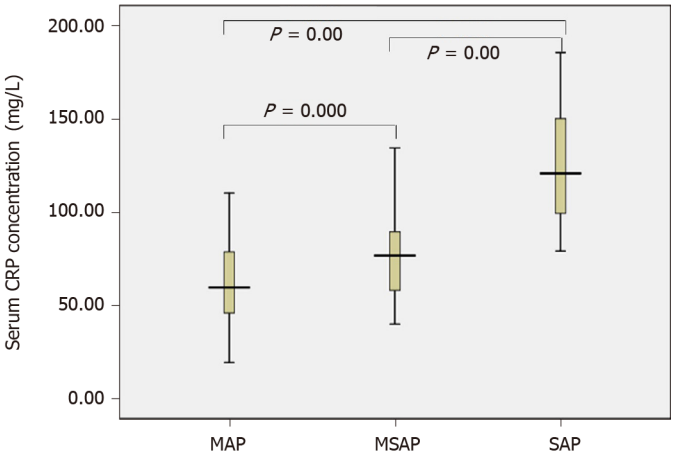

Figure 3 Serum C-reactive protein level in acute pancreatitis patients. AP: Acute pancreatitis; CRP: C-reactive protein; MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis.

correlated with worsened outcome of AP. Thus, the normal function of IL-33 may be inhibited in AP.

SAP can cause severe systemic inflammation and multiple organ dysfunction. It is reported that endogenous danger signals, such as tissue damage or necrosis, and exogenous danger signals, such as microbial pathogens and endotoxins, can enhance the production of sST2 and stimulate the secretion of inflammatory cytokines, thereby weakening the immune response of organs exposed to the danger signals and leading to adverse outcomes[19]. It has been suggested that serum levels of sST2 are significantly increased in inflammatory diseases and serve as a prognostic biomarker of multiple organ dysfunction syndrome (MODS), cardiovascular events, heart failure, acute hypoxemic respiratory failure, and progression of kidney failure. A study based on cardiopulmonary resuscitation showed that plasma levels of sST2 were associated with higher risk of MODS and early death[20]. A role of sST2 in acute hypoxemic respiratory failure indicates that using sST2 concentrations to guide ventilator management may more accurately reflect underlying lung injury and outperform traditional measures of readiness for ventilator liberation[21]. Higher sST2 Levels predict mortality in severe sepsis[12]. These findings agree with our results showing that sST2 was a predictor of any organ failure and mortality during AP.

The IL-33/ST2L pathway has been shown to mediate the modulation of inflammation in different diseases by promoting Th2 response and inhibiting Th1 response [22,23]. Mice that lack IL-33 signaling develop significantly more severe pancreatitis [6]. Based on the regulatory effect of the IL-33/ST2 signaling pathway on Th1 and Th2 cells, we further measured the function of Th1 and Th2 cells in patients with AP. An important finding in our study was the relationship between Th1- and Th2-related cytokines and the severity of AP. Cytokine levels in Th1 cells in patients with SAP were significantly higher than those in patients with MAP and MSAP, and cytokines in

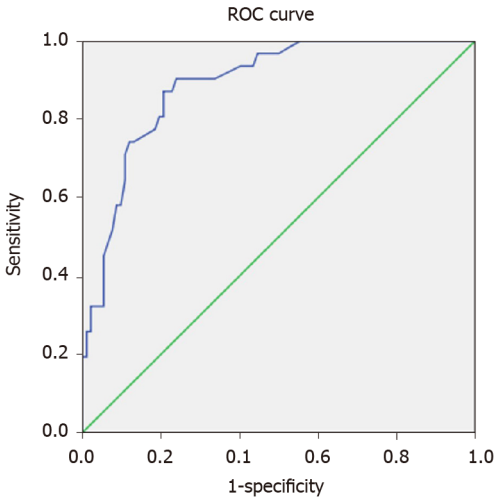

Figure 4 Receiver operating characteristic curve demonstrating sensitivity as a function of 1 – specificity for predicting severe acute pancreatitis based on serum soluble suppression of tumorigenicity 2 levels. ROC: Receiver operating characteristic.

Th2 cells in patients with MAP and MSAP were significantly increased compared with patients with SAP. In our study, we observed that both sST2 and the Th1 cytokines IFN-γ and TNF-α were increased and the Th2 cytokine IL-4 was decreased in SAP. We speculate that increased sST2 in SAP might cause increases in IFN-γ and TNF-α, and decreases in IL-4 by antagonizing the IL-33/ST2 signaling pathway, whereas this inference still needs to be verified by further animal research in the future.

The serum levels of Th1-related cytokines IFN-γ and TNF-α in the SAP group were higher than those in the MAP and MSAP groups, and expression of Th2-related cytokine IL-4 in the SAP group was significantly lower than in the MAP and MSAP groups. The expression of Th1-related inflammatory factors IFN-γ and TNF-α was positively correlated with the length of total hospital stay and total ICU stay, and Th2-cell-associated inflammatory factor IL-4 expression was negatively correlated with the length of hospital and ICU stays. The results suggested that Th1-cell-related immune response was an important factor that aggravated the development of AP, and Th2 cells might have a protective role in the development of AP. Our findings indicate that Th1 and Th2 cells were involved in cytokine-dependent pathways in severity of AP. The results proved that a change in immune response played an important role in the pathogenesis of AP.

In this study, we found an immune imbalance in patients with SAP. Th1-cell-related cytokines IFN-γ and TNF-α in the serum of patients with SAP were higher than those in patients with MSAP. We also found that the expression levels of Th1-cell-associated inflammatory factors IFN-γ and TNF-α were positively correlated with the length of total hospital stay and total ICU stay. This finding is consistent with the latest research showing that inflammatory factors play a consistent role in the pathogenesis of AP[24-26]. IFN-γ and TNF-α could stimulate B cells to produce antibodies, activate macrophages and $CD8^+$ T cells, promote cell-mediated immunity and cytotoxic T cell responses[27], and could induce Th1 cell differentiation and inhibit Th2 cell proliferation. Previous research findings and the present study suggest that Th1 cells are involved in the pathogenesis of AP, especially in the development of SAP. Th1 cells exacerbate the pathophysiological process of AP by releasing Th1-related inflammatory factors.

In humans, serum concentrations of sST2 are increased in several diseases, such as heart disease, pulmonary disease, burn injury, and graft-versus-host disease[28-33]. Few studies have reported the influence of the IL-33/ST2 signaling pathway on the development of AP[6,34-36] and less attention has been paid to Th1 and Th2 cells in the pathogenesis of AP[24]. In this study, we found that the expression of the Th2-related cytokine IL-4 in SAP was significantly lower than that in MAP and MSAP. Expression of the Th2-related inflammatory factor IL-4 was negatively correlated with the length of hospital and ICU hospital stays, suggesting that Th2 cells are involved in the pathogenesis of AP with the tendency to improve symptoms of acute pancreatitis. Collectively, Th2 cells might have a protective effect in the development of AP, tending to delay its development.

Our research had some limitations. Firstly, the main limitation was the small sample size, which may have reduced the statistical power, the accuracy, and effectiveness of identifying true positive results. In future, the results need to be confirmed with larger sample sizes. Secondly, this study was a single-center study, which might have resulted in selection bias. Thirdly, in cases of pancreatitis combined with cholecystitis, cholecystitis might affect the results of the study. Fourthly, we did not examine the numbers of Th1 and Th2 cells. Finally, establishing the initial relationship between AP and elevated sST2 concentrations in our study is novel and useful, but it is necessary to determine the long-term predictive value of sST2 for the prognosis of AP in subsequent longitudinal studies.

CONCLUSION

In conclusion, sST2 may be used as a novel inflammatory marker in predicting AP severity and may regulate the function and differentiation of IL-33/ST2-mediated Th1 and Th2 Lymphocytes in AP homeostasis.

ARTICLE HIGHLIGHTS

Research background

The clinical severity of acute pancreatitis (AP) is related to its prognosis. Excessive activation of inflammatory cells and their cytokines is one of the main mechanisms of pathogenesis of AP. Research on changes in inflammatory mediators in patients with different clinical severity of AP is important clinically. The interleukin (IL)-33/ST2L functional pathway is involved in the pathological process of AP. Soluble suppression of tumorigenicity 2 (sST2) is a secreted form of the ST2 receptor and acts as a decoy receptor for IL-33. In this study, we investigated whether the sST2 could serve as a novel inflammatory marker predicting the severity of acute pancreatitis.

Research motivation

In this study, the authors focused on the function of sST2 in predicting the severity of AP. The key issue to be solved is the association between IL-33/ST2L pathway and AP. The significance of solving these problems may constitute a new therapeutic target for regulating immune activation during the AP inflammatory storm.

Research objectives

The objective of this study was to investigate the role of sST2 in AP.

Research methods

The authors assessed the association between sST2 and severity of AP in 123 patients enrolled in this study. The serum levels of sST2, C-reactive protein (CRP) and Th1- and Th2-related cytokines interferon (IFN)-γ, tumor necrosis factor (TNF)-α ,IL-2, IL-4, IL-5 and IL-13 were measured by highly sensitive ELISA and the severity of AP patients was evaluated by the 2012 Atlanta Classification Criteria.

Research results

The serum sST2 Level was significantly increased in AP patients and significantly elevated in severe acute pancreatitis (SAP) patients compared to moderate severe acute pancreatitis and mild acute pancreatitis patients. The cutoff point of sST2 at 1190pg/mL was associated with SAP.

Research conclusions

This study suggests that sST2 may be used as a novel inflammatory marker in predicting the severity of acute pancreatitis and that sST2 might regulate the function and differentiation of IL-33/ST2L mediated Th1 and Th2 lymphocytes in the homeostasis of acute pancreatitis.

Research perspectives

It is necessary to determine the long-term predictive value of sST2 for the prognosis of AP in subsequent longitudinal studies. Additionally, the role of IL-33/ST2L in acute pancreatitis needs to be further verified in both *in vivo* and *in vitro* experiments.

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CASE REPORT

Monomorphic epitheliotropic intestinal T-cell lymphoma presenting as melena with long-term survival: A case report and review of literature

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Author contributions: Ozaka S cared for the patient, performed endoscopic treatment, and wrote and corrected the manuscript; Inoue K, Okajima T, Tasaki T, Ariki S, and Ono H cared for the patient and reviewed and corrected the manuscript; Daa T interpreted the pathological findings and contributed to manuscript drafting; Ando T performed the chemotherapy and contributed to manuscript drafting; Murakami K provided oversight for the manuscript and revised it for important intellectual content; all authors issued final approval for the version to be submitted.

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Abstract

BACKGROUND

Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) is a rare primary intestinal T-cell lymphoma, previously known as enteropathy-associated T-cell lymphoma type II. MEITL is an aggressive T-cell lymphoma with a poor prognosis and high mortality rate. The known major complications of MEITL are intestinal perforation and obstruction. Here, we present a case of MEITL that was diagnosed following upper gastrointestinal bleeding from an ulcerative duodenal lesion, with recurrence-free survival for 5 years.

CASE SUMMARY

A 68-year-old female was admitted to our hospital with melena and mild anemia. An urgent esophagogastroduodenoscopy (EGD) revealed bleeding from an ulcerative lesion in the transverse part of the duodenum, for which hemostatic treatment was performed. MEITL was diagnosed following repeated biopsies of the lesion, and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy was administered. She achieved complete remission after eight full cycles of CHOP therapy. At the last follow-up examination, EGD revealed a scarred ulcer and ^{18}Fluorodeoxyglucose (^{18}FDG) positron emission tomography/computed tomography showed no abnormal FDG accumulation. The patient has been in complete remission for 68 mo after initial diagnosis.

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CONCLUSION
To rule out MEITL, it is important to carefully perform histological examination when bleeding from a duodenal ulcer is observed.

Key Words: Monomorphic epitheliotropic intestinal T-cell lymphoma; Enteropathy-associated T-cell lymphoma type II; Gastrointestinal bleeding; Intestinal lymphoma; Duodenal ulcer; Case report

Core Tip: Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) is a primary intestinal T-cell lymphoma with the known major complications of intestinal perforation and obstruction. We experienced a case of MEITL that was diagnosed after gastrointestinal bleeding from an ulcerative duodenal lesion, who survived for a long time after treatment. MEITL can present as gastrointestinal bleeding. In addition, although MEITL has a poor prognosis, patients with MEITL might survive for a long time if effective treatment is administered at an early stage. Therefore, it is important to perform thorough histological examination for the early diagnosis of MEITL in cases with bleeding duodenal ulcers.

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INTRODUCTION

Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) is a rare primary intestinal T-cell lymphoma newly defined by the 2016 revision of the World Health Organization, that was previously known as enteropathy-associated T-cell lymphoma (EATL) type II[1]. It arises from intestinal intraepithelial T lymphocytes and tends to behave aggressively[2]. EATL was originally categorized into two major groups, EATL type I and EATL type II. EATL type I is now simply classified as EATL, and it is strongly associated with celiac disease and occurs in Western countries. In this type, tumor cells are positive for CD3 and CD30 on immunohistochemistry staining, but negative for CD8 and CD56. On the other hand, EATL type II has been renamed MEITL, shows no definite association with celiac disease, and occurs in an Asian population. In this type of malignancy, tumor cells are positive for CD3, CD8 and CD56, but negative for CD30[3,4]. MEITL is most frequently found in the jejunum and ileum[5,6], and often presents with gastrointestinal perforation or obstruction[2]. MEITL is known to have a very poor prognosis due to treatment resistance and perforation or obstruction of the bowel at diagnosis or during the course of treatment [7]. Herein, we present a case of MEITL that was diagnosed following upper gastrointestinal bleeding from an ulcerative duodenal lesion, and who enjoyed recurrence-free survival for 5 years following treatment.

CASE PRESENTATION

Chief complaints

A 68-year-old female presented to the emergency department with melena.

History of present illness

The melena started a day before she consulted us. She had no past history of chronic abdominal symptoms suggesting the presence of celiac disease.

History of past illness
The patient had a history of hypertension and hyperlipidemia.

Personal and family history
She had no significant personal and family history.

Physical examination
Her body temperature was 36.4 °C, blood pressure was 128/85 mmHg, and heart rate was 98 bpm with sinus rhythm. She had mild abdominal tenderness, but there was no obvious hepatosplenomegaly or lymphadenopathy. Digital rectal examination revealed melena.

Laboratory examinations
Laboratory tests indicated a hemoglobin level of 11.3 g/dL, blood urea nitrogen level of 26.0 mg/dL, and creatinine level of 1.64 mg/dL. Her serum lactate dehydrogenase level was 232 U/L (106- 211 U/L) and soluble interleukin-2 receptor level was 213 U/mL (145- 519 U/mL) (Table 1).

Imaging examinations
Urgent esophagogastroduodenoscopy (EGD) showed an ulcerative lesion with fresh blood clots in the transverse part of the duodenum (Figure 1A). Based on the location and shape of the lesion, we suspected not only a peptic ulcer, but also an ulcer caused by vascular malformation or malignancy. Therefore, we decided to interrupt the endoscopy and perform contrast-enhanced computed tomography (CT) scan, which showed slight localized contrast enhancement on the wall of the transverse part of the duodenum in the early phase of contrast injection (Figure 1B). No vascular lesions were observed, and there was no extravasation of contrast agent in the delayed phase. EGD was immediately resumed again for further observation of the lesion. When we removed the blood clots, a protruding vessel was seen at the base of the ulcer, which was coagulated using hemostatic forceps (Coagrasper; Olympus Corp., Tokyo, Japan) (Figure 1C).

Further diagnostic work-up
Following this hemostatic treatment, the patient was discharged from the hospital without re-bleeding. The lesion that caused the bleeding was suspected to be a malignant tumor of the duodenum based on its location. EGDs, including forceps biopsies from the ulcerative lesion, were performed three times after the initial hemostatic treatment. While the first and second biopsies revealed no malignancy, the third biopsy showed findings suggestive of malignant lymphoma. On pathological evaluation, diffuse proliferation of atypical medium-sized lymphoid cells was seen in the entire mucosa, along with a few intraepithelial lesions (Figure 2A and B). No necrosis was observed. Immunohistochemical analysis revealed that the cells were positive for CD3 and CD56, and negative for CD4, CD5, CD8, CD20 and EBER (Figure 2C-E).

At this point, MEITL was suspected, and examinations for systemic lesions were subsequently performed. No abnormal lymphocytes were found on iliac bone marrow examination. ^{18}Fluorodeoxyglucose (^{18}FDG) positron emission tomography/CT (^{18}FDG-PET/CT) showed nodular FDG accumulation in the wall of the transverse part of the duodenum, consistent with the findings of contrast-enhanced CT (Figure 3). There was no abnormal FDG accumulation in the systemic lymph nodes or other parts of the gastrointestinal tract. The results of total colonoscopy and random biopsies of the gastrointestinal tract were unremarkable.

MULTIDISCIPLINARY EXPERT CONSULTATION

Ayako Gamachi, MD, PhD, Chief of the Department of Diagnostic Pathology, Almeida Memorial Hospital; and Tsutomu Daa, MD, PhD, Professor, Department of Diagnostic Pathology, Oita University
Pathological evaluations of the duodenal biopsy samples were performed by expert pathologists. The results of immunostaining of the duodenal biopsy specimen were consistent with MEITL.

Table 1 Laboratory data on admission

Items	Data	Reference	Items	Data	Reference	Items	Data	Reference
WBC	6720 /μL	3500-9100	TP	7.5 g/dL	6.4-8.4	γ-GTP	12 U/L	16-73
RBC	393 × 10^4 /μL	376-500	Alb	4.5 g/dL	3.6-5.2	Na	140 mEq/L	135-146
Hb	11.3 g/dL	11.3-15.2	BUN	26.0 mg/dL	6-22	Cl	107 mEq/L	96-108
Hct	34.2%	33.4-44.9	Cre	0.81 mg/dL	0.40-0.80	K	4.4 mEq/L	3.5-5.0
MCV	87.0 fL	79-100	T-bil	0.5 mg/dL	0.2-1.2	CRP	0.08 mg/dL	0-0.23
MCHC	33.0%	13.0-36.9	CK	140 U/L	43-165	sIL-2R	213 U/mL	145-519
Plt	21.4 × 10^4 /μL	13.0-16.9	AST	31 U/L	13-33	HBs Ag	(-)	(-)
PT	68%	70-130	ALT	12 U/L	6-30	HCV Ab	(-)	(-)
APTT	38.5 s	24-39	LDH	232 U/L	106-211	HTLV-1 Ab	(-)	(-)
			ALP	220 U/L	100-340			

WBC: White blood cell; RBC: Red blood cell; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; Plt: Platelets; PT: Prothrombin; APTT: Activated partial thromboplastin time; TP: Total protein; Alb: Albumin; BUN: Blood urea nitrogen; Cre: Creatinine; T-bil: Total bilirubin; CK: Creatine Kinase; AST: Aspartate transaminase; ALT: Alanine transferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase; γ-GTP: γ-glutamyl transpeptidase; Na: Sodium; Cl: Chloride; K: Potassium; CRP: C-reactive protein; sIL-2R: Soluble interleukin-2 receptor; HBs Ag: HBs antigen; HCV Ab: HCV antibody; HTLV-1 Ab: HTLV-1 antibody.

FINAL DIAGNOSIS

Based on the clinical course, features of the tumor and imaging evaluations, we diagnosed MEITL (previously EATL type II) confined to the duodenum.

TREATMENT

Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy was administered without a dosage reduction.

OUTCOME AND FOLLOW-UP

EGD performed after three cycles of CHOP therapy clearly showed that the ulcerative lesion in the duodenum had reduced in size (Figure 4A), and no atypical lymphoid cells were detected in biopsy specimens taken from the lesion. The patient tolerated the chemotherapy well and received eight full cycles of CHOP chemotherapy. ^{18}FDG-PET/CT after the final cycle revealed no abnormal FDG accumulation in the transverse part of the duodenum. Therefore, we concluded that the patient had achieved complete remission (CR). EGD at the last follow-up examination revealed a scarred ulcer (Figure 4B), and ^{18}FDG-PET/CT showed no abnormal FDG accumulation (Figure 5). The patient has been in CR for 68 mo after the initial diagnosis.

DISCUSSION

Our experience in this case highlights two important clinical points. First, MEITL can present as gastrointestinal bleeding, and not just as intestinal perforation and obstruction. Second, patients with MEITL can survive for a long time if effective treatment is administered early.

MEITL is a primary intestinal T-cell lymphoma previously known as EATL type II [1]. It is a rare intestinal tumor, accounting for 1.7% of all malignant lymphomas[8] and less than 10%-16% of gastrointestinal lymphomas[9]. MEITL differs from EATL (previously EATL type I) in that it predominantly affects Asian populations and is not associated with celiac disease[10]. Histologically, it consists of monomorphic small- to

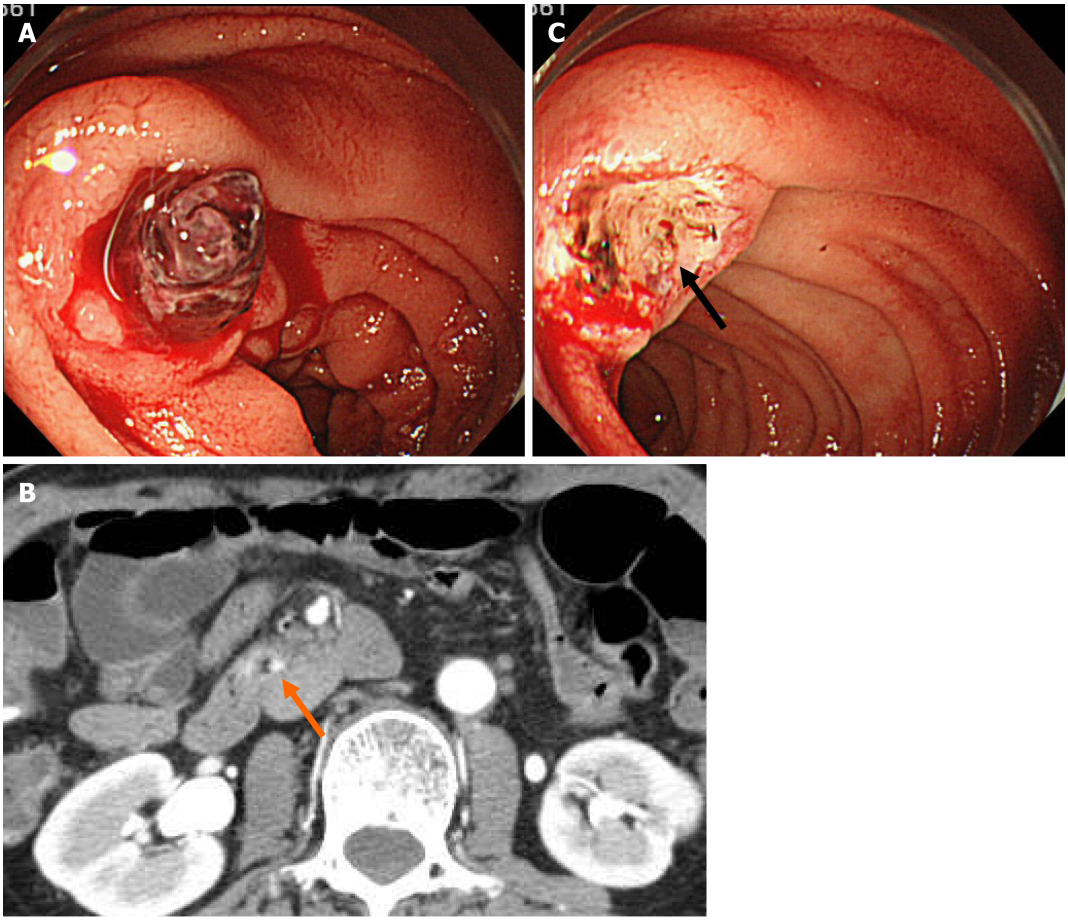

Figure 1 Endoscopic images and abdominal contrast-enhanced computed tomography scan images on initial examination. A: Esophagogastroduodenoscopy showed an ulcerative lesion with fresh blood clots in the transverse part of the duodenum; B: Contrast-enhanced computed tomography scan showed a slight localized contrast effect on the wall of the transverse part of the duodenum (arrow); C: When the blood clots were removed, a protruding vessel was observed at the base of the ulcer (arrow).

medium-sized cells that usually express CD3, CD8 and CD56, but not CD30[3,4]. Positive rates for CD8 and CD56 were reported to be 63-79% and 73-95%, respectively [8,11]. The present case had no past history of chronic abdominal symptoms suggestive of the presence of celiac disease. Histopathological examination revealed an infiltration of atypical medium-sized lymphoid cells in the entire mucosa, along with intraepithelial lesions, and immunostaining was positive for CD3 and CD56 and negative for CD5 and CD8, leading to the diagnosis of MEITL.

Our MEITL patient presented with gastrointestinal bleeding and not intestinal perforation or obstruction. More than 50% of MEITL cases are diagnosed as a result of intestinal perforation or obstruction, and emergency surgery is required in about 40% of them[9,12]. In contrast, MEITL is less frequently detected as a result of gastrointestinal bleeding. A search of the PubMed database found four case reports of MEITL or EATL that presented with gastrointestinal bleeding at the first diagnosis[13-16] (Table 2). There was a male preponderance (80%) among the five cases, and all the patients were aged above 60 years including our patient. Two cases had lesions in the jejunum, two in the stomach, and only our case had a lesion in the duodenum. Most of them showed ulcerative lesions. Only one case had a perforated lesion. Four of the five patients were diagnosed with MEITL and one with EATL. Chemotherapy was administered in four cases, and surgery was performed in two cases. To the best of our knowledge, this is the first case of MEITL that was treated with an endoscopic procedure for upper gastrointestinal bleeding. In terms of the outcomes, two cases, including our case, survived for more than 24 mo, and the two cases with gastric involvement died at 13 mo. Although it was previously reported that the median overall survival of all types of MEITL is 7 mo[11,17], the cases diagnosed after gastrointestinal bleeding had a relatively good prognosis.

In a histopathological study of a case of MEITL with perforation, tumor cells positive for TIA-1 or Granzyme B, a cytotoxic molecule, were reported to have markedly infiltrated all layers of the intestinal wall[18,19]. This suggests that MEITL

Table 2 Case reports of enteropathy-associated T-cell lymphoma and monomorphic epitheliotropic intestinal T-cell lymphoma presenting gastrointestinal bleeding

Case	Ref.	Age/Sex	Symptom	Location	Macroscopic finding	Perforation	Diagnosis	Treatment	Outcome
1	[13]	76/M	Gastrointestinal bleeding	Jejunum	Ulcer	Yes	EATL	Operation → chemotherapy	24 mo/alive
2	[14]	77/M	Positive for fecal occult bleeding	Jejunum	Ulcer, stenosis	No	MEITL	Operation	NA
3	[15]	68/M	Melena	Stomach	Ulcer (type 3 tumor)	No	MEITL	Chemotherapy	13 mo/death
4	[16]	65/M	Gastrointestinal bleeding	Stomach	NA	No	MEITL	Chemotherapy	13 mo/death
5	Our case	68/F	Melena	Duodenum	Ulcer	No	MEITL	Chemotherapy	66 mo/alive

NA: Not available; MEITL: Monomorphic epitheliotropic intestinal T-cell lymphoma; EATL: Enteropathy-associated T-cell lymphoma.

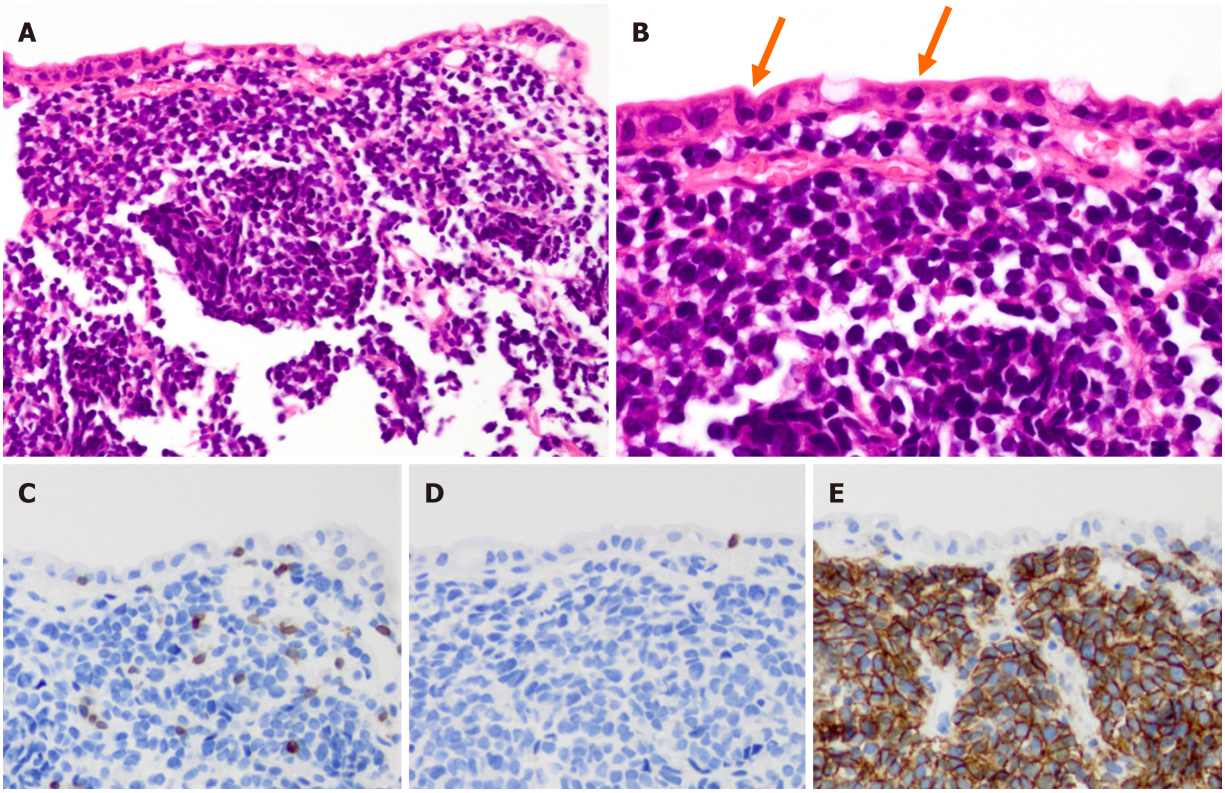

Figure 2 Histological and immunohistochemical analyses. A: Hematoxylin-eosin staining showed infiltration of medium-size atypical lymphoid cells in the mucosa; B: Lymphoid cell infiltration was also observed in the epithelium (arrow); C: Lymphoid cells expressed CD3; D: The lymphoid cells were CD8 negative; E: Lymphoid cells were CD56 positive. Magnification 200× (A, C-E) and 400× (B).

easily penetrates the gastrointestinal tract by growing destructively through all its layers, resulting in a relatively lower incidence of gastrointestinal bleeding.

Our case suggests that patients with MEITL might survive for a long time if effective treatment is administered at an early stage. MEITL is an aggressive T-cell lymphoma with a very poor prognosis and high mortality rate[20]. The median overall survival was previously reported as 7 mo[11,17], and the 5-year survival rate was reported as 20%, with a 5-year failure-free survival rate of 4%[8]. Surgery, chemotherapy and radiotherapy are all used to treat MEITL, although these treatments have shown poor overall outcomes[21]. Although several studies have demonstrated the benefit of autologous stem cell transplantation[22-25], there is no standardized treatment strategy. Moreover, MEITL often requires emergency surgery due to

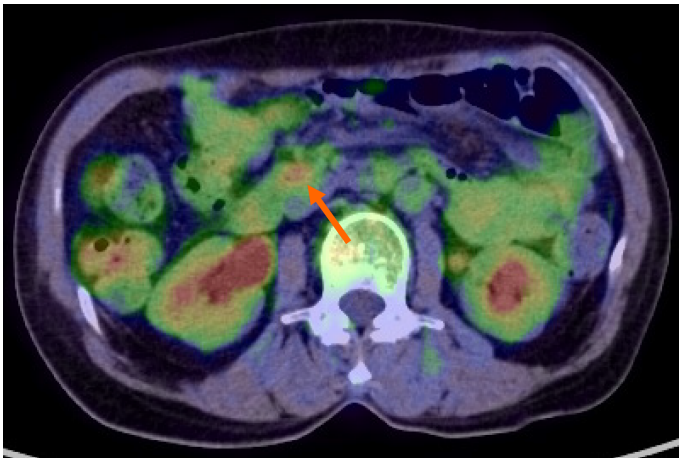

Figure 3 Pre-treatment ^{18}Fluorodeoxyglucose positron emission tomography/computed tomography images. ^{18}Fluorodeoxyglucose positron emission tomography/computed tomography showed abnormal nodular accumulation in the wall of the transverse part of the duodenum (arrow).

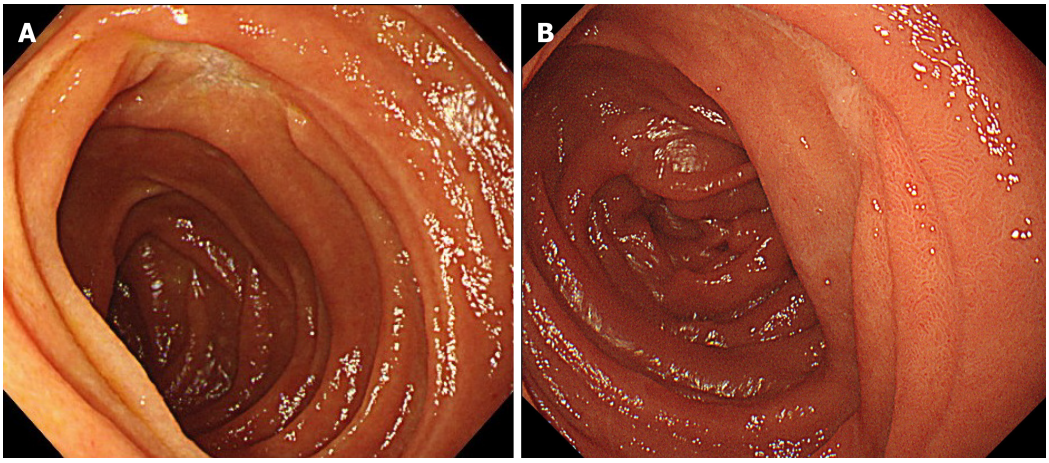

Figure 4 Follow-up endoscopic examinations. A: Esophagogastroduodenoscopy (EGD) after three cycles of cyclophosphamide, doxorubicin, vincristine, and prednisone therapy revealed that the ulcerative lesion had clearly reduced in size; B: EGD performed 60 mo after complete remission showed a scarred ulcer.

intestinal perforation or obstruction, and the lesion has usually spread before it is diagnosed[26]. Unfortunately, most patients are unable to receive effective chemotherapy due to their poor performance status and severe malnutrition at the time of diagnosis[27]. On the other hand, in a study of 26 patients with MEITL, the prognosis of non-perforated cases was significantly better than that of perforated cases, suggesting that early treatment before perforation might improve the prognosis of MEITL[28]. The present case has shown the most favorable prognosis to date, because we were able to diagnose MEITL at an early stage due to her presentation with upper gastrointestinal bleeding, and hence performed chemotherapy while the patient was in good general condition. In the previously reported cases shown in Table 2, as well, the prognosis tended to be relatively good in cases that were diagnosed after hemorrhage. This is probably because the lesion was perforated in only one case and the other cases were able to start chemotherapy before perforation. This suggests that gastrointestinal bleeding is an important sign that can lead to early diagnosis and treatment of MEITL.

So far, only one other case of MEITL with a failure-free survival for more than 5 years has been reported[29]. This case was diagnosed early by the finding of only chorionic changes (white villi) without a mass or ulcer, and CHOP therapy was started before perforation. Ishibashi *et al*[30] reported that edematous and granular mucosae with or without villous atrophy are characteristic findings of prodromal lesions of MEITL in the small intestine or duodenum. Even in the absence of obvious masses or ulcers, it is important not to overlook such micro-changes in the gastrointestinal mucosa to facilitate the early diagnosis of MEITL.

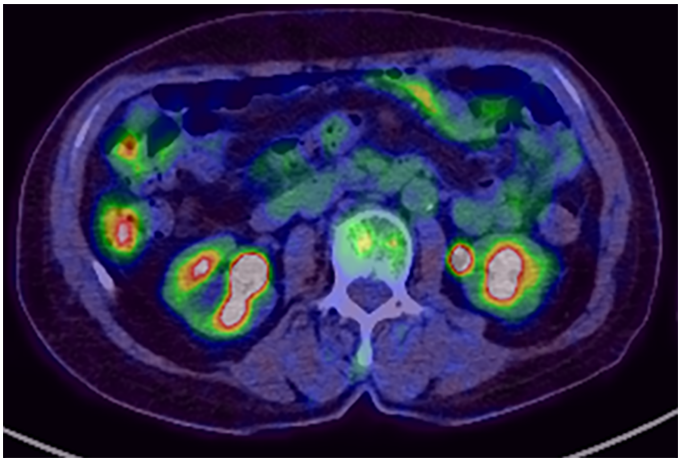

Figure 5 Follow-up ^{18}Fluorodeoxyglucose positron emission tomography/computed tomography at 60 mo after complete remission. ^{8}Fluorodeoxyglucose positron emission tomography/computed tomography showed no abnormal fluorodeoxyglucose accumulation.

Another point to note is that the diagnostic ratio of intestinal T-cell lymphoma (ITCL) by endoscopy, including tissue biopsy, is low. Sun *et al*[31] reported that out of 34 ITCL patients who underwent endoscopy, eight patients (23.5%) were definitively diagnosed with ITCL by histology. Daum *et al*[32] also reported that only 21% of intestinal non-Hodgkin lymphomas were diagnosed endoscopically. In the present case as well, a total of three tissue biopsies were required before the final diagnosis of MEITL. The following are some of the reasons for this: (1) Tissue specimens from endoscopic biopsy are usually not sufficiently large to allow correct diagnosis; (2) ITCL is known to be primarily located in the submucosa and smooth muscle, and it is difficult to detect the lesion from biopsy specimens through the mucosal layer; and (3) The disease is easy to overlook because of its rarity. Hence, a tissue biopsy of ulcerative gastrointestinal lesions should be performed carefully from the base of the ulcer, while considering the possibility of malignant lymphoma.

CONCLUSION

We report a case of MEITL diagnosed after upper gastrointestinal bleeding from an ulcerative duodenal lesion, with recurrence-free survival for 5 years after chemotherapy. MEITL can present as gastrointestinal bleeding, and not only as intestinal perforation and obstruction. In addition, although MEITL is an aggressive T-cell lymphoma with a poor prognosis, patients with MEITL can survive for a long time if effective treatment is administered early. Therefore, it is important to carefully perform histological examination to rule out MEITL when bleeding from a duodenal ulcer is observed.

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CORRECTION

Correction to "Effect of probiotic Lactobacillus plantarum Dad-13 powder consumption on the gut microbiota and intestinal health of overweight adults". *World J Gastroenterol* 2021; 27(1): 107-128 [PMID: 33505154 DOI: 10.3748/wjg.v27.i1.107]

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Abstract

We corrected the wrong name of the primers used in this study. The correct name should be Bakt_341F (5′-CGCTCTTCCGATCTCTGCCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-TGCTCTTCCGATCTGACGACTACHVGGGTATCT-AATCC-3′).

Key Words: Correction; Primers; Wrong name; Error; Suggestions

Core Tip: This revision includes correction of the name of primer used in the study and added content based on reviewer suggestion, such as title revision, commentary on female group's weight loss, and explanations regarding the reduced average daily energy intake in the last month of ingestion period.

Citation: Rahayu ES. Correction to "Effect of probiotic Lactobacillus plantarum Dad-13 powder consumption on the gut microbiota and intestinal health of overweight adults". *World J Gastroenterol* 2021; 27(1): 107-128 [PMID: 33505154 DOI: 10.3748/wjg.v27.i1.107]. *World J Gastroenterol* 2021; 27(38): 6511-6512

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TO THE EDITOR

Correction to: Rahayu ES, Mariyatun M, Putri Manurung NE, Hasan PN, Therdtatha P, Mishima R, Komalasari H, Mahfuzah NA, Pamungkaningtyas FH, Yoga WK, Nurfiana DA, Liwan SY, Juffrie M, Nugroho AE, Utami T. "Effect of probiotic Lactobacillus plantarum Dad-13 powder consumption on the gut microbiota and intestinal health of overweight adults". *World J Gastroenterol* 2021; 27(1): 107-128 [PMID: 33505154 DOI: 10.3748/wjg.v27.i1.107].

The name of the primers used in this study[1], Bakt_341F (5′-CGCTCTTCCGATCTCTGCCTACGGGGGGGCWGCAG-355) GGCTATICCCACCATTCCCCATTCCACCACCACCACCACCACCACCACCACCAUTAA was spelled incorrectly. The correct name should be Bakt_341F (5′-CGCTCTTCCGATCTCTGCCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-TGCTCTTCCGATCTGACGACTACHVGGGTATCTAATCC-3′). We apologize for this error.

The title of this article[1] was "Effect of probiotic Lactobacillus plantarum Dad-13 powder consumption on the gut microbiota and intestinal health of overweight adults". As the focus of the manuscript was on Lombok, Indonesia, the title should be "Effect of probiotic Lactobacillus plantarum Dad-13 powder consumption on the gut microbiota and intestinal health of Indonesian overweight adults".

Regarding the weight loss in females, it may be caused by probiotics influence. This finding also found in the study conducted by Sanchez *et al*[2] that the *Lactobacillus rhamnosus* CGMCC1.3724 (LPR) supplementation was significantly reduce weight on female subjects and had no effect on male subjects.

About the reduced average daily energy intake in the last month of ingestion period, we did not know the exact cause of this case. When the study was carried out, we let the subjects ate as their usual diet. There was no emphasis on reducing the daily intake from us.

We express our gratitude toward reviewer for these suggestions.

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LETTER TO THE EDITOR

Preservation of the superior rectal artery in laparoscopic colectomy for slow transit constipation: Is it really associated with better outcomes?

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Abstract

Few patients with slow-transit constipation refractory to conservative treatment can benefit with a subtotal colectomy with ileorectal anastomosis with the preservation of the superior rectal artery. In this letter to the editor some important issues were discussed. First, the study did not include a comparison group. Second, they did not present the functional results in the short or long term related to the bowel function of these patients after surgery. Finally, the authors showed that this surgical procedure was safe, and no cases of leakage were found.

Key Words: Laparoscopy; Colorectal surgery; Constipation; Colectomy

Core Tip: Subtotal colectomy remains a treatment option for few patients with slow-transit constipation (STC) refractory to conservative treatment. A careful patient selection is important to improve benefits and reduce risk of adverse outcomes. Laparoscopically assisted subtotal colectomy with ileorectal anastomosis and preservation of the superior rectal artery may be effective for STC and can be the best surgical option in these situation.

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TO THE EDITOR

Wu *et al*[1] recently published an observational study on the largest patient sample with vascular preservation with slow transit constipation (STC) who were submitted to laparoscopically assisted subtotal colectomy with ileorectal anastomosis and preservation of the superior rectal artery (SRA). The authors concluded that this surgical approach could significantly improve bowel function with careful patient selection and that sparing the SRA may protect against anastomosis leakage. Despite the relevant information described in the study, some important issues need to be discussed.

First, the study did not include a comparison group, *e.g.*, patients undergoing the same surgical laparoscopic procedure without SRA preservation, to find out whether one procedure is advantageous over the other. Would the SRA preservation status affect the length of hospital stay, first time to flatus, leakage volume, or postoperative complication rate? Second, based on the results, the authors cannot state that the surgery "can significantly improve bowel function with careful patient selection". The authors did not present the functional results in the short or long term related to the bowel function of these patients after surgery, as other authors have[2]. Third, they cannot state that sparing the SRA may protect against anastomosis leakage. What the authors showed is that this surgery was safe, and no cases of leakage were found. Finally, why did the surgeons make a 4-5 cm Pfannenstiel incision and bring out the mobilized bowel segment? The key limitation of the work was the absence of a control group, which would enrich the important and relevant findings of their study. In our clinical practice, we try to preserve the SRA in nononcological procedures, such as for endometriosis, diverticular disease and STC, as described by other authors[3]. However, we remove the surgical specimen using a trocar in the lower right abdominal quadrant. In the other aspects of the manuscript, we agree with the authors and believe that the best surgical option in STC could be laparoscopic subtotal colectomy with ileorectal anastomosis with preservation of the SRA.

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