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Myeloid-derived suppressor cells in gastrointestinal cancers: A systemic review

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Abstract

Gastrointestinal (GI) cancers are one of the most common malignancies worldwide, with high rates of morbidity and mortality. Myeloid-derived suppressor cells (MDSCs) are major components of the tumor microenvironment (TME). MDSCs facilitate the transformation of premalignant cells and play roles in tumor growth and metastasis. Moreover, in patients with GI malignancies, MDSCs can lead to the suppression of T cells and natural killer cells. Accordingly, a better understanding of the role and mechanism of action of MDSCs in the TME will aid in the development of novel immune-targeted therapies.

Key Words: Myeloid-derived suppressor cells; Gastrointestinal cancers; Immune checkpoint inhibitors; Tumor progression

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Core Tip: In patients with cancer, the levels of myeloid-derived suppressor cells (MDSCs) are presumed to be of prognostic and predictive value. Recent studies have shown that MDSCs appear to be independent prognostic factors in gastrointestinal cancer. In addition, therapeutics that target MDSCs have been shown to enhance anti-tumor immune responses in animal models. Consequently, a better understanding of the role and mechanism of action of MDSCs in the tumor microenvironment may aid in the development of novel immune-targeted therapies.

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INTRODUCTION

In 2018, 4.8 million new diagnoses of gastrointestinal (GI) cancer and 3.4 million related deaths were reported globally. The incidence of GI cancer is 26% worldwide, accounting for 35% of all cancer-associated mortalities^[1]. Numerous stromal and immune cells and soluble markers are related to the immunosuppressive network in the tumor microenvironment (TME)^[2]. This network is involved in tumor cell growth and the blockade of anti-tumor immune responses, which subsequently promote the progression and invasion of tumor cells^[3]. Macrophages, monocytes, and dendritic cells (DCs) represent a subgroup of leukocytes called myeloid cells, which are generated from polymorphonuclear granulocytes^[4]. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that arise from myeloid progenitor cells^[5]. They play a key role in tumor-associated immune evasion, angiogenesis, and tumor metastasis^[6,7].

In patients with cancer, the levels of MDSCs are thought to have prognostic and predictive significance^[8]. Recent studies have examined the role of MDSCs in solid tumors, and discovered that they appear to be independent prognostic factors in GI cancer^[8,9]. In a meta-analysis of 17 studies with 1115 patients with GI malignancies, patients with a higher number of MDSCs at tumor sites and peripheral blood had higher mortality rates (hazard ratio: 3.35, 95% confidence interval: 1.46-7.68; $P = 0.0004$), risk of relapse, and tumor progression. The authors concluded that MDSC levels have prognostic and predictive value in cancer patients^[10]. Additionally, higher levels of MDSCs in patients with cancer are associated with advanced tumor stage and a poor clinical prognosis^[11]. Shibata *et al*^[12] evaluated 123 patients with advanced GI malignancy, including 62 with colorectal cancer (CRC), 43 with gastric cancer (GC), and 18 with esophageal malignancies, and found that overall survival (OS) was significantly shorter in stage IV GI cancer patients with high MDSC levels than in those with low MDSC levels ($P < 0.05$). Because MDSCs have a significant role in modulating cancer progression and metastasis by inhibiting the anti-tumor reactivity of T cells and natural killer (NK) cells, targeting MDSCs with immune checkpoint inhibitors (ICIs) can alleviate their pro-tumorigenic functions. Therefore, in this systematic review, we summarize the characteristics and proposed function of MDSCs in the TME and their relationship to prognosis in patients with GI cancers.

MATERIALS AND METHODS

PubMed/MEDLINE databases were explored with search strategies using search keywords "MDSCs," "gastrointestinal cancers," "prognosis," "tumor progression," and "mortality rate," to categorize studies published between 2006 and 2020. A total of 128 articles were reviewed by the authors for relevance to MDSCs and GI cancers, including retrospective, cross-sectional, case reports, and cohort studies, of which 85 papers were selected that met our selection criteria.

MDSC MECHANISM OF ACTION

MDSCs are myeloid-derived heterogeneous cells with potent immune regulatory functions. They are derived from the myeloid lineage of immune cells that give rise to macrophages, granulocytes, and immature DCs^[13]. Monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs) are the two major myeloid subsets of MDSCs^[14]. Phenotypically and morphologically, they are equivalent to monocytes and neutrophils, respectively (Table 1)^[15].

The process of myelopoiesis is driven by granulocyte-macrophage colony-stimulating factor (GM-CSF) in normal physiological conditions. Ultimately, GM-CSF and M-CSF induce the differentiation of granulocytes and macrophages from a common myeloid precursor that transforms into a common myeloblast^[16]. Myeloid

Table 1 Two main categories of myeloid-derived suppressor cells and their immunosuppressive functions^[59]

Type of MDSC	Markers in humans	Immunosuppression mediator	Mechanism of immunosuppression
PMN-MDSCs	CD11b ⁺ CD14 ⁺ CD15 ⁺ HLADR ⁺ or CD11b ⁺ CD14 ⁺ CD66b ⁺ or LOX-1 ⁺	ARG1, ROS	Suppressing immune responses mainly in an antigen-specific manner; ROS production
M-MDSCs	CD11b ⁺ CD14 ⁺ CD15 ⁺ HLADR ^{low/-}	NO, ARG1, and cytokines such as TGF- β and IL-10	Suppressing T cell responses, both in antigen-specific and non-specific manners; production of NO and cytokines

ARG1: Arginase 1; IL-10: Interleukin-10; M-MDSCs: Monocytic myeloid-derived suppressor cells; NO: Nitric oxide; PMN-MDSCs: Polymorphonuclear myeloid-derived suppressor cells; ROS: Reactive oxygen species; TGF- β : Transforming growth factor-beta; MDSC: Myeloid-derived suppressor cell.

DCs eventually arise from monocytic as opposed to granulocytic lineages^[17]. However, the hypersecretion of these mediator factors during chronic inflammation and cancer leads to the generation of MDSCs^[18]. For example, inflammatory cytokines such as interleukin 6 (IL-6), IL-1 β , and IL-3 and C-X-C chemokine receptor type 4 (CXCR4) and CXCL12 can lead to the induction and proliferation of MDSCs in peripheral blood and tumor sites in cancer patients^[7,19]. The most important function of MDSCs is immunosuppression, mainly of target T cells (Figure 1)^[14].

Several *in vitro* and *in vivo* studies have documented the mechanisms underlying the immunosuppressive actions of MDSCs. Arginase 1 (ARG1), inducible nitric oxide (iNOS), reactive oxygen species, and reactive nitrogen species are important suppressive factors produced by MDSCs^[20,21]. Cao *et al*^[22] reported that PMN-MDSCs store ARG1 and secrete it to the TME. ARG1 and NOS activities lead to cellular depletion of L-arginine (referred to as L-arg), which is an essential substrate for T cell proliferation^[22,23]. Similar to T cells, depletion of L-arg also impairs the function of NK cells^[24].

In addition to immunosuppressive factors, MDSCs can overpower T cell functions by directly engaging with T cell inhibitory and apoptotic receptors. Activated MDSCs express high levels of Fas ligand (referred to as Fas L), programmed death-ligand 1 (PD-L1), and galectin-9. Subsequently, the interaction between these ligands with their receptors on T cells leads to T cell exhaustion *via* PD-L1/programmed cell death protein 1 (PD-1) or T cell apoptosis through the Fas L/Fas and galectin-9/T cell immunoglobulin and mucin domain-3 pathways^[25]. Generally, M-MDSCs have more suppressive effects than PMN-MDSCs^[26]. Moreover, MDSCs can stimulate and recruit regulatory T cells (Tregs) to the TME^[27]. Tregs suppress anti-tumor immunity, and the interaction between MDSCs and Tregs create a strong blockade preventing cytotoxic immune cells from mounting an anti-tumor attack^[28]. Elevated levels of Tregs are associated with poor survival in patients with hepatocellular carcinoma (HCC) and pancreatic cancer^[29,30]. Regarding metastasis, MDSCs can promote angiogenesis by secreting IL-28 [interferon lambda (IFN- λ)] and matrix metalloproteinase (MMP)-9, promoting the invasion and migration of tumor cells^[31].

MARKERS OF MDSCS IN PERIPHERAL BLOOD

MDSCs are categorized according to their phenotype, which includes several recognized surface markers, such as cluster of differentiation 33 (CD33), CD11b, or human leukocyte antigen-DR isotype (HLA-DR), as well as by the lack of expression of markers distinctive of mature lymphoid cells, such as CD3, CD19, and CD56^[32]. Typically, flow cytometry is performed to isolate MDSCs from peripheral mononuclear blood cells (PBMCs)^[33]. Fluorescent-labeled monoclonal antibodies are used to distinguish M-MDSCs and granulocytic MDSCs (G-MDSCs). M-MDSCs are identified as CD11b⁺CD14⁺CD33^{high}HLA-DR^{low} and CD66b, whereas G-MDSCs are recognized as CD11b⁺CD14⁺CD33^{low}HLA-DR⁺CD66b⁺^[34]. MDSCs can be distinguished from other immune suppressor cells within the myeloid lineage, *e.g.*, tumor-associated macrophages and macrophage type 2, by other specific surface markers, including CD163 and F4/80^[35].

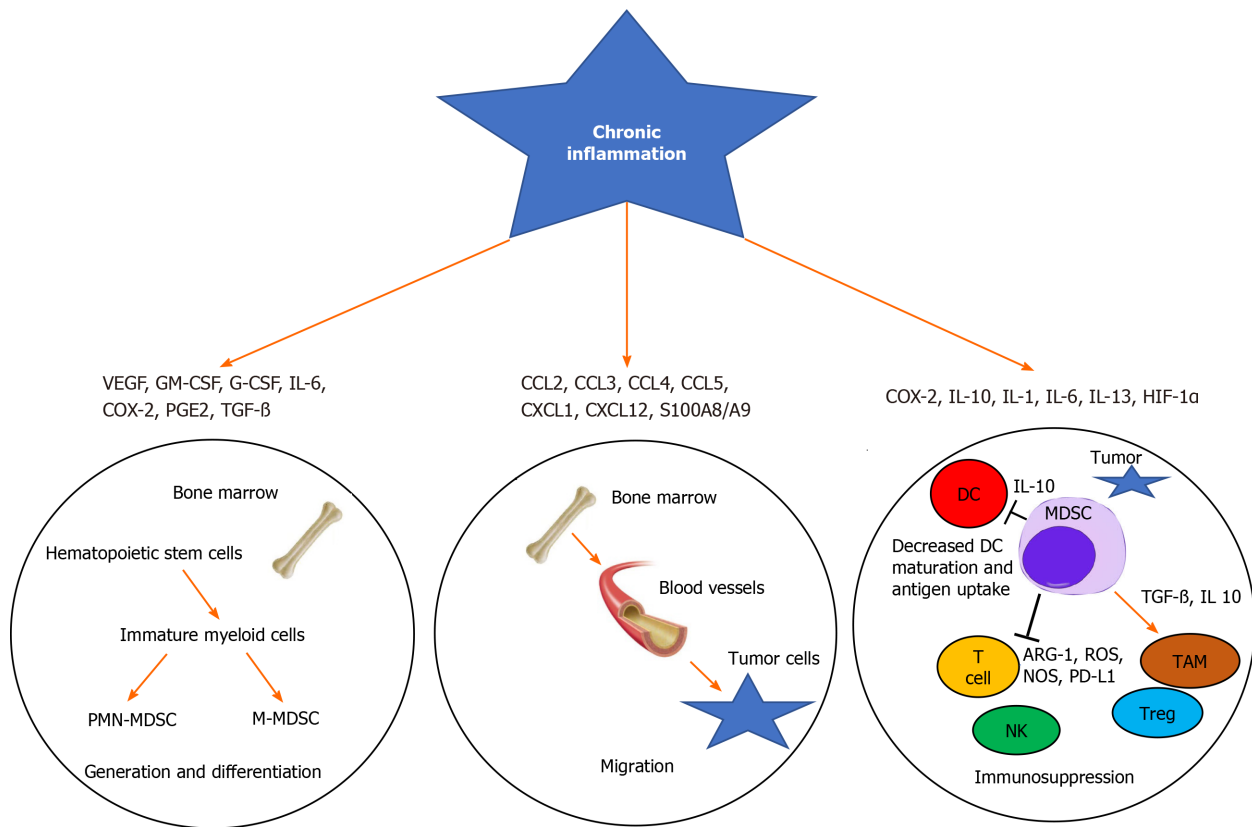


Figure 1 Chronic inflammation activates myeloid-derived suppressor cell generation, migration, and immunosuppression in the tumor microenvironment. Several cytokines and stimulator factors secreted by stroma and tumor cells (e.g., VEGF, granulocyte-macrophage colony-stimulating factor, IL-1, IL-6, HIF-1 α , TGF- β , COX-2) trigger myeloid-derived suppressor cell (MDSC) generation and migration. Cytokines (e.g., CCL2, CCL3, CCL4, CCL5, CXCL1) stimulate the migration of MDSCs into the tumor microenvironment. At the tumor site, MDSCs undergo activation (via TNF, IL-10, IL-1, IL-6, IFN- γ , COX-2, HIF-1 α , etc.) and suppress the anti-tumor reactivity of T and natural killer cells. Cross-talk between MDSCs and dendritic cells (DCs) impairs DC function and promotes tumor progression^[81]. GM-CSF: Granulocyte-macrophage colony-stimulating factor; PMN-MDSC: Polymorphonuclear myeloid-derived suppressor cells; M-MDSC: Monocytic myeloid-derived suppressor cells; MDSC: Myeloid-derived suppressor cell; DC: Dendritic cell; ARG-1: Arginase 1; NOS: Nitric oxide; PD-L1: Programmed death-ligand 1; NK: Natural killer; ROS: Reactive oxygen species.

MDSCS IN GI MALIGNANCIES

Esophageal cancer

High levels of circulating MDSCs in esophageal cancer are associated with a poor prognosis^[5]. Elevated MDSCs in the blood are correlated with elevated numbers of immunosuppressive cells, including Tregs^[5]. Jiao *et al*^[36] evaluated 31 esophageal cancer patients and 26 healthy controls (HCs), and found that MDSC numbers in the peripheral blood were increased 15-fold in esophageal cancer patients compared to HCs. The authors also showed that the plasma levels of ARG1 were 3-fold higher in cancer patients than in HCs. Xu *et al*^[37] showed that 178 patients with esophageal cancer had a high level of G-MDSCs (> 82.5%), which were correlated with high morbidity due to the development of sepsis postoperatively after esophageal cancer surgery. The authors suggested that the level of G-MDSCs may be used to determine the incidence of sepsis in preoperative esophageal cancer patients postoperatively, and could improve the mortality of cancer-associated sepsis by targeting the level of MDSCs.

Additionally, a study by Chen *et al*^[38] found that the levels of IL-6 and MDSCs predicted the prognosis and treatment response in mice with esophageal squamous cell carcinoma (SCC). The levels of MDSCs induced by IL-6 were linked to tumor growth and a poor prognosis. The authors concluded that targeted therapy against IL-6 with rapamycin or casein kinase 2 inhibitors might be a potential treatment modality for esophageal SCC^[38,39].

GC

According to the Global Cancer Observatory (GLOBOCAN) 2018 database, GC is the fifth most common cancer and third most deadly cancer worldwide, with an estimated

783000 deaths in 2018^[40], Li *et al*^[41] documented the levels of MDSCs in the peripheral blood of 21 GC patients who had not previously received treatment, and noted that the levels of MDSCs in these patients were about 4-fold higher than in the control groups. The authors concluded that cancer cell differentiation and lymph node metastasis are mostly related to the presence of M-MDSCs. They also showed that treatment with epirubicin and paclitaxel regimens can reduce the level of MDSCs in these patients, potentially leading to better outcomes for patients due to inhibition of cancer progression. Moreover, in 29 patients with GC and 18 HCs, MDSCs were increased in stage IV patients compared with HCs, and the 2-year survival rate of patients with higher levels of MDSCs was significantly poorer (median OS: 498 d *vs* 473 d; $P = 0.048$), but no significant difference was observed in survival among patients with stage I, II, and III GC^[42]. Previously, we reported that schlafen (SLFN) 4-expressing myeloid cells recruited to the stomach during *Helicobacter* infection undergo a phenotypic shift to G-MDSCs under the influence of damage-associated molecular pattern (DAMP) signaling and the production of IFN- α ^[43,44]. SLFN4 is a myeloid cell differentiation factor that controls myelopoiesis^[45]. These SLFN-expressing MDSCs secrete factors including microRNAs, which can be detected in the peripheral blood as a biomarker and promote epithelial cell growth. This sustained immune dysregulation creates a microenvironment capable of supporting GC development^[46].

HCC

HCC is a leading cause of death in cirrhotic patients. Per the GLOBOCAN 2018 database, 841000 new cases of primary liver cancer and 782000 deaths due to HCC occurred that year^[40]. In a prospective case-control study, Elwan *et al*^[47] demonstrated a higher number of MDSCs in the peripheral blood of cirrhotic groups without HCC than in patients with cirrhosis and HCC compared to patients in control groups. They showed that mean MDSC counts in the peripheral blood of cirrhotics without HCC group and cirrhotics with HCC group were about 3.5-fold and 5-fold higher compared to the control groups, respectively. Although not statistically significant, the authors reported a low number of MDSCs in the ascitic fluid of patients with both cirrhosis and HCC. Additionally, they investigated the correlation of levels of IFN- γ and alpha-fetoprotein with MDSC level. Their data showed that alpha-fetoprotein was positively and INF- γ was negatively correlated with MDSC count in the HCC group^[47]. A high frequency of MDSCs in the PBMCs of patients with HCC has been linked to more aggressive forms of HCC and poor clinical outcomes following local ablation, hepatectomy, or hepatic arterial infusion chemotherapy^[48,49]. A cohort study by Bayik *et al*^[50] showed an upsurge in circulating MDSC frequency in 114 patients with a secondary liver cancer, including CRC with liver metastases and neuroendocrine tumors, compared to individuals with benign lesions.

Data from animal models have shown that myeloid cells secrete MMPs, serine proteases, and cysteine cathepsins, which facilitate tumor cell invasion and metastasis by disrupting cell adhesions^[51]. Tumor angiogenesis in HCC can be promoted by MDSCs by producing high levels of MMP-9 in HCC^[52].

Pancreatic cancer

The incidence of pancreatic ductal adenocarcinoma has significantly increased worldwide over the last 30 years, with a 5-year survival time less than 8%^[53]. Khaled *et al*^[54] demonstrated that G-MDSCs, but not M-MDSCs, are much higher in circulation and in the tumor tissue of patients with pancreatic cancer compared to HCs or those with chronic pancreatitis. These results suggest that the high level of G-MDSCs in pancreatic cancer plays a key factor in tumor development and progression. A cohort study reported that the percentages of all subpopulations of MDSCs were higher in patients with intraductal papillary mucinous neoplasm (IPMN) than in HCs, and were even higher in those with pancreatic adenocarcinoma. Although there was a trend towards higher MDSC levels in pancreatic cancer *vs* IPMN, it was not statistically significant ($P = 0.33$)^[55].

CRC

A recent study reported that CRC cells induce an increase in the number of MDSCs by producing inflammatory factors, such as transforming growth factor-beta, IL-10, and ARG1^[56,57]. Consequently, T cell proliferation can be suppressed by tumor-derived MDSCs and promote tumor cell growth *via* oxidative metabolism. Previously, it was shown that the numbers of circulating Tregs and MDSCs are significantly reduced following tumor resection in patients with CRC. These data indicate that immunosuppression can be mitigated by reducing the number of MDSCs and Tregs in

patients with CRC after reducing the tumor burden^[57]. Tada *et al*^[58] showed that patients with unresectable metastatic CRC with high M-MDSC, low CD4⁺, or low CD8⁺ effector memory T cell levels had significantly shorter progression-free survival.

MDSC-TARGETED THERAPY

Many studies have examined MDSCs as the core of targeted therapeutic strategies to improve tumor control in experimental animal models. These targeted therapies could be achieved by reducing MDSC numbers, hindering their trafficking and migration, or inhibiting their immunosuppressive function (Table 2)^[59].

Because of variances in immunophenotype and mechanisms of suppression in the TME and diverse nature of human MDSCs, it is challenging to target human MDSCs^[60]. Wang *et al*^[61] treated pancreatic cancer patients with cytokine-induced killer (CIK) cell immunotherapy, CIK plus gemcitabine, and 5-fluorouracil (5-FU), and analyzed the levels of MDSCs in the peripheral blood pre- and post-treatment. The OS of metastatic pancreatic patients was increased with the combination of CIK and chemotherapy (gemcitabine and 5-FU) compared to patients treated with only CIK. Also, Jiang *et al*^[62] reported that the quality of life and 2-year survival rate improved in patients with advanced GC following combining chemotherapy (5-FU and oxaliplatin) with CIK cell treatment compared to treatment with chemotherapy alone. Tadalafil, the Federal Drug Administration-approved phosphodiesterase-5 inhibitor, can suppress MDSCs through downregulation of ARG1 and iNOS activities in several preclinical models^[63-65]. Rawat *et al*^[66] showed in aflatoxin-induced HCC rats, that tadalafil reduced the level of glutamic oxaloacetic transaminase, an important enzyme that facilitates carbohydrate and protein metabolism in cancer cells. A previous study showed that treatment with tyrosine kinase inhibitors, such as sunitinib, reduced the number of MDSCs and Tregs in animals with intrahepatic colorectal metastases^[67]. The authors also showed that the number of MDSCs was significantly reduced from 53.9% in phosphate-buffered saline-treated mice to 39% in sunitinib-treated mice. Sunitinib has established efficacy against advanced GI stromal tumors^[68].

Treatment of esophageal SCC with 1 α ,25-dihydroxyvitamin D3 (calcitriol) has been shown to inhibit MDSC proliferation induced by IL-6 stimulation in C57 mice. Therefore, it has been proposed that this treatment may be a promising strategy for the prevention and treatment of esophageal SCC^[69].

ICIs such as cytotoxic T-lymphocyte-associated protein 4 (ipilimumab and tremelimumab), PD-1 (pembrolizumab and nivolumab), and PD-L1 (atezolizumab, avelumab, and durvalumab) are promising treatment strategies that can be applied across numerous solid tumors^[70]. Pembrolizumab is a therapeutic antibody that blocks PD-1, and has shown promising anti-tumor activity in advanced GC^[71]. Although ICIs show great therapeutic benefits, substantial GI side effects such as colitis and GI bleeding can limit their use^[72]. However, due to immunosuppression, which is regulated by MDSCs, some patients with cancer may develop resistance to ICIs^[73]. Therefore, it is important to inhibit MDSC proliferation and migration to the TME by different strategies, such as anti-CXCR2 monoclonal antibody, to enhance PD-1 efficacy^[74].

CORONAVIRUS-19 AND MDSCS

In this age of coronavirus-19 (COVID-19), we would be remiss not to address what is currently known about activation of the host's immune response, in particular MDSCs, by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). Plasmacytoid DCs (pDCs) are the major source of tissue-derived type 1 IFNs in response to tissue antigens and activation of DAMPs. Typically, intracellular Toll-like receptors (TLRs) found on endosomes that mediate this pathway evolve to defend the cell against viral pathogens^[75]. Thus, we queried whether coronavirus infections might be modulated by pDCs residing in the GI tract. Apparently, SARS-CoV-2 induces a massive anti-viral response by secretion of IFN- α from pDCs *via* TLR7^[76]. The severity of COVID-19 infection might correlate with the activation of endosomal TLRs on pDCs in the GI tract and increased myeloid cell polarization to MDSCs. If this occurs, the cellular immune response to the virus could be rendered ineffective, suggesting that those with severe disease exhibit higher levels of MDSCs than those with mild disease. Indeed, the limited studies available of COVID-19 patients have shown that the MDSC population expands in those with severe disease^[77,78]. Although T cell exhaustion from

Table 2 Potential therapeutic strategy for targeting myeloid-derived suppressor cells

Strategy	Agents
Blocking TDFs from being produced or from reaching the bone marrow Key cytokines, such as IL-6 or S100A8/A9, could be directly targeted ^[82,84]	Targeting the IL-6 receptor (tocilizumab) ^[83]
Inhibiting generation of MDSCs from bone marrow progenitors or inducing apoptosis of circulating MDSCs ^[6]	Gemcitabine, 5-fluorouracil, sunitinib, and zoledronate ^[84]
Preventing trafficking of myeloid cells from the marrow to peripheral lymphoid organs or to the tumor microenvironment ^[6]	Drugs targeting chemokines CXCR2, CXCR4, and CSF1R ^[14]
Directly blocking MDSC suppression of T cells ^[85]	Phosphodiesterase type 5 inhibitors, <i>e.g.</i> , sildenafil and tadalafil, or cyclooxygenase 2 inhibitors ^[63]
Drugs that would promote differentiation of MDSCs into proficient antigen-presenting cells that can stimulate tumor-specific T cells and/or into mature leukocytes ^[85]	All-trans retinoic acid, vitamin D3, and the DNA-methylating agent 5-azacytidine ^[85]

MDSCs: Myeloid-derived suppressor cells; CSF1R: Colony-stimulating factor 1 receptor; CXCR2: C-X-C motif receptor 2; CXCR4: C-X-C motif receptor 4; IL-6: Interleukin-6; TDFs: Tumor-derived factors.

the cytokine storm that COVID-19 patients display is the leading cause for severe disease, massive production of immune suppressor cells also explains the lymphopenia occurring in many of these patients^[79,80]. A better understanding of what controls MDSCs will facilitate not only therapeutic treatments but may ultimately help to predict who will respond to vaccination. Whether patients who recover from these infections are predisposed to chronic disorders, such as autoimmune diseases and cancer, will require long-term follow up of these patients over decades.

CONCLUSION

This review article provides a better understanding of the role and mechanism of action of MDSCs in GI malignancies. MDSCs are one of the most important elements in the TME. In patients with GI cancer, MDSCs can lead to immunosuppression, and they play an important role in premalignant cell transformation, tumor growth, and metastasis. A higher number of MDSCs at tumor sites and peripheral blood is correlated with higher mortality rates, risk of relapse, and tumor progression. Therefore, monitoring circulating MDSC levels might have prognostic and predictive value in patients with GI malignancies. The benefit of targeting treatment against MDSCs as a combination therapy has been shown. Consequently, a better comprehension of the role and mechanism of action of MDSCs in the TME may aid in the development of novel immune-targeted therapies. Further prospective studies are needed to understand the characterization and clinical value of MDSCs and more selective anti-MDSC therapies with improved therapeutic outcomes.

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Clinical and Translational Research

Laparoscopy-assisted transanal total mesorectal excision for lower rectal cancer: A feasible and innovative technique

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Abstract

BACKGROUND

Transanal total mesorectal excision (taTME) is a new technique with many potential technical advantages. Laparoscopy-assisted taTME is a combination of transabdominal taTME and transluminal endoscopic surgery taTME. Laparoscopy-assisted taTME is a combination of techniques such as minimally invasive surgery, intersphincter-assisted resection, natural orifice extraction, ta minimally invasive surgery, and ultralow-level preservation of the anus.

AIM

To verify the feasibility and safety of an innovative technique of taTME for treatment of cancer located in the lower rectum.

METHODS

From January 2016 to March 2018, we attempted to perform laparoscopy-assisted taTME surgery in 24 patients with lower rectal cancer.

RESULTS

The new technique of laparoscopy-assisted taTME was successfully performed in all 24 patients. Mean operating time was 310.0 min and mean intraoperative blood loss was 69.1 mL. The mean time to passing of first flatus was 3.1 d, and mean postoperative hospital stay was 9.2 d. Two patients were given postoperative analgesics due to anal pain. Twenty-three patients were able to walk in first 2 d, and five patients had postoperative complications.

CONCLUSION

written consent prior to study enrollment.

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

Data sharing statement: No additional data are available.

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Laparoscopy-assisted taTME is suitable for selected patients with lower rectal cancer, and this technique is worthy of further recommendation.

Key Words: Laparoscopy-assisted; Total mesorectal excision; Technique; Lower rectal cancer; Trans-abdominal; Trans-anus

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Core Tip: We report our initial experience with transanal total mesorectal excision for distal rectal cancer, with a 100% success rate of intraoperative preservation of the anal sphincter. The patients in this study had a narrow pelvis, mild obesity, and distal rectal lesions, making the operation extremely difficult. Most of the patients had undergone neoadjuvant chemoradiation. We believe that this procedure is feasible for selected patients with lower rectal cancer, and is worthy of further recommendation.

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INTRODUCTION

The introduction of total mesorectal excision (TME) in combination with advanced neoadjuvant and adjuvant oncological treatment regimens has improved overall survival and local recurrence in rectal cancer patients^[1]. Although minimal invasive procedures are being used more frequently and generally add to this positive development, dissection in the pelvic cavity remains a technical challenge^[2,3]. Concerns about adequate distal resection margin, bulky tumors and inadequate view of the operating field in the lower pelvis may negatively affect the surgical as well as oncological quality of the procedure, which reduces the advantages of laparoscopy-assisted approaches^[4]. Laparoscopy-assisted transanal (ta) TME has been developed as a means of reducing the above disadvantages. This approach is reasonable in lower rectal cancer patients with a strong desire for preserving anus, narrow pelvic, or obesity, or male patients^[5-8]. Between January 2016 and March 2018, 24 patients with lower rectal cancer were treated by laparoscopy-assisted taTME.

MATERIALS AND METHODS

Patients

From January 2016 to March 2018, we attempted to perform laparoscopy-assisted taTME for 24 patients with lower rectal cancer (Table 1). Currently, there is no international standard surgical indication for taTME. The suggested indications are: Low rectal cancer, distance 0-8 cm from anal verge (defined by magnetic resonance imaging [MRI]), histological biopsy showing adenocarcinoma stage I-III (by MRI and abdominal computed tomography). All patients underwent a standard clinical examination including rigid proctoscopy, MRI of the rectum, and thoracoabdominal computed tomography. Distant metastasis was excluded by imaging examination. All operations were performed by a stationary surgical team. The patients were selected to have a detailed understanding of the taTME surgical procedure and risks, have a strong desire to retain the anus and choose the surgical method, and take risks.

The training phase included cadaver dissection, technical and practical courses, and participation as an assistant in a human taTME procedure.

All patients received standard mechanical bowel preparation and followed a standard postoperative enhanced recovery program for colorectal resection.

TaTME technique

After induction of general anesthesia, proctoscopy was performed to ensure adequate

Table 1 Patient characteristics

Cases	Age in yr	Gender	BMI, kg/m ²	Tumor height from anorectal junction in cm	Clinical stage	CRT	Pathological stage	Tumor size in cm	Technique	Operation time in min	Blood loss in mL	POHS	Time to first flatus in d	Complications
1	64	F	21.94	5.0	T3N2b	Yes	ypT0N0	7.0	taTME	198	100	9	2	N
2	48	M	30.78	5.0	T3N2b	Yes	ypT3N1b	1.5	taTME	270	100	12	3	N
3	64	M	30.06	5.0	T3N2b	Yes	ypT3N0	1.5	taTME (Hartmann)	364	100	9	2	N
4	67	M	25.24	6.0	T3N1	Yes	ypT2N1a	2.0	taTME	270	100	11	2	Fever
5	57	M	27.11	5.0	T3N2	Yes	ypT2N0	2.0	taTME	392	100	9	3	N
6	66	M	20.44	3.0	T3N+	Yes	ypT2N0	1.0	taTME	262	50	9	2	N
7	45	M	26.67	4.0	T3N0	Yes	ypT3N0	1.2	taTME	317	200	8	2	N
8	38	M	25.82	3.0	T3bN2a	Yes	ypT2N0	2.0	taTME	266	200	9	2	N
9	47	M	24.39	5.0	T3N+	Yes	ypT2N1	2.5	taTME	375	100	14	10	Obstruction
10	61	M	26.20	4.0	T3N2b	Yes	ypT3N1a	1.3	taTME	293	100	9	3	N
11	62	M	29.41	6.0	T4aN+	Yes	ypT3N2b	2.0	taTME	337	100	10	2	N
12	72	M	36.37	3.0	T4N+	Yes	ypT0N1a	0.5	taTME	405	200	12	2	Fever
13	65	M	24.0	7.0	T3N+	Yes	ypT3N1a	2.0	taTME	290	100	7	2	N
14	51	M	25.76	2.0	T3N2b	Yes	ypT1N1b	2.0	taTME	364	200	9	2	N
15	55	M	23.66	8.0	T3N+	Yes	ypT3N0	4.0	taTME	400	100	10	2	N
16	30	M	26.17	3.0	T3N0	Yes	ypT0N0	0.0	taTME	283	100	10	2	N
17	61	M	26.99	5.0	T2N2	Yes	ypT2N0	1.5	taTME	245	50	7	2	Fever
18	49	F	26.34	2.0	T3N+	Yes	ypT2N0	1.1	taTME	226	100	7	2	N
19	58	M	20.96	2.0	T3N+	Yes	ypT3N0	1.0	taTME	342	200	10	2	N
20	60	M	26.90	5.0	T3N1	No	ypT3N0	5	taTME	271	100	7	2	N
21	71	M	25.3	5.0	T3N1	Yes	ypT2N0	0.5	taTME	310	100	8	2	Anastomotic fracture
22	69	M	23.9	3.0	T3N2	Yes	ypT1N0	1.5	taTME	317	50	8	2	N
23	39	F	24.2	4.0	Neuroendocrine tumor G2	No	Neuroendocrine tumor G2	1.0	taTME	235	50	12	2	N

24	59	M	23.39	4.0	T3N+	Yes	ypT2N0	1.0	taTME	266	50	8	2	N
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BMI: Body mass index; CRT:chemoradiotherapy; POHS: Post-operative hospital stay; F: Female; M: Male; taTME: Transanal total mesorectal excision; N: None.

preoperative bowel preparation and to perform washout of the rectum with Povidone-iodine (PVP-I).

The procedure started with the laparoscopic transabdominal part of the dissection according to TME principles and high ligation of the inferior mesenteric artery. To ease the transanal extraction of the specimen, the splenic flexure was mobilized entirely to the midline in some patients. The mobilization continued caudally/posteriorly to the sacral promontory and anteriorly to the peritoneal reflection of the rectum, and anteriorly to the level of vagina/seminal vesicles (Figure 1).

We commenced by inserting an Applied Medical STARPORT Path Trans Anal Access Platform and closed the rectum with a purse string suture under direct visualization of the tumor (Figure 2). This provided optimal conditions for identifying the appropriate distal margin at least 1 cm below the tumor.

A full thickness incision of the entire rectum wall provided access to the anatomical planes. Mobilization of the mesorectum started posteriorly and was then continued anteriorly. Finally, the dissection progressed laterally, to avoid the specimen blocking the operating field during the posterior dissection and to ensure the correct lateral plane to avoid nerve injury (taTME video).

When communication to the peritoneal cavity was achieved and the ta and transabdominal dissection planes met, one surgeon returned to the abdominal group and assisted with the final mobilization of the rectum. Depending on the tumor size, the specimen was extracted either *via* a Pfannenstiel incision or transanally, using the Wound Protector.

The remaining part of the colonic mesentery was divided extracorporeally above the division of the inferior mesenteric artery, testing the marginal artery for sufficient blood flow by division and subsequent ligation. A purse string was made and the anvil of the circular stapler was inserted and fastened. The end of the colon could now be repositioned into the abdomen and the anal distal stamp was pre-closed with a purse string suture, stitching the anvil before connecting with the central rode of the stapler. The anastomosis was performed as end-to-end or side-to-end using a 28-33 mm circular stapling device depending on the colonic caliber.

Diverting loop ileostomy was usually done,as well as full-thicken strengthen of the anastomosis. Anastomotic leaks were graded according to the classification system proposed by the International Study Group of Rectal Cancer and complications by the Clavien-Dindo classification. The quality of the specimens was assessed by the surgeon and pathology department.

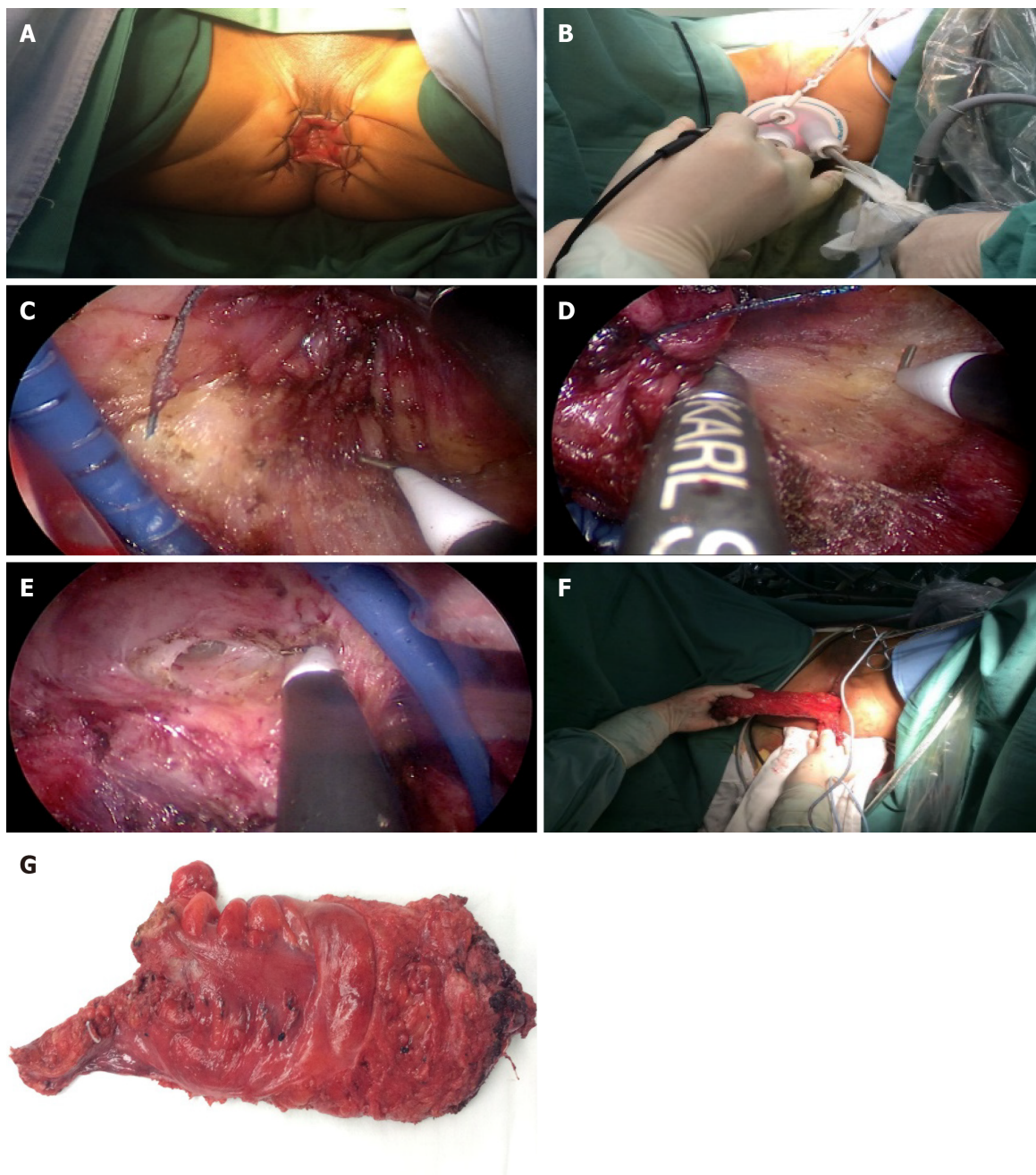


Figure 1 Transanal total mesorectal excision surgical procedure presentation. A: Anal exposure (anal and perianal skin suture); B: Place the operation platform (Star port); C: Left lateral dissection; D: Right lateral dissection; E: Anterior dissection; F: Specimen extraction.

Follow-up

We followed up patients every 3 mo with physical examination and laboratory tests, including tumor markers (carcinoembryonic antigen and carbohydrate antigen 19-9). Abdominal computed tomography was performed every 6 mo after the operation, and endoscopy was performed 1 year postoperatively. To evaluate the ano(neo)rectal function after taTME, the low anterior resection syndrome (LARS) questionnaire^[9] was used. The LARS questionnaire of taTME group was sent 6 mo after ileostomy closure. The LARS score was categorized into no LARS (0-20 points), minor LARS (21-29 points), and major LARS (30-42 points) (Table 2)^[9]. The follow-up time was 3-13 mo, and the last follow-up date was March 30, 2018.

RESULTS

We successfully completed laparoscopy-assisted taTME in 24 patients with lower

Table 2 Low anterior resection syndrome questionnaire and scoring^[16]

LARS Score: Scoring instructions
Add the scores from each 5 answers to one final score.
Do you ever have occasions when you cannot control your flatus (wind)?
<input type="checkbox"/> No, never
<input type="checkbox"/> Yes, less than once per week
<input type="checkbox"/> Yes, at least once per week
Do you ever have any accidental leakage of liquid stool?
<input type="checkbox"/> No, never
<input type="checkbox"/> Yes, less than once per week
<input type="checkbox"/> Yes, at least once per week
How often do you open your bowels?
<input type="checkbox"/> More than 7 times per day (24 h)
<input type="checkbox"/> 4-7 times per day (24 h)
<input type="checkbox"/> 1-3 times per day (24 h)
<input type="checkbox"/> Less than once per day (24 h)
Do you ever have to open your bowels again within 1 hour of the last bowel opening?
<input type="checkbox"/> No, never
<input type="checkbox"/> Yes, less than once per week
<input type="checkbox"/> Yes, at least once per week
Do you ever have such a strong urge to open your bowels that you have to rush to the toilet?
<input type="checkbox"/> No, never
<input type="checkbox"/> Yes, less than once per week
<input type="checkbox"/> Yes, at least once per week
Total Score:
Interpretation:
0-20: No LARS
21-29: Minor LARS
30-42: Major LARS

LARS: Low anterior resection syndrome.

rectal cancer. Among them, one underwent additional lateral lymph node dissection and one Hartmann surgery. In 24 patients, mean operation time was 310.0 min, mean intraoperative blood loss was 69.1 mL, and mean time to passing of first flatus was 3.1 d. The mean postoperative hospital stay was 9.2 d. Postoperative pain associated with taTME surgery was scored by the patient according to the subjective simulated pain scale (numeric rating scale) ranging from 0 to 10, with 0 representing no pain at all and 10 the worst pain imaginable. Pain was also assessed by a rehabilitation physician at 24 h and 72 h after surgery. Patients suffering from 4-7 score pain were given analgesics. Twenty patients experienced slight anal pain after operation, and only 4 patients received analgesics. Twenty-three patients were able to walk within 2 d. Five patients had postoperative complications. One patient had anastomotic fracture, followed by Hartmann operation (the patient developed an anastomotic rupture followed by a pelvic infection. Despite the prophylactic ileostomy, the patient developed proximal colonic retraction, which was followed by Hartmann operation). One patient had intestinal obstruction, which was confirmed by abdominal X-ray on the postoperative day 8, and he was cured by indwelling gastric tube methods. Three patients developed fever within 7 d after taTME operation, and were diagnosed as pelvic infection. After antibiotic treatment, pelvic infection was cured. No patient had

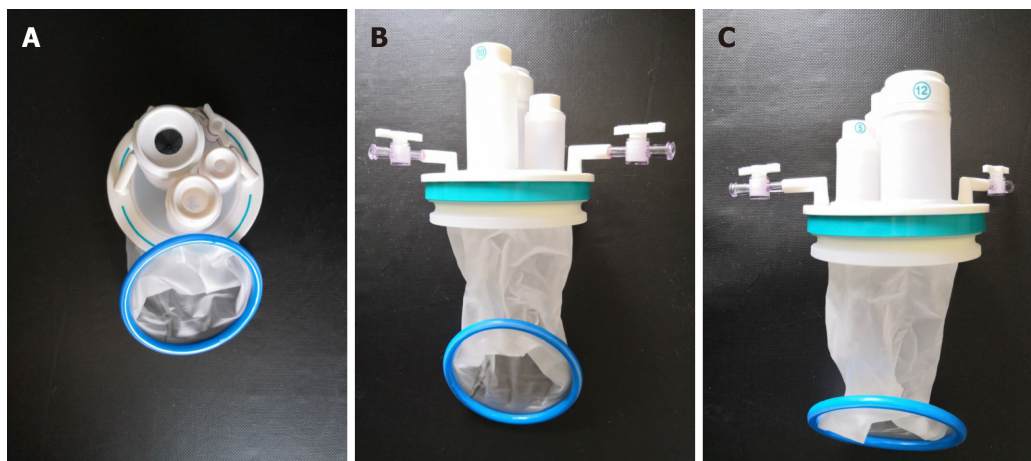


Figure 2 Photos of the transanal STARPORT used in transanal total mesorectal excision surgery. The transanal STARPORT consists of an air-tight cover and an anal dilator. A: Positive view; B: Back view; C: Side view.

cancer recurrence during the follow-up period. Two of the 24 patients had fecal incontinence after the operation; the other patients could control their defecation easily.

DISCUSSION

Our primary aim for the taTME technique was to gain experience with this novel surgical approach, especially in patients with lower and midrectal cancer, as we believe the technique will potentially improve the surgical and oncologic outcome in selected cases (obesity, male patients, narrow pelvis, neoadjuvant therapy). TaTME is a new technique that seems to provide technical advantages. Laparoscopy-assisted taTME is also known as trans-abdominal taTME or hybrid-natural orifice transluminal endoscopic surgery (NOTES) taTME. Laparoscopy-assisted taTME is a combination of techniques, such as minimally invasive surgery, intersphincteric resection (ISR), natural orifice specimen extraction (NOSE), minimally invasive surgery, and ultralow-level preservation of the anus^[6].

The quality of our specimens seems to be equivalent to the series by Nagtegaal *et al*^[10]. All the specimens were intact and achieved good quality. Shortterm results were satisfactory with a low morbidity and consequently a median length of stay of 9.2 d and no mortality.

The results of our initial cases presented in this paper were obtained during our learning curve and as we continue to learn by each procedure performed, we do not expect to have reached the top of the curve yet. Our initial experience confirmed our impression during the preparation phase that the technique presents many challenges both operatively and in the use of the necessary equipment. Having tried out the procedure in more recent cases with an instrument that provides continuous low flow carbon dioxide gas inflation, we found that it eases the procedure remarkably.

We presented the case of a young patient with lower bulky rectal cancer who highlighted the possibility of abdominoperineal resection (Figure 3). A 30-year-old stout man was diagnosed in October 2017 with stage IIIA lower rectal bulky mucinous adenocarcinoma and treated with preoperative chemoradiotherapy and subsequently with four cycles of capecitabine and oxaliplatin. After neoadjuvant therapy, the tumor size remained unchanged with mucus components. It was difficult to preserve the anus using the routine surgical technique. We successfully completed taTME operation for this patient, and found that the tumor was 3 cm from the anus and the anus was retained. Postoperative evaluation of the anal pressure function and urinary and sexual function was normal. Postoperative pathology indicated PCR of the tumor. Recently, the patient had reversal of an ileostomy operation. The advantages of taTME for patients with lower rectal cancer are significant, especially those with obesity, male, narrow pelvis and neoadjuvant therapy. TaTME is helpful for preservation of the anal sphincter.

TaTME can be relatively easy to complete free and low rectum and mesorectum excision to guarantee the quality of TME-resected specimens, and may have

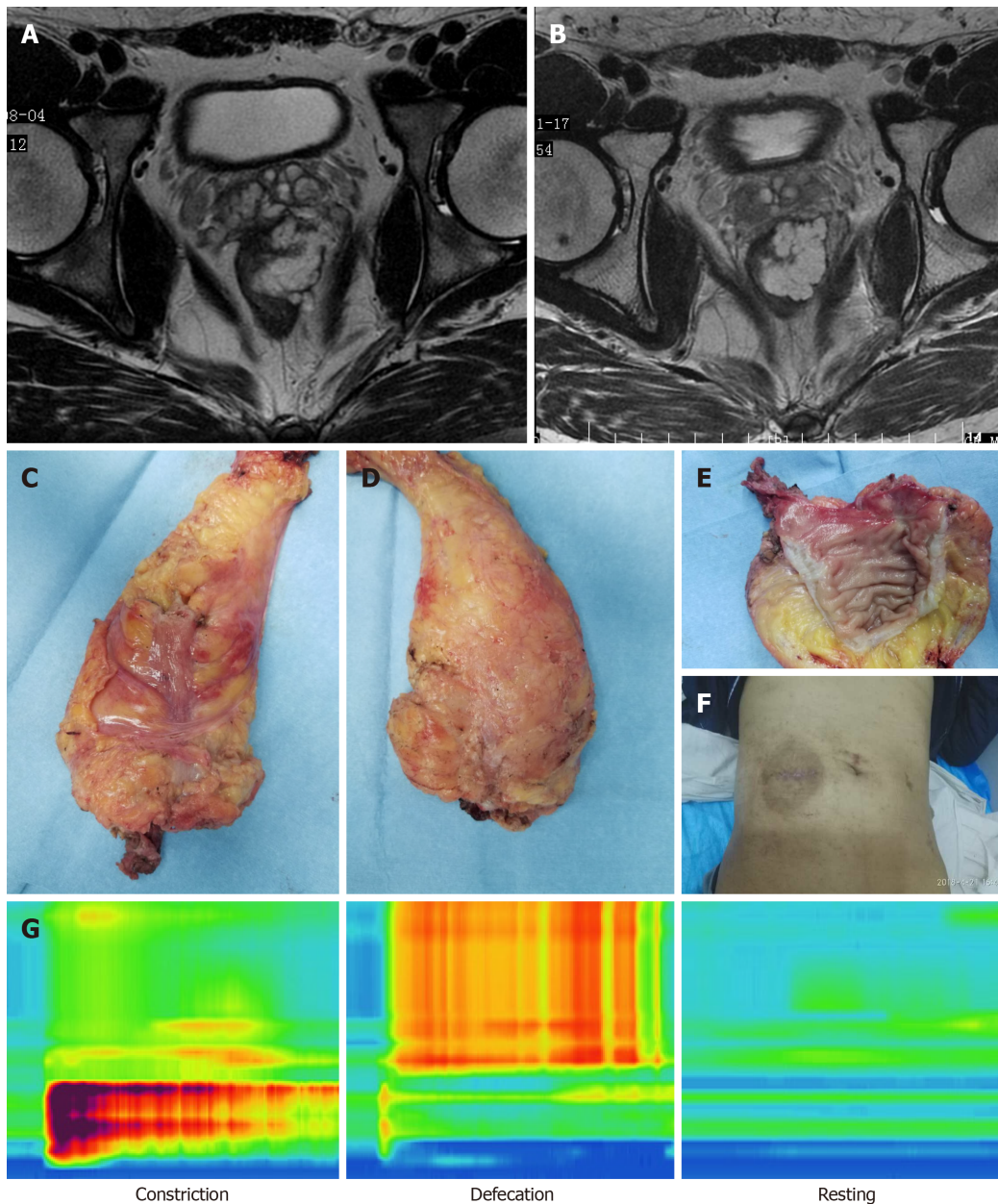


Figure 3 One case of a 30-year-old male patient. A: Magnetic resonance image before neoadjuvant therapy; B: Magnetic resonance image after neoadjuvant therapy; C: Front view of specimen; D: Back view of specimen; E: Specimen; F: Abdominal appearance; G: Anal pressure measurement and pressure variation diagram (constriction pressure $[121.0 \pm 11.6 \text{ mmHg}]$, defecation pressure, resting pressure $[41.5 \pm 8.6 \text{ mmHg}]$).

advantages of NOTES, NOSE without abdominal incision, ISR, and single-port laparoscopic minimally invasive technique^[11,12]. However, the technological requirements of taTME are high, the learning curve is long, and the operation is difficult. It has been reported that taTME has an effect on postoperative short-term voiding function and anal function^[13,14]. Whether there are long-term effects needs further observation. Notably, the longest duration of taTME surgery in this study was 402 min. The main reason is that the operation was performed by one group of doctors between transabdominal and transanal parts, and the operation platform was constantly changed. However, theoretically taTME can be performed by the transabdominal group and the ta group at the same time, so it is possible to reduce the operation time. After we adjusted the procedure (Figure 4), the overall operation time was reduced. In the abdominal operation group pelvic autonomic nerve preservation was performed, and the nerves were better protected than using the pure NOTES taTME. According to the 24 patients with operation time, average operation time was 310 min. After optimizing the operation process and adjusting the surgical approach, the average operation time was shortened to 240 min, and the learning curve was stable.

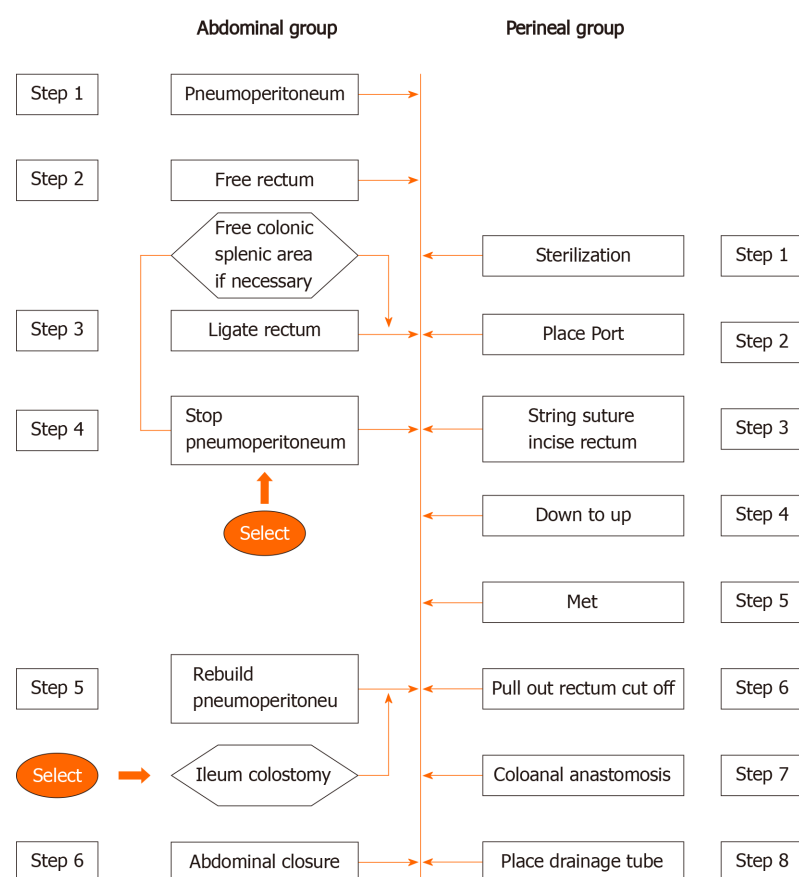


Figure 4 The laparoscopy-assisted transanal total mesorectal excision was divided into the transabdominal and transanal parts. This operation can make full use of the advantages of transabdominal and transanal surgery. Laparoscopic surgery can complete laparoscopic exploration, vascular ligation and lymph node dissection, middle and upper mesentery dissociation, while transanal surgery can complete the lower mesentery migration and specimen removal, and then complete abdominal and transanal anastomosis reconstruction.

Concerns about this technique focus on bacterial contamination and tumor cell contamination^[15,16]. However, several studies can help to remove these doubts^[12,16]. For example, it has been confirmed that intracorporeal bowel opening for anastomosis completion does not increase the risk of infection during colorectal surgery. Tumor cell contamination can be prevented by suturing predetermined margins and closing the intestinal cavity to isolate the tumor. From our follow-up data, we confirmed that surgical procedures on the anus had no influence on bacterial and tumor cell contamination.

As a new technique applied in clinic, safety is the most important. Even at the beginning of the learning curve, when the risk of complications is high, safety is particularly important. The same is true for taTME. In this study, the safety of taTME was verified by the amount of blood loss, postoperative complications, postoperative mortality and postoperative hospital stay. In the taTME group, the average blood loss of patients undergoing surgical treatment was less than 100 mL, which was not significantly different from the traditional laparoscopic surgery group and the open surgery group. In the first 20 taTME surgeries performed by Atallah *et al*^[15], the average patient lost 153 mL of blood. In 720 samples enrolled study, 61.2% of patients had intraoperative blood loss less than 100 mL, and 1% had intraoperative blood loss greater than 1 L^[11]. From the perspective of complications, there were 5 complications in this group. The most serious patient had anastomotic rupture and therefore received sigmoidostomy (Hartmann operation) for treatment; 1 patient had postoperative intestinal obstruction which was improved by conservative treatment and 3 patients had fever and other infection symptoms which were controlled by antibiotic treatment. Burke *et al*^[14] studied 50 patients in their early taTME study and found that 12% needed a second operation within 30 d of surgery. The main causes of secondary operation were ileostomy dysfunction, pelvic effusion and anastomotic leakage^[15]. In this study, no postoperative death occurred in the taTME group. Penna *et al*^[11] conducted a study with 720 samples, showing that the postoperative mortality rate was 0.5%, suggesting that taTME is a safe surgical method.

At present, taTME is mainly suitable for malignant tumors requiring accurate anatomy and resection of the middle and lower rectum and mesangial. The indications of taTME for the treatment of malignant rectal tumors should be limited to low and medium rectal cancers, especially low rectal cancers. TaTME may be more advantageous for rectal cancer patients with “difficult pelvis,” such as male, prostatic hypertrophy, obesity, tumor diameter of > 4 cm, rectal mesangial hypertrophy, lower anterior rectal wall tumor, pelvic stenosis, and unclear tissue plane caused by neoadjuvant radiotherapy.

CONCLUSION

Laparoscopy-assisted taTME is suitable for selected patients with lower rectal cancer. Bulky tumor, obesity, male, narrow pelvis, neoadjuvant therapy, and the lower position of the tumor may be the indications to perform this technique. The short-term outcomes of this technique are adjudged to be satisfactory. Laparoscopy-assisted taTME is safe and feasible for patients with lower rectal cancer, and this technique is worthy of further recommendation. Of course, our data are preliminary and the clinical outcome of taTME technique must be confirmed through more cases.

ARTICLE HIGHLIGHTS

Research background

Transanal total mesorectal excision (taTME) is a new technique that might have many technical advantages. Laparoscopy-assisted taTME is also known as transabdominal taTME or hybrid-natural orifice transluminal endoscopic surgery taTME. Laparoscopy-assisted taTME is a combination of techniques, such as minimally invasive surgery, intersphincter-assisted resection, natural orifice extraction, ta minimally invasive surgery, and ultralow-level preservation of the anus.

Research motivation

Laparoscopy-assisted taTME surgery was reported by literature with relatively small amount of cases. However, there has been little published data on laparoscopy-assisted taTME surgery on the Chinese population. The safety and feasibility of laparoscopy-assisted taTME is still lack of report.

Research objectives

This study was designed to investigate the utility of laparoscopy-assisted taTME technique with both favorable and unfavorable factors.

Research methods

Laparoscopy-assisted taTME surgery was done by a standard laparoscopic platform (STARPORT Port). Patients' characteristics, surgery duration, pathological diagnosis and postoperative complications (Clavien-Dindo classification) were collected.

Research results

Laparoscopy-assisted taTME could be safe and feasible technique to rectal tumor. Laparoscopic surgeons would be proficient for laparoscopy-assisted taTME with approximately 20 cases. Laparoscopy-assisted taTME may provide an alternative to traditional surgical methods for accurate anal retention. This study demonstrated the first piece of evidence of peri-operative data and short-term outcome in patients treated with laparoscopy-assisted taTME in Chinese tertiary hospital.

Research conclusions

Laparoscopy-assisted taTME is suitable for selected patients with lower rectal cancer, and this technique is worthy of further recommendation.

Research perspectives

At present, taTME is mainly suitable for malignant tumors requiring accurate anatomy and resection of the middle and lower rectum and mesangial. The indications of taTME for the treatment of malignant rectal tumors should be limited to low and medium rectal cancers, especially low rectal cancers. TaTME may be more

advantageous for rectal cancer patients with “difficult pelvis,” such as male, prostatic hypertrophy, obesity, tumor diameter of > 4 cm, rectal mesangial hypertrophy, lower anterior rectal tumor, anterior rectal wall tumor, narrow pelvic, and unclear tissue, and unclear tissue plane caused by neoadjuvant radiotherapy. In addition, taTME can be performed in combination with sphincter resection (ISR) for ultra-low rectal cancer patients. TaTME surgery may have indications for the treatment of colorectal benign diseases: Large benign tumors of the middle and lower rectum that cannot be removed locally, inflammatory bowel disease requiring rectal excision, familial adenomatous polyposis, and radioactive proctitis.

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Retrospective Study

Survival outcomes and prognostic indicators for gastric cancer patients with positive peritoneal wash cytology but no peritoneal metastasis after radical gastrectomy

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Abstract

BACKGROUND

Positive peritoneal wash cytology with no peritoneal metastasis (CY1P0) is a special type of distant gastric cancer metastasis, which describes a patient with positive peritoneal lavage cytology, but no definitive peritoneal metastasis, and there are no widely accepted treatment guidelines. We enrolled 48 primary CY1P0 gastric cancer patients treated by radical gastrectomy in this study. Our study illustrated the efficacy of radical gastrectomy for CY1P0 gastric cancer patients, and suggested that the pathological N factor and vascular invasion were significant independent risk factors for overall survival (OS).

AIM

To assess the survival of CY1P0 gastric cancer patient post-radical gastrectomy, and to identify factors associated with long-term prognosis.

METHODS

Our study included 48 patients with primary CY1P0 gastric cancer who had radical gastrectomies at the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China between 2013 and 2018. R0 resection was achieved in all 48 patients. Twelve patients received neoadjuvant chemotherapy. Thirty patients

were anonymously analyzed.

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at tyt67@163.com.

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received adjuvant chemotherapy and four received adjuvant chemoradiotherapy. OS statistics were available for 48 patients. Follow-up continued through March 2020. Univariate and multivariate analyses were performed using a Cox proportional hazards model to identify prognostic factors.

RESULTS

Median OS was 22.0 mo (95% confidence interval: 13.366-30.634 mo) post-surgery. Univariate analyses demonstrated that tumor site ($P = 0.021$), pathological N factor ($P = 0.001$), pathological T factor ($P = 0.028$), vascular invasion ($P = 0.046$), and the level of CA199 prior to initiating therapy ($P = 0.002$) were significant risk factors for OS. Multivariate analyses demonstrated that pathological N factor ($P = 0.001$) and vascular invasion ($P = 0.031$) were significant independent risk factors for OS.

CONCLUSION

This study suggested that radical gastrectomy may be efficient for CY1P0 gastric cancer patient post-radical gastrectomy and the pathological N factor and vascular invasion are significant independent risk factors for OS.

Key Words: Gastric cancer; Overall survival; R0 resection; Prognostic factors; Lymph node metastasis

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Core Tip: This is a retrospective study to investigate the survival of gastric cancer patients with positive peritoneal wash cytology but no peritoneal metastasis post-radical gastrectomy and to identify factors associated with long-term prognosis. Our study included 48 such patients and demonstrated that more effective treatment should be established for patients who are diagnosed with pN3b disease and vascular invasion.

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INTRODUCTION

Gastric cancer is one of the most common malignant tumors worldwide. There are no specific symptoms in early-stage gastric cancer, and when patients are diagnosed, the disease is usually advanced, and may even have metastasized. Advanced gastric cancer often metastasizes to the peritoneum, and metastasis is the main cause of disease-related death^[1]. Peritoneal lavage cytology has been widely used to stage gastric cancer^[2]. Positive peritoneal wash cytology with no peritoneal metastasis (CY1P0) is a special type of distant gastric cancer metastasis, which describes a patient with positive peritoneal lavage cytology, but no definitive peritoneal metastasis. The Japanese Classifications of Gastric Carcinoma define this as stage IV disease^[3]. Although the American Joint Committee on Cancer guidelines for gastric cancer (eighth edition) clearly state that CY1P0 is equivalent to M1 disease^[4], it was still potentially treatable. Positive intraperitoneal free cancer cells are an important risk factor for postoperative intraperitoneal recurrence and metastasis in patients with gastric cancer^[5]. Positive peritoneal lavage cytology is a predictor of peritoneal dissemination^[6] and poor prognosis^[7-10].

Currently, there are no widely accepted treatment guidelines for CY1P0 gastric cancer patients^[11]. Some retrospective studies have demonstrated the efficacy of radical surgery combined with intraoperative chemotherapy and systemic chemotherapy; however, larger, randomized, controlled clinical studies are needed to standardize the treatment of CY1P0 patients and to develop relevant guidelines. The aim of this study

was to evaluate the effect of radical gastrectomy on the survival of CY1P0 gastric cancer patients and to identify risk factors associated with prognosis.

MATERIALS AND METHODS

Patients

This retrospective study included 48 patients with primary CY1P0 gastric cancer who had radical gastrectomy at the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China between 2013 and 2018. All patients were diagnosed with gastric adenocarcinoma (with no peritoneal metastasis or distant metastasis). All patients underwent abdominal lavage before surgery, and in all cases, they had positive peritoneal lavage cytology, but no definitive peritoneal metastasis. R0 resection was achieved in all 48 patients. Patients who had undergone palliative surgery or received only chemoradiotherapy were excluded from the study.

Treatment

All patients underwent gastroscopy and computed tomography (CT) examination to assess their condition. Because of advanced disease or suspected lymph node metastasis, 12 patients received neoadjuvant chemotherapy. All 48 patients had radical surgery and D2 lymph node dissection. The cytological examination of the peritoneal lavage samples was performed before surgery. Thirty patients received adjuvant chemotherapy and four received adjuvant chemoradiotherapy.

Followup

Patients reported for follow-up every 3 mo to the out-patient department. Follow-up included physical examination, routine blood work, blood biochemistry, and tumor biomarkers including CEA, CA724, CA242, AFP, and CA19-9. CT examination and endoscopy were performed every 6 mo. Hematological tests were performed at least every 2 wk during chemoradiotherapy. Disease progression, unacceptable adverse events, and patient death were recorded. We regularly followed the patients by telephone to ensure that we had up-to-date information for all patients. Follow-up continued through March 2020. Overall survival (OS) was measured from the time of surgery.

Statistical analysis

Cumulative survival rates were obtained using the Kaplan-Meier method and were compared using the log-rank test to evaluate statistically significant differences. Cox proportional hazard regression analysis was employed to evaluate factors affecting OS. A *P* value < 0.05 was considered statistically significant. Statistical analyses were performed with statistic package for social science for windows, version 22.0.

RESULTS

The clinical characteristics of the patients are shown in Table 1. Neoadjuvant chemotherapy was administered to 12 patients (Table 2) and the types of surgery are listed in Table 3. Thirty-four patients received adjuvant therapy; 30 patients received adjuvant chemotherapy and 4 received adjuvant chemoradiotherapy (Table 4). A flow diagram of the 48 patients who underwent radical gastrectomy is shown in Figure 1. OS was measured from the time of surgery. For the 48 CY1P0 patients, median OS was 22.0 mo [95% confidence interval (CI): 13.366–30.634 mo] (Figure 2). The 1-, 2-, 3-, and 5-year OS rates were 72.4%, 47.8%, 32.9%, and 20.5%, respectively. Median recurrence-free survival was 16.5 mo (95%CI: 5.141–27.859 mo) (Figure 3). Median follow-up was 35.0 mo. Univariate analysis showed that tumor site (*P* = 0.021), pathological N factor (*P* = 0.001), pathological T factor (*P* = 0.028), vascular invasion (*P* = 0.046), and the level of CA199 prior to initiating therapy (*P* = 0.002) were significant risk factors for OS (Table 5). Compared with gastric cardia cancer and gastric body cancer, gastric antrum tumors had better prognosis [odds ratio (OR): 0.427; 95%CI: 0.207–0.880; *P* = 0.021]. Pathological N factor in 3b (OR: 4.194; 95%CI: 1.870–9.406; *P* = 0.001) and T factor in 4a–4b (OR: 5.008; 95%CI: 1.190–21.072; *P* = 0.028) correlated with poor OS rate. Patients with vascular invasion had a poor prognosis (OR: 2.554; 95%CI: 1.017–6.413; *P* = 0.046). Patients with normal CA199 levels before treatment had a better prognosis (OR: 0.267; 95%CI: 0.118–0.604; *P* = 0.002). Multivariate analysis was performed based on factors

Table 1 Patient characteristics (n = 48)

Characteristic	Patients
Age	
< 60 yr	23
≥ 60 yr	25
Smoking history	
Yes	26
No	22
Drinking history	
Yes	26
No	22
Family history	
Yes	18
No	30
Treatment	
Surgery	48
Neoadjuvant chemotherapy	12
Adjuvant chemotherapy	30
Adjuvant chemoradiotherapy	4
Site of tumor	
Upper	5
Middle	16
Lower	27
Bormann classification	
Type 1	3
Type 2	9
Type 3	20
Type 4	12
Lauren's classification	
Type 1	9
Type 2	22
Type 3	13
Pathological N factor	
0-3a	25
3b	23
Pathological T factor	
0-3	8
4a-4b	38

Lauren's classification: Type 1: Intestinal-type adenocarcinoma; Type 2: Diffuse adenocarcinoma; Type 3: Mixed adenocarcinoma.

with $P < 0.1$ in the univariate analysis (Table 6). Pathological N factor ($P = 0.001$) and vascular invasion ($P = 0.031$) were identified to be significant independent risk factors for OS (Figures 4 and 5).

DISCUSSION

CY1P0 is a special type of distant gastric cancer metastasis, which describes a patient with positive peritoneal lavage cytology, but no definitive peritoneal metastasis. Currently, there are no widely accepted treatment guidelines for CY1P0 gastric cancer patients^[11].

The positive rate of peritoneal lavage cytology of Japanese patients with gastric carcinoma is approximately 5%–20%^[12,13]. In this study, we assessed the survival of CY1P0 gastric cancer patients and sought to identify prognostic risk factors. We performed surgery on 48 patients with positive peritoneal lavage cytology but without peritoneal metastases. Median OS was 22.0 mo. The 1-, 2-, 3-, and 5-year OS rates were 72.4%, 47.8%, 32.9%, and 20.5%, respectively. It was reported in another study that the 5-year OS was 17.6% for CY1 gastric cancer patients^[11], while the OS of patients who received chemotherapy alone was 9.9–12.6 mo^[7,14]. These results suggest that radical gastrectomy is effective, and surgery is the most crucial component of this conversion therapy^[15]. However, OS of the 36 patients who received neoadjuvant or adjuvant therapy combined with surgery was no better than that of the 12 patients who had surgery alone ($P = 0.112$). We hypothesize that this may be because the disease had progressed further in patients who received combined therapy; however, since CY1 represents peritoneal seeding, we believe that chemotherapy after surgery is warranted. The univariate and multivariate Cox proportional hazard analyses of the clinicopathological factors associated with OS showed that the lymph node metastasis status affected the OS of CY1-only gastric cancer patients who underwent radical gastrectomy. In addition, pathological pN3b is an indicator of distant nodal metastasis^[6]. A more effective treatment should be established for patients who are diagnosed with CY1 and pN3b disease. Similarly, vascular invasion is an important prognostic factor, and these patients require further treatment and regular review. Several previous publications demonstrated that Borrmann type-4 tumors are negatively associated with survival and prognosis of this population. Noda *et al.*^[16] evaluated the survival of 91 CY1P0 patients with Borrmann type-4 tumors. They found that the 5-year OS rate of these patients was 6.3%, while that of patients with other types of tumors was 27.7%. In another study, researchers assessed clinicopathological features associated with prognosis in 37 CY1P0 gastric cancer patients^[10]. A multiple linear regression analysis revealed that Borrmann type-4 tumors were an independent predictor of poor prognosis; however, Borrmann type-4 tumors were not prognostic in the current study ($P = 0.416$). In the univariate analysis, tumor site ($P = 0.021$) and the level of CA199 before therapy ($P = 0.002$) were risk factors for OS. These two factors were not statistically significant in the multivariate analysis. In general, cardia cancer and gastric body cancer have worse prognoses and require more difficult surgical procedures, and postoperative tumor marker levels are useful during follow-up. Changes in the levels of tumor markers may be associated with tumor recurrence. At present, radical gastrectomy, regional radiotherapy, and adjuvant antitumor chemotherapy have been proven effective for the treatment of advanced gastric cancer^[17]. Standard treatment for gastric cancer patients with distant metastasis is systemic chemotherapy. Conversion therapy provides a new approach for the treatment of patients with advanced gastric cancer^[18]. In one study, Japanese researchers treated 41 patients with peritoneal metastasis (30 of whom were positive for free abdominal cancer cells) with S-1 combined with cisplatin. The treatment eradicated peritoneal metastasis in 19 patients. After radical surgery, median survival of these patients increased from 12.6 mo to 43.2 mo^[7]. Patients with good therapeutic effect may selectively benefit from radical surgery. Another study came to the same conclusion. Patients received systemic chemotherapy combined with S-1+ paclitaxel intraperitoneal infusion chemotherapy. Then, if free tumor cells were not detected in the abdominal cavity, the patients had radical surgery. The safety of surgeries was acceptable and postoperative prognosis of the patients improved^[15]. Radical surgery combined with postoperative adjuvant chemotherapy has been widely used in patients with advanced gastric cancer for some time. Some clinical studies have confirmed the significance of S-1 adjuvant chemotherapy in CY1P0 patients after radical surgery. Kano *et al.*^[2] found that the median OS survival of 36 CY1P0 patients who underwent radical surgery and postoperative S1 monotherapy was 22.3 mo. In

Table 2 Neoadjuvant chemotherapy

Neoadjuvant chemotherapy regimen	Patients
SOX	5
DOS	1
XELOX	1
PTX + L-OHP + S-1	2
Paclitaxel liposome + L-OHP + S-1	1
PTX + DDP + S-1	1
DXT + S-1/5-Fu + L-OHP + CPT-11	1

Table 3 Types of surgery

Surgery type	Patients
Laparoscopic assist distal gastrectomy + D2	16
Laparoscopic assist total gastrectomy + D2	9
Traditional distal gastrectomy + D2	11
Traditional total gastrectomy + D2	10
Laparoscopic assisted proximal gastrectomy + splenectomy + D2	1
Laparoscopic assisted proximal gastrectomy + D2	1

Table 4 Adjuvant chemotherapy

Adjuvant chemotherapy regimen	Patients
SOX	12
SOX + radiotherapy	4
XELOX	3
Paclitaxel liposome + L-OHP + S-	1
S-1	2
PTX + 5-Fu + L-OHP	1
DXT + S-1	1
PTX + CAP	1
DXT + L-OHP + CAP	1
S-1 + DDP	2
Unknown	6

another study, long-term follow-up of CY1P0 patients who underwent D2 radical surgery and postoperative S1 monotherapy demonstrated a 2-year survival rate of 46%, a 5-year OS rate of 26%, and a relapse-free survival rate of 21%, which exceeded the researchers' expectations^[19]. Therefore, further studies were conducted. The effect of preoperative neoadjuvant chemotherapy was assessed in CY1P0 patients who had radical surgery and S1 single-drug adjuvant chemotherapy after surgery. The 5-year survival rate was 15%, with or without preoperative neoadjuvant chemotherapy. This study also showed that preoperative chemotherapy efficacy and lymph node involvement significantly impacted patient prognosis^[20]. As researchers explore more aggressive treatments, hyperthermic intraperitoneal chemotherapy (HIPEC) shows unique application prospects, and multiple basic and clinical studies have confirmed the safety and effectiveness of HIPEC. HIPEC is a highly selective regional chemotherapy, characterized by high local drug concentrations, long duration of action, direct effects on tumor cells, synergy of chemotherapy and thermal effect, and

Table 5 Univariate analysis of the risk factors for overall survival

Patient characteristic		OR	95%CI	P value
Age				0.588
< 60 yr	23	1.000		
≥ 60 yr	25	0.822	0.404-1.671	
Smoking history				0.935
Yes	26	1.000		
No	22	0.971	0.476-1.979	
Drinking history				0.137
Yes	26	1.000		
No	22	1.726	0.841-3.540	
Site of tumor				0.021
Upper/Middle	21	1.000		
Lower	27	0.427	0.207-0.880	
Signet-ring cell				0.229
Yes	19	1.000		
No	28	0.640	0.309-1.325	
Bormann classification				0.416
Type1/2/3	32	1.000		
Type4	12	1.431	0.603-3.392	
Lauren's classification				0.080
Type 1	9	1.000		
Type 2/Type 3	35	2.588	0.892-7.508	
Pathological N factor				0.001
0-3a	24	1.000		
3b	22	4.194	1.870-9.406	
Pathological T factor				0.028
0-3	9	1.000		
4a-4b	37	5.008	1.190-21.072	
Vascular invasion				0.046
Negative	12	1.000		
Positive	34	2.554	1.017-6.413	
CA199				0.002
Elevate	13	1.000		
Normal	31	0.267	0.118-0.604	
CEA				0.837
Elevated	13			
Normal	32	0.917	0.403-2.089	
Therapy				0.112
Surgery along	12			
Combined therapy	36	0.540	0.252-1.154	

OR: Odds ratio; CI: Confidence interval.

negligible systemic toxicity and side effects, which has obvious advantages over traditional, peripheral venous chemotherapy. Results of a meta-analysis also showed that surgery combined with intraperitoneal chemotherapy increased the 5-year survival rate of CY1P0 patients and reduced the risk of recurrence compared to surgery alone. These benefits could be further increased when combined with extensive intraoperative peritoneal lavage therapy^[21]. Extensive intraoperative peritoneal lavage therapy is another effective means to reduce the number of free cancer cells in the abdomen, which can significantly improve the postoperative survival rate of CY1P0 gastric cancer patients^[22]. A study of 37 CY1P0 patients showed a 5-year survival rate of 46.5% after radical surgery and extensive intraoperative peritoneal lavage. This prognosis is similar to that of gastric cancer patients receiving the same treatment at stage III B and III C, which means that these patients achieved a reduction in tumor staging^[23]. Phase II clinical studies have shown that the combination of intravenous and abdominal injections of paclitaxel and S-1 to treat gastric cancer patients with peritoneal metastasis is effective, providing a new idea for the treatment of CY1P0 patients^[24].

Our study has several potential limitations. First, different types of surgery may result in different OS; however, we did not investigate the effect of different surgical procedures on prognosis. Second, we did not assess the effects of postoperative complications. The safety of such procedures and the incidence of complications associated with these procedures should be evaluated. Third, since this was a retrospective study, there were no data available that recorded the effect of neoadjuvant chemotherapy prior to surgery. Some studies report that surgery after response to systemic chemotherapy is safe and may prolong the survival of gastric cancer patients^[15]. A prospective, randomized controlled trial or a large cohort study should be conducted to verify the efficacy of surgery for gastric cancer patients with positive peritoneal cytology findings. Yamashita *et al*^[11] reported that preoperative serum albumin levels may be a predictive factor for CY1 gastric cancer patients. Finally, in this study, we did not consider the effects of preoperative nutritional status, biochemical indicators, and complications on prognosis. Currently, most studies evaluating the treatment of CY1P0 patients are retrospective and have small sample sizes. Some guidelines recommend treating these patients using guidelines for patients with recurrent or metastatic gastric cancer. We found that for eligible CY1P0 gastric cancer patients, radical surgery combined with intraoperative chemotherapy and systemic chemotherapy is effective; however, the precise timing, indications, and surgical methods for patients undergoing translational therapy have not been determined. Usually, abdominal lavage fluid is assessed for the presence of free tumor cells after neoadjuvant chemotherapy. The absence of tumor cells in the lavage fluid reflects effective conversion therapy, and patients with this result should be considered for radical surgical resection. For eligible CY1P0 gastric cancer patients, we recommend multidisciplinary MDT discussions, the development of individualized treatment regimens, and participation in clinical studies. With a growing list of new drugs and the maturation of HIPEC and other technologies, cancer cells found during abdominal lavage may not always prognose disease-related mortality for CY1P0 gastric cancer patients.

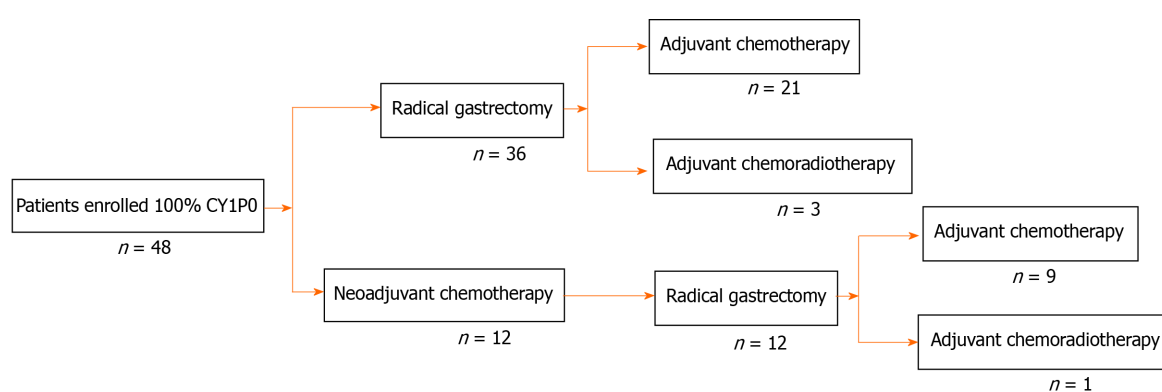
CONCLUSION

In conclusion, this study illustrated the efficacy of radical gastrectomy for CY1P0 gastric cancer patients. More effective treatment should be established for patients who are diagnosed with pN3b disease and vascular invasion. We look forward to the insights offered by future, prospective, randomized, controlled clinical studies with larger sample sizes to verify the efficacy of radical surgery and to standardize the recommendations for the treatment of patients with CY1P0 gastric cancer.

Table 6 Multivariate analysis of the risk factors for overall survival ($N = 39$, $n = 25$)

Patient characteristic		OR	95%CI	P value
Site of tumor				0.105
Upper/Middle	18			
Lower	21			
Lauren's classification				0.476
Type 1	8			
Type 2/Type 3	31			
Pathological N factor				0.001
0-3a	19	1.000		
3b	20	5.365	1.971-14.609	
Pathological T factor				0.146
0-3	9			
4a-4b	30			
Vascular invasion				0.031
Negative	10	1.000		
Positive	29	3.660	1.124-11.917	
CA199				0.789
Elevated	12			
Normal	27			

n : The number of subjects who died; OR: Odds ratio; CI: Confidence interval.

**Figure 1** Flow diagram of the 48 patients who underwent radical gastrectomy.

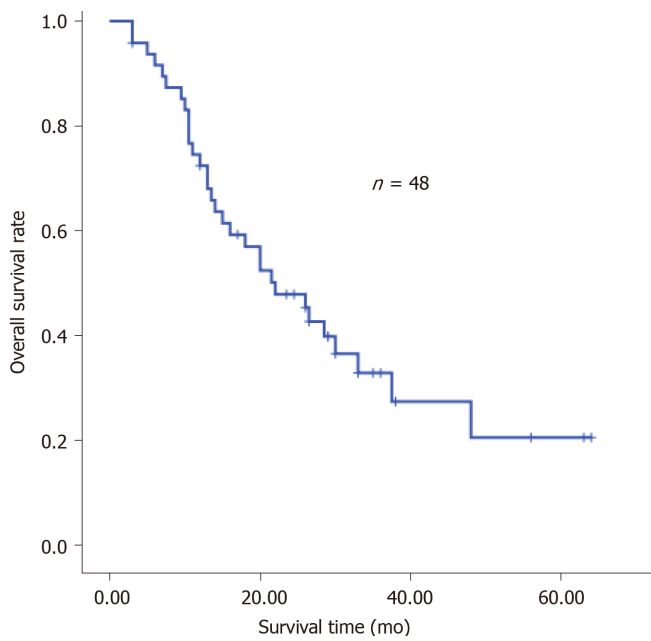


Figure 2 Overall survival for the 48 patients who underwent radical gastrectomy.

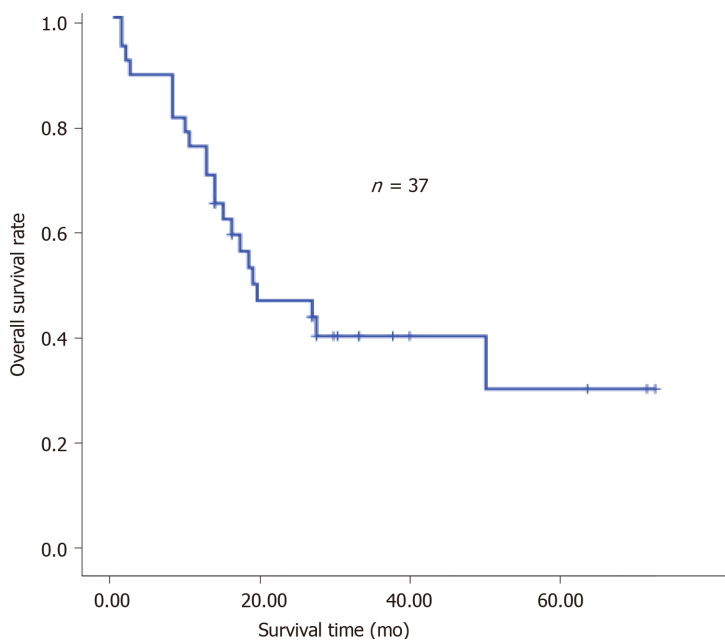


Figure 3 Recurrence free survival.

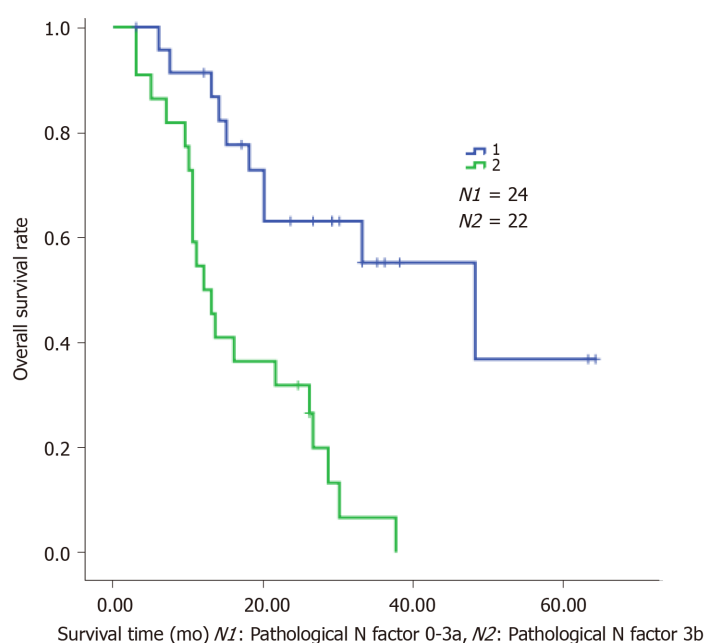


Figure 4 Pathological N factor: 0-3a/3b. *N1*: Pathological N factor 0-3a, *N2*: Pathological N factor 3b.

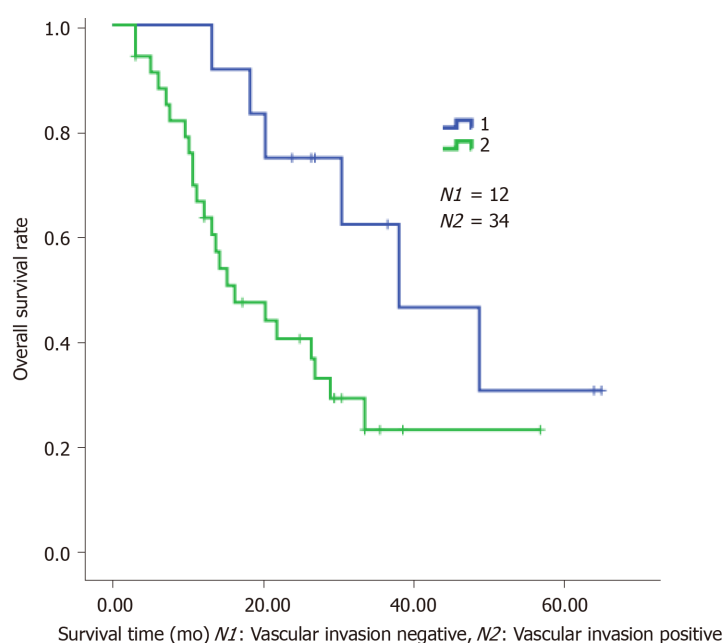


Figure 5 Vascular invasion: Negative/Positive. *N1*: Vascular invasion Negative, *N2*: Vascular invasion Positive.

ARTICLE HIGHLIGHTS

Research background

Positive peritoneal wash cytology with no peritoneal metastasis (CY1P0) is a special distant metastasis of gastric cancer, and currently there are no extensive treatment guidelines for patients with CY1P0 gastric cancer.

Research motivation

To assess survival after radical gastrectomy for CY1P0 gastric cancer and to identify factors associated with long-term prognosis.

Research objectives

To evaluate the effect of radical gastrectomy on survival in patients with CY1P0 gastric cancer, and to identify prognostic risk factors.

Research methods

Our study included 48 patients with primary CY1P0 gastric cancer who had radical gastrectomies. Overall survival (OS) statistics were available for 48 patients. Follow-up continued through March 2020. Univariate and multivariate analyses were performed using a Cox proportional hazards model to identify prognostic factors.

Research results

For the 48 CY1P0 patients, median OS was 22.0 mo, while the OS of patients who received chemotherapy alone was 9.9-12.6 mo. Pathological N factor ($P = 0.001$) and vascular invasion ($P = 0.031$) were significant independent risk factors for OS.

Research conclusions

This study illustrated the efficacy of radical gastrectomy for CY1P0 gastric cancer patients. More effective treatment should be established for patients who are diagnosed with pN3b disease and vascular invasion.

Research perspectives

To formulate the standard treatment plan for CY1P0 gastric cancer.

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Observational Study

Mining The Cancer Genome Atlas database for tumor mutation burden and its clinical implications in gastric cancer

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Author contributions: Zhao DY conceived and designed the study and wrote the manuscript; Sun XZ took part in analyzing the data; Yao SK designed the study, revised the manuscript, and obtained the funding; all authors read and approved the final manuscript.

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Institutional review board statement: This study was approved by the Ethics Committee of China-Japan Friendship Hospital (No. 2018-116-K85-1).

Informed consent statement: All the data were obtained from The Cancer Genome Atlas (TCGA, <http://portal.gdc.cancer.gov/repository>) database in our study. TCGA database is freely available open to the public, so there is no requirement for additional informed consent statement.

Conflict-of-interest statement: All authors report no conflicts of interest.

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Abstract

BACKGROUND

Tumor mutational burden (TMB) is an important independent biomarker for the response to immunotherapy in multiple cancers. However, the clinical implications of TMB in gastric cancer (GC) have not been fully elucidated.

AIM

To explore the landscape of mutation profiles and determine the correlation between TMB and microRNA (miRNA) expression in GC.

METHODS

Genomic, transcriptomic, and clinical data from The Cancer Genome Atlas were used to obtain mutational profiles and investigate the statistical correlation between mutational burden and the overall survival of GC patients. The difference in immune infiltration between high- and low-TMB subgroups was evaluated by Wilcoxon rank-sum test. Furthermore, miRNAs differentially expressed between the high- and low-TMB subgroups were identified and the least absolute shrinkage and selection operator method was employed to construct a miRNA-based signature for TMB prediction. The biological functions of the predictive miRNAs were identified with DIANA-miRPath v3.0.

RESULTS

C>T single nucleotide mutations exhibited the highest mutation incidence, and the top three mutated genes were *TTN*, *TP53*, and *MUC16* in GC. High TMB values (top 20%) were markedly correlated with better survival outcome, and multivariable regression analysis indicated that TMB remained prognostic independent of TNM stage, histological grade, age, and gender. Different TMB levels exhibited different immune infiltration patterns. Significant differences

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between the high- and low-TMB subgroups were observed in the infiltration of CD8+ T cells, M1 macrophages, regulatory T cells, and CD4+ T cells. In addition, we developed a miRNA-based signature using 23 differentially expressed miRNAs to predict TMB values of GC patients. The predictive performance of the signature was confirmed in the testing and the whole set. Receiver operating characteristic curve analysis demonstrated the optimal performance of the signature. Finally, enrichment analysis demonstrated that the set of miRNAs was significantly enriched in many key cancer and immune-related pathways.

Key Words: Tumor mutational burden; Gastric cancer; Prognosis; Immune infiltration; microRNA; Immunotherapy

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Core Tip: Whether tumor mutation burden (TMB) is associated with a favorable prognosis remains controversial in various cancers. Accumulating evidence highlights that it is necessary to explore clinical impact of TMB in gastric cancer (GC). We defined the highest mutation load quintile (top 20%) in GC as the high-TMB group and found that high TMB values were associated with improved clinical outcomes, which might be attributed to the induction of antitumor immune responses in the microenvironment. We developed a microRNA-based signature to predict TMB values, which might serve as a surrogate biomarker for TMB in GC and aid physicians in clinical medical decision-making.

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INTRODUCTION

Gastric cancer (GC) represents the fifth most frequent malignant disease around the globe. GC alone accounts for 8.2% of cancer-related deaths worldwide and represents a heavy economic burden and a serious public health concern, especially in China^[1,2]. Despite the considerable benefits of current therapies, such as targeted biological agents and combination therapies, prognosis for advanced GC is still poor with a median survival of less than 12 mo^[3,4]. Thus, it is urgently important to develop new therapeutic approaches to prolong patient life. More recently, immunotherapy with immune checkpoint inhibitors (ICIs) has emerged as one of the most promising therapeutic approaches for various solid tumors^[5-7]. ICIs, specifically programmed death receptor-1/ligand 1 (PD-1/L1) antibodies, have been approved for the treatment of advanced and refractory GC^[8,9]. However, only a small subset of these patients have shown a response to ICIs owing to the complexity of immunosuppressive mechanisms and genetic heterogeneity among tumors^[10-13]. Therefore, a reliable biomarker is required to determine which patients can respond to ICIs and guide the selection of GC patients for immunotherapy.

Currently, tumor mutational burden (TMB), referring to the amount of nonsynonymous mutations per one million bases, is in the spotlight as a novel biomarker and a rational target for predicting response to ICIs. High TMB may be a response biomarker for immunotherapy, based on the established notion that high mutational burden could facilitate neoantigen accumulation on tumor cells, enhancing immune cell activities in the microenvironment, subsequently eliciting T-cell-dependent immune responses, and thereby inhibiting tumor development^[14,15]. ICIs can restore neoantigen-mediated antitumor immune responses, thus patients with high TMB are more likely to respond to immunotherapy and exhibit improved clinical outcomes. For example, patients with high mutational load, including bladder cancer, melanoma, and lung adenocarcinoma, have appeared to benefit from ICIs^[16-18]. A positive correlation between high incidence of TMB and overall survival benefit has

been found in a small cohort of patients suffering from refractory GC treated with PD-1 antibody^[19]. However, studies of TMB in GC patients treated with ICIs are limited in number to date^[19,20]. The association of mutational load with clinical characteristics/outcomes and immune infiltration in the microenvironment also remains lacking. Continued research is needed to delineate the somatic mutation profile of GC and explore the correlations of TMB with immune cell fractions.

There are two traditional approaches used to assess TMB in formalin-fixed, paraffin-embedded tissue of patients in most studies to date, including whole genome sequencing and whole exome sequencing (WES). WES is generally regarded as the definitive standard for mutation load assessment, but it is currently still clinically impractical because of high cost, turnaround time, and tissue heterogeneity^[21,22]. Furthermore, WES requires the analysis of the matched normal tissue to remove germline mutations, and it is difficult for clinical doctors to use complex bioinformatics algorithms to quantify TMB^[23]. Several targeted gene panels focusing on cancer-related regions have recently developed as new methods to determine TMB; however, the demand for larger amounts of tumor DNA limits their use in clinical practice^[24]. Thus, to find surrogate biomarkers that can predict TMB status accurately in GC is highly desirable. Lv *et al.*^[25] have identified a classifier based on microRNA (miRNA) expression patterns to predict mutational load in lung adenocarcinoma. MiRNAs are endogenous non-coding RNAs with the capacity to modulate many biological processes through gene regulation and have the potential to be biomarkers in GC immunotherapy^[26,27]. Therefore, we hypothesized that miRNAs can also be surrogate biomarkers that highly correlate with the TMB status in GC.

In the current study, we determined the tumor mutational profiles of patients with GC by using the Cancer Genome Atlas (TCGA) data (<https://portal.gdc.cancer.gov/repository>). Specifically, we attempted to answer the following questions: Is the TMB an independent predictive biomarker for GC patients? Is the abundance of immune cell fractions in the microenvironment of high mutational load subgroup different from that of low subgroup? Do miRNAs have the potential to predict TMB values in GC?

MATERIALS AND METHODS

Data acquisition

Data on somatic mutations, RNA-seq, and miRNA expression profiles for GC were obtained from the TCGA database. For mutation data, we chose the “Masked Somatic Mutation” data which were based on VarScan software and subsequently applied the Maftools package for mutational analysis and comprehensive visual presentation^[28]. The TCGA database is freely available and open to the public; therefore, there is no requirement for additional ethical approval.

Estimation of TMB values

The TMB values for each sample were determined by measuring the total amount of nonsynonymous mutations, including somatic substitutions, coding deletions and insertions, and coding errors of genes *via* Perl scripts and represented as the amount of mutations per mega-base (Mb) of the genomic region being sequenced. Based on a previous study, we used a cut-off of the top 20% of the TMB (9 mutations/Mb) in this study as the cut-off value. Samples with TMB \geq 9 mutations/Mb were defined as high mutational burden (TMB-H), whereas samples with TMB < 9 mutations/Mb were defined as low mutational burden (TMB-L)^[19].

Prognosis analysis

Based on the requirement for the prognosis analysis, we excluded the GC patients having a survival time of less than 1 mo, potentially implying death caused by other disease. The Kaplan-Meier curve was utilized to explore the association of somatic mutation count with overall survival, and the survival difference between the TMB-H and TMB-L group was assessed by log-rank test. We also performed Wilcoxon rank-sum tests to evaluate the relationships of TMB levels with clinicopathological parameters, including TNM status, histological grade, age, gender, and American Joint Committee on Cancer (AJCC) stage. In addition, in order to determine whether the prognostic value of TMB was independent of other clinicopathological parameters, Cox proportional hazards regression analyses (univariate and multivariable) were utilized to determine statistical significance, representing results as hazard ratios (HR) and 95% confidence intervals (95% CI).

Immune infiltration analysis

Based on RNA-seq expression data, the R package “CIBERSORT” was utilized to quantify the levels of 22 immune cells in GC patients with a threshold P value < 0.05 . CIBERSORT is a deconvolution algorithm that requires an input matrix of a known reference set and accurately determines the relative levels of leukocyte subtypes from their gene expression profiles^[29]. Next, we used the R package “pheatmap” to visualize the distributions of immune cell fractions in the high- and low-TMB subgroups. The difference in immune cell abundance between the high- and low-TMB groups was compared by Wilcoxon rank-sum test and visualized with the R package “vioplot”.

Construction of a miRNA-based signature

The patients diagnosed as GC were randomly sorted into a training dataset (60%) and a testing dataset (40%). The training set was used to construct a miRNA-based signature for TMB prediction. First, the miRNAs differentially expressed between the high mutation load and low mutation load groups were identified by analyzing the training set using the R package ‘limma’, and only the miRNAs with a P value < 0.01 and fold change (FC) > 1.5 were selected for subsequent analysis. We illustrated the differential expression patterns of miRNAs in the TMB-L and TMB-H subgroups by performing bidirectional hierarchical clustering and generating a heatmap plot. To construct the miRNA-based signature for predicting TMB values, we used the package “glmnet” in R software to conduct least absolute shrinkage and selection operator (LASSO) regression analysis. LASSO analysis is a powerful method that can improve prediction accuracy by constructing a penalty function, shrinking some coefficients to zero, reducing the number of variables, and finally selecting only a subset of variables into the model^[30]. Then, ten-fold cross-validation was performed with $\text{type.measure} = \text{“auc”}$ to identify the optimal tuning parameters (minimum value of lambda) in the LASSO model. The final model retained all predictors with coefficients not equal to zero. Finally, the individual index score was constructed as a linear combination of the expression level of miRNA multiplied with a regression coefficient (β) for the corresponding miRNA obtained from the LASSO regression model: The index = $(\beta_{\text{miRNA1}} * \text{expression level of miRNA1}) + (\beta_{\text{miRNA2}} * \text{expression level of miRNA2}) + (\beta_{\text{miRNA3}} * \text{expression level of miRNA3}) + (\beta_{\text{miRNA}n} * \text{expression level of miRNA}n)$.

Performance of the miRNA-based signature

The testing set and the whole set were utilized to validate the robustness and predictive performance of this signature. The ability of the signature to predict TMB value was assessed by using receiver operating characteristic (ROC) curve methodology and calculating the area under the curve (AUC) with the R package “survival ROC”. Sensitivity and specificity of this signature, as well as positive predictive value (PPV) and negative predictive value (NPV), were determined in all the sets. The relationship between the signature index of each sample and TMB value was determined by the Spearman method. With the aim to investigate the association between this signature and immune checkpoint molecules, we explored the correlation of the signature index with gene expression levels of immune checkpoints including cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), PD-L1, and PD-1.

Determination of miRNA functions

To identify the biological functions of the list of miRNAs generated to construct the predictive signature, pathway enrichment analysis including KEGG pathways and GO was conducted using DIANA-miRPath v3.0, an online software suite capable of deciphering miRNA function with experimental support (<http://www.microrna.gr/miRPathv3>)^[31]. The results of GO and KEGG pathway analyses were considered to indicate significance at a threshold of P value < 0.01 and were visualized with the R package “ggplot2”.

Statistical analysis

R software (version 3.6.1) was employed to implement the statistical analyses in the study. P values < 0.05 were considered significant unless otherwise specified.

RESULTS

Mutational genomic landscape in GC

We included 433 GC patients for mutational analysis in this study. As depicted in

Figure 1A-F missense mutation was the most common mutational category, and single nucleotide polymorphism accounted for the most frequent variant type. We classified single nucleotide variants into six classes and the results revealed that C>T mutations exhibited the highest incidence (91939) in GC. The number of variants per sample ranged from 0 to 5612, and the median number was 89. The waterfall map presented the top 30 mutated genes and their status with respect to mutational categories (Figure 1G). The top 20 mutated genes were as follows: *TTN*, *TP53*, *MUC16*, *ARID1A*, *LRP1B*, *SYNE1*, *FLG*, *FAT4*, *CSMD3*, *PCLO*, *DNAH5*, *KMT2D*, *FAT3*, *OBSCN*, *HMCN1*, *RYR2*, *ZEFX4*, *SPTA1*, *CSMD1*, and *PIK3CA*. We used the interaction plot to display the co-occurrence and exclusive correlations among the top 20 mutated genes (Supplementary Figure 1). Blackish green represents the coincident associations across mutated genes, whereas yellow represents the exclusive associations. A gene-cloud plot is used to present mutation information for genes in Supplementary Figure 2.

Prognosis value of TMB in GC

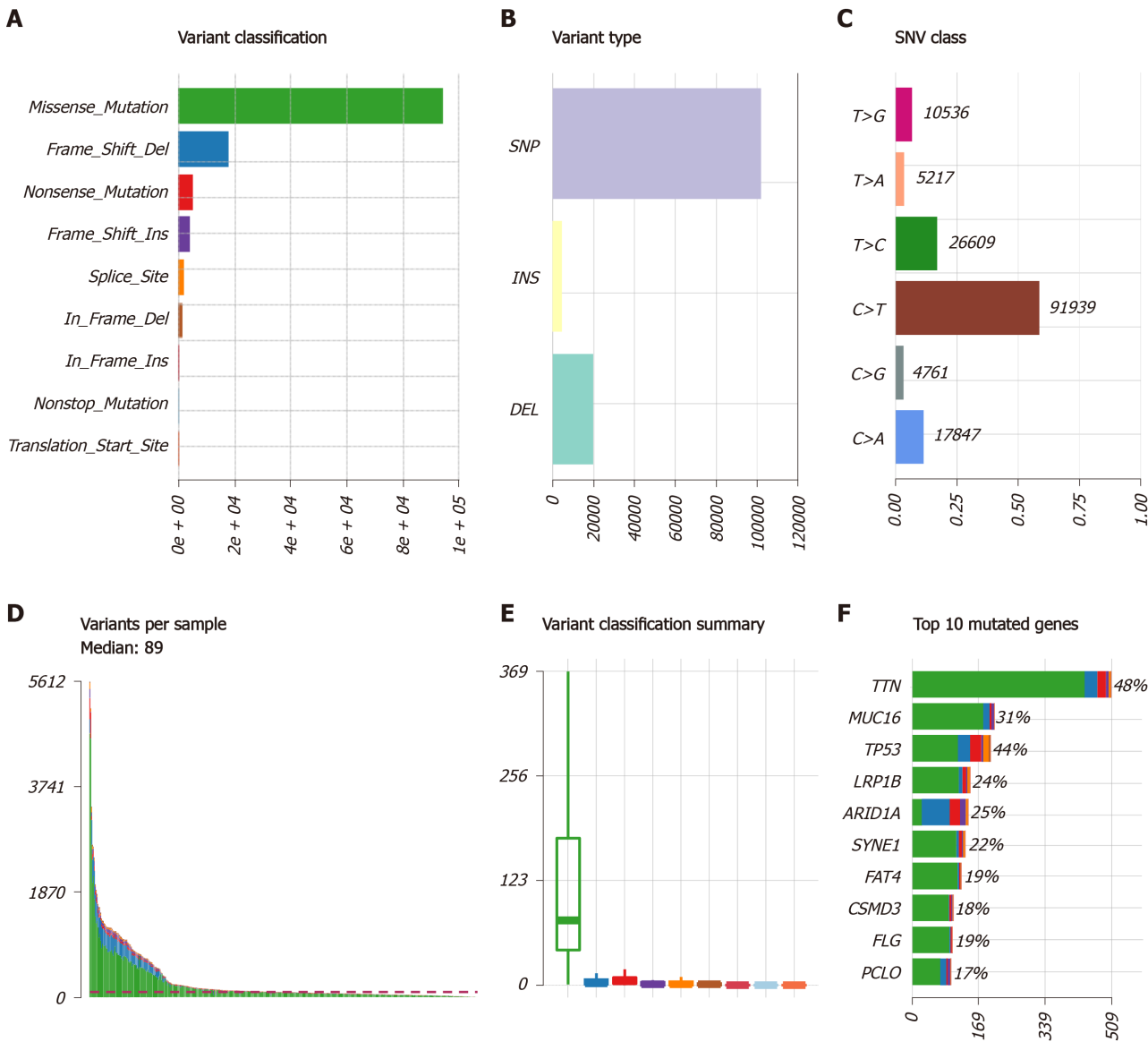
First, the value of TMB was calculated for each sample in the whole set and patients were classified into the TMB-H ($n = 87$) and TMB-L group ($n = 346$) on the basis of the cut-off of the top 20% of TMB value. Then, the prognosis capacity of TMB was determined by Kaplan-Meier analysis and log-rank test, which indicated that patients in the high mutational burden subgroup showed a significantly better overall survival than those in the low subgroup ($P = 0.020$, Figure 2A). In addition, we included age as a continuous variable and gender, histological grade, and TNM stage as categorical variables for univariate and multivariable Cox regression analyses to further investigate the clinical value of TMB (Figure 3). Results of the Cox regression indicated that TMB value was an independent and favorable prognostic biomarker for overall survival in GC (HR = 0.982, 95%CI: 0.967–0.997, $P = 0.021$), as were gender (HR = 1.693, 95%CI: 1.119–2.561, $P = 0.013$) and lymph node metastasis (HR = 1.280, 95%CI: 1.022–1.604, $P = 0.032$) (Figure 3B). High TMB was considered a favorable prognostic factor, while other parameters were confirmed to be unfavorable prognostic factors for GC. We further compared the differences of TMB among different subgroups. High levels of TMB were observed in the GC patients with the following characteristics (Figure 2): Over the age of 65 years ($P < 0.001$, Figure 2B), female gender ($P = 0.033$, Figure 2C), early AJCC stage ($P = 0.044$, Figure 2E), AJCC T1-2 stage ($P = 0.039$, Figure 2F), and lack of lymph node metastasis ($P = 0.018$, Figure 2G). However, there were no significant differences observed in the correlations of TMB value with AJCC M stage ($P = 0.104$, Figure 2H) or histological grade ($P = 0.051$, Figure 2D).

TMB and immune cell infiltration

To further explore the potential relationships between TMB value and immune infiltration in the tumor microenvironment, we used a deconvolution algorithm to calculate the 22 immune cell fractions and present the immune infiltration landscape for each sample in Figure 4A. We further used Wilcoxon rank-sum tests to compare the difference in immune infiltration between the high mutational load and low mutational load subgroups, and the results demonstrated that patients with high mutational load exhibited significantly increased abundance of CD8+ T cells ($P = 0.023$), T follicular helper cells (Tfh, $P < 0.001$), M1 macrophages ($P < 0.001$), and activated CD4+ T memory cells ($P < 0.001$). However, resting CD4+ memory T cells ($P = 0.026$), regulatory T cells (Tregs, $P < 0.001$), and naïve B cells ($P < 0.001$) displayed notably decreased proportions in the TMB-H group (Figure 4B).

Identification of a miRNA-based signature for TMB prediction from the training dataset

Patients with complete miRNA expression information were enrolled for subsequent study ($n = 425$) and randomly sorted into a training dataset ($n = 255$) and a testing dataset ($n = 170$). No clinicopathological characteristics, including age, gender, histological grade, or AJCC stage, were significantly different between the training and testing cohorts, as shown in Supplementary Table 1. Next, we conducted differential expression analysis in the training set, and 70 miRNAs were identified based on a cut-off point ($|\log_2FC| > 0.585$ and $P < 0.01$) to be differentially expressed between the TMB-L and TMB-H groups. Among these miRNAs, 22 differentially expressed miRNAs were downregulated and 48 miRNAs were upregulated. A heatmap of the top 50 differentially expressed miRNAs is shown in Figure 5. Next, the differentially expressed miRNAs were input into LASSO analysis. Ten-fold cross-validation was used for selecting parameters in the LASSO model by minimum criteria. At the optimal values $\log(\lambda)$, dotted vertical lines were set *via* the minimum criteria, where 23



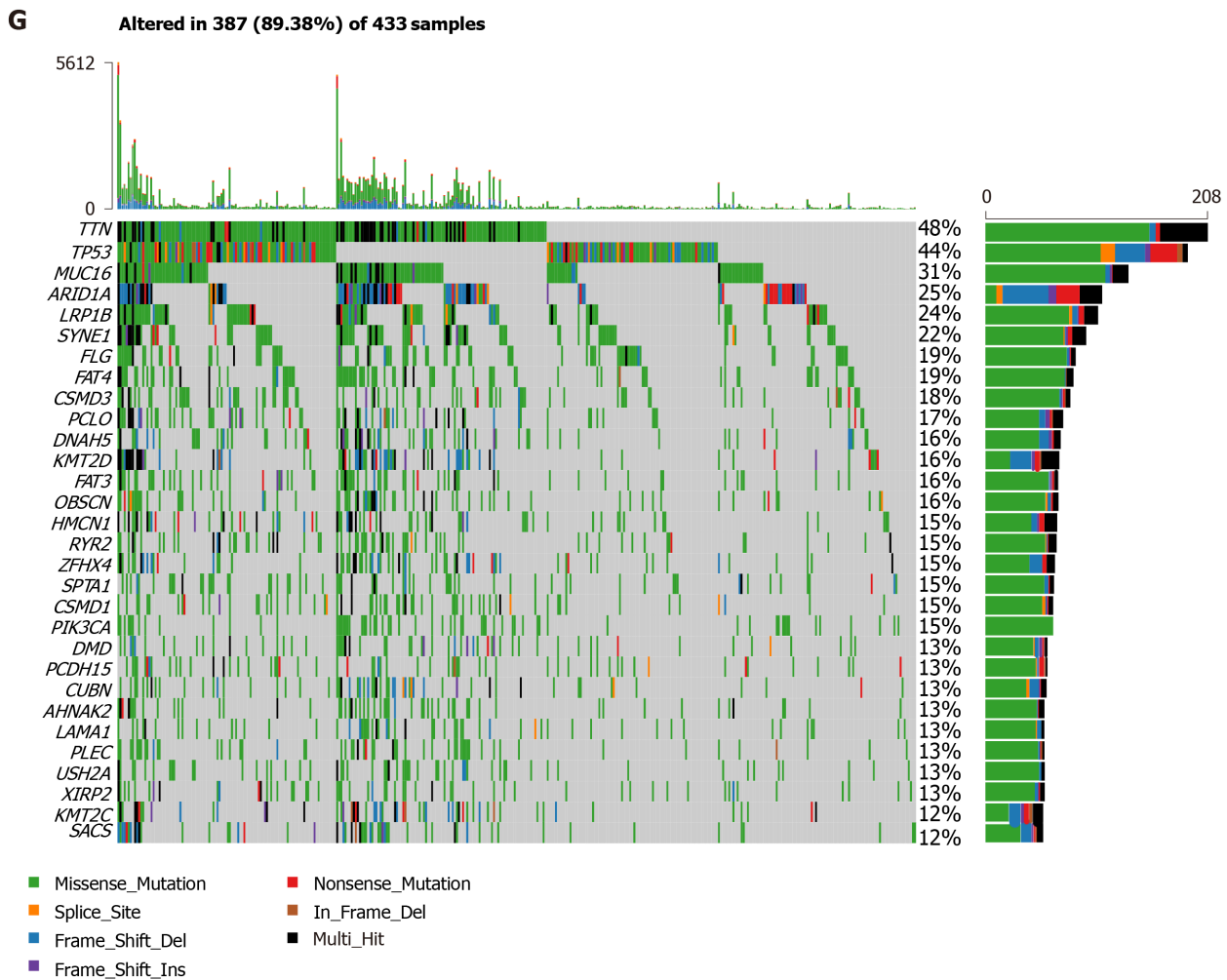


Figure 1 Mutational genomic landscape in The Cancer Genome Atlas gastric cancer cohort. A: Variant categories; B: Variant types; C: Single nucleotide variant types; D: Number of variants per sample; E: Summary of variant categories; F: Top 10 mutated genes. G: Waterfall map of the top 30 mutated genes and their status of variant categories. Various colors with annotations at the bottom represent the different variant categories while the barplot above the legend exhibits the value of tumor mutational burden. SNV: Single nucleotide variants; SNP: Single nucleotide polymorphism; INS: Insertion; DEL: Deletion; TCGA: The Cancer Genome Atlas.

features were selected to establish the prognostic model (Figure 6A). Subsequently, the index of each patient was measured to predict the value of TMB as follows: Index = $-4.801 + \text{miR-452-5p}^*(-0.773) + \text{miR-203b-3p}^*(0.081) + \text{miR-582-3p}^*(0.194) + \text{miR-582-5p}^*(0.140) + \text{miR-27a-5p}^*(0.032) + \text{miR-651-5p}^*(0.057) + \text{miR-508-3p}^*(0.273) + \text{miR-410-3p}^*(0.139) + \text{miR-181d-5p}^*(-0.760) + \text{miR-96-5p}^*(0.467) + \text{miR-30a-3p}^*(-0.238) + \text{miR-155-5p}^*(0.773) + \text{miR-4662a-5p}^*(-0.130) + \text{miR-196b-5p}^*(0.264) + \text{miR-3913-5p}^*(-0.205) + \text{let-7g-3p}^*(-0.338) + \text{miR-210-3p}^*(0.352) + \text{miR-497-5p}^*(-0.727) + \text{miR-9-5p}^*(-0.037) + \text{miR-625-5p}^*(-0.061) + \text{miR-181b-5p}^*(-0.869) + \text{miR-100-5p}^*(0.527) + \text{miR-338-5p}^*(0.453)$.

Performance of the miRNA signature for TMB prediction

In the study, we used AUC, sensitivity, specificity, PPV, and NPV to describe the performance of the miRNA signature. The performance of the signature for predicting TMB in each set is displayed in Figure 6B and Table 1. The AUCs reached 0.982, 0.887, and 0.947 for the training set, the testing set, and the whole set, respectively, demonstrating the competitive power of the signature for predicting the TMB values of GC patients. We obtained an accuracy of 0.934 with a sensitivity of 0.802, specificity of 0.968, PPV of 0.863, and NPV of 0.951 for the whole set. The accuracy in the training set was 0.953 and 0.906 in the testing set, respectively, with a sensitivity of 0.750 and specificity of 0.937. These results revealed that the miRNA-based signature predicted TMB values with high efficiency and could be used as a predictor. Previous studies have shown PD-L1 expression and TMB to be independent of each other in most cancer subtypes^[32,33]. Hence, it would be meaningful to explore the association between the miRNA signature index and immune checkpoint molecules. Clearly, the signature

Table 1 Performance of the miRNA-based signature to predict tumor mutation burden in gastric cancer

Type	Training set	Testing set	Whole set
sensitivity	0.828	0.750	0.802
specificity	0.990	0.937	0.968
PPV	0.960	0.700	0.863
NPV	0.951	0.950	0.951
Accuracy	0.953	0.906	0.934
AUC	0.982	0.887	0.947

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under curve.

index exhibited a significant strong correlation with the level of TMB ($r = 0.57$, $P < 0.001$). However, the index showed a low correlation with the gene expression of *PD-1* ($r = 0.13$, $P = 0.013$) and *CTLA4* ($r = 0.13$, $P = 0.011$) and a moderate correlation with that of *PD-L1* ($r = 0.33$, $P < 0.001$) (Figure 7).

Determination of miRNA function

Functional enrichment analysis showed that 48 KEGG pathways (Supplementary Table 1) and 104 GO terms (Supplementary Table 2) were enriched for the 23 miRNAs ($P < 0.01$). It was noted that several KEGG pathways are involved in the development and progression of GC, including the HIPPO, PI3K-Akt, WNT, ERBB, and transforming growth factor-beta (TGF- β) signaling pathways (Figure 8A). GO enrichment analysis demonstrated significant enrichment of immune related pathways, such as Toll-like receptor signaling pathway, Fc-epsilon receptor signaling pathway, immune system process, innate immune response, and Fc-gamma receptor signaling pathway involved in phagocytosis (Figure 8B). Taken together, the functional enrichment analysis revealed the potential roles of the miRNAs in cancer-related immune processes.

DISCUSSION

Biological behaviors of cancers, including tumor initiation, angiogenesis, tumor invasion, and metastasis, are driven by genome instability, expression-level modulation, and immune cells present in the tumor microenvironment^[34]. With the widespread use of high-throughput molecular technologies, TMB, one of the manifestations of genetic instability, has attracted the attention of researchers. Currently, TMB is considered an innovative biomarker of immunotherapy response for multiple cancers, including GC. Further understanding of TMB and its relationship with immune cells becomes more important in the field of personalized medicine. Therefore, the present study focused on the clinical implications of TMB in GC.

In the current study, we summarize the mutational genomic landscape in GC patients based on the TCGA dataset. The top 3 mutated genes were *TTN*, *TP53*, and *MUC16*. *TP53* is one of the most extensively studied tumor suppressor genes, and its mutation not only inhibits the suppression of tumor development, but also produces certain cancer-promoting proteins^[35]. *TTN*, the longest known gene, originally known for its effects on the development and regulation of cardiac and skeletal muscles, has been confirmed to be highly correlated with TMB levels and the responsiveness to ICIs in solid tumors^[36]. Despite the length of the gene contributing to a greater number of somatic mutations, the molecular mechanisms of *TTN* in the production of tumor mutations require further study. *MUC16* is one of the most frequently mutated genes in various cancers and contributes to tumor proliferation and metastasis by regulating immune response to cancer^[37]. In a recent study, it has been suggested that *MUC16* was associated with high TMB and favorable prognosis in GC^[38].

Our study analyzed the correlation between TMB values and overall survival rates in patients with GC. The Kaplan-Meier analysis in the GC cohort from the TCGA dataset was performed by defining the highest TMB quintile (top 20%) as the high TMB group. Based on this approach, we found that GC patients with high mutational burden had a favorable prognosis. In addition, higher mutational load in GC was also

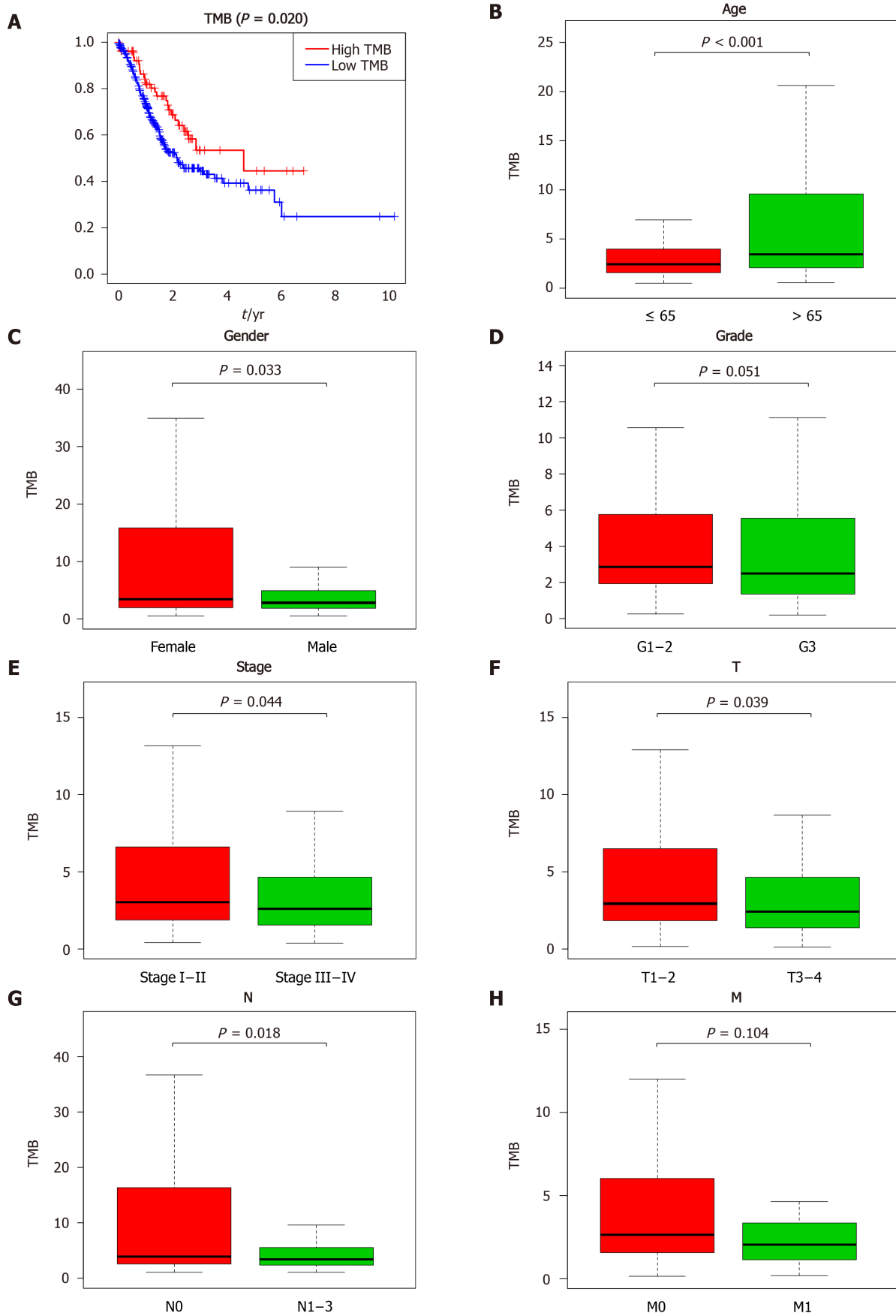


Figure 2 Clinical significance of tumor mutation burden in gastric cancer patients. A: Survival analysis to explore the overall survival of gastric cancer patients between the high and low tumor mutation burden groups; B-H: Correlation between tumor mutation burden values and clinical characteristics in gastric cancer. TMB: Tumor mutation burden.

significantly associated with older ages, female gender, earlier tumor stage, and lack of lymph node metastasis. However, the prognostic impact of mutational burden in different types of cancer remains controversial. Yuan *et al.*^[39] demonstrated that patients with higher mutational load had a worse prognosis in esophageal cancer^[39], consistent with previous studies in clear cell renal cell carcinoma and prostate cancer^[40,41], whereas bladder cancer with higher mutational load was associated with better outcomes^[42]. In these previous studies, they used the median value as the cut-off to distinguish TMB-H and TMB-L groups.

Currently, a universal cut-off value for defining high TMB is lacking. Previous studies have revealed that there are several factors that influence the threshold for high TMB, such as cancer types, sample types, pre-analytic variables, and detection methods^[43]. Some studies have been exploratory, distributing TMB into three groups. For example, a study on a total of 908 resected lung cancer species measured TMB levels by using targeted gene panels and divided TMB into terciles: High (> 8 mutations/Mb), low (4 mutations/Mb), and intermediate subgroup (> 4 and ≤ 8 mutations/Mb)^[44]. In a large cohort focusing on diverse cancers, TMB levels was separated into three subgroups: Low-(50% of patients), intermediate-(40% of patients), and high-TMB subgroup (10% of patients)^[23]. Most published clinical studies were likely to distribute TMB into two groups. In a study with small sample size, researchers defined the higher mutation load quintile (top 20%, 14.31 mutations/Mb) in advanced GC treated with ICIs as the high TMB group and found that TMB was correlated with clinical outcomes^[20]. Wang *et al.*^[19] have studied the correlation of TMB with survival in chemo-refractory GC under treatment with toripalimab and demonstrated that high TMB (top 20%, 12 mutations/Mb) was significantly correlated with improved survival^[19]. A large-scale study also used an upper 20th percentile cut-off to define high TMB and analyzed the association between tumor mutational load and the clinical responses to ICIs across multiple cancer types^[45]. Therefore, we took the same approach of separating TMB-H and TMB-L groups (top 20%, 9 mutations/Mb) in order to dichotomize the data, but this was not a universal number of high TMB in GC and might not have any clinical significance. When we set the cut-off at the median of mutational load (2.671 mutations/Mb) in the current study, the difference of overall survival rates between the high mutational load and low mutational load subgroups was still statistically significant ($P = 0.016$, **Supplementary Figure 3**). Thus, it is critical and urgent to develop a satisfactory and reproducible definition of the predictive TMB cut-off value before implementing TMB as a biomarker of immunotherapy response in individualized treatment. To further clarify whether TMB is a favorable dependent factor in GC, Cox proportional hazards regression analysis was implemented and demonstrated that TMB was significantly correlated with overall survival as a continuous variable, indicating that it was able to predict the survival of GC patients without consideration of other conventional clinicopathological variables.

Immune cells, a large proportion of infiltrating cells in the tumor microenvironment, interact with tumor cells by releasing inflammatory cytokines and chemokines, which drive biological behaviors of cancers and influence their therapeutic responses to immunotherapy. In the present study, we explored the potential relationships between TMB values and immune infiltration in GC patients. The abundance of CD8⁺ T cells, CD4⁺ T cells, and Tfh cells were markedly higher in the TMB-H group than in the TMB-L group. Accumulating evidence has shown that high mutational load tends to cause neoantigens to accumulate in cancer and results in the activation of CD8⁺ cytotoxic T cells and subsequent initiation of tumor cell lysis, consistent with our results^[14,15]. CD4⁺ T cells were considered a component of anticancer immunomechanisms and an independent indicator of favorable prognosis in multiple cancers^[46]. It is to be noted that not all mutations would generate high neoantigens on the surfaces of tumor cells, and further research is warranted to determine which gene mutations are responsible for the induction of immune response. Different from the conventional view on immune cells, TAMs play a dual role in tumor development depending on their polarization status. The M1 macrophages are involved in antitumor response whereas M2 macrophages have pro-tumorigenic properties^[47]. In our study, M1 macrophages, but not M2, were significantly elevated in the tumor microenvironment with high TMB levels. Furthermore, we found that patients with high mutational load showed significantly decreased infiltrating naïve B cells and Tregs. Tregs have been considered to have a central role in inhibiting effective antitumor immunity and correlate with an unfavorable prognosis in many cancers^[48]. The mechanisms underlying the decreased level of naïve B cells in the TMB-H group remain unclear. We hypothesize that B-cell differentiation factors secreted by tumor cells may be responsible for decreased infiltration of naïve B cells. In summary, these results

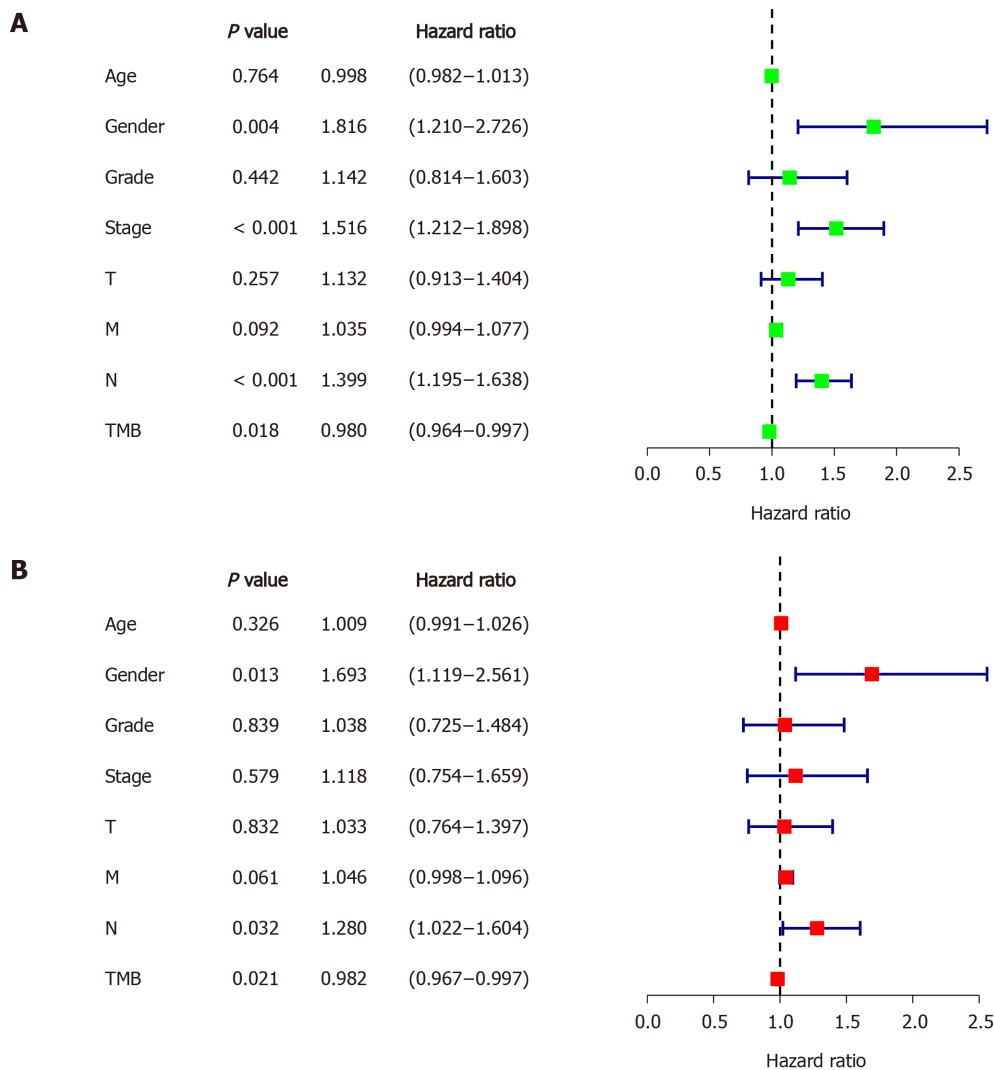


Figure 3 Identifying independent prognostic parameters in gastric cancer. A: Forrest plot of the univariate Cox regression analysis in gastric cancer; B: Forrest plot of the multivariate Cox regression analysis in gastric cancer. Solid squares indicate the hazard ratios of death, and close-ended horizontal lines represent the 95% confidence intervals. TMB: Tumor mutation burden.

indicate that tumor microenvironment exhibited a significant antitumor response in patients with high TMB.

Growing evidence has revealed that aberrant expression of miRNAs can be found in various cancer types and plays a crucial role in the carcinogenesis, migration, and invasion of tumor cells by regulating adaptive and innate immune responses in the tumor microenvironment^[26,27]. It has been suggested that miRNA-targeted immunotherapeutics have great potential in clinical practice. However, the relationship between miRNA and mutational load in GC has not previously been explored. In the present study, we screened the differentially expressed miRNAs between the TMB-H and TMB-L groups in the GC cohort and found that different mutational load values were correlated with different miRNA profiles. Next, we conducted LASSO analysis to select parameters from differentially expressed miRNAs and established a 23-miRNA classifier to predict TMB values based on the training dataset, which was further validated in the whole dataset and testing dataset. The accuracies of this predictive model for the training set, the testing set, and the whole set were 0.951, 0.906, and 0.934, respectively, and specificities were 0.990, 0.937, and 0.968, respectively, which implied that this signature was very effective with a high accuracy and specificity in predicting TMB values. The efficiency of this signature was also evaluated by ROC analysis and the results revealed that this signature was credible in predictive performance throughout the testing set and whole set. Moreover, the strong correlation between the signature index and TMB values in GC patients further confirmed the robustness of this signature. However, PPV in the testing set was lower than that in the training and whole set, indicating that the ability of the signature to recognize high mutational load needs to be improved. We also explored

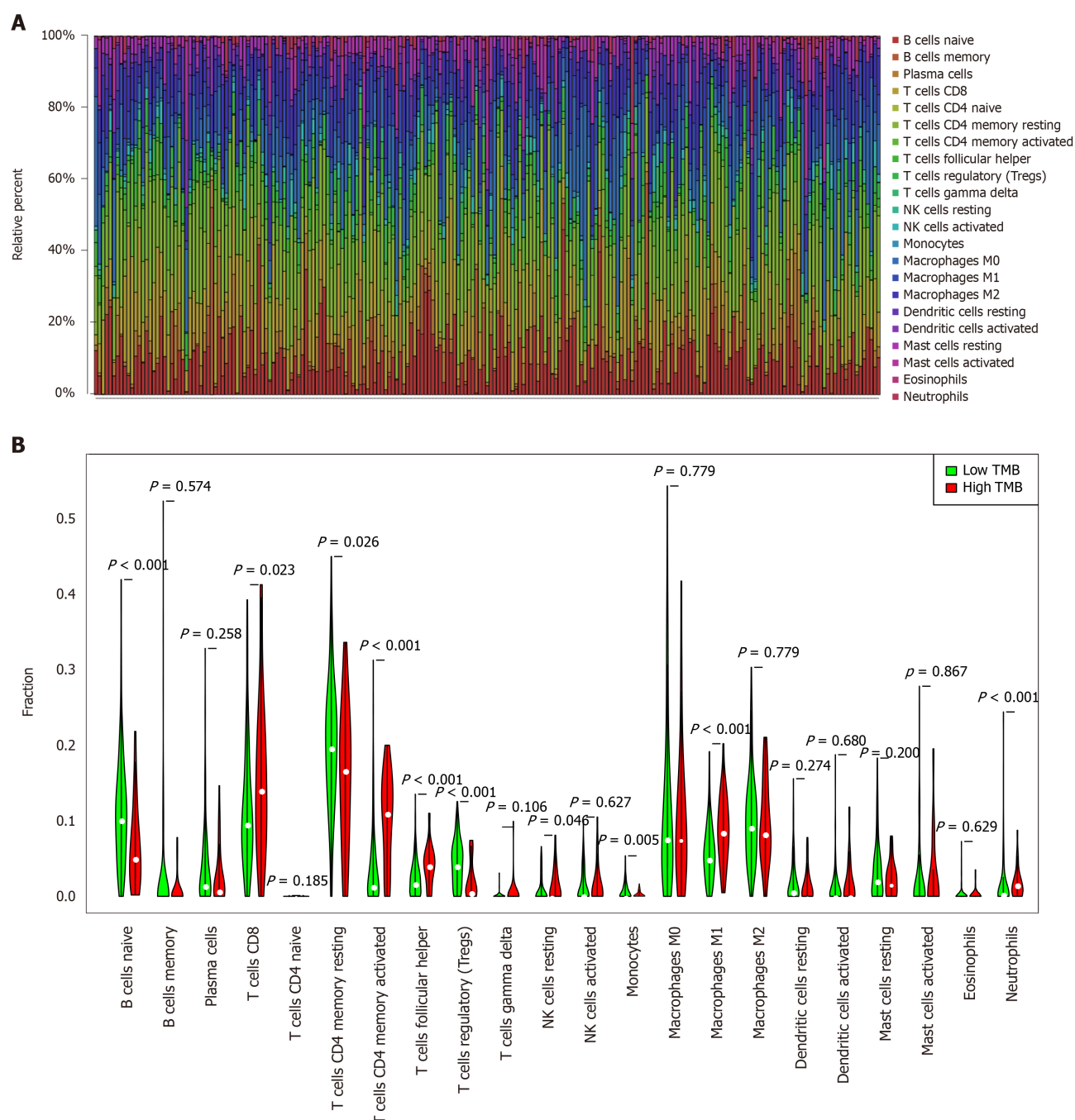


Figure 4 Quantitative analysis of immune infiltration in two groups based on tumor mutation burden status. A: The box plot of 22 tumor-infiltrating immune cells in each sample; B: Expression comparison of 22 tumor-infiltrating immune cells between the high tumor mutation burden (TMB) and low TMB groups. TMB: Tumor mutation burden.

the association of the signature with immune checkpoint molecules and demonstrated that the signature showed a low correlation with PD-1, PD-L1, and CTLA4. These results were consistent with previous studies, which demonstrated that TMB was independent of the expression of immune checkpoint molecules^[32,33].

With respect to the biological functions of the miRNAs in the predictive signature, functional annotation was conducted. KEGG pathway analysis indicated that the functions of the 23 miRNAs were potentially associated with HIPPO, PI3K-Akt, WNT, ERBB, and TGF- β signaling pathways, and those were supposed to play a critical role in the tumorigenesis of GC^[49]. Moreover, the miRNA sets were found to be involved in immune-related pathways, including immune system process and innate immune response. Increasing evidence has demonstrated that high mutational burden might lead to the activation of antitumor immune responses^[14,15]. The enrichment analysis indicated that the 23 miRNAs contributed to vital cancer and immune pathways, which might provide strong biological evidence for the feasibility of the miRNA-based

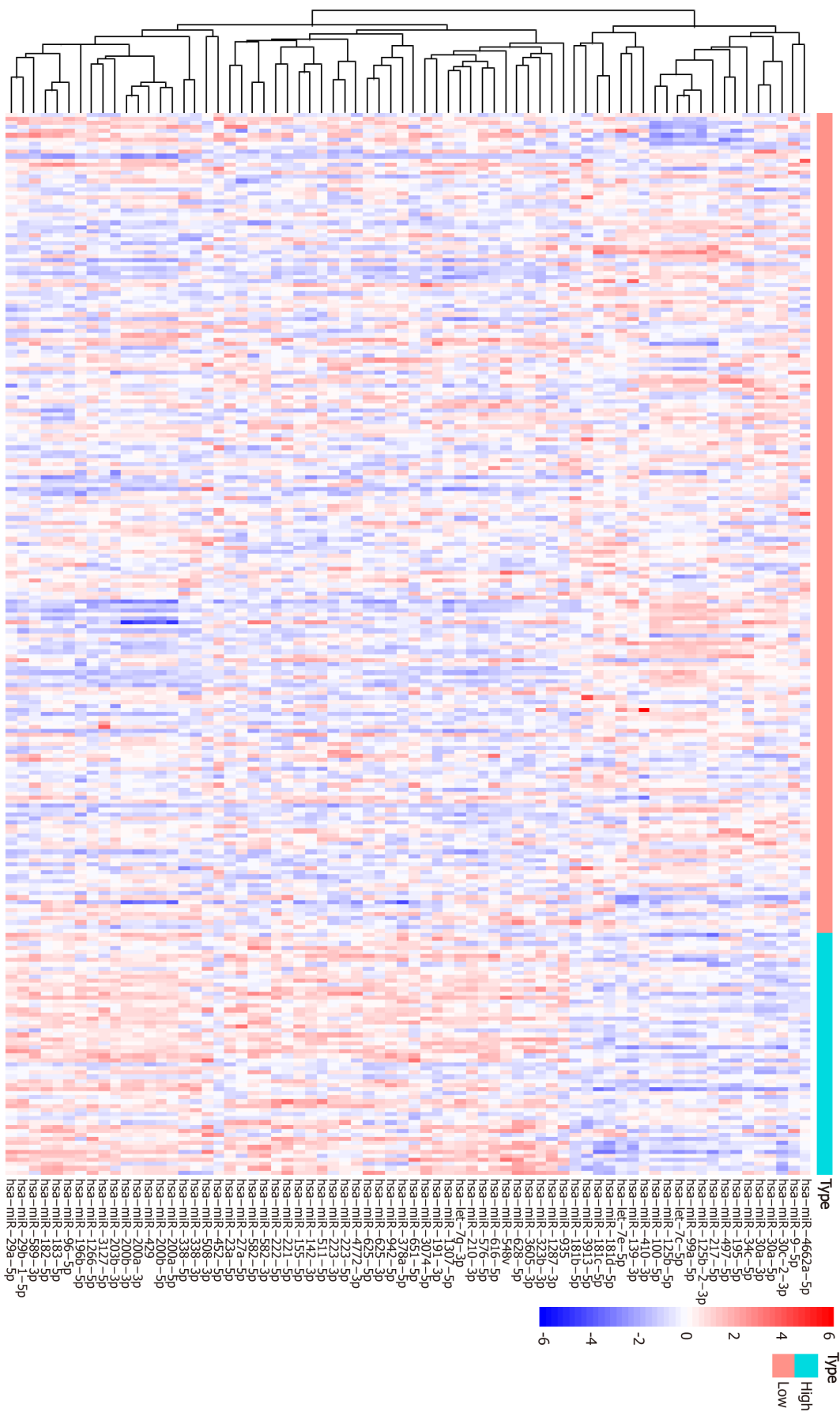


Figure 5 Heatmap of top 50 differentially expressed miRNAs between the high and low tumor mutation burden groups.

signature in predicting TMB values.

This study has several limitations. First, all of our samples and clinical data were based on the TCGA dataset, and most of patients were Westerners. Cohorts with larger sample sizes from other regions are needed to confirm our results, and external validation of the miRNA-based signature is necessary in the future. Second, the cut-off definition used in the present study to distinguish the TMB-H and TMB-L groups was not uniform, and multi-center randomized controlled studies focusing on immunotherapy in GC to identify TMB cut-off values are proposed. Third, all the results in the present study were based on a bioinformatics analysis and description. Mechanistic investigation should be performed to clarify the underlying mechanism of high mutation load in the activation of antitumor immune responses in GC patients. In addition, the functions of 23 miRNAs in immune responses were not characterized using *in vitro* or *in vivo* experimentation.

CONCLUSION

Taken together, mutational burden is considered an independent and favorable prognostic biomarker in patients suffering from GC. High TMB is notably correlated with a good survival and might lead to the activation of antitumor immune cells in the tumor microenvironment. Moreover, different mutational load is associated with different miRNA expression patterns, and a miRNA-based signature was established to predict TMB values in GC, which might aid physicians in clinical medical decision-making.

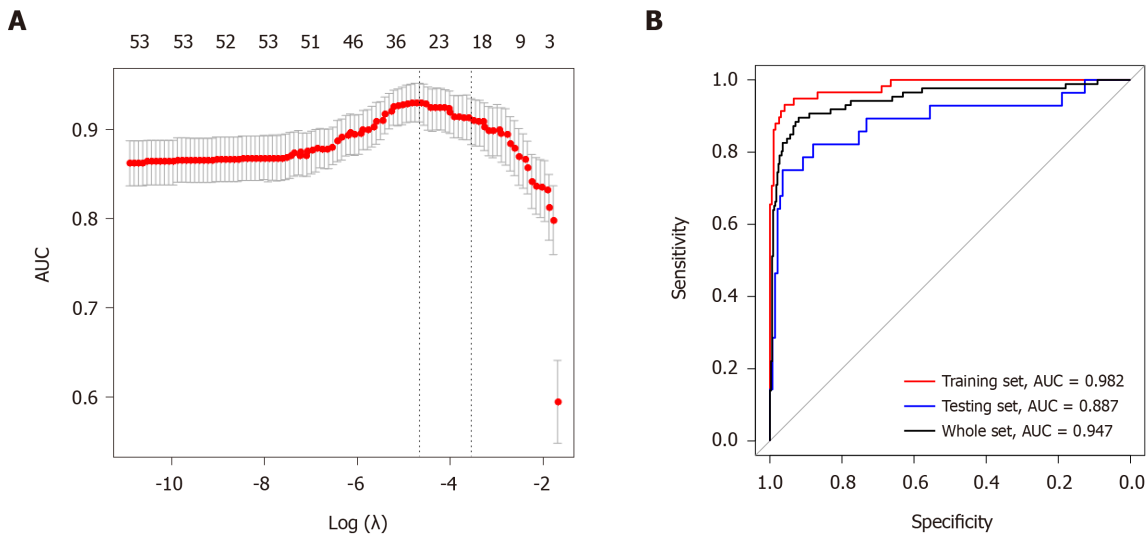


Figure 6 Least absolute shrinkage and selection operator analysis for prognostic features screening and receiver operating characteristic curves for the miRNA-based signature. **A:** Least absolute shrinkage and selection operator regression with ten-fold cross-validation obtained 23 prognostic parameters using minimum lambda value; **B:** Receiver operating characteristic analysis showed that the areas under curves for the training set, the testing set, and the whole set were 0.982, 0.887, and 0.947, respectively. λ : Lambda; AUC: Area under curve.

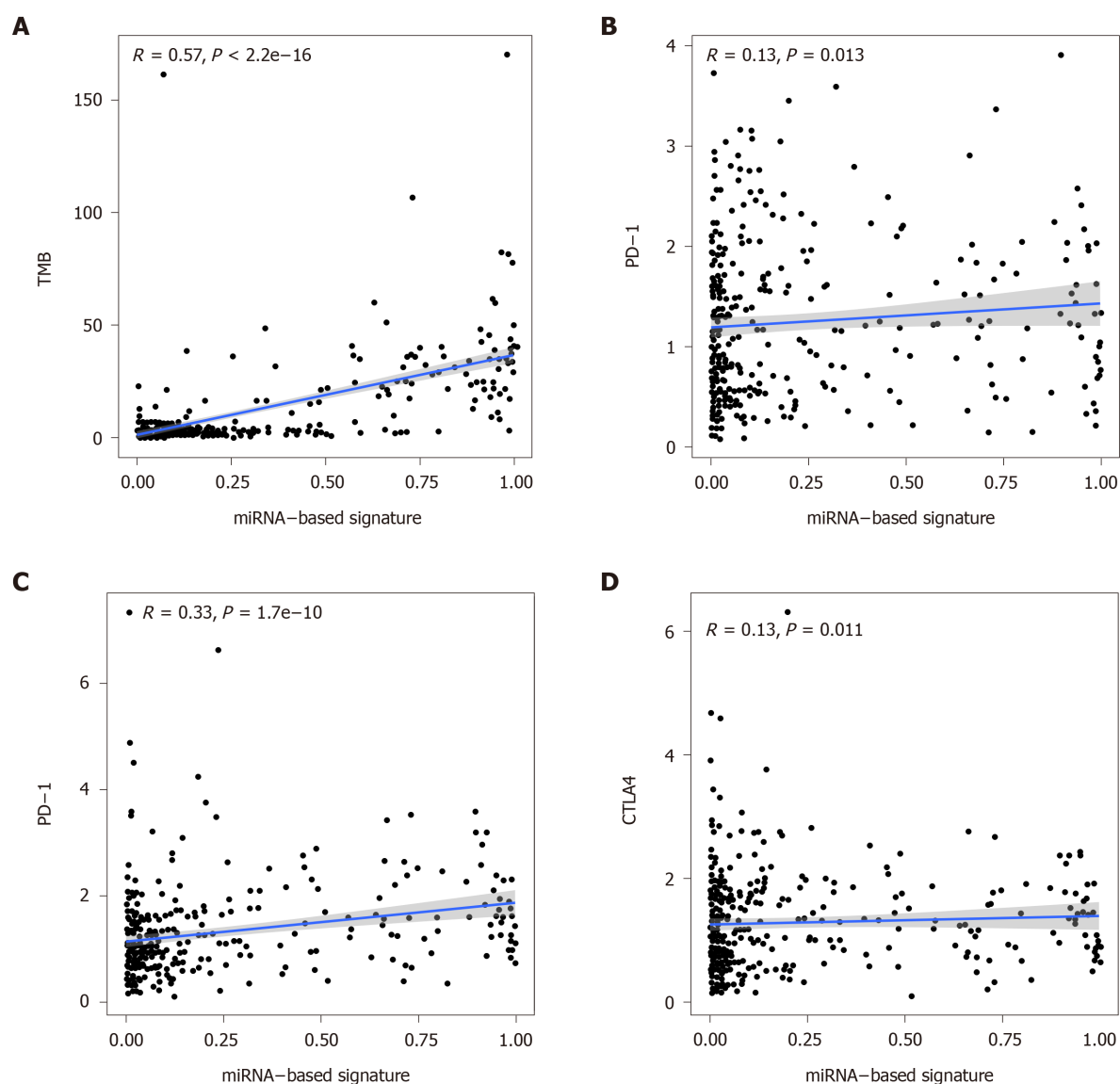


Figure 7 Correlation of the miRNA-based signature with tumor mutation burden values and immune checkpoint molecules (programmed death-1, programmed death ligand-1, and cytotoxic T lymphocyte associated antigen 4). TMB: Tumor mutation burden; PD-1: Programmed death-1; PD-L1: Programmed death ligand-1; CTLA4: Cytotoxic T lymphocyte associated antigen 4.

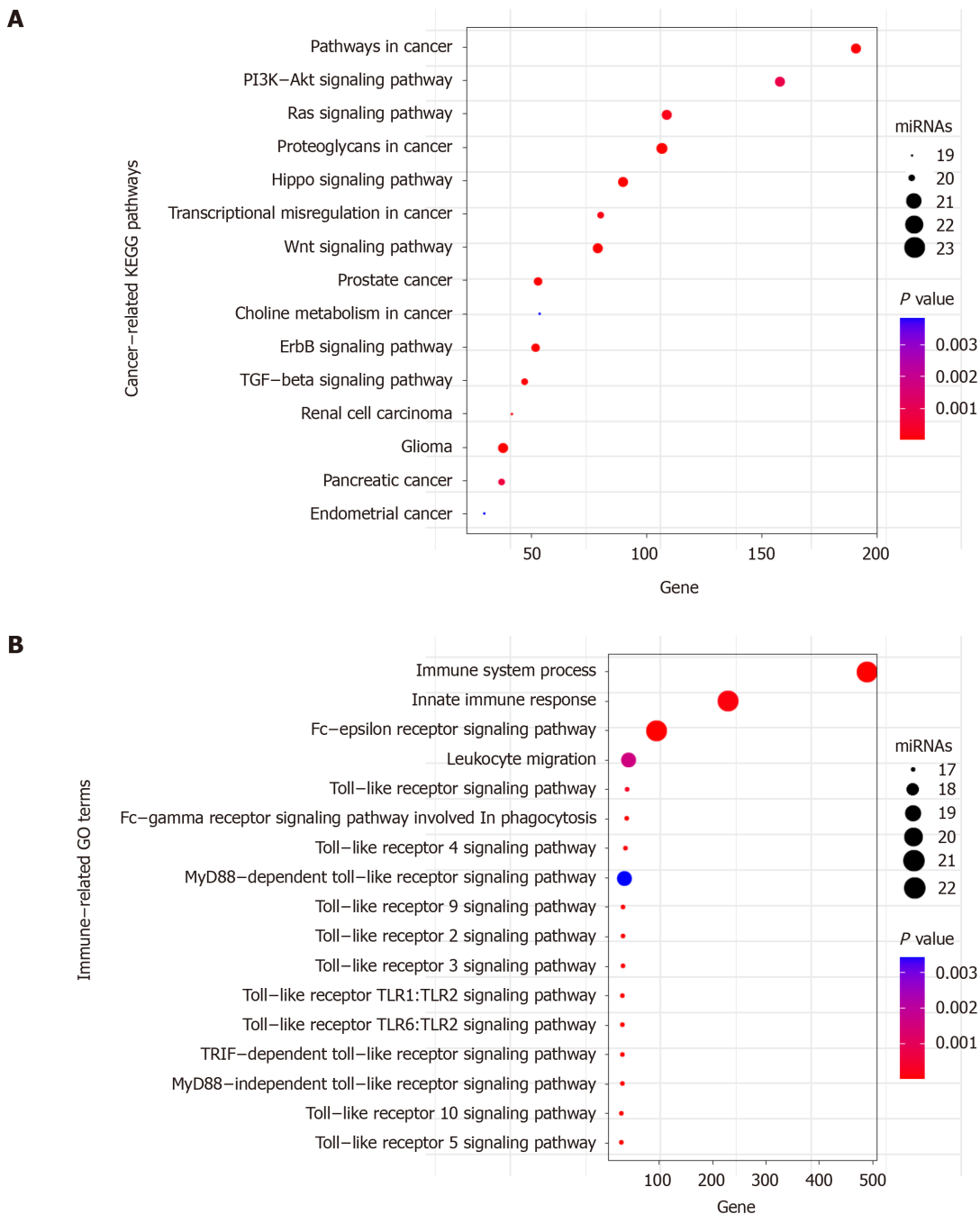


Figure 8 Functional enrichment analysis of the 23 miRNAs. A: Significant enriched cancer-related KEGG pathways; B: Significant enriched immune-related GO terms. X axis refers to the number of targeted genes. Y axis refers to GO or KEGG entry name. Bubble color refers to the enrichment *P* value. Bubble size refers to the number of targeting miRNAs.

ARTICLE HIGHLIGHTS

Research background

Tumor mutational burden (TMB) is in the spotlight as a novel biomarker and a rational target for predicting response to immunotherapy in multiple cancers. Gastric cancer (GC) is one of the most common gastrointestinal malignant tumors worldwide. Accumulating evidence highlights that it is necessary to further explore clinical impact of TMB in GC.

Research motivation

The association of TMB with clinical outcomes and immune infiltration in the tumor microenvironment in GC patients has not yet been elucidated. MicroRNAs (miRNAs) have a crucial role in the carcinogenesis, migration, and invasion of tumor cells by

regulating adaptive and innate immune responses, but the relationship between miRNA expression patterns and mutational load is not clear in GC.

Research objectives

This study aimed to explore the clinical impact of TMB and establish a miRNA-based signature for TMB prediction in GC patients.

Research methods

The Kaplan-Meier analysis in the GC cohort from The Cancer Genome Atlas dataset was performed by defining the highest TMB quintile (top 20%) as the high-TMB group. The difference in immune infiltration between the high- and low-TMB subgroups was evaluated by Wilcoxon rank-sum test. The least absolute shrinkage and selection operator analysis was conducted to select parameters from differentially expressed miRNAs between the high- and low-TMB subgroups and construct a miRNA-based signature classifier for TMB prediction.

Research results

Higher mutational load in GC was significantly associated with better prognosis, older ages, female gender, earlier tumor stage, and lack of lymph node metastasis. Different mutational load levels exhibited different immune infiltration patterns and different miRNA expression patterns. In addition, we developed a miRNA-based signature using 23 differentially expressed miRNAs to predict TMB values of GC patients.

Research conclusions

High TMB is notably correlated with good survival and might lead to the activation of antitumor immune cells in the tumor microenvironment in GC. The miRNA-based signature might be developed as a surrogate biomarker for TMB in GC.

Research perspectives

The miRNA-based signature for TMB prediction might help develop treatment strategies for GC patients and have an impact on the clinical practice in the course of GC.

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Observational Study

Diagnostic performance of narrow-band imaging international colorectal endoscopic and Japanese narrow-band imaging expert team classification systems for colorectal cancer and precancerous lesions

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Abstract

BACKGROUND

In recent years, two new narrow-band imaging (NBI) classifications have been proposed: The NBI international colorectal endoscopic (NICE) classification and Japanese NBI expert team (JNET) classification. Most validation studies of the two new NBI classifications were conducted in classification setting units by experienced endoscopists, and the application of use in different centers among endoscopists with different endoscopy skills remains unknown.

AIM

To evaluate clinical application and possible problems of NICE and JNET classification for the differential diagnosis of colorectal cancer and precancerous lesions.

METHODS

Six endoscopists with varying levels of experience participated in this study. Eighty-seven consecutive patients with a total of 125 lesions were photographed during non-magnifying conventional white-light colonoscopy, non-magnifying NBI, and magnifying NBI. The three groups of endoscopic pictures of each lesion

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were evaluated by the six endoscopists in randomized order using the NICE and JNET classifications separately. Then we calculated the six endoscopists' sensitivity, specificity, accuracy, positive predictive value, and negative predictive value for each category of the two classifications.

RESULTS

The sensitivity, specificity, and accuracy of JNET classification type 1 and 3 were similar to NICE classification type 1 and 3 in both the highly experienced endoscopist (HEE) and less-experienced endoscopist (LEE) groups. The specificity of JNET classification type 1 and 3 and NICE classification type 3 in both the HEE and LEE groups was > 95%, and the overall interobserver agreement was good in both groups. The sensitivity of NICE classification type 3 lesions for diagnosis of SM-d carcinoma in the HEE group was significantly superior to that in the LEE group (91.7% *vs* 83.3%; $P = 0.042$). The sensitivity of JNET classification type 2B lesions for the diagnosis of high-grade dysplasia or superficial submucosal invasive carcinoma in the HEE and LEE groups was 53.8% and 51.3%, respectively. Compared with other types of JNET classification, the diagnostic ability of type 2B was the weakest.

CONCLUSION

The treatment strategy of the two classification type 1 and 3 lesions can be based on the results of endoscopic examination. JNET type 2B lesions need further examination.

Key Words: Narrow-band imaging international colorectal endoscopic; Japanese narrow-band imaging expert team; Colorectal neoplasms; Precancerous lesions; Colorectal endoscopy; Narrow-band imaging

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Core Tip: We evaluated the clinical application and possible problems of the narrow-band imaging international colorectal endoscopic (NICE) classification and Japanese NBI expert team (JNET) classification in our unit, which is a tertiary hospital in China. We found that the treatment strategy of NICE type 1 and 3 and JNET type 1, 2A and 3 lesions can be determined based on the results of endoscopic examination. Compared with other types of JNET classification, the diagnostic ability of type 2B is the weakest. The JNET type 2B lesions still needs further examinations, such as magnifying chromoendoscopy or endoscopic ultrasonography.

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INTRODUCTION

Colorectal cancer (CRC) was the third most common malignancy and the second leading cause of cancer-related death globally in 2018^[1]. The morbidity and mortality of CRC are still rising rapidly in many low- and middle-income countries^[2]. The outcome and prognosis of patients with CRC are closely related to the stage of the disease. Miller *et al*^[3] reported that the 5- and 10-year relative survival rates in CRC patients were 65% and 58%, respectively, but the 5-year relative survival rate was 90% when CRC was detected at a localized stage^[3]. Therefore, it is important to improve the detection rate of early stage CRC and precancerous lesions.

Colorectal endoscopy can directly observe intestinal lesions, so it is irreplaceable in the examination of intestinal disease, especially CRC. To improve the detection rate of early-stage CRC and precancerous lesions, many new assistive techniques have been

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used in clinical practice such as chromoendoscopy, magnifying endoscopy, fluorescence endoscopy, confocal laser endoscopy, and electronic staining endoscopy. However, the process of chromoendoscopy is complicated and time-consuming, fluorescence endoscopy and confocal laser endoscopy are expensive, and these disadvantages limit the application of these new techniques.

Compared with these new techniques, electronic staining endoscopy is more convenient and practical, and its sensitivity and specificity in distinguishing colorectal neoplastic lesions from non-neoplastic lesions are about 90% and 85%, respectively^[4]. Electronic staining endoscopy includes narrow-band imaging (NBI), flexile spectral imaging color enhancement, and i-scan, of which NBI is the most widely used. Since the emergence of NBI in 1999, it has been a reliable tool that has contributed to improving diagnostic accuracy, such as differentiation of neoplastic from non-neoplastic lesions and characterization of colorectal neoplasia^[4,5]. Through the analysis of capillary vessel structure, surface structure and lesion color under NBI, researchers have proposed a variety of classifications to judge the nature of lesions accurately and select treatment strategy appropriately. In recent years, colorectal NBI magnifying classifications such as Hiroshima, Sano, Showa and Jikei classifications have been widely used in clinical practice and play an important role clinically^[6,7]. However, magnifying endoscopy has not yet been widely applied outside of Japan.

The Colon Tumor NBI Interest Group put forward a new NBI classification called the NBI international colorectal endoscopic (NICE) classification in 2009^[8], and validation studies of this new NBI classification were conducted in 2012^[9,10]. It is the first NBI classification that can be used for both non-magnifying and magnifying NBI endoscopy^[8,11]. The NICE classification has a high diagnostic accuracy in detecting non-neoplastic lesions that do not require resection and deep submucosal invasive (SM-d) carcinoma that needs to be treated surgically^[12,13]. However, it is difficult to differentiate high-grade dysplasia (HGD) or superficial submucosal invasive (SM-s) carcinoma from low-grade dysplasia (LGD)^[8,14] using NICE classification. To solve this problem, the Japanese NBI expert team (JNET) composed of Japanese magnifying colonoscopists was organized in 2011, and a new NBI colorectal magnifying classification, the JNET classification was put forward in 2014^[15].

To the best of our knowledge, most validation studies of the two new NBI classifications were conducted in originating centers by experienced endoscopists, but application in different centers among endoscopists with varying endoscopic skills remains unknown. To achieve external validity, in our study, we evaluated the clinical application and possible problems of NICE and JNET classifications in our unit, which is a tertiary hospital in China, and six endoscopists with varying levels of experience participated in this study.

MATERIALS AND METHODS

Patients

From September 2014 to December 2019, we enrolled consecutive patients who received white-light colonoscopy, NBI colonoscopy, and magnifying NBI colonoscopy at the same time in Beijing Shijitan Hospital (Beijing, China). Informed consent was obtained from all patients before their examinations. Patients with inflammatory bowel disease, familial adenomatous polyposis, or incomplete clinical data were excluded from this study.

Endoscopic examination

Patients drank 4 L of polyethylene glycol solution for their bowel preparation. A complete colonoscopy was performed by two experienced endoscopists, each of whom had previously performed > 1000 colonoscopies annually. All examinations were performed using magnifying colonoscopy (CF-H260AZI; Olympus Optical, Tokyo, Japan) and a standard videoendoscopic system (EVIS LUCERA; Olympus Optical), and magnifying images were taken with moderate-to-high-level power zoom. When a lesion was detected, the mucus and liquid feces on the surface of the lesion were washed away with lukewarm water. Endoscopic images of each lesion were taken in the following order: Non-magnifying conventional white-light colonoscopy, non-magnifying NBI, and magnifying NBI. The size of each lesion was estimated using the open-biopsy forceps method, with an open diameter of 7 mm (Radial Jaw 3; Boston Scientific Corp., Natick, MA, United States). The locations of the lesions were divided into three groups (proximal colon, distal colon, and rectum). Lesions were resected by biopsy, endoscopic mucosal resection (EMR) or endoscopic submucosal dissection

(ESD), and the biopsy was analyzed histopathologically. The histopathological diagnosis was based on World Health Organization criteria.

NICE and JNET classification

The NICE classification^[9,10] and JNET classification^[15], and typical examples of the endoscopic images used in our study are shown in Figures 1 and 2.

Interpretation of endoscopic images

Six endoscopists with varying levels of experience participated in the present study. The endoscopists were divided into two groups: A group of less-experienced endoscopists (LEE group) who had carried out colonoscopies for > 5 years but not with magnifying NBI, and a group of highly experienced endoscopists (HEE group) who had routinely used magnifying NBI for > 5 years^[16]. The three groups of endoscopic pictures of each lesion (non-magnifying white-light colonoscopy, non-magnifying NBI, and magnifying NBI) were evaluated by the six endoscopists in a randomized order using the NICE and JNET classifications separately. The non-magnifying white-light colonoscopy and non-magnifying NBI images demonstrated an overview of each lesion in order to mimic real-time endoscopic examination, whereas the magnifying NBI images showed crucial findings to evaluate the histopathological features. Patients information such as age, sex, clinical diagnosis, and histopathological results was not disclosed to any of the evaluators, and discussions were not permitted among the endoscopists individually or in groups.

Statistical analysis

We calculated sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) for each category of the two classifications. We received histology of colorectal lesions as the gold standard. Sensitivity, specificity, accuracy, PPV, and NPV of each category of the two classifications were compared between the two groups by using the Mann-Whitney *U* test. $P < 0.05$ was considered statistically significant. In addition, the interobserver agreement in each group was assessed using *k* values as follows: < 0.4, poor agreement; 0.41-0.60, fair agreement; 0.61-0.80, good agreement; and > 0.80, excellent agreement. All statistical analyses were conducted using SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, United States). The statistical methods of this study were reviewed by Qing-Kun Song from Beijing Shijitan Hospital, Capital Medical University.

RESULTS

Ninety-six patients received white-light colonoscopy, NBI colonoscopy and magnifying NBI colonoscopy at the same time between September 2014 and December 2019, and 137 lesions were resected. Nine patients with inflammatory bowel disease, familial adenomatous polyposis, or incomplete clinical data were excluded. Finally, 87 consecutive patients were enrolled for endoscopic evaluation, and 125 lesions were photographed during non-magnifying conventional white-light colonoscopy, non-magnifying NBI, and magnifying NBI. Bowel preparation was achieved perfectly and complete colonoscopy was performed to the cecum in every patient. Demographic data and characteristics of the lesions such as size, location and pathology are shown in Table 1.

Diagnostic characteristics of NICE classification

The diagnostic characteristics of each category among the two groups are shown in Table 2. The sensitivity, specificity, accuracy, PPV and NPV of type 1 lesions for the diagnosis of hyperplastic lesions (HPLs) and sessile serrated lesions (SSLs) in the HEE group were 84.6%, 94.9%, 93.9%, 65.9%, and 98.2%, respectively, and 82.1%, 93.8%, 92.5%, 60.4%, and 97.8%, respectively, in the LEE group. The sensitivity, specificity, accuracy, PPV and NPV of type 2 lesions for the diagnosis of adenoma in the HEE group were 91.4%, 86.3%, 90.7%, 97.7%, and 61.2%, respectively, and 89.8%, 84.3%, 89.1%, 97.3%, and 56.6%, respectively, in the LEE group. The sensitivity, specificity, accuracy, PPV and NPV of type 3 lesions for the diagnosis of SM-d carcinoma in the HEE group were 91.7%, 97.0%, 96.8%, 54.0%, and 99.7%, respectively, and 83.3%, 96.4%, 96.0%, 45.8%, and 99.4%, respectively, in the LEE group. Except for sensitivity of type 3 lesions for diagnosis of SM-d carcinoma in the HEE group was significantly superior to that in the LEE group (91.7% *vs* 83.3%; $P = 0.042$), the diagnostic characteristic of each category of the NICE classification was comparable, and there

Table 1 Demographic characteristics of 87 patients with 125 colorectal lesions

Variables	Number
Patients	87
Lesions	125
Sex, male/female	61; 26
Age in yr, mean \pm SD; range	59.9 \pm 10.6; 34-89
Location	
Proximal colon	42
Distal colon	61
Rectum	22
Size in mm, mean \pm SD; range	14.3 \pm 0.7; 4-45
5	9
6-10	44
11-20	58
≥ 20	14
Morphology	
Ip	22
Isp	39
Is	30
Ila	31
Ilb	3
Pathology	
Hyperplastic or sessile serrated lesion	13
Tubular adenoma	67
Tubulovillous adenoma	27
Low-grade intramucosal neoplasia	1
High-grade intramucosal neoplasia	3
Superficial submucosal invasive cancer	10
Deep submucosal invasive cancer	4

were no significant differences between the two groups. The overall interobserver agreement was good in both groups ($\kappa = 0.751$ in HEE group, and $\kappa = 0.744$ in LEE group).

Diagnostic characteristics of JNET classification

The diagnostic characteristics of each category between the two groups are shown in Table 3. The sensitivity, specificity, accuracy, PPV and NPV of type 1 lesions for the diagnosis of HPLs and SSLs in the HEE group were 87.1%, 97.3%, 95.5%, 74.1%, and 98.5%, respectively, and 84.6%, 96.4%, 95.2%, 73.4%, and 98.2%, respectively, in the LEE group. The sensitivity, specificity, accuracy, PPV and NPV of type 2A lesions for the diagnosis of LGD in the HEE group were 82.5%, 90.0%, 81.9%, 93.3%, and 58.5%, respectively, and 82.5%, 91.1%, 84.5%, 96.7%, and 62.1%, respectively, in the LEE group. The sensitivity, specificity, accuracy, PPV and NPV of type 2B lesions for the diagnosis of HGD-SM-s carcinoma in the HEE group were 53.8%, 84.2%, 81.4%, 31.5%, and 92.2%, respectively, and 51.3%, 84.8%, 81.3%, 28.3%, and 93.8%, respectively, in the LEE group. The sensitivity, specificity, accuracy, PPV and NPV of type 3 lesions for the diagnosis of SM-d carcinoma in the HEE group were 91.7%, 98.1%, 97.9%, 63.2%, and 99.7%, respectively, and 83.3%, 98.4%, 97.9%, 63.3%, and 99.4%, respectively, in the LEE group. The overall interobserver agreement was good in both groups ($\kappa = 0.747$ in HEE group, $\kappa = 0.759$ in LEE group).

Table 2 Performance characteristics of each type of the narrow-band imaging international colorectal endoscopic classification

NICE	Group ¹	Sensitivity, % (95%CI)	Specificity, % (95%CI)	Accuracy, % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)
Type 1	HEE	84.6 (65.5-100.0)	94.9 (93.7-96.2)	93.9 (90.8-96.9)	65.9 (55.6-76.3)	98.2 (95.9-100.0)
	LEE	82.1 (60.0-100.0)	93.8 (91.5-96.0)	92.5 (88.4-96.7)	60.4 (46.2-74.5)	97.8 (95.2-100.0)
		<i>P</i> = 0.637	<i>P</i> = 0.105	<i>P</i> = 0.275	<i>P</i> = 0.275	<i>P</i> = 0.376
Type 2	HEE	91.4 (88.7-94.0)	86.3 (77.8-94.7)	90.7 (89.5-91.8)	97.7 (96.4-99.0)	61.2 (56.0-66.4)
	LEE	89.8 (87.5-92.1)	84.3 (75.9-92.8)	89.1 (86.8-91.4)	97.3 (96.0-98.7)	56.6 (50.6-62.6)
		<i>P</i> = 0.105	<i>P</i> = 0.456	<i>P</i> = 0.043 0.1	<i>P</i> = 0.121	<i>P</i> = 0.100
Type 3	HEE	91.7 (55.8-100.0)	97.0 (92.2-100.0)	96.8 (91.5-100.0)	54.0 (-2.7-100.0)	99.7 (99.5-100.0)
	LEE	83.3 (47.5-100.0)	96.4 (92.1-100.0)	96.0 (90.7-100.0)	45.8 (0.7-91.0)	99.4 (98.2-100.0)
		<i>P</i> = 0.042	<i>P</i> = 0.487	<i>P</i> = 0.367	<i>P</i> = 0.376	<i>P</i> = 0.346

¹Three endoscopists in each group.

CI: Confidence interval; HEE: Highly experienced endoscopist; LEE: Less-experienced endoscopist; NPV: Negative predictive value; PPV: Positive predictive value; NICE: Narrow-band imaging international colorectal endoscopic.

Table 3 Performance characteristics of each type of the Japanese narrow-band imaging expert team classification

JNET	Group ¹	Sensitivity, % (95%CI)	Specificity, % (95%CI)	Accuracy, % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)
Type 1	HEE	87.1 (76.2-98.2)	97.3 (95.1-99.5)	95.5 (92.4-98.5)	74.1 (60.0-88.1)	98.5 (97.2-99.8)
	LEE	84.6 (65.5-100.0)	96.4 (94.2-98.6)	95.2 (91.8-98.6)	73.4 (58.8-88.0)	98.2 (96.0-100.0)
		<i>P</i> = 0.369	<i>P</i> = 0.261	<i>P</i> = 0.637	<i>P</i> = 0.822	<i>P</i> = 0.500
Type2A	HEE	82.5 (78.5-86.5)	90.0 (81.7-98.3)	81.9 (72.7-91.1)	93.3 (77.6-108.9)	58.5 (47.0-69.9)
	LEE	82.5 (81.0-84.0)	91.1 (86.3-95.9)	84.5 (83.4-85.7)	96.7 (95.0-98.4)	62.1 (60.3-63.9)
		<i>P</i> = 0.817	<i>P</i> = 0.637	<i>P</i> = 0.099	<i>P</i> = 0.376	<i>P</i> = 0.077
Type 2B	HEE	53.8 (34.7-73.0)	84.2 (73.1-95.4)	81.4 (69.2-93.5)	31.5 (16.4-46.6)	92.2 (81.5-100.0)
	LEE	51.3 (29.2-73.4)	84.8 (81.0-88.7)	81.3 (75.6-87.1)	28.3 (14.2-42.3)	93.8 (90.8-96.7)
		<i>P</i> = 0.817	<i>P</i> = 0.825	<i>P</i> = 0.825	<i>P</i> = 0.268	<i>P</i> = 0.825
Type 3	HEE	91.7 (55.8-100.0)	98.1 (94.9-100.0)	97.9 (93.7-100.0)	63.2 (16.4-100.0)	99.7 (98.5-100.0)
	LEE	83.3 (47.5-100.0)	98.4 (96.3-100.0)	97.9 (94.8-100.0)	63.3 (25.4-100.0)	99.4 (98.3-100.0)
		<i>P</i> = 0.456	<i>P</i> = 0.822	<i>P</i> = 0.856	<i>P</i> = 0.891	<i>P</i> = 0.817

¹Three endoscopists in each group.

CI: Confidence interval; HEE: Highly experienced endoscopist; LEE: Less-experienced endoscopist; NPV: Negative predictive value; PPV: Positive predictive value; JNET: Japanese narrow-band imaging expert team.

DISCUSSION

Colorectal adenoma is a precancerous lesion of CRC, and its resection can reduce the incidence and mortality of CRC; therefore, in western countries, removal of all adenomatous polyps has been standardized^[17,18]. In clinical practice, the pathological diagnosis of all resected polyps is routinely performed, and the final pathological result determines the intervention of endoscopic follow-up^[19]. However, the removal of all polyps and routine pathological diagnosis not only increase the risks associated with the resection process, but also the cost of both the operation and the pathological diagnosis. Therefore, the resect and discard policy has been proposed^[20-22]. The policy states that the HPL do not need treatment, and the treatment of these lesions may increase the adverse events of polypectomy and cost of medical care^[19,23-25]. As reported previously, the NICE classification is simple and practical in identifying HPL that should be left^[8,26]. In our study, the sensitivity, specificity and accuracy of NICE


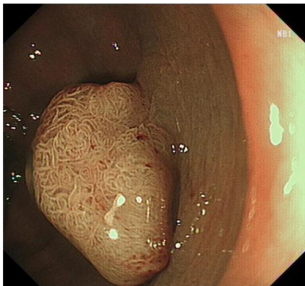

	Type 1	Type 2	Type 3
Color	Same or lighter than background	Browner relative to background (verify color arises from vessels)	Brown to dark brown relative to background; sometimes patchy whiter areas
Vessels	None, or isolated lacy vessels may be present coursing across the lesion	Brown vessels surrounding white structures	Has area(s) with disrupted or missing vessels
Surface pattern	Dark or white spots of uniform size, or homogenous absence of pattern	Oval, tubular, or branched white structures surrounded by brown vessels	Amorphous or absence of pattern
Most likely pathology	Hyperplastic/SSL	Adenoma	Deep submucosal invasive cancer
Example			

Figure 1 Narrow-band imaging international colorectal endoscopic classification. SSL: Sessile serrated lesion.

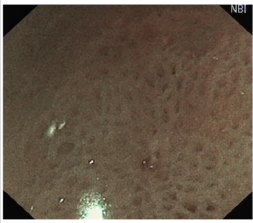
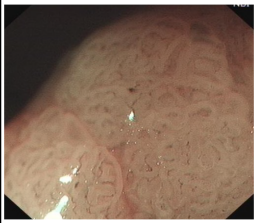
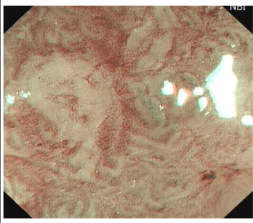
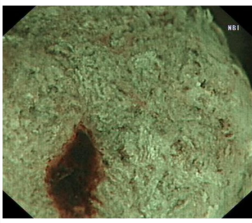
	Type 1	Type 2A	Type 2B	Type 3
Vessel pattern	Invisible	Regular caliber regular distribution (meshed/spiral pattern)	Variable caliber irregular distribution	Loose vessel areas Interruption of thick vessels
Surface pattern	Regular dark or white spots similar to surrounding normal mucosa	Regular (tubular/branched/papillar)	Irregular or obscure	Amorphous areas
Most likely histology	Hyperplastic/SSL	Low-grade intramucosal neoplasia	High-grade intramucosal neoplasia/superficial submucosal invasive cancer	Deep submucosal invasive cancer
Example				

Figure 2 Japanese Narrow-band Imaging Expert Team classification. SSL: Sessile serrated lesion.

classification type 1 lesions for the diagnosis of HPLs and SSLs in both the HEE and LEE groups were > 80%, with specificity and accuracy > 90%, with no significant difference between the two groups. This result shows that endoscopists can choose the treatment plan based on the NICE classification, which may improve the resect and discard strategy better promote.

The HEE group still had high specificity and accuracy > 95% when using NICE type 3 to diagnose SM-d carcinoma, and the sensitivity was 91.7%, but in the LEE group the sensitivity was only 83.3%. The *P* value of the sensitivity between the two groups was 0.042 by the Mann-Whitney *U* test, and the difference between the two groups for diagnosis of SM-d carcinoma was significant. Hayashi *et al*^[10] found that the sensitivity of NICE type 3 for the diagnosis of SM-d carcinoma was 94.9%^[10]. Compared with the study above, the sensitivity of the LEE group in the diagnosis of SM-d carcinoma was still low. This result may be related to the lack of experience in the diagnosis of SM-d carcinoma in the LEE group. Therefore, endoscopists in the LEE group should receive more training to avoid missed diagnosis of SM-d carcinoma.

To obtain a precise histological diagnosis, HGD or SM-s carcinoma should be resected by *en bloc* EMR/ESD or surgery rather than piecemeal EMR (pEMR). However, in clinical practice, we cannot determine the strategy of endoscopic treatment, such as pEMR, *en bloc* EMR/ESD or surgery, because NICE type 2 is difficult to differentiate HGD or SM-s carcinoma from LGD^[8,14]. To solve this problem and unify the current NBI classifications, the JNET classification with magnification was proposed^[15]. The principles and characteristics of the JNET classification are as follows: Magnification is essential and the basis is the NICE classification; NICE type 2 is divided into 2A and 2B subtypes using magnifying findings; Because magnification does not need estimation of color, the classification does not include the finding of color; and basic findings are composed of both vessel and surface patterns^[27].

Our results suggested that the sensitivity, specificity and accuracy of JNET classification types 1 and 3 were similar to NICE classification types 1 and 3 in both the HEE and LEE groups, and the specificity of JNET classification types 1 and 3 and NICE classification type 3 in both the HEE and LEE groups was > 95%. The sensitivity, specificity, accuracy, PPV and NPV of JNET classification type 2A lesions for the diagnosis of LGD in the HEE group were 82.5%, 90.0%, 81.9%, 93.3%, and 58.5%, respectively, and 82.5%, 91.1%, 84.5%, 96.7%, and 62.1%, respectively, in the LEE group. The results are similar to those of Sumimoto *et al.*^[27]. In order to avoid missed diagnosis of lesions, the sensitivity of the classification is important. However, before treatment of the lesion, the specificity of the classification is more important, because only by accurately determining the nature of the lesion can the appropriate treatment strategy be selected. In our study, the specificity of NICE types 1 and 3 and JNET types 1, 2A and 3 in both the HEE and LEE groups was > 90%. So, when the endoscopist's diagnostic confidence level is high (> 95%)^[27], the treatment strategy for NICE types 1 and 3 and JNET types 1, 2A and 3 lesions can be determined based on the results of endoscopic examination. Of course, if the confidence level is low, an additional examination should be performed.

The JNET type 2B lesions are the most important for curation and the most difficult to be diagnosed endoscopically. In our study, the sensitivity of JNET classification type 2B lesions for the diagnosis of HGD-SM-s carcinoma in the HEE group was 53.8% and 51.3% in the LEE group. As our result, even in the HEE group the sensitivity was not more than 60%. Previous studies showed that the sensitivity of JNET classification Type 2B lesions for diagnosis of HGD-SM-s carcinoma was 44.9%-61.9%^[27,28]. Compared with other types of JNET classification, the diagnostic ability of type 2B is the weakest. Although Sumimoto *et al.*^[29] further divided JNET type 2B into 2B-low and 2B-high^[29], the ability to diagnose HGD-SM-s carcinoma has not been significantly improved. The original intention of the JNET classification introduce the type 2A and 2B lesion was to distinguish LGD and SM-d carcinoma from HGD-SM-s carcinoma, and then to choose an appropriate treatment strategy, such as pEMR, *en bloc* EMR/ESD or surgery. However, due to poor diagnostic capabilities of type 2B, this goal cannot be achieved, and the type 2B lesions is still the biggest challenge for the endoscopists. So, lesions of type 2B need a further pit pattern diagnosis using magnifying chromoendoscopy or endoscopic ultrasound^[29-31].

Our study had some limitations. First, although six endoscopists with varying levels of experience participated in the study, they all belonged to the same institution and would be following similar guidelines, which may produce high interobserver agreement and threaten the external validity of the results. These results might be different when endoscopists belong to different units. Second, we initially presented non-magnifying conventional white-light overview images of entire lesion in order to mimic real-time endoscopic examination. For some endoscopists, the first observation of an entire lesion may affect their diagnosis of the lesion, especially when evaluating the lesion using the JNET classification. It is necessary for us to conduct a further study where we evaluate JNET classification using only magnifying NBI images of the lesions. Third, as a retrospective study, we enrolled as many cases as possible. However, in clinical practice, most SM-d carcinoma can be correctly diagnosed under white-light colonoscopy without further magnifying examination, so there were only four cases of deep-submucosal invasive cancer. The diagnostic accuracy and reliability of NICE and JNET classifications should be validated in a multicenter prospective study.

CONCLUSION

In conclusion, NICE types 1 and 3 and JNET types 1, 2A and 3 lesions showed

excellent diagnostic ability in both the HEE and LEE groups. When the confidence level is high, the treatment strategy of the NICE types 1 and 3 and JNET types 1, 2A and 3 lesions can be determined based on the results of endoscopic examination. JNET type 2B lesions require extra examination, such as magnifying chromoendoscopy or endoscopic ultrasound, to make an accurate assessment of the invasion depth for selecting an appropriate treatment strategy.

ARTICLE HIGHLIGHTS

Research background

Detecting and treating early stage colorectal cancer (CRC) and precancerous lesions is the most effective method to reduce the morbidity and mortality of CRC. Narrow-band imaging (NBI) endoscopy has been a very useful technique that has contributed to improving the detection rate of early stage CRC and precancerous lesions. Researchers have proposed a variety of NBI classifications to judge the nature of lesions accurately and select treatment strategy appropriately.

Research motivation

For the past few years, two new NBI classifications have been proposed: The NBI international colorectal endoscopic (NICE) classification and Japanese NBI expert team (JNET) classification. Most validation studies of the two new NBI classifications were conducted in originating centers by experienced endoscopists, but application in different centers among endoscopists with varying endoscopic skills remains unknown.

Research objectives

To achieve external validity, we evaluated the clinical application and possible problems of the NICE and JNET classifications for differential diagnosis of colorectal cancer and precancerous lesions.

Research methods

Six endoscopists with varying levels of experience were divided into two groups: Highly experienced endoscopists (HEEs) and less-experienced endoscopists (LEEs). Eighty-seven consecutive patients with a total of 125 lesions were photographed during non-magnifying conventional white-light colonoscopy, non-magnifying NBI, and magnifying NBI. The three groups of endoscopic pictures of each lesion were evaluated by the six endoscopists in a randomized order using the NICE and JNET classifications separately. We calculated sensitivity, specificity, accuracy, positive and negative predictive value for each category of the two classifications.

Research results

In both the HEE and LEE groups, the specificity of JNET classification type 1 and 3 and NICE classification type 3 was > 95%, and the overall interobserver agreement was good in both groups. However, the sensitivity of JNET classification type 2B lesions for the diagnosis of high-grade dysplasia or superficial submucosal invasive carcinoma in both the HEE and LEE groups was < 55%. Compared with other types of NICE and JNET classification, the diagnostic ability of JNET type 2B was the weakest.

Research conclusions

Due to the poor diagnostic capabilities of JNET type 2B, the type 2B lesions is still the biggest challenge for the endoscopists. So, lesions of type 2B need an additional examination to choose an appropriate treatment strategy.

Research perspectives

The JNET type 2B lesions are the most important for curation and the most difficult to be diagnosed endoscopically, and accurate diagnosis of JNET 2B lesions still requires further efforts.

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Efficacy and safety of intraoperative radiotherapy in rectal cancer: A systematic review and meta-analysis

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Abstract

BACKGROUND

In recent years, intraoperative radiotherapy (IORT) has been increasingly used for the treatment of rectal cancer. However, the efficacy and safety of IORT for the treatment of rectal cancer are still controversial.

AIM

To evaluate the value of IORT for patients with rectal cancer.

METHODS

We searched PubMed, Embase, Cochrane Library, Web of Science databases, and conference abstracts and included randomized controlled trials and observational studies on IORT *vs* non-IORT for rectal cancer. Dichotomous variables were evaluated by odds ratio (OR) and 95% confidence interval (CI), hazard ratio (HR) and 95% CI was used as a summary statistic of survival outcomes. Statistical analyses were performed using Stata V.15.0 and Review Manager 5.3 software.

RESULTS

In this study, 3 randomized controlled studies and 12 observational studies were included with a total of 1460 patients, who are mainly residents of Europe, the

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United States, and Asia. Our results did not show significant differences in 5-year overall survival (HR = 0.80, 95%CI = 0.60-1.06; $P = 0.126$); 5-year disease-free survival (HR = 0.94, 95%CI = 0.73-1.22; $P = 0.650$); abscess (OR = 1.10, 95%CI = 0.67-1.80; $P = 0.713$), fistulae (OR = 0.79, 95%CI = 0.33-1.89; $P = 0.600$); wound complication (OR = 1.21, 95%CI = 0.62-2.36; $P = 0.575$); anastomotic leakage (OR = 1.09, 95%CI = 0.59-2.02; $P = 0.775$); and neurogenic bladder dysfunction (OR = 0.69, 95%CI = 0.31-1.55; $P = 0.369$). However, the meta-analysis of 5-year local control was significantly different (OR = 3.07, 95%CI = 1.66-5.66; $P = 0.000$).

CONCLUSION

The advantage of IORT is mainly reflected in 5-year local control, but it is not statistically significant for 5-year overall survival, 5-year disease-free survival, and complications.

Key Words: Intraoperative radiotherapy; Rectal cancer; Systematic review; External beam radiation therapy; Randomized controlled trials; Meta-analysis

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Core Tip: Rectal cancer is one of the malignant tumors with a high fatality rate in the world. Intraoperative radiotherapy (IORT) allows for direct administration of high-dose radiation and the area that is at the greatest risk after resection. Although research reports on IORT for rectal cancer have been published, there is still a lack of reliable evidence regarding treatment efficacy and safety. Therefore, we conducted a systematic review and meta-analysis to evaluate the efficacy and safety of IORT for the treatment of rectal cancer.

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INTRODUCTION

Rectal cancer is a common malignant tumor of the digestive tract^[1]. Because of its characteristics of being difficult to locate, high mortality, and poor prognosis, it is a killer, thereby threatening human health^[2]. Surgical resection is one of the main clinical treatment methods, and tumor tissue can be removed as much as possible to achieve good clinical treatment results^[3]. Currently, laparoscopic surgery is commonly used in the clinical treatment of rectal cancer^[4]. For advanced or recurrent rectal cancer, the combination of surgery and radiotherapy can prolong the survival rate of patients, but external beam radiation therapy (EBRT) alone has a poor response to treatment and a high recurrence rate^[5,6].

Intraoperative radiotherapy (IORT) involves the precise delivery of large doses of ionizing radiation to a tumor or tumor bed during surgery^[7,8]. Direct visualization of the tumor bed and the ability to separate healthy tissue from the tumor bed maximize the radiation dose to the tumor, while minimizing the dose to healthy tissue, thereby leading to an increased treatment rate for IORT^[9,10]. Although IORT was introduced in the 1960s^[5], its popularity increased with the introduction of self-shielding mobile linear accelerators and low-voltage IORT devices^[11]. In May 2019, the American Society of Brachytherapy reached a consensus on IORT: IORT can be considered at the time of surgical resection of locally advanced or recurrent colorectal cancer in cases with concern for a positive margin, particularly when pelvic EBRT has already been delivered^[12]. The National Comprehensive Cancer Network guidelines for the treatment of rectal cancer (Version 4.2020) described the following: IORT, if available, may be considered for very close or positive margins after resection, as an additional boost, especially for patients with T4 or recurrent cancers^[13]. At present, the number of studies that focus on IORT is increasing and includes breast cancer, colorectal cancer,

pancreatic cancer, gastric cancer, head and neck cancer, glioma, and gynecological tumors^[14-16].

In the past 10 years, cases of rectal cancer patients receiving IORT have gradually increased^[17]. In previous studies^[18], it was demonstrated that adding IORT to traditional treatment of rectal cancer not only reduces the local recurrence rate of advanced rectal cancer but also influences the local control (LC) rate of locally recurrent rectal cancer. However, a recent randomized controlled trial (RCT) showed that IORT cannot be recommended as a standard therapy to compensate less radical resection for advanced lower rectal cancer^[19]. Although several research reports on IORT for the treatment of rectal cancer have been published, due to the small sample size, there is still a lack of reliable evidence regarding the efficacy and safety of IORT.

Therefore, to draw more reliable conclusions, we conducted a systematic review and meta-analysis to evaluate the effectiveness and safety of IORT *vs* non-IORT in the treatment of rectal cancer.

MATERIALS AND METHODS

This systematic review and meta-analysis are reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis statement^[20].

Search strategy

Up to November 2020, PubMed, Embase, Cochrane Library, Web of Science, letters to the editor and abstracts of conferences were searched to compare the efficacy and safety of IORT and non-IORT for the treatment of rectal cancer. The following medical subject heading terms and keywords were used: “intraoperative radiotherapy”, “IORT”, “intra-operative radiation therapy”, “intraoperative radiation therapy”, “rectal neoplasms”, and “rectal cancer”. The search strategy for PubMed is revealed in the supplementary material (Item 1).

Selection criteria

Studies were included if the RCT and observational study published compared IORT and non-IORT treatment for rectal cancer, and at least 20 patients were included in the study. Studies were excluded if the study was a review, expert opinion, or meta-analysis, lack of original data, no control group, duplicate studies, and animal studies.

Titles and abstracts of retrieved studies were screened by two independent reviewers, and any conflicts were resolved by discussion. Any potentially eligible study was retrieved for further reviewer.

Data extraction and quality assessment

Two reviewers (BL and LG) independently assessed the eligibility of each trial and extracted the data (first author name, publication date, country/region, study type, number of patients per group, age, tumor site, stage, pre-operative radiotherapy; chemotherapy; post-operative radiotherapy and IORT dose from each study. The main results were 5-year overall survival (OS), 5-year disease-free survival (DFS), 5-year LC, and complications (abscess, fistulae, wound complications, anastomotic leakage, and neurogenic bladder dysfunction).

The Cochrane risk of bias tool^[21] was used to evaluate the quality of RCTs including selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias through high-risk, low-risk, and unknown risk. The quality of the study was assessed by the Newcastle-Ottawa Scale^[22] for observational studies. We analyzed the representativeness of the exposed observational, selection of the non-exposed observational, ascertainment of exposure, demonstration that outcome of interest was not present at the start of the study, comparability of cohorts based on the design and analysis, assessment of outcome, whether follow-up time was long enough for outcomes to occur, and the adequacy of follow-up of the cohorts. A score of 0-9 was assigned to each study. In general, studies were considered of high quality if a score of 6 was reached. Disagreements were resolved by discussion and consultation with the senior investigator.

Statistical analysis

Dichotomous variables were evaluated by odds ratio (OR) and 95% confidence interval (CI), including LC and complication results. In addition, hazard ratio (HR) was used as a summary statistic of survival outcomes (5-year OS and 5-year DFS). Heterogeneity was evaluated using the Higgins I^2 value, and values < 25, 25 to 50, and

> 50 were defined as corresponding to low, moderate, and high heterogeneity, respectively. The OR and HR values are reported with the 95% CIs. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with Review Manager 5.3 software (Cochrane Collaboration's Information Management System) and Stata version 15.0 software (STATA, College Station, TX, United States).

Subgroup analysis was performed on the basis of study type, and sensitivity analysis was performed on the outcome indicators of more than 10 studies to explore their potential sources and assess the robustness of these results. The Begg's test and Egger's test were used to test publication bias.

RESULTS

Search results

Initially, 645 studies were included in the study through electronic retrieval. A total of 169 duplicate studies were removed and 448 articles were excluded after reading the title and abstract; thus, a total of 28 studies were obtained. After reading the full text, another 15 studies were excluded. Finally, 15^[19,23-36] studies were included, involving 1460 patients (687 in the IORT group, 773 in the non-IORT group). The studies included 3 RCTs^[19,30,32] and 12 observational studies^[23-29,31,33-36] comparing IORT with non-IORT for rectal cancer. Tables 1 and 2 summarize the baseline characteristics of the included studies. Basic characteristics of the included studies were as follows. (1) There was a large sample size gap between the studies, with the largest being 163 cases^[34] and the smallest being 43 cases^[27]; (2) The publication year of the literature varied greatly, and the time span ranged from 1991 to 2020; (3) The literature was mainly obtained from European, American, and Asian countries; and (4) The literature mostly consisted of observational studies and a few RCTs.

The quality of RCTs showed that attrition bias was at high risk and the quality of all observational studies showed that two studies received nine stars, four received eight stars, and four received seven stars (Table 1). Figure 1 presents the screening flow chart of the included studies. Figure 2 shows the quality assessment of the three RCTs, which indicates that the overall quality of the three RCTs was sufficient.

Meta-analysis results

The results of the meta-analysis were arbitrated by the study type subgroup (RCTs and observational studies) (Table 3).

Five-year OS and five-year DFS

A total of 9^[19,25,26,28,30-32,35,36] of the 15 studies included the 5-year OS results reported in their results (Figure 3A). We did not observe statistically significant differences in the meta-analysis (HR = 0.80, 95% CI = 0.60-1.06; $P = 0.189$). The meta-analysis of RCTs (HR = 0.68, 95% CI = 0.29-1.63; $P = 0.390$) and observational studies (HR = 0.81, 95% CI = 0.60-1.11; $P = 0.189$) also showed similar results. Furthermore, the results showed no heterogeneity in the subgroup of observational studies ($\chi^2 = 2.21$, $I^2 = 0.0\%$; $P = 0.819$).

In 6^[23,28,30,32,35,36] of the 13 studies, a 5-year DFS period was reported (Figure 3B). No significant differences were observed in the data: Totality (HR = 0.94, 95% CI = 0.73-1.22; $P = 0.650$). The meta-analysis of RCTs (HR = 1.61, 95% CI = 0.74-3.53; $P = 0.231$) and observational studies (HR = 0.89, 95% CI = 0.68-1.16; $P = 0.378$) showed similar results. The results showed no heterogeneity in the subgroup of observational studies ($\chi^2 = 1.72$, $I^2 = 0.0\%$; $P = 0.633$).

Five-year local control

In 14^[19,23-33,35,36] cases, the meta-analysis of 5-year LC revealed statistically significant differences (OR = 3.07, 95% CI = 1.66-5.66; $P = 0.000$) (Figure 4). However, the meta-analysis of RCTs (OR = 1.37, 95% CI = 0.35-5.35; $P = 0.655$) and observational studies (OR = 3.45, 95% CI = 1.54-7.73; $P = 0.000$) showed different results. High heterogeneity was found in the subgroup of observational studies ($\chi^2 = 41.31$, $I^2 = 73.4\%$; $P = 0.000$).

Abscess

In 6^[19,24,26,30,33,34] of the 13 studies, abscess results reported were included in the study (Figure 5). No statistical significance was observed (OR = 1.10, 95% CI = 0.67-1.80; $P = 0.833$). The meta-analysis of RCTs (OR = 1.83, 95% CI = 0.65-5.11; $P = 0.252$) and observational studies (OR = 0.94, 95% CI = 0.53-1.66; $P = 0.833$) also showed similar results. The results showed no heterogeneity in the subgroup of observational studies (

Table 1 Baseline characteristics of the included studies

Ref.	Year	Location	Time frame	Type	Patients, <i>n</i> IORT/ non-IORT	Age in yr, mean IORT non-IORT	Follow-up in mo, mean IORT non-IORT	Resection margin	Clinical stages, %	PR- RT, %	CT, %	PO- RT, %	IORT dose in Gy mean	NOS score
Willett <i>et al</i> ^[23]	1991	United States	1978-1988	Observational studies	20; 21/22; 2	64 v	26 v	R0; R1/R2	NA	100	NA	0	15	7
Suzuki <i>et al</i> ^[24]	1995	Germany	1981-1988	Observational studies	42/64	64.3 v	44 v	R1/R2	NA	NA	NA	98	20	8
Huber <i>et al</i> ^[25]	1996	Germany	1989-1993	Observational studies	36/18	NA	25.5 v	R0/R1/R2	T3 (50); T4 (50)	50	100	50	15	8
Wiig <i>et al</i> ^[26]	2002	Norway	1990-1999	Observational studies	59/48	NA	NA	R0/R1/R2	NA	100	NA	NA	15-20	8
Ratto <i>et al</i> ^[27]	2003	Italy	1990-1997	Observational studies	19/24	62	74 v	NA	T3 (7); T4 (93)	NA	NA	NA	10-15	7
Sadahiro <i>et al</i> ^[28]	2004	Japan	1991-2001	Observational studies	99/68	60 61	67 v	NA	T1/T2 (29); T3 (59); T4 (12)	100	53	0	17.3	7
Ferenschield <i>et al</i> ^[29]	2006	Netherlands	1987-2001	Observational studies	30/93	66 v	25 v	R0	T2 (14); T3 (57); T4 (25)	100	NA	0	10	9
Masaki <i>et al</i> ^[30]	2008	Japan	2000-2007	RCT	19/22	NA	34 v	NA	T1/T2 (11); T3 (89)	NA	37	NA	18-20 f	RCT
Valentini <i>et al</i> ^[31]	2009	Italy	1991-2006	Observational studies	11/35	62 v	80 v	R0	T4 (100)	NA	NA	NA	10-15 f	7
Dubois <i>et al</i> ^[32]	2011	France	1993-2001	RCT	72/68	62.5 64.5	60 v	NA	T3/T4 (100)	100	25	NA	18	RCT
Zhang <i>et al</i> ^[35]	2014	China	1996-2007	Observational studies	45/46	61 61	72.9 v	NA	T3 (100)	NA	100	51	20	8
Alberda <i>et al</i> ^[33]	2014	Netherlands	1996-2012	Observational studies	21; 22/31; 17	66 59/61 56	38 39/23 12	R0/R1	T3 (41); T4 (59)	NA	NA	NA	10	8
Klink <i>et al</i> ^[34]	2014	Germany	2004-2012	Observational studies	52/111	62 63	NA	R0	T3/T4 (100)	NA	NA	NA	10-20 f	9
Zhang <i>et al</i> ^[36]	2015	China	1994-2007	Observational studies	71/77	58 63	72.3 v	R0/R1/R2	T2 (6); T3 (52); T4 (42)	NA	100	100	15	7
Masaki <i>et al</i> ^[19]	2020	Japan	2000-2017	RCT	38/38	NA	72 v	R0/R1	T1/T2 (17); T3 (80); T4 (3)	NA	NA	NA	18-20 f	RCT

CT: Chemotherapy; f: Range reported with no mean/median; IORT: Intraoperative radiotherapy; NA: Not reported; non-IORT: Non-intraoperative radiotherapy; PO-RT: Post-operative radiotherapy; PR-RT: Pre-operative radiotherapy; RCT: Randomized controlled trial; v: Reported for IORT and non-IORT combined.

Table 2 Outcome indicators of the included studies

Ref. %	Surgery, %	Resection margin %	5-yr OS, % IORT non-IORT %	5-yr DFS, % IORT non-IORT %	5-yr LC, % IORT non-IORT %	Complications, % IORT non-IORT	
Willett <i>et al</i> ^[23]	NA	R0; R1/R2	NA	53 60; 32 NA	88 71; 60 0	Abscess (5); Fistulae (7) Wound (5); Anastomotic leakage (2) Ureteric obstruction (2) Sacral necrosis (2)	NA
Suzuki <i>et al</i> ^[24]	LAR (57); APR (35); Hartmann (6)	R1/R2	19 7.3	30 5.9	60 7	Pelvic abscess (12) Fistula (2) Perineal wound (7); Small bowel obstruction (14) Ureteral obstruction (7)	Pelvic abscess (11) Fistula (6) Perineal wound (2); Small bowel obstruction (5) Ureteral obstruction (2)
Huber <i>et al</i> ^[25]	LAR (84); APR (16)	R0/R1/R2	40 20	28 NA	80 24	Wound (45) Sacral wound dehiscence (21); Neurogenic bladder dysfunction (8)	Wound (58) Sacral wound dehiscence (26); Neurogenic bladder dysfunction (11)
Wiig <i>et al</i> ^[26]	LAR (31); APR (10); Hartmann (19)	R0/R1/R2	30 35	NA	44 28	Abscess (24) wound (3) Anastomotic leakage (3); Late perineal healing (10)	Abscess (29) wound (13) Anastomotic leakage (13); Late perineal healing (2)
Ratto <i>et al</i> ^[27]	LAR (33); APR (56)	NA	NA	47 39	91 57	NA	
Sadahiro <i>et al</i> ^[28]	LAR (54); APR (46)	NA	79 58	71 54	98 84	Anastomotic leakage (6) Wound (23) Bleeding (3) Neurogenic bladder dysfunction (2)	Anastomotic leakage (3) Wound (12) Bleeding (1) Neurogenic bladder dysfunction (4)
Ferenschild <i>et al</i> ^[29]	NA	R0	56 66	NA	71 72	NA	
Masaki <i>et al</i> ^[30]	TME (100)	NA	64 NA	60 NA	95 95	Anastomotic breakdown (25) Intrapelvic abscess (14)	Anastomotic breakdown (14) Intrapelvic abscess (21)
Valentini <i>et al</i> ^[31]	APR (56)	R0	19.4 16.3	41.1 16.8	79.5 23.7	NA	
Dubois <i>et al</i> ^[32]	APR (20)	NA	77 75	62 66	92 93	Anastomotic leakage (8.5) Re-operation (11.3) Infectious complications (9.9) Medical complications (7.0) Sacral necrosis (1.5)	Anastomotic leakage (4.4) Re-operation (8.8) Infectious complications (11.8) Medical complications (2.9)
Zhang <i>et al</i> ^[35]	TME (80)	NA	84 86	71 73	84 86	Grade 3 diarrhea (3) numbness and motor weakness (4.4)	Leukopenia (10.9) Grade 3 diarrhea (14) incomplete intestinal obstruction (6.5) acute mucositis of the anal verge (23.9)
Alberda <i>et al</i> ^[33]	TME (100)	R0; R1	63 81; 41 13	NA	70 79; 84 41	Abdominal/perineal wound infections (31) abscess (6) Anastomotic leakage (2) Urinary tract infection (8) Cardiac (6)	Abdominal/perineal wound infection (23) abscess (13) Anastomotic leakage (3) Urinary tract infection (8) Cardiac (3)
Klink <i>et al</i> ^[32]	NA	R0	NA	NA	NA	Postoperative bleeding (0) Anastomotic leakage (11) Surgical site infection (15) Abscess (10) Fistula (2) Stenosis (4) Bladder dysfunction (8) Urethral leakage (0) Sexual dysfunction (2)	Postoperative bleeding (4) Anastomotic leakage (14) Surgical site infection (9) Abscess (5) Fistula (0) Stenosis (1) Bladder dysfunction (10) Urethral leakage (1) Sexual dysfunction (3)
Zhang <i>et al</i> ^[36]	TME (100)	R0/R1/R2	74.6 66.2	69 58.5	89.7 79	Incomplete intestinal obstruction (4) Hydronephrosis (7)	Incomplete intestinal obstruction (2.6) Hydronephrosis (10)

Masaki <i>et al</i> ^[19]	TME (100)	R0/R1	71.5 81.8	NA	87.6 91.7	Anastomotic leakage (29) abscess (18) Small bowel obstruction (13)	Anastomotic leakage (13) abscess (11) Small bowel obstruction (18)
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APR: Abdominoperineal resection; DFS: Disease-free survival; LAR: Low anterior resection; LC: Local control; NA: Not reported; non-IORT: Non-Intraoperative radiotherapy; OS: Overall survival; TME: Total mesorectal excision.

$\chi^2 = 1.99$, $I^2 = 0.0\%$; $P = 0.575$).

Fistulae

In 3^[24,32,34] of the 13 studies, the fistulae results were included in the study (Figure 6). The results were not statistically significant (OR = 0.79, 95%CI = 0.33-1.89; $P = 0.600$). The meta-analysis of RCTs (OR = 0.75, 95%CI = 0.30-1.88; $P = 0.542$) and observational studies (OR = 1.22, 95%CI = 0.08-18.77; $P = 0.888$) showed similar results. High heterogeneity was found in the subgroup of observational studies ($\chi^2 = 2.02$, $I^2 = 50.4\%$; $P = 0.156$).

Wound complications

In 8^[24-26,28,32-35] of the 13 cases, wound complications results were included in the study (Figure 7) and were not statistically significant (OR = 1.02, 95%CI = 0.52-2.02; $P = 0.948$). The meta-analysis of RCTs (OR = 0.75, 95%CI = 0.30-1.88; $P = 0.542$) and observational studies (OR = 1.06, 95%CI = 0.47-2.37; $P = 0.893$) also showed similar results. High heterogeneity was found in the subgroup of observational studies ($\chi^2 = 16.09$, $I^2 = 62.7\%$; $P = 0.013$).

Anastomotic leakage

In 7^[19,26,28,30,32-34] of the 13 cases, the anastomotic leakage results were not statistically significant (OR = 1.09, 95%CI = 0.59-2.02; $P = 0.775$) (Figure 8). RCTs (OR = 2.18, 95%CI = 0.89-5.33; $P = 0.087$) and observational studies (OR = 0.66, 95%CI = 0.31-1.41; $P = 0.283$) The results showed no heterogeneity in the subgroup of observational studies ($\chi^2 = 2.46$, $I^2 = 0.0\%$; $P = 0.482$).

Neurogenic bladder dysfunction

In 3^[25,28,34] of the 13 cases, the neurogenic bladder dysfunction results were included in the study (Figure 9). No statistically significant differences were observed (OR = 0.69, 95%CI = 0.31-1.55; $P = 0.369$). The results showed no heterogeneity in the subgroup of observational studies ($\chi^2 = 0.25$, $I^2 = 0.0\%$; $P = 0.874$).

Publication bias and sensitivity analysis

Our data showed that Begg's tests ($P = 0.855$) and Egger's tests ($P = 0.483$) did not have publication bias (Figure 10). Sensitivity analysis on the primary outcomes was performed with high and moderate heterogeneity (5-year LC, fistulae, and wound complications) to explore their potential source and assess the robustness of these

Table 3 Results of meta-analysis and subgroup analysis by intraoperative radiotherapy compared with non-intraoperative radiotherapy for rectal cancer

Outcome indicators	Study type	NO of study	Patients, <i>n</i> IORT non-IORT	HR/OR/WMD (95% CI)	<i>P</i> value	Heterogeneity, χ^2 / <i>I</i> / <i>P</i> value
5-yr overall survival	RCT	3	129 95	0.68 (0.29-1.63)	0.390	2.92/31.4%/0.233
	Observational studies	6	321 292	0.81 (0.66-1.11)	0.189	2.21/0.0%/0.819
	Totality	9	450 387	0.80 (0.60-1.06)	0.126	5.16/0.0%/0.740
5-yr disease free survival	RCT	2	91 57	1.61 (0.74-3.53)	0.231	0.60/0.0%/0.440
	Observational studies	4	235 212	0.89 (0.68-1.16)	0.374	1.72/0.0%/0.633
	Totality	6	326 269	0.94 (0.73-1.22)	0.650	4.33/0.0%/0.503
5-yr local control	RCT	3	129 95	1.37 (0.35-5.35)	0.655	1.33/24.8%/0.249
	Observational studies	11	487 511	3.38 (1.73-6.57)	0.000	41.31/73.4%/0.000
	Totality	14	616 606	3.07 (1.66-5.66)	0.000	43.42/70.9%/0.000
Abscess	RCT	2	57 60	1.83 (0.65-5.11)	0.252	0.01/0.0%/0.905
	Observational studies	4	205 262	0.94 (0.53-1.66)	0.833	1.99/0.0%/0.575
	Totality	6	262 322	1.10 (0.67-1.80)	0.713	3.22/0.0%/0.665
Fistulae	RCT	1	72 68	0.75 (0.30-1.88)	0.542	0.00/NA/NA
	Observational studies	2	94 175	1.22 (0.08-18.77)	0.888	2.02/50.4%/0.156
	Totality	3	166 243	0.79 (0.33-1.89)	0.600	2.07/3.2%/0.356
Wound complications	RCT	1	72 68	0.75 (0.30-1.88)	0.542	0.00/NA/NA
	Observational studies	7	385 393	1.06 (0.47-2.37)	0.893	16.09/62.7%/0.013
	Totality	8	457 461	1.21 (0.62-2.36)	0.575	17.01/58.8%/0.017
Anastomotic leakage	RCT	3	129 95	2.18 (0.89-5.33)	0.087	0.11/0.0%/0.946
	Observational studies	4	262 266	0.66 (0.31-1.41)	0.283	2.46/0.0%/0.482
	Totality	7	391 361	1.09 (0.59-2.02)	0.775	6.57/8.7%/0.363
Neurogenic bladder dysfunction	Observational studies	3	187 197	0.69 (0.31-1.55)	0.369	0.27/0.0%/0.874

HR: Hazard ratio; IORT: Intraoperative radiotherapy; NA: Not reported; non-IORT: Non-Intraoperative radiotherapy; OR: Odds ratio; RCT: Randomized controlled trial; WMD: Weighted mean difference.

outcomes. After ignoring each included study in turn for each outcome, the results of 5-year LC, fistulae, and wound complications were stable after testing.

DISCUSSION

For the treatment of rectal cancer, total mesorectal excision is a treatment method that clearly improves the condition; however, recurrence is a major challenge for the prognosis of patients^[37]. Multidisciplinary treatment methods including surgery, chemotherapy and radiotherapy significantly improve the prognosis of patients^[38]. The total dose of radiotherapy may be an important determinant of LC of advanced and recurrent tumors; however, treatment with EBRT alone has not achieved sufficient results^[39]. Therefore, IORT allows for the direct administration of high-dose radiation and the area that is at the greatest risk after resection^[40]. Although research reports on IORT for rectal cancer have been published, the sample sizes were small, and

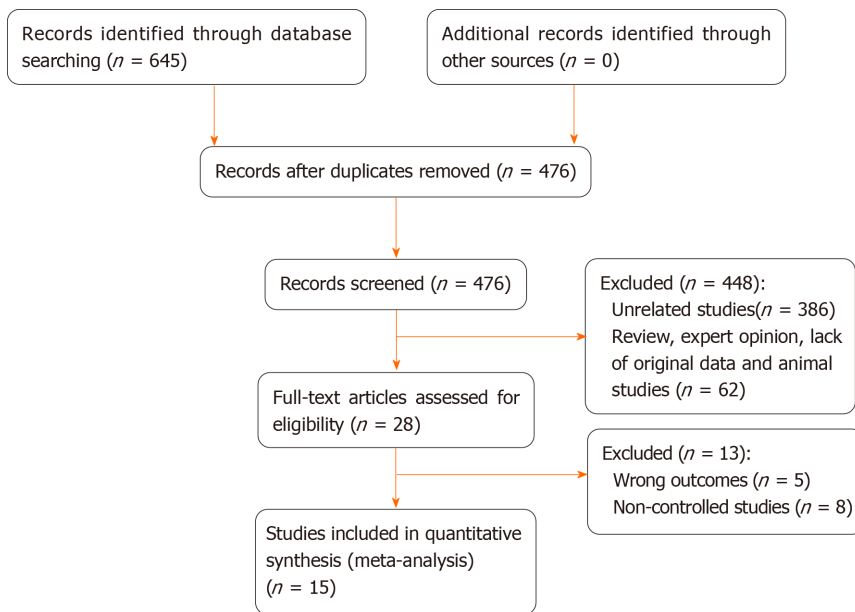


Figure 1 Study identification and selection flow chart.

therefore, there is still a lack of reliable evidence regarding treatment efficacy and safety. The systematic review and meta-analysis of this study show that IORT is associated with improved LC after resection.

When subgroup analysis was conducted by study type (RCTs or observational studies), the 5-year survival rate, fistulae, and wound complications showed moderate heterogeneity, which were likely to be different from the original research design, racial various, and inconsistent measurement methods^[41]. Concerning the 5-year survival rate, whether or not to undergo preoperative radiotherapy, postoperative radiotherapy, and chemotherapy regimen may be influencing factors in all studies included. In addition, differences in the dose of IORT will likely lead to a shift in survival rates in each study^[42]. The difference in complication results may be due to the longer IORT time compared with simple surgery and more blood loss^[43]. The studies included primary rectal cancer and recurrent rectal cancer. Due to the destruction of the anatomical plane in cases with recurrent rectal cancer^[44] as well as the limitation of the pelvic area, achieving an R0 resection is more complicated. In addition, compared with primary rectal cancer, a more systematic radiotherapy and chemotherapy regimen was received before surgery, thereby leading to differences in both outcomes and bias^[45].

The benefit of IORT after R0 resection is a potential confounding factor between studies^[46]. Many reports have confirmed that the 5-year DFS rate of the IORT group and non-IORT group after R0 resection is equivalent^[31,36]. In addition, in a recent RCT^[19], IORT and non-IORT treatment were compared and the 5-year overall survival rates were 71.5% and 81.8%, respectively, and support the view that IORT may not be beneficial after complete tumor resection. When compared with patients who did not receive adjuvant therapy (preoperative radiotherapy, postoperative radiotherapy, chemotherapy), patients who received adjuvant therapy clearly showed beneficial effects of the treatment^[28,32,36], which indicates the importance of adjuvant therapy for IORT.

In this study, we present the first pooled analysis of the impact of IORT on long-term oncology outcomes after rectal cancer resection. In a previous study, the safety and effectiveness of IORT in the treatment of colorectal cancer was systematically evaluated in 2011^[47], and in one study, the benefits of IORT treatment for colorectal cancer were reported in 2013^[48]. However, due to the differences in anatomical location and biological function between colon cancer and rectal cancer, we analyzed rectal cancer separately. By contrast to previous studies, our research incorporated more original studies and detailed subgroup analysis and sensitivity analysis were performed. Despite the inherent limitations of meta-analysis using observational studies, our findings suggested that the use of IORT during rectal cancer surgery may improve LC and has a more moderate impact on disease prognosis and survival. The application of IORT in the treatment of various types of tumors has significant benefits. Indeed, early breast cancer patients who received IORT during breast-

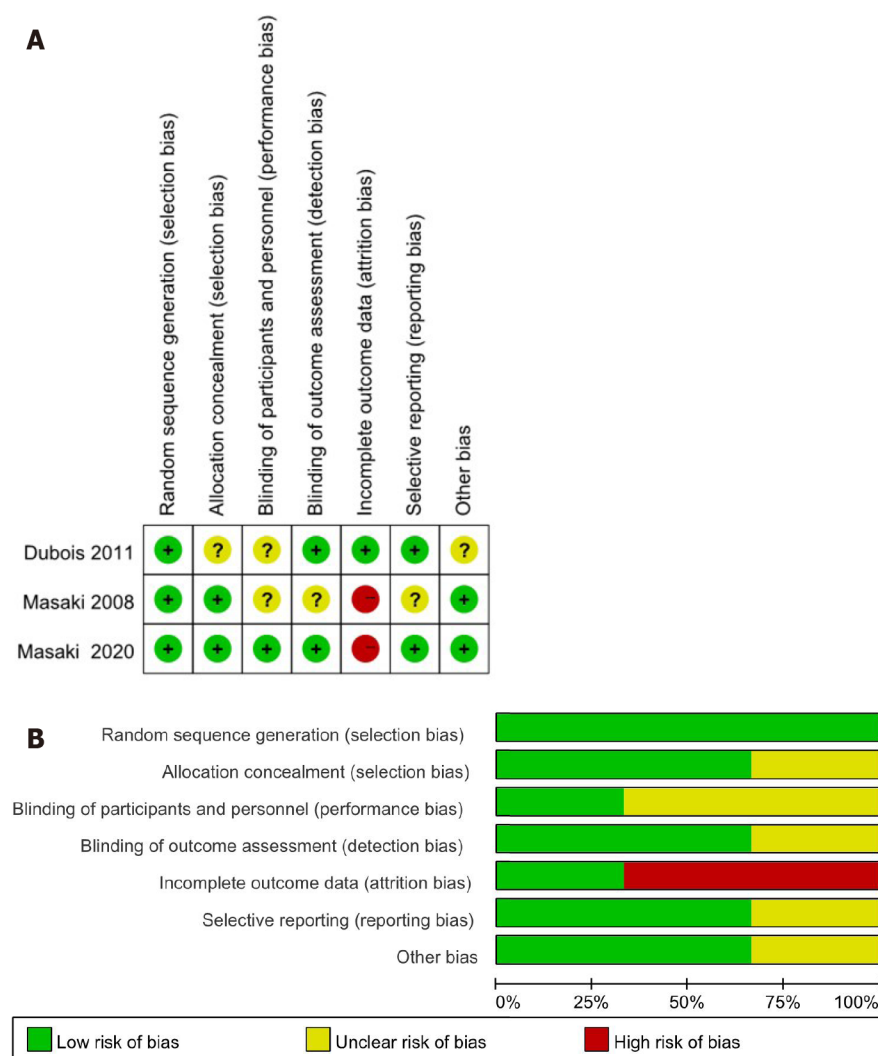


Figure 2 Quality assessment of three randomized controlled trials. A: Risk of bias summary; B: Risk of bias graph.

conserving surgery had a better survival period^[49], and in patients with brain metastases, using IORT can deliver auxiliary radiation to the resection cavity with a high LC rate and low incidence of radiation necrosis^[50].

This research also had limitations. At first, the randomization in the original research was limited. There were few controlled experiments and the sample size was irregular. Second, although most patients were treated in large tertiary cancer centers, the inclusion criteria for patients were different. Moreover, during treatment, the assessment methods of the outcome index was related to the proficiency of the surgeon. In addition, there were differences in the surgical procedures in this research, which may be a confounding factor for the results. Finally, our research is a secondary study and differences in the original data cannot be controlled for, including experimental design, inclusion criteria, and the original study included, which may affect the reliability of the results.

CONCLUSION

Our findings demonstrate that in patients with rectal cancer, adding IORT to traditional multimodal treatment strategies can improve LC but does not significantly improve the survival rate and complications of patients. In the future, well-designed prospective RCTs are warranted to better define the treatment effects using IORT.

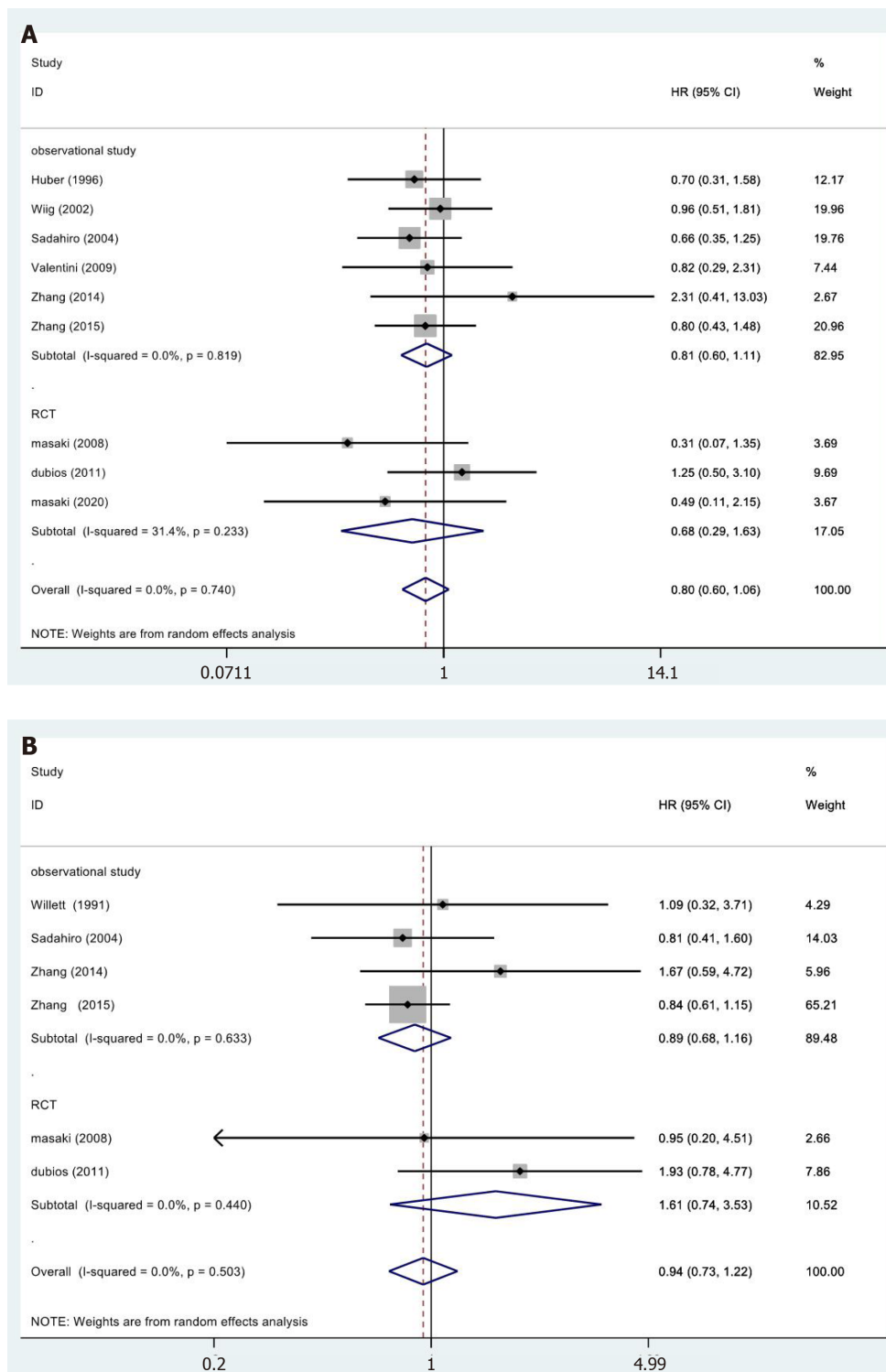


Figure 3 Results of meta-analysis. A: 5-yr overall survival; B: 5-yr disease-free survival.

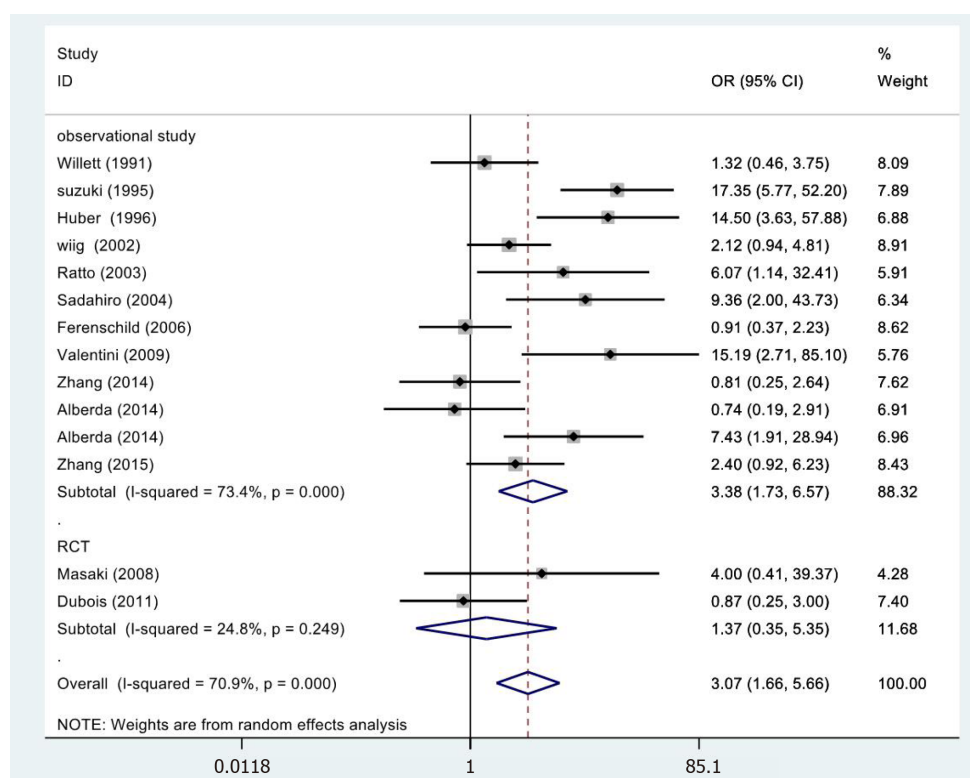


Figure 4 Results of meta-analysis: 5-year local control.

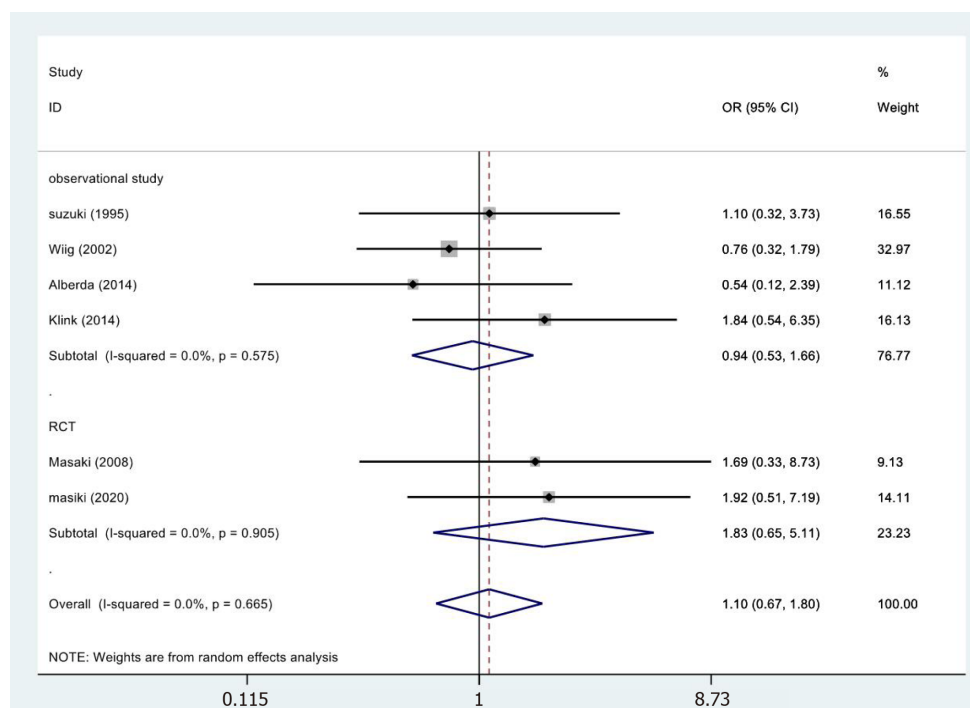


Figure 5 Results of meta-analysis: Abscess.

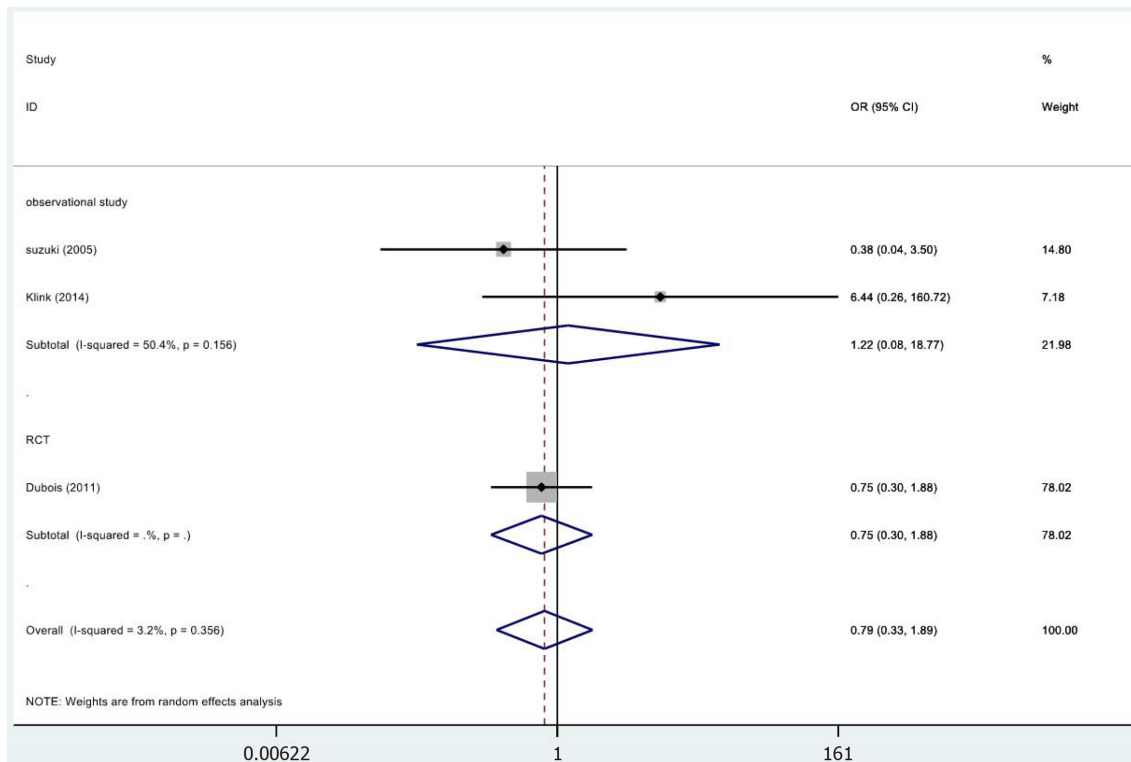


Figure 6 Results of meta-analysis: Fistulae.

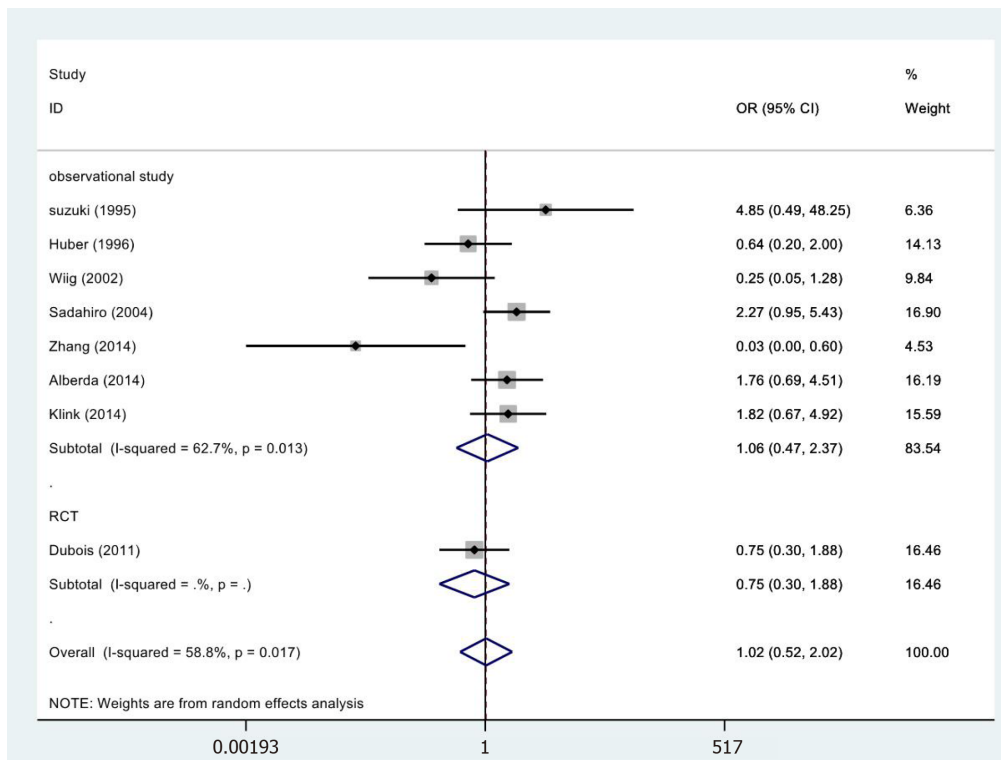


Figure 7 Results of meta-analysis: Wound complications.

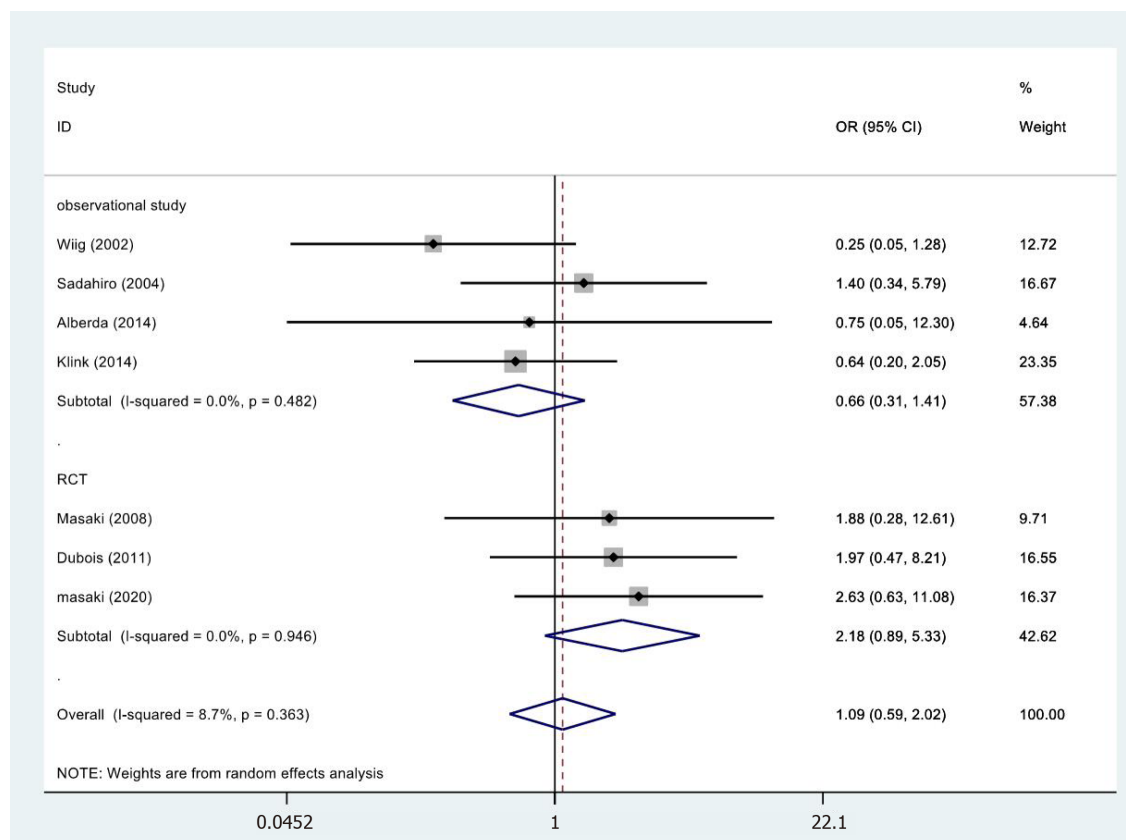


Figure 8 Results of meta-analysis: Fistulae anastomotic leakage.

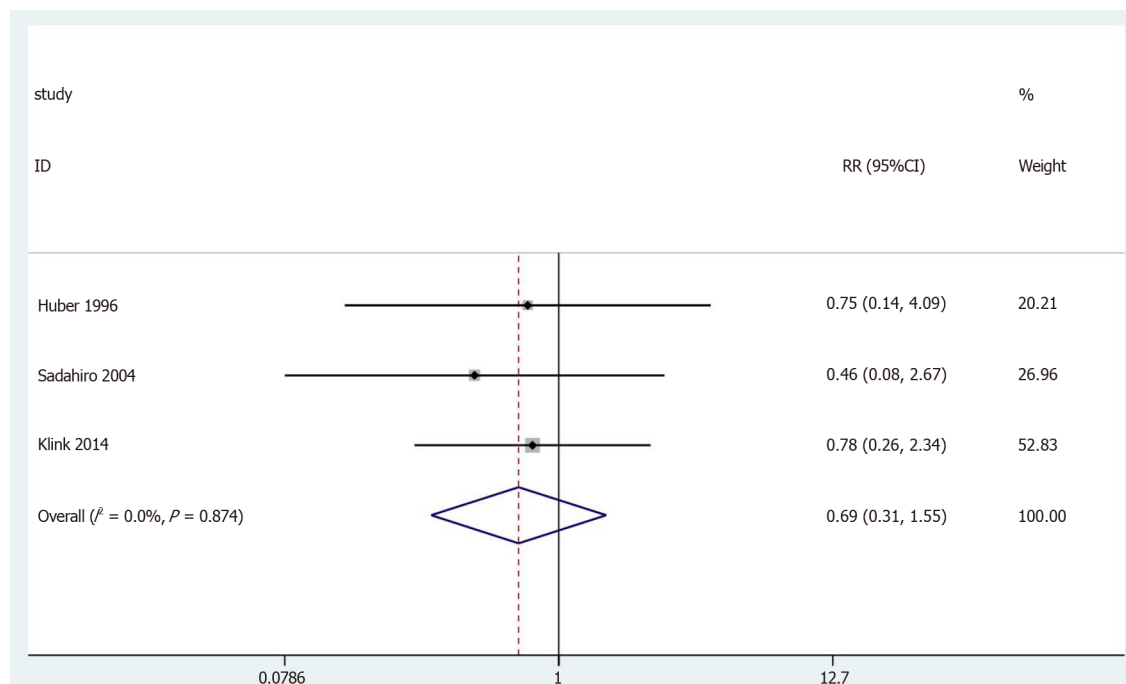


Figure 9 Results of meta-analysis: Neurogenic bladder dysfunction.

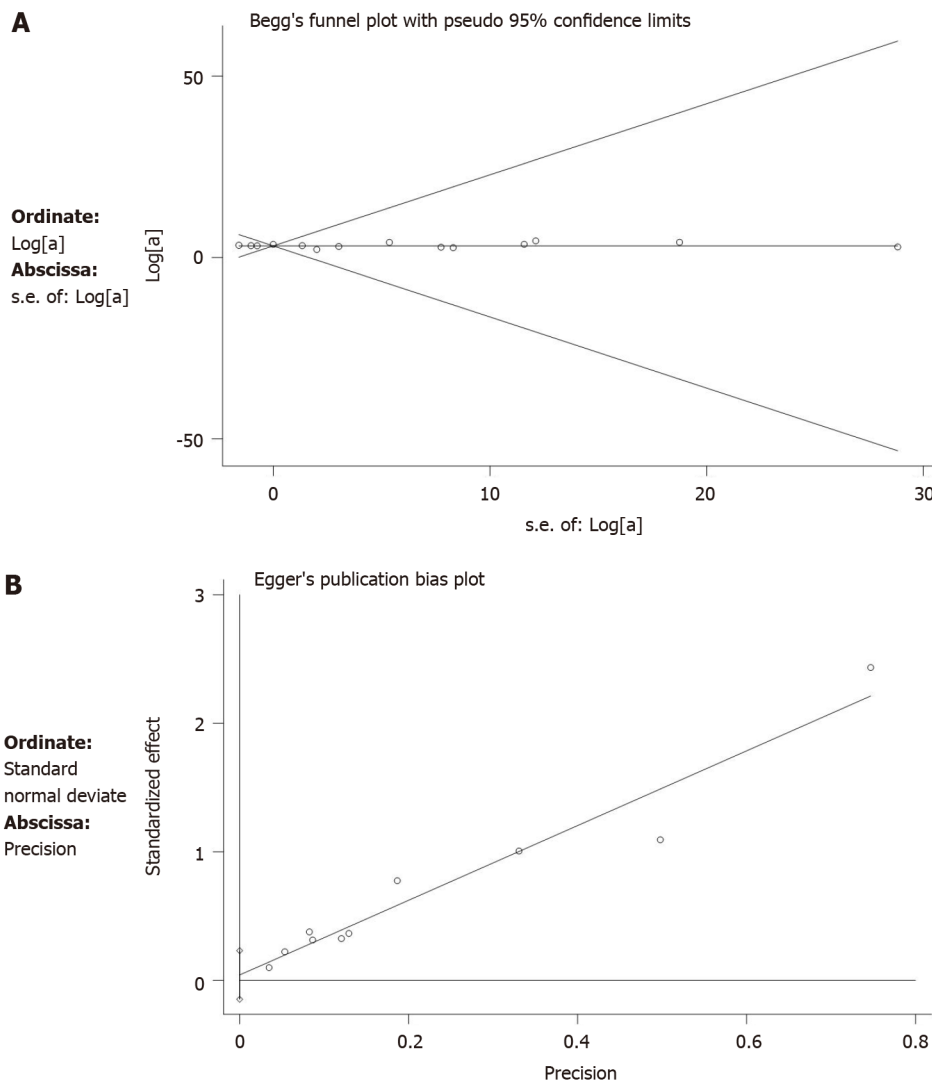


Figure 10 Publication bias. A: Results of Begg's funnel plot; B: Egger's publication bias plot for assessing publication bias of local control.

ARTICLE HIGHLIGHTS

Research background

The prognosis of patients with rectal cancer is poor and the mortality rate is high. The effectiveness and safety of intraoperative radiotherapy (IORT) for rectal cancer still controversial.

Research motivation

Previous studies have demonstrated that adding IORT to traditional treatment of rectal cancer not only reduces the local recurrence rate of advanced rectal cancer but also influences the local control rate of locally recurrent rectal cancer. However, a recent randomized controlled trial (RCT) showed that IORT cannot be recommended as a standard therapy to compensate less radical resection for advanced lower rectal cancer. It is necessary to perform a meta-analysis to systematically and comprehensively investigate the effectiveness and safety of IORT in the treatment of rectal cancer.

Research objectives

A systematic review and meta-analysis to evaluate the value of IORT for patients with rectal cancer.

Research methods

We searched PubMed, Embase, Cochrane Library, Web of Science databases and conference abstracts and included RCTs and observational studies on IORT *vs* non-

IORT for rectal cancer. Dichotomous variables were evaluated by odds ratio (OR) and 95% confidence interval (CI), hazard ratio (HR) and 95%CI was used as a summary statistic of survival outcomes. Statistical analyses were performed using Stata V.15.0 and Review Manager 5.3 software.

Research results

In this study, 3 RCTs and 12 observational studies were included with a total of 1460 patients, who were mainly residents of Europe, the United States, and Asia. Our results did not show significant differences in 5-year overall survival (HR = 0.80, 95%CI = 0.60-1.06; $P = 0.126$), 5-year disease-free survival (HR = 0.94, 95%CI = 0.73-1.22; $P = 0.650$); abscess (OR = 1.10, 95%CI = 0.67-1.80; $P = 0.713$); fistulae (OR = 0.79, 95%CI = 0.33-1.89; $P = 0.600$); wound complication (OR = 1.21, 95%CI = 0.62-2.36; $P = 0.575$); anastomotic leakage (OR = 1.09, 95%CI = 0.59-2.02; $P = 0.775$); and neurogenic bladder dysfunction (OR = 0.69, 95%CI = 0.31-1.55; $P = 0.369$). However, the meta-analysis of 5-year local control was significantly different (OR = 3.07, 95%CI = 1.66-5.66; $P = 0.000$).

Research conclusions

The advantage of IORT is mainly reflected in 5-year local control but it is not statistically significant for 5-year overall survival, 5-year disease-free survival, and complications.

Research perspectives

Several limitations in this analysis should be carefully addressed. First, the randomization in the original research was limited. There were few controlled experiments and the sample size was irregular. Second, although most patients were treated in large tertiary cancer centers, the inclusion criteria for patients were different. Moreover, during treatment, the assessment methods of the outcome index was related to the proficiency of the surgeon. In addition, there were differences in the surgical procedures in this research, which may be a confounding factor for the results. Finally, our research is a secondary study and differences in the original data cannot be controlled for, including experimental design, inclusion criteria, and the original study included, which may affect the reliability of the results.

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Internal hemorrhoid harboring adenocarcinoma: A case report

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Abstract

BACKGROUND

The incidence of carcinoma found within an internal hemorrhoid specimen is exceptionally rare. Further, the presence of primary anal canal adenocarcinoma within internal hemorrhoids is even more infrequent. We describe a case in which anal canal adenocarcinoma was found within an internal hemorrhoidectomy specimen and perform a review of the current literature.

CASE SUMMARY

The patient was a 79-year-old male who presented with rectal bleeding and was found to have large thrombosed internal hemorrhoids during screening colonoscopy. The patient subsequently underwent a three-column hemorrhoidectomy. Pathologic analysis revealed one of three specimens containing a 1.5 cm moderate-to-poorly differentiated adenocarcinoma of anal origin with superficial submucosal invasion. At three-month follow up, he was taken to the operating theatre for biopsy and re-excision of his non-healing wound, which showed no recurrence. His wound has since healed and he was cancer free at ten-month follow up.

CONCLUSION

When faced with primary anal canal adenocarcinoma an interdisciplinary approach to treatment should be considered. Routine pathological analysis of hemorrhoidectomy specimens may be beneficial due to the severity of anal canal carcinomas if left undiagnosed and untreated in a timely manner.

Key Words: Hemorrhoidectomy; Adenocarcinoma; Colorectal; Anal canal; Oncology; Hematochezia; Case report

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Core Tip: The presence of primary anal canal adenocarcinoma within internal hemorrhoids is extremely rare and can be easily missed if hemorrhoidectomy specimens are not sent for routine pathology. When faced with primary anal canal adenocarcinoma an interdisciplinary approach to treatment should be considered. Routine pathological analysis of hemorrhoidectomy specimens may be beneficial due to the severity of anal canal carcinomas if left undiagnosed and untreated in a timely manner.

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INTRODUCTION

Pathologic analysis of a hemorrhoidectomy specimen rarely results in carcinoma^[1]. Current literature cites an incidence of 1%-2%; however, objective data is lacking^[2-4]. Cataldo and Mackeigan^[2] reviewed a data set that revealed only 1 of 21527 hemorrhoidectomies (0.0046%) contained an unsuspected carcinoma and did not specify whether this particular specimen contained adeno- or squamous cell carcinoma. Neoplasms of the anal canal are most commonly squamous cell carcinoma, followed by cloacogenic (or basaloid or transitional cell) carcinoma, and rarely adenocarcinoma^[4,5]. Additionally, adenocarcinomas of the anal canal are often considered to be the result of the downward extension of a primary tumor of distal rectal origin or from the columnar epithelium in the upper anal canal, and are therefore considered to be of rectal origin and not true anal carcinomas^[1]. We describe the case of an unsuspecting hemorrhoidectomy specimen that was found to contain adenocarcinoma of the anal canal.

CASE PRESENTATION

Chief complaints

Rectal bleeding.

History of present illness

A 79-year-old male who presented with rectal bleeding and discovery of large thrombosed internal hemorrhoids during screening colonoscopy. He reported intermittent hematochezia and denied rectal pain or changes in the frequency, consistency or caliber of bowel movements. He is a self-reported never smoker who adheres to a high-fiber diet. The patient subsequently underwent an uneventful three-column hemorrhoidectomy. The internal hemorrhoids were identified, excised, and sent for routine pathologic evaluation. Pathologic analysis revealed the left lateral hemorrhoid column positive for a 1.5 cm moderate-to-poorly differentiated adenocarcinoma. The tumor showed superficial invasion into the submucosa along with a focus that was suspicious for lymphatic invasion (Figure 1).

History of past illness

The patient has a history of atrial fibrillation, diabetes, and remote history of hemorrhoids. The patient underwent a previous laser ablation 10 years prior for bleeding internal hemorrhoids. However, there was no procedure note to denote the location of the bleeding hemorrhoid or pathology report to suggest biopsy in the electronic medical record.

Physical examination

Digital rectal examination prior to hemorrhoidectomy revealed one small skin tag, but was otherwise unremarkable. He had no inguinal lymphadenopathy. Anoscopy revealed a single large inflamed and prolapsing internal hemorrhoid.

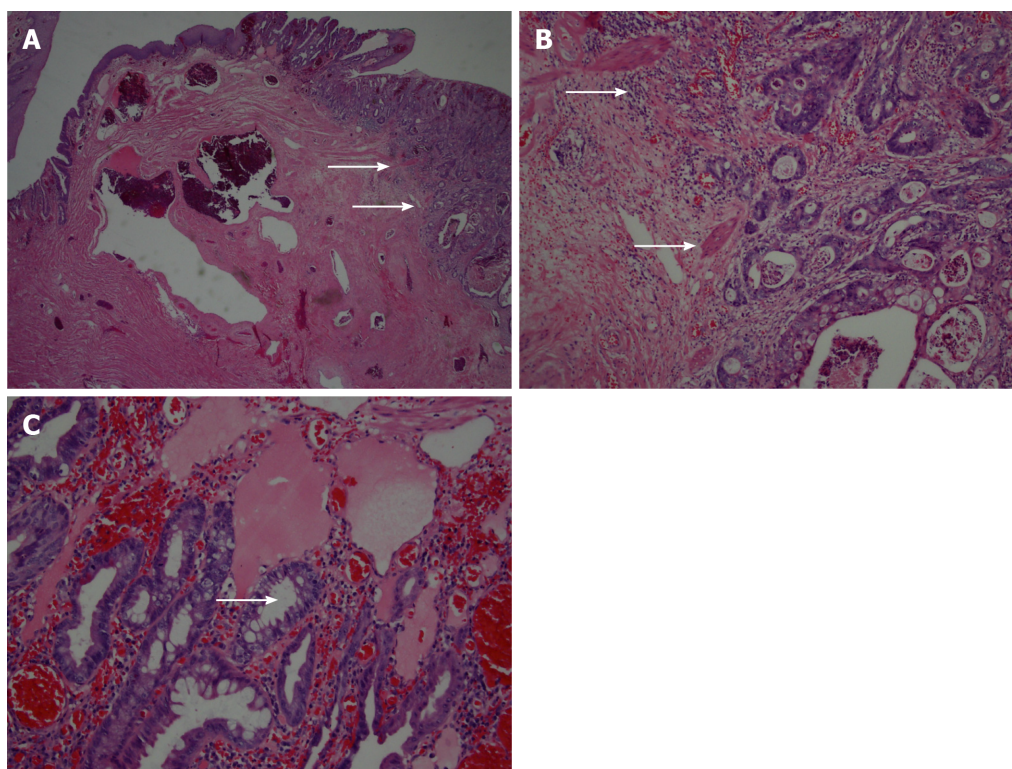


Figure 1 Hematoxylin and eosin staining results. A and B: Hematoxylin and eosin (H/E) stains (2 × and 20 × lenses) showing classic anorectal hemorrhoidal vascular ectasia, but with associated moderately differentiated adenocarcinoma invading through the muscularis mucosa (arrows); C: H/E stain (40 × lens) showing benign glands.

Laboratory examinations

Hemoglobin A1c (HbA1c), 6.3%; and albumin, 4.4 g/dL.

Imaging examinations

Computed tomography (CT) chest/abdomen/pelvis-negative, endorectal ultrasound-negative.

MULTIDISCIPLINARY EXPERT CONSULTATION

The case was presented to the institution's Interdisciplinary Tumor Board, at which time the recommendation was made to pursue wide local excision of the area.

FINAL DIAGNOSIS

Moderate-to-poorly differentiated adenocarcinoma of the anal canal.

TREATMENT

A wide local excision of the previous left lateral hemorrhoidectomy column was pursued. The excision was carried out with a wide margin from the previous scar - at least 2 cm in either direction. The distal and proximal margins were 3 cm onto the perianal skin, and 5 cm into the anal canal and distal rectum. The lateral margins were at least 2 cm from previous scar. The deep margin was into the ischioanal fat. No frozen section was planned at the time of reoperation.

OUTCOME AND FOLLOW-UP

Pathologic analysis of the transanal excision specimen revealed chronic inflammation, fibrosis, and foreign body reaction with no residual neoplasm identified. The patient experienced some post-operative bleeding requiring chemical cauterization of granulation tissue, but has otherwise continued along an uncomplicated trajectory of recovery. At three-month follow up, he was taken to the operating theatre for biopsy and re-excision of his non-healing wound, which showed no recurrence. His wound has since healed and he was cancer free at ten-month follow up. It is unclear why the wound initially did not heal. The patient was a non-smoker, had well-controlled diabetes (HbA1c, 6.3%), and albumin (4.4 g/dL) at the time of operation. Close observation and surveillance will be kept, including a full colonoscopy 1 year from diagnosis of adenocarcinoma.

DISCUSSION

The incidence of a hemorrhoidectomy specimen harboring any type of malignancy is exceptionally rare. Adenocarcinoma represents approximately six percent of anal carcinomas overall and is even more rare within a hemorrhoidectomy specimen as one would expect squamous cell carcinoma to occur with higher frequency in the anal canal. It is also more common to have a primary rectal carcinoma within a hemorrhoid specimen from downward extension or metastasis than to have a hemorrhoid harboring a primary anal canal carcinoma. Additionally, there have been reports of implantation of primary rectal adenocarcinoma on a hemorrhoidectomy specimen and occurrences of metastatic rectal adenocarcinoma found within a hemorrhoid^[6,7].

Our case is unique because we describe a primary anal canal adenocarcinoma within a hemorrhoidectomy specimen. To our knowledge, there have been less than ten cases reported in the literature. Our patient presented with rectal bleeding, which is consistent with anal cancer but can often be attributed to internal hemorrhoids. Interestingly, he did not experience any anorectal pain, a symptom, which occurs in approximately thirty percent of patients with anal canal cancer. Additional epidemiologic factors that contribute to anal cancers are human papillomavirus, chronic immunosuppression and smoking^[8-10]. These risk factors are typically associated with anal squamous cell carcinomas, and were not demonstrated by our patient.

Due to the rarity of primary adenocarcinoma in the anal canal, staging workup follows that of a rectal adenocarcinoma^[11]. This should include full colonoscopy, carcinoembryonic antigen level, CT chest/abdomen/pelvis and either endorectal ultrasound or magnetic resonance imaging pelvis, as would be performed for rectal adenocarcinoma. Consideration should be given to an interdisciplinary approach to treatment due to the rarity of this tumor. Our patient underwent complete evaluation and was discussed at our institution's tumor board. The staging workup for our patient's lesion was consistent with a T1N0M0 anal cancer. Management of adenocarcinomas arising in the anal canal typically follows the same principles as those applied to rectal cancer. Since our patient's tumor was limited to the superficial submucosa, transanal excision was adequate and has spared the patient from the morbidity of an abdominoperineal resection (APR).

Another key point to be made in this case is that pathologic evaluation of the hemorrhoidectomy specimen was performed. It has been suggested that pathologic evaluation should be limited to patients who are at high risk of lesions with anal intraepithelial neoplasia (immunocompromised, papillomatous lesions) or if there is concern for malignancy based on preoperative, intraoperative or inspection of excised tissue^[4]. This has been suggested as the cost of detecting one cancer would be uneconomic, given its rarity. Had this standard approach been followed, we would have missed a potentially life-threatening anal canal cancer, or it may have manifested months later and resulted in an APR. At our institution, hemorrhoidectomy specimens are routinely sent for pathologic analysis.

CONCLUSION

Adenocarcinoma within a hemorrhoidectomy specimen is an exceptionally rare event, but should not be overlooked. Consideration for routine pathological analysis of hemorrhoidectomy specimens may be beneficial due to the severity of anal canal

carcinomas if left undiagnosed and untreated in a timely manner.

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