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2016 Colorectal Cancer: Global view

Therapeutic options for peritoneal metastasis arising from colorectal cancer

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Abstract

Peritoneal metastasis is a common sign of advanced tumor stage, tumor progression or tumor recurrence in patients with colorectal cancer. Due to the improvement of systemic chemotherapy, the development of targeted therapy and the introduction of additive treatment options such as cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), the therapeutic approach to peritoneal metastatic colorectal cancer (pmCRC) has changed over recent decades, and patient survival has improved. Moreover, in contrast to palliative systemic chemotherapy or best supportive care, the inclusion of CRS and HIPEC as inherent components of a multidisciplinary treatment regimen provides a therapeutic approach with curative intent. Although CRS and HIPEC are increasingly accepted as the standard of care for selected patients and have become part of numerous national and international guidelines, the individual role, optimal timing and ideal sequence of the different systemic, local and surgical treatment options remains a matter of debate. Ongoing and future randomized controlled clinical trials may help clarify the impact of the different components, allow for further improvement of patient selection and support the standardization of oncologic treatment regimens for pmCRC. The addition of further therapeutic options such as neo-adjuvant intraperitoneal chemotherapy or pressurized intraperitoneal aerosol chemotherapy, should be investigated to optimize therapeutic regimens and further improve the oncological outcome.

Key words: Peritoneal metastasis; Colorectal cancer; Systemic chemotherapy; Intraperitoneal chemotherapy; Cytoreductive surgery

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Core tip: Beyond diverse systemic, interventional and

surgical palliative treatment options for peritoneal metastasis arising from colorectal cancer, the combination of systemic chemotherapy, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy provides a therapeutic approach with curative intent for selected patients. Nevertheless, the treatment regimens, the sequence of therapy and the impact of the different components of the multidisciplinary treatment concept on clinical and oncological outcomes remain a matter of debate. Moreover, the addition of further therapeutic options to the existing treatment regimens might allow for higher complete resection rates and improved survival rates.

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INTRODUCTION

Colorectal cancer (CRC) remains one of the leading causes of cancer-related death worldwide^[1]. Peritoneal metastasis (PM) is common in patients with advanced stage primary and recurrent colorectal cancer^[2,3]. The natural course of this disease is associated with poor prognosis and led to a mean overall survival of 5.2 mo in the prospective European multicenter EVOCAPE I study ($n = 118$)^[4]. A retrospective analysis of 3000 patients with pmCRC reported a median survival of 7 mo without specific treatment^[5]. Although peritoneal metastases develop avascular tumor nodules within the abdominal cavity that often cannot be efficiently addressed by systemic chemotherapy^[6], advances in the development of cytostatic agents, targeted therapy and combined treatment regimens has led to significant improvement in survival rates. Moreover, additive treatment options such as cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) might be performed with curative intent in selected patients^[7]. Thus, pmCRC currently requires a multidisciplinary treatment approach that considers the available treatment options and modalities (Figure 1).

STAGING SYSTEMS FOR PM

The estimation of the extent of peritoneal tumor dissemination plays an important role in choosing therapeutic options for patients with pmCRC. Different staging systems allow for the standardization of PM classification and facilitate prognosis estimation. The most commonly used classification system for peritoneal tumor dissemination is the peritoneal cancer index (PCI). This numerical score combines the lesion size (LS) and tumor localization in 13 abdomino-pelvic regions including four small bowel regions (region 0-12) and

ranges from 0 to 39^[8]. The PCI was initially introduced for intraoperative determination of the extent of peritoneal carcinomatosis but the extent can also be determined by staging laparoscopy or diagnostic imaging. Elias *et al*^[9] showed that the PCI is easy to use and reproducible with high inter-surgeon concordance. Although the PCI is underestimated by computed tomography compared to intraoperative findings, the clinical impact of the inaccuracies of CT-PCI is modest^[10]. Thus, the (CT-)PCI is a helpful tool to determine and communicate the extent of peritoneal disease and to select patients for different therapeutic options. Moreover, the PCI correlates with overall and progression-free survival in patients with pmCRC^[11-14]. Nevertheless, the predictive value is limited with respect to PM and does not include other prognostic factors. Therefore, prognostic scores for patients with pmCRC have been developed. The Peritoneal Surface Disease Severity Score (PSDSS) is based on the following three important prognostic indicators: (1) clinical symptoms; (2) PCI; and (3) tumor histopathology. The PSDSS ranges from 2 to 22 and divides patients into four prognostic groups (stage I = PSDSS 2-4, stage II = PSDSS 4-7, stage III = PSDSS 8-10 and stage IV = PSDSS > 10)^[15]. Several retrospective analyses show a high correlation between PSDSS and the survival of patients with pmCRC. The score might be helpful for determining survival probability and resectability of peritoneal disease in the context of therapeutic decision-making^[16-18]. Another recently developed prognostic score for patients with pmCRC is the Colorectal Peritoneal Score (COREP). COREP includes signet cell histology, hemoglobin, white blood cell count and the value and status of serum tumor markers and ranges from 0 to 18. The cut-off value for the poor-prognosis group is COREP > 6. In the first published evaluation of 77 patients the predictive value of COREP for open/close-procedure, R1 resection and one-year survival was superior to that of PSDSS^[19]. Based on the Japanese classification of pmCRC, which divides peritoneal tumor dissemination into four groups (P0: no PM, P1: local PM, P2: limited distant PM and P3: extended distant PM)^[20,21] Noura *et al*^[22] proposed a new simple classification system that includes the colorectal liver metastases (CLM) status. Patients without CLM and local (P1) or limited distant PM (P2) are classified as Grade A and Grade B, respectively. Patients with extended distant PM and all patients with CLM have been defined as Grade C. Initial data shows significant stratification of the survival and R0 resection rates^[22]. However, new scores considering different histological and clinical factors might be helpful for decision-making and allow for further improvement of the selection of appropriate therapeutic options within a multidisciplinary treatment approach.

SYSTEMIC CHEMOTHERAPY FOR pmCRC

Although there are multiple prospective randomized trials and retrospective analyses about systemic chemotherapy in patients with advanced stage and metastatic CRC, data regarding the subgroup of patients with pmCRC

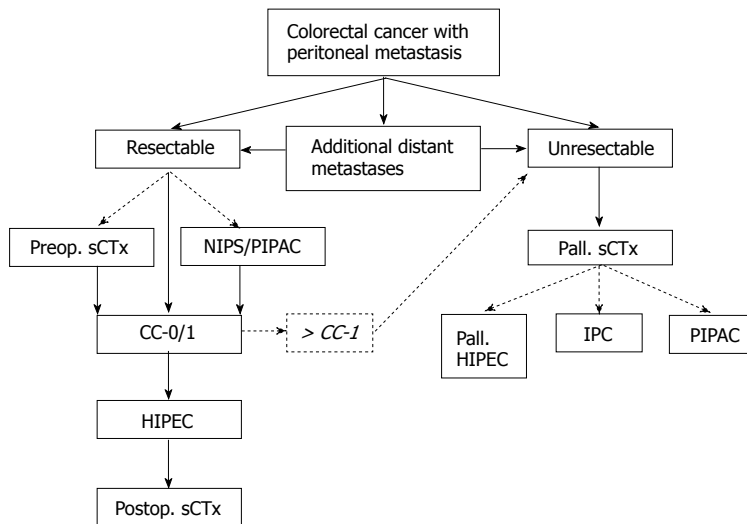


Figure 1 Proposed algorithm for treating peritoneal metastatic colorectal cancer. CC: Completeness of cytoreduction; CC-0/1: Complete macroscopic cytoreduction; IPC: Intraperitoneal chemotherapy; NIPS: Neoadjuvant intraperitoneal/systemic chemotherapy; PIPAC: Pressurized intraperitoneal aerosol chemotherapy; sCTx: Systemic chemotherapy; scattered lines indicate additional therapeutic options; HIPEC: Hyperthermic intraperitoneal chemotherapy.

are limited. Franko *et al.*^[23] analyzed 364 patients with PM out of 2095 patients enrolled in the two prospective randomized NCCTG phase III trials N9741 and N9841 and showed a 30% relative reduction in overall survival in this subgroup. The 5-year OS rates were 4.1% and 6% and the median survival was 12.7 mo and 17.6 mo in the pmCRC and the non-pmCRC group, respectively. In this analysis infusional oxaliplatin-based chemotherapy was superior to irinotecan-based regimens irrespective of the PM status^[23]. The subgroup analysis of patients with pmCRC enrolled in the prospective randomized CAIRO and CAIRO2 trials showed a significant impairment in the overall survival of these patients. Klaver *et al.*^[24] published a median OS of 10.4 and 17.3 mo in the CAIRO trial and 15.2 and 20.7 mo in the CAIRO2 trial. An Asian prospective single-arm phase II study investigating FOLFOX-4 in patients with pmCRC reported median overall survival of 21.5 mo. The median time to progression was 4.4 mo^[25]. Consistent with these reported survival rates, Elias *et al.*^[26] reported a median OS of 23.9 mo under modern multidrug systemic chemotherapy in 48 patients with pmCRC from the French registry.

Considering the promising results of the first line treatment of patients with metastatic colorectal cancer using systemic polychemotherapy plus targeted therapy with median OS ranging from 25 mo to 41.3 mo^[27-30] these regimens have also been used for treating pmCRC. In a retrospective analysis of 65 consecutive patients with pmCRC, Adachi *et al.*^[31] reported an improvement in the survival rate in response to systemic chemotherapy after incomplete cytoreduction. The oxaliplatin-based regimen and addition of targeted therapy was superior to irinotecan-based chemotherapy^[31]. Razenberg *et al.*^[32] analyzed 1235 patients treated with palliative systemic chemotherapy for pmCRC. In 436 patients (35%) bevacizumab has been added to the treatment regimen. The median OS was 7.5 mo vs 11 mo in the bevacizumab group^[32]. In a population-based study patients with metachronous colorectal PM were analyzed with respect to their treatment as follows: 94 patients received palliative

systemic chemotherapy, 36 patients had the addition of bevacizumab and 92 did not receive therapy and the median survival was 13 mo, 20.3 mo and 3.4 mo, respectively^[33]. Comparable results are reported by van Oudheusden in 82 patients who underwent open/close procedures for unresectable colorectal PM. The median OS was 11.2 mo with palliative systemic chemotherapy and 2.7 mo with best supportive care^[34].

These data demonstrate the efficacy of modern systemic chemotherapy regimens with or without targeted therapy in improving the survival of patients with unresectable pmCRC. Based on these findings systemic chemotherapy should be considered the standard of care in patients with unresectable pmCRC and should be the backbone of a multimodal treatment regimen in patients who qualify for a multidisciplinary therapeutic approach. In the absence of contraindications, infusional oxaliplatin-based regimens, such as FOLFOX with or without monoclonal antibodies like bevacizumab, cetuximab or panitumumab, might be preferred as first-line therapies for these patients. Moreover, based on the results of the RAISE trial, ramucirumab might also be considered for the second-line treatment of patients with pmCRC^[35]. Nevertheless, reliable data for this subgroup are not available.

SURGERY FOR pmCRC

CRS

In contrast to palliative surgery, such as fecal diversion, intestinal bypass, primary tumor resection, *etc.*, CRS followed by HIPEC provides an additive treatment option for selected colorectal PM patients with a curative intent. Although disease recurrence is common^[36], cure rates between 16% and 28% are reported after complete CRS and HIPEC^[37,38]. The aim of surgical cytoreduction, which may consist of multiple peritonectomy procedures and visceral resections is the removal of all visible tumor deposits within the abdominal cavity^[8,39,40]. Despite extensive and aggressive surgery, most patients return to baseline in terms of their quality of life within 6 mo

after surgery^[41-43]. The success of surgery is classified according to the completeness of cytoreduction (CC) score^[13,44]. Complete macroscopic cytoreduction (CC-0/1), defined as no visible tumor or single tumor nodules < 2.5 mm, is a precondition for the efficient application of HIPEC. Therefore, consistent preoperative patient selection is crucial for the efficacy of the multimodal treatment concept. A PCI > 20 might be considered a relative contraindication for CRS and HIPEC^[45]. Da Silva *et al.*^[11] reported a median OS of 41 mo in patients with PCI < 20 and 16 mo in patients with PCI > 20 after complete macroscopic cytoreduction. Comparable results are published by Hompes *et al.*^[12] for patients with a PCI higher or lower than 15. A recently published analysis of 180 patients defined a cut-off PCI value of 17^[14].

CRS in patients with additional CLM

There are limited published data regarding cytoreductive surgery in patients with additional resectable CLM. In a retrospective matched-pair analysis, hepatobiliary procedures during CRS and HIPEC did not lead to increased perioperative complication rates and/or overall mortality^[46]. According to the Milan consensus statement of the Peritoneal Surface Malignancy Group International cytoreductive surgery (and HIPEC) should not be routinely recommended in patients with more than three peripheral resectable liver metastases^[45]. However, two retrospective studies demonstrated median survival rates of approximately 36 mo after CRS, including mostly minor liver resections followed by HIPEC^[47,48]. As expected, liver involvement is associated with decreased overall survival rates. Berger *et al.*^[49] reported a median overall survival of 45.1 mo in 108 patients with additional liver involvement and 73.5 mo in 166 patients with isolated PM after CRS and HIPEC. Nevertheless, patients with malignancies other than CRC were included in the analysis. There was no significant difference regarding the morbidity and mortality between the two groups^[49]. Allard *et al.*^[50] reported a median survival of 42 mo in patients who underwent complete resection of CLM and unexpected limited CPM with a median PCI of 2. In a multivariate analysis Delhorme *et al.*^[51] identified the size of liver metastasis and grade II/III toxicity of preoperative chemotherapy as poor prognostic factors. Response to preoperative chemotherapy significantly increased overall survival. These data are supported by a recently published meta-analysis that identified concurrent CLM as an independent negative prognostic factor for overall survival in patients with pmCRC after CRS and HIPEC^[52]. Noura *et al.*^[22] showed that the presence of CLM impairs survival and R0 resection rates. The 5-year overall survival rates of patients without CLM and local or limited distant PM were 25.6% and 12.0%, respectively. The 5-year survival rate of patients with extended distant PM and/or additional CLM was 5.6%. R0 resection rates were 65.9%, 44.6% and 8.1%^[22]. However, the combination of extended liver surgery for CLM and extended cytoreductive surgery in patients

with high PCI should be avoided because of the impaired clinical and oncological outcomes. Moreover, there are no reliable data on patients with pmCRC and additional isolated resectable lung metastases. Therefore, lung metastasis should be considered a contraindication of CRS and HIPEC.

HIPEC

The aim of intraoperative HIPEC is to consolidate complete surgical resection by destroying scattered (and residual) tumor cells within the abdominal cavity. In a prospective randomized phase III trial comparing CRS and HIPEC plus systemic chemotherapy with 5-FU/FA to systemic chemotherapy with 5-FU/FA in selected patients with pmCRC there was a significant survival benefit for the treatment group. The median survival was 22 and 12.6 mo for the treatment and non-treatment groups. In the subgroup of patients with complete macroscopic cytoreduction (CC-0/1), the median survival reached 42.9 mo. The low survival rates might be explained by the use of 5-FU-based systemic chemotherapy in both groups in the pre-oxaliplatin era^[53,54].

Elias *et al.*^[26] reported a median survival of 62.7 mo and a 5-year survival rate of 51% after complete macroscopic cytoreduction and bidirectional oxaliplatin-based HIPEC. All patients additionally received modern systemic chemotherapy^[26]. A prospective phase II study investigating complete macroscopic cytoreduction and bidirectional oxaliplatin-based HIPEC showed a 2-year overall survival rate of 88.7% and a median disease-free survival (DFS) of 19.8 mo^[12]. Based on the promising results of the FOLFOXIRI protocol in the systemic treatment of mCRC, irinotecan has been added to the bidirectional oxaliplatin-based HIPEC regimen, leading to increased morbidity without improving the survival. Quenet *et al.*^[55] reported a median overall survival of 47 mo and a 5-year survival rate of 42.4%. Goéré *et al.*^[37] reported a cure rate, defined as the 5-year disease-free survival, of 16% after CRS and HIPEC in 107 patients with pmCRC. Another retrospective analysis of 342 patients with pmCRC from a prospective database showed a 10-year recurrence-free survival rate of 10% after CRS and HIPEC^[56].

Although there are only few prospective RCTs, several studies and retrospective analyses show that the integration of CRS and HIPEC into a multidisciplinary treatment approach that includes systemic chemotherapy can improve the survival of selected patients with pmCRC^[7,57]. Nevertheless, the exact role of the HIPEC procedure and components remains unclear. A comparative analysis published by Hompes *et al.*^[58] investigated different HIPEC regimens and their effects on patient survival. There was no statistically significant difference between bidirectional oxaliplatin-based HIPEC and MMC-based HIPEC after complete macroscopic cytoreduction. The median RFS was 12.2 mo in the oxaliplatin-group and 13.8 mo in the MMC group ($P = 0.87$). The median OS was 37.1 mo in the oxaliplatin group and 26.5 mo in the MMC

group ($P = 0.45$)^[58]. A matched-pair analysis showed no significant differences in morbidity and mortality by HIPEC regimen. The grade 3/4 morbidity rates according to CTCAE were 42.5% in the OX group and 37.5% in the MMC group ($P = 0.648$) and the mortality rates of the OX and MMC groups were 2.5% and 0%, respectively^[59]. Consistent with these findings the American Society of Peritoneal Surface Malignancies reported an OS of 32.7 mo in patients with MMC-based HIPEC and 31.4 mo for oxaliplatin-based HIPEC in 539 patients with pm CRC after complete macroscopic cytoreduction ($P = 0.925$). After stratification to PSDSS there was a statistically significant survival benefit for the MMC-subgroup with PSDSS I / II ($P = 0.012$)^[60]. A retrospective analysis of a limited number of patients compared bidirectional oxaliplatin-based HIPEC to bidirectional irinotecan-based HIPEC. The 3-year survival rates were 65.0% in the OX group vs 41.7% in the IRI group ($P = 0.295$)^[61].

In a recently published retrospective analysis of 50 consecutive patients with pmCRC, Désolneux *et al.*^[62] reported a median survival of 34.2 mo and a 5-year survival rate of 29.6% after complete macroscopic cytoreduction and systemic chemotherapy alone. These findings are supported by a retrospective Japanese multicenter database analysis of 564 patients who underwent surgery without HIPEC for pmCRC. In patients with R0 resection, the median overall survival was 30 mo and 5-year survival rate was 32.4%. The 5-year survival rate after R0 resection and adjuvant chemotherapy was 31.7% compared to 24.6% without adjuvant treatment. R0 resection and adjuvant chemotherapy were independent positive prognostic factors for survival^[63]. This concept and the role of HIPEC is investigated by the French prospective randomized PRODIGE 7 trial that compares CRS and HIPEC plus systemic chemotherapy with CRS alone plus systemic chemotherapy. However, survival data are not yet available. Cashin *et al.*^[64] published survival data of a prematurely terminated prospective randomized trial evaluating CRS followed by normothermic intraperitoneal chemotherapy (IPC) with 5-FU vs CRS followed by systemic oxaliplatin-based chemotherapy. Both treatments were continued for 6 mo. The median overall survival times were 25 mo vs 18 mo ($P = 0.04$) and the 2-year survival rates were 54% vs 38% ($P = 0.04$)^[64]. However, the optimal therapeutic regimen of IPC after complete CRS remains a matter of debate^[65].

Prophylactic and palliative HIPEC

Another therapeutic concept that is evaluated by the ongoing French ProphylCHIP trial is the prophylactic application of HIPEC in patients with CRC and high risk of developing PM, such as tumor perforation, isolated ovarian metastases or removal of localized PM during resection of primary tumor resection. The enrolled patients were randomized eight months after adjuvant chemotherapy to the control arm with follow-up or to the treatment arm with explorative laparotomy and prophylactic HIPEC (NCT01226394). The COLOPEC

trial evaluates the effect of adjuvant HIPEC during or shortly after resection of primary CRC with a high risk of metachronous PM. A risk reduction from 25% to 10% and, therefore, improvement in the long-term survival is assumed^[66].

HIPEC without cytoreductive surgery, also applied by the laparoscopic approach, might be considered in patients with unresectable PM (Figure 2) and symptomatic therapy for refractory malignant ascites. Several retrospective studies showed significant reduction of ascites production and efficient symptom control after HIPEC. Nevertheless, the number of reported patients and procedures is limited and data from prospective randomized trials are not available^[67-69].

PERIOPERATIVE SYSTEMIC CHEMOTHERAPY

The importance of systemic chemotherapy in the context of CRS and HIPEC has been demonstrated. Postoperative systemic chemotherapy has been shown to be an independent positive prognostic marker in all registries and retrospective analyses^[13,70]. In a recently published database analysis of 5516 patients with PM arising from colorectal adenocarcinoma, mucinous adenocarcinoma and signet ring cell carcinoma, Simkens *et al.*^[71] showed that systemic chemotherapy improved survival independent of the histological subtype. In contrast to these findings, a multicenter study, including 221 patients with pmCRC reported no significant difference in the OS after CRS and HIPEC between postoperative systemic chemotherapy and surveillance. The median OS was 43.3 mo. Nevertheless, during the first year the rates of progression and recurrence were significantly lower in the chemotherapy group^[72]. However, the optimal sequence of the therapeutic modalities remains a matter of investigation. Elias *et al.*^[13] reported no significant impact on the prognosis of neoadjuvant systemic chemotherapy in patients undergoing CRS and HIPEC for pmCRC. Passot *et al.*^[73] showed an overall response rate of 36% and a disease progression rate of 21% in patients who received different regimens of modern neoadjuvant systemic chemotherapy before CRS and HIPEC. Interestingly, the response to neoadjuvant treatment was not a significant prognostic factor, therefore, it might not be considered a contraindication for CRS and HIPEC. The median survival of patients with disease progression was 31.4 mo^[73]. Further analysis of different preoperative chemotherapy regimens consisting of 5-FU, oxaliplatin, irinotecan and/or monoclonal antibodies showed a 9.7% complete response rate, 20.2% major response and 70.1% rate of minor or no response. In the multivariate analysis the pathohistological response was an independent predictor of survival ($P = 0.01$)^[74]. Devilee *et al.*^[75] compared patients with pmCRC who received neoadjuvant systemic chemotherapy before CRS and HIPEC with patients who were treated with adjuvant

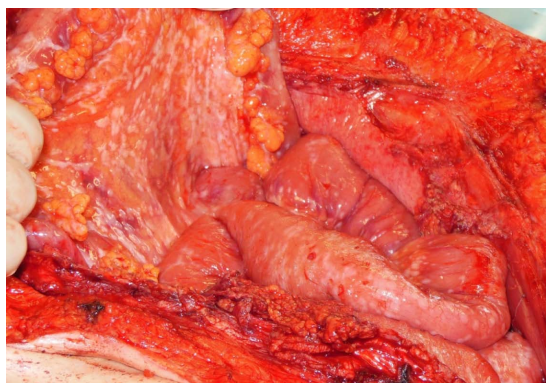


Figure 2 Diffuse peritoneal tumor dissemination.

systemic chemotherapy after CRS and HIPEC. All patients underwent complete or nearly complete macroscopic cytoreduction. The 3-year survival rates were 89% and 50% for the neoadjuvant and adjuvant groups. Although the PCI was lower and operation time was shorter for patients who received preoperative chemotherapy, neoadjuvant treatment was still independently associated with improved survival after correcting for other significant prognostic factors^[75]. Kuijpers *et al.*^[76] analyzed a prospective database regarding the effect of systemic chemotherapy on survival of patients with lymph-node positive pm CRC undergoing CRS and HIPEC. There was a statistically significant increase in the median PFS (15 mo vs 4 mo, $P = 0.024$) and median OS (30 mo vs 14 mo, $P = 0.015$) in patients who received perioperative systemic chemotherapy. Interestingly, the timing of systemic chemotherapy had no influence on survival^[76]. The prospective multicenter phase II COMBATAC study evaluates CRS and bidirectional oxaliplatin-based HIPEC plus perioperative cetuximab-containing polychemotherapy^[77]. The first safety data showed no increase in the morbidity or mortality when using the perioperative treatment approach^[78]. There is another ongoing prospective phase II study (BEV-IP) evaluating perioperative systemic chemotherapy plus bevacizumab in combination with CRS and oxaliplatin-based HIPEC^[79]. However, survival data from both studies are not yet available.

IPC AND PRESSURIZED INTRAPERITONEAL AEROSOL CHEMOTHERAPY

Except for the case of early postoperative IPC (EPIC) instead of or in addition to HIPEC after cytoreductive surgery there are no reliable published data for normo-thermic IPC without cytoreductive surgery for local treatment of pmCRC. The concept of sequential intraperitoneal treatment, which could be applied over a peritoneal port system, has been demonstrated for ovarian cancer^[80]. Yonemura *et al.*^[81] developed a protocol consisting of neoadjuvant systemic and intraperitoneal

chemotherapy (NIPS) for gastric cancer. Clinical trials are needed to evaluate the potential role of IPC in patients with pmCRC, especially in the neoadjuvant setting. Preoperative IPC or NIPS may allow for higher rates of CC-0/1 resection and may further improve the outcome after CRS and HIPEC. Moreover, sequential IPC with or without palliative systemic chemotherapy might improve response rates and local tumor control in patients with unresectable PM arising from CRC.

Pressurized intraperitoneal aerosol chemotherapy (PIPAC) is a new technique for the local application of cytostatics as aerosol under pressure that allows for improved drug distribution and tumor tissue penetration. The feasibility and safety of the procedure has been demonstrated^[82,83]. Most data published for ovarian cancer show local anticancer activity after sequential application of PIPAC^[84]. A recently published retrospective analysis of 48 applications of PIPAC given every six weeks in 17 patients with pretreated pmCRC reported a median OS of 15.7 mo. The overall response rate was 71%^[85]. Quality of life analysis accessed by the EORTC-QLQ30 questionnaire in 48 patients with PM arising from different tumor entities (PCI: 16 ± 10) that received at least two PIPAC applications showed an impairment of the global physical score and pain score after the first treatment improved after the second PIPAC application. Gastrointestinal symptoms remained stable with PIPAC therapy^[86]. Based on the promising preliminary data, PIPAC might become an additional therapeutic option for the palliative local treatment of pmCRC in the future. Moreover, it might be interesting as a neoadjuvant local treatment with or without the addition of systemic chemotherapy beyond CRS and HIPEC. Several prospective clinical trials evaluating this therapy approach are ongoing. The results may help to determine the role of PIPAC within a multidisciplinary treatment concept and allow for further improvement of patient selection.

CONCLUSION

The therapeutic approach to PM of colorectal cancer has changed in recent decades. There are multiple treatment options for patients with pmCRC that must be integrated in an individualized multidisciplinary treatment approach (Figure 1). Consistent diagnostics and patient selection are crucial to obtaining optimal oncologic outcome. Thus, the therapeutic approach should be discussed by an interdisciplinary tumor board, and, if necessary, patients should be referred to specialized treatment centers. In addition to multiple palliative treatment options, CRS and HIPEC provide an additive treatment modality with curative intent for selected patients with pmCRC. The integration of further treatment options such as repeated preoperative intraperitoneal chemotherapy or PIPAC in current treatment regimens should be discussed and evaluated in randomized controlled clinical trials. Prognostic factors, such as peritoneal tumor distribution, lymph node status, hematogenous metastasis, histology, tumor mutation status, tumor immunology, numerous

patient-related factors and the resection status must be considered during patient selection and should be further investigated. The development and clinical use of the prognostic scores may help tailor individual treatment regimens that consider all available therapeutic options. Further prospective randomized trials focussed on patients with pmCRC are highly recommended to optimize and standardize the multimodal treatment regimens.

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2016 Inflammatory Bowel Disease: Global view

Overview of cytokines and nitric oxide involvement in immuno-pathogenesis of inflammatory bowel diseases

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Abstract

Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis are complex disorders with

undetermined etiology. Several hypotheses suggest that IBDs result from an abnormal immune response against endogenous flora and luminal antigens in genetically susceptible individuals. The dysfunction of the mucosal immune response is implicated in the pathogenesis of IBD. The balance between pro-inflammatory cytokines [tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-8, and IL-17A], anti-inflammatory cytokines (IL-4 and IL-13), and immunoregulatory cytokines (IL-10 and transforming growth factors β) is disturbed. Moreover, evidence from animal and clinical studies demonstrate a positive correlation between an increased concentration of nitric oxide (NO) and the severity of the disease. Interestingly, proinflammatory cytokines are involved in the up-regulation of inducible nitric oxide synthase (iNOS) expression in IBD. However, anti-inflammatory and immunoregulatory cytokines are responsible for the negative regulation of iNOS. A positive correlation between NO production and increased pro-inflammatory cytokine levels (TNF- α , IL-6, IL-17, IL-12, and interferon- γ) were reported in patients with IBD. This review focuses on the role of cytokines in intestinal inflammation and their relationship with NO in IBD.

Key words: Inflammatory bowel disease; Cytokines; Nitric oxide; Inducible nitric oxide synthase; Immuno-pathogenesis

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Core tip: Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis are an immunologically mediated disease with undetermined etiology. Evidence from animal and clinical studies demonstrate a positive correlation between an increased concentration of nitric oxide (NO) and the severity of the disease. Moreover, a positive correlation between NO production and increased pro-inflammatory cytokine levels [tumor necrosis factor- α , interleukin (IL)-6, IL-17, IL-12, and interferon- γ] were reported in patients with IBD. This review focuses on

the role of cytokines in intestinal inflammation and their relationship with NO in IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD), represented primarily by ulcerative colitis (UC) and Crohn's disease (CD) is a multifactorial condition characterized by the chronic inflammation of the gastrointestinal tract. It is widely accepted that IBD results from an uncontrolled mucosal immune response to intestinal microflora in genetically susceptible hosts^[1,2]. The mechanisms underlying the deregulated immune response in IBD continue to be extensively investigated to understand the etio-physiopathology of this disease further and to identify new therapeutic strategies. The inflamed intestine of patients with IBD is massively infiltrated by inflammatory cells that release a large number of pro-inflammatory mediators, such as cytokines and nitric oxide (NO)^[3].

NO is a free radical which has several physiological and pathological functions. It is generated from the oxidation of the amino acid L-arginine by a family of enzymes called the nitric oxide synthases (NOS). Three distinct isoforms of NOS are known: (1) two isoforms constitutively expressed in neuronal (nNOS); and (2) endothelial (eNOS) tissues; as well as an inducible isoform (iNOS) expressed primarily by immune cells (e.g., macrophages)^[4,5]. The constitutively expressed isoforms release low levels of NO that exert physiological functions, whereas iNOS releases a high output of NO production under immunogenic and inflammatory stimuli^[6,7].

iNOS is highly expressed upon activation of the transcription factor nuclear factor-kappa B (NF-κB) in response to many stimuli including tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin (IL)-6, interleukin-1α (IL-1α), lipopolysaccharide (LPS), bacterial and viral components^[8,9]. The protective actions of inducible NO have clearly been demonstrated. Its actions include protection against pathogens, reduction of leukocyte adherence, inhibition of macrophage activation, and the inhibition of Th1 type cytokines^[10]. Substantial evidence suggests that iNOS-induced NO exerts protective effects during acute experimental colitis^[11]. However, in IBD, the high levels of NO released in the mucosa appear to be strongly implicated in the maintenance of the chronic inflammation. In this context, it has been shown that NO can cause tissue damage and an exacerbation of inflammation indirectly through the generation of peroxynitrite^[6,12].

The dysregulated balance between pro- and anti-inflammatory cytokines, as well as the immuno-regulatory cytokines observed in IBD distinguish a distinct T cell profile in CD and UC. Classically, CD is described as Th1 type immune response characterized by the secretion of IFN-γ, IL-12, and TNF-α. In contrast, UC is viewed similar to an atypical Th2 type immune response which generates high levels of IL-5, IL-4, and IL-13^[13,14]. In addition, several studies have shown the involvement of Th17 type cytokines (i.e., IL-17, IL-23, IL-22, and IL-6) in the pathogenic process of both CD and UC^[15,16]. Interestingly, Both Th1 and Th17 cytokines are involved in the up-regulation of iNOS expression in IBD. Indeed, a positive correlation between NO production and increased pro-inflammatory cytokine levels (e.g., TNF-α, IL-6, IL-17 IL-12 and IFN-γ) have been reported in IBD plasma^[16,17].

Considerable research has been conducted over the past year to better understand the pathogenesis of IBD, and has led to the development of novel therapeutic strategies based on targeting cytokines, their receptors, as well as the modulation of NO. The assessment of NO production in IBD might be a useful inflammatory marker to predict the stage and the progression of disease^[18]. Unfortunately, some of the current strategies have shown limited efficacy. Hence, a better understanding of the underlying mechanisms of the inflammation and the immune response in IBD may give rise to new alternative, complementary therapeutic strategies.

This review will address the cytokine involvement and relationship with NO in the immuno-pathogenesis of IBD.

NO AND IBD

NO is a lipophilic free radical which plays a key role in regulating the homeostasis of many biological systems. It is synthesized by NOS which catalyzes the oxidation of the terminal nitrogen of the amino acid L-arginine and produces L-citrulline and NO. Three NOS isoforms have been identified and characterized in humans and mice; their nomenclature respects the chronological order in which they were purified: (1) the neuronal form (nNOS or NOS1); (2) the inducible form (iNOS or NOS2); and (3) the endothelial form (eNOS or NOS3). nNOS and eNOS are termed constitutive NOS (cNOS) as they are calcium-dependent, and are respectively expressed constitutively in neuronal and endothelial tissues^[3,4,6]. The effects of NO differ depending on the rate, duration, place of production, and the nature of the target molecules. Under physiological conditions, cNOS generate low levels of NO which have direct regulatory effects (e.g., neurotransmission and the regulation of blood vessels)^[18,19]. In contrast, iNOS generates high levels of NO which mediates antimicrobial and antitumoral activities^[19]. This isoform was first isolated in murine macrophages and was subsequently found in several other cell types, including epithelial cells, hepatocytes, endothelial cells, and fibroblasts. It is expressed after the

induction by immunologic and inflammatory stimuli^[6,20-22]. However, when NO is produced in excess, it becomes noxious. It causes deleterious effects indirectly through the creation of reactive nitric oxygen species (RNOS), such as peroxynitrite anion (OONO⁻), the nitroxyl anion (NO⁻) and dioxide nitrogen (NO₂), responsible for the oxidative stress^[7,23]. Peroxynitrite, is a molecule with high oxidative potential that can trigger cytotoxic processes, such as lipid peroxidation and DNA damage leading to tissue damage and inflammation^[24]. NO has been implicated as a pathogenic mediator in a variety of conditions, such as Alzheimer's disease, rheumatoid arthritis (RA), Behçet disease, multiple sclerosis (MS), Sjogren's syndrome, and IBD^[25].

The deleterious role of NO in IBD was proposed after clinical studies reported the presence of a high levels of nitrite/nitrate in the plasma, urine, and the lumen of the colon^[26-28]. Moreover, a correlation between the overexpression of iNOS, the increased concentration of NO, and the severity of diseases was shown^[29]. In fact, increased levels of NO were found in the serum, stool, and urine of patients in the active phase of UC and CD compared to those in the inactive phase^[26,29]. Our study^[16,17] showed significantly higher serum levels of NO in CD patients compared to UC patients. However, data from previous studies reported no significant differences between these two categories of disease, whereas higher systemic levels of NO in UC compared to CD was found^[16,17,26,29]. A significant difference was observed in the NO concentrations between the active and inactive phase of the disease. This observation suggests a possible use of serum NO levels for monitoring disease activity in both types of IBD^[16,28,29].

While several studies conducted using animal models indicate the deleterious effect of NO, recent studies have shown that NO may also exert a protective effect against colitis^[29-32]. One study conducted using a DSS-induced colitis model found that nitrite administration exerts both preventive and therapeutic effects in colonic inflammation^[33]. More recently, iNOS deficiency enhanced the inflammation aggravation in an animal model of colitis through enhancing a Th17 differentiated subset^[34].

CYTOKINES IMPLICATED IN IBD

The dysfunction of the mucosal immune response in IBD is characterized by abnormalities in both the innate and adaptive immune systems. The final common pathway of this dysregulated immune activation is an abundant infiltration of immune cells in the intestinal mucosa^[15,35-39]. These cells were found to release excessive proinflammatory mediators that amplify the inflammatory cascade through the activation of mitogen-activated protein kinases (MAPK) and NF- κ B. Several studies have reported evidence of the contribution of cytokines, adhesion molecules, reactive oxygen metabolites (ROMs), and NO in mucosal inflammation and injury in triggering IBDs^[40,41]. Cytokines are small soluble peptides which are produced

by diverse immune and non-immune cells. They exert their biological functions through specific receptors activating the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway that controls gene expression in target cells^[42]. In IBDs, the balance between pro-inflammatory cytokines (e.g., TNF- α , IL-1 β , IL-8, and IL-17), anti-inflammatory cytokines (e.g., IL-4 and IL-13), and immunoregulatory cytokines (e.g., IL-10 and TGF α) is disrupted^[43]. According to the cytokine environment found in IBDs patients, CD and UC were conventionally associated with a different CD4⁺ helper T cell profile based on the Th2/Th1 paradigm. Thus, CD was described as Th1 type immune response promoted by the transcription factors STAT-4 and T-bet and characterized by the secretion of IFN- γ , IL-12, and TNF- α ^[13,44]. Indeed, the studies conducted by our group and other teams produced high levels of IL-12 and IFN- γ in CD patients with active disease^[17,45]. IL-12 produced by macrophages/monocytes and dendritic cells plays a pivotal role in enhancing natural killer (NK) cell-mediated cytotoxicity. Moreover, it has been shown that both IL-12 and IL-18 induce a high levels of IFN- γ production leading to the reinforcement of the Th1 immune response^[16,45-47]. In addition, TNF- α plays a pivotal role in the production of NO and enhances the production of metalloproteinases (MMP) leading to the loss of epithelial integrity^[48,49]. In contrast, UC is viewed as a Th2 type immune response promoted by the expression of the transcription factors STAT-6 and Gata-3, as well as the secretion of IL-5, IL-4, and IL-13. Furthermore, Fuss *et al.*^[50] demonstrated that UC patients, unlike CD patients, have atypical natural killer T (NKT)-cells. These cells produce high levels of IL-13 and have cytotoxic activity toward epithelial cells. Similarly, studies using the experimental model of colitis induced by oxazolone have demonstrated that IL-13 produced by NKT cells is the driving cytokine of the disease. Indeed, IL-13 causes alterations of the epithelial barrier function by stimulating epithelial cell apoptosis and the downregulation of tight junction proteins.

Currently, the aforementioned classical concept of the pathogenesis of IBDs is reconsidered with the strong involvement of Th17 cells. This subset of CD4⁺ T helper cells is promoted by the activation of the transcription factors STAT-3 and retinoid-related orphan receptor gamma (ROR- γ t) and is characterized by the production of IL-17A, IL-17F, IL-22, IL-21, IL-6, and IL-26, as well as the chemokine CCL20^[51,52]. Several pieces of evidence support the implication that Th17 cells in the intestinal mucosa provide protection against invading pathogens (e.g., *Candida* and *Salmonella*), through the chemotaxis of neutrophils and the stimulation of antimicrobial peptide production by epithelial cells^[53]. However, both in CD and UC, high levels of Th17 cytokines have been found in the serum and inflamed mucosa. Increased IL-17A production can drive and aggravate the chronic inflammatory response^[17,54,55]. More recently, another subset of Th17 cells, Th1/Th17 cells producing both IFN- γ and IL-17 has been identified in the ileal form of active CD and experimental models of colitis^[56-58]. In addition, it

has been reported that Th17 induces the production of a high levels of TNF- α , IL-1 β , chemokines (IL-8), and matrix metalloproteinases (MMP) (e.g., MMP-9). Moreover, the expression of the cytokine IL-23 and chemokine CCL20, a chemoattractant for Th17 cells expressing the receptor CCR6, is highly up-regulated in CD lesions. Additionally, IL-23 is a crucial effector cytokine necessary for the stabilization and expansion of Th17 cells. It enhances the expression of the master transcription factor (ROR γ t) following IL-6 and tumor growth factor-beta (TGF- β) stimulation^[46]. Moreover, it plays an important role in the development and propagation of the inflammatory response in the gut by inhibiting the expression of the transcription factor Forkhead box P3 (Foxp3) and the development of T regulatory cells (Treg)^[15,46].

The Th17/Treg balance plays an essential role in maintaining intestinal homeostasis. The immunoregulatory cytokine, TGF- β orchestrates the differentiation of Th17 and Treg cells in a dose-dependent manner. In the presence of high levels of IL-6 and inflammatory mediators, TGF- β promotes the differentiation of Th17 cells. Conversely, high levels of TGF- β and low levels of IL-6 and inflammatory mediators promote the development of inducible Foxp3+Treg cells (iTreg)^[59-61]. Regarding the pro-inflammatory role of IL-6, elevated levels of this cytokine and its soluble receptor, sIL-6R were found in the colonic mucosa and sera of patients with IBD. Compelling evidence in human and in animal models has shown that IL-6 plays an important role in maintaining a chronic response by promoting the accumulation of T cells resistant to apoptosis. In addition, IL-6 induces the production of IFN- γ , TNF- α , and IL-1 β , and increases the expression of adhesion proteins, such as intercellular adhesion molecule-1 (ICAM-1) protein which participates in the migration and activation of inflammatory cells to the intestine^[62,63].

It is well established that ongoing inflammation in CD and UC is mediated by uncontrolled T cell responses. Altered Treg regulatory mechanisms have been documented in IBD. However, it remains unclear whether this defect is due to a numerical lack of Treg or a defective TGF- β and IL-10 immunoregulatory activity^[64,65]. Interestingly, it has been shown that in the inflamed colon of CD patients, there is a common CD4⁺T cell population which co-expresses both Foxp3 and ROR γ t. This resident Treg population exhibits plasticity towards Th17 in an inflammatory environment. The Treg/Th17 balance is tightly regulated by intestinal factors, such as endogenous microflora as well as the presence of retinoic acid. Indeed, it has been reported that the vitamin A metabolite, retinoic acid promotes Treg differentiation while inhibiting the formation of Th17 cells^[66]. Thus, these data support the involvement of an altered intestinal microenvironment in the development of IBD and the rupture of gut homeostasis.

Other studies conducted on IBD experimental models reported the implication of other cytokines with an immunomodulatory role [e.g., IL-25, thymic stromal lymphopoietin (TSLP), and IL-22], thereby paving the way for

new therapeutic strategies in IBD^[67-69].

CYTOKINE REGULATION OF NO IN IBD

The inflamed tissue of patients with active IBD is characterized by a massive infiltration of immune cells that release several pro-inflammatory mediators and produce high, *de novo* levels of NO. The expression of iNOS is highly regulated at both the transcriptional and post-transcriptional level by several pro-inflammatory cytokines and immunogenic stimuli (e.g., LPS)^[6,70].

In both patients and animal models of IBD, a positive correlation between the overproduction of pro-inflammatory cytokines (e.g., IL-1 β , TNF- α , IFN- γ) and an overexpression of iNOS was found. This expression was primarily detected in the lamina propria mononuclear cells and the colon epithelial cells of the inflamed mucosa^[6,16,17,27,30,71,72]. Several studies conducted on a dextran sulfate sodium (DSS)-induced experimental model of colitis in BALB/c mice indicated that the neutralization of endogenous TNF- α and/or IFN- γ ameliorated the chronic colitis and concomitantly decreased the generation of NO. These data support the fact that IFN- γ and TNF- α are both involved in the exacerbation of DSS-induced colitis and may exert their detrimental role in the colonic mucosa partly through the induction of the high output of NO. These cytokines had an additive effect on the severity of histological damage and NO colonic levels. However, it seems that IFN- ϕ is the most potent inducer of iNOS in macrophages and epithelial cells than TNF- α , since its neutralization was more effective in attenuating the experimental colitis^[30].

Moreover, our studies reported an up-regulation of iNOS expression in the inflamed colonic mucosa which correlates with high systemic levels of NO, IFN- γ , and IL-12. These observations suggest that IFN- γ and IL-12 may play a pivotal role in IBD pathogenesis through the NO pathway^[16]. Human peripheral blood mononuclear cells (PBMC) from IBD patients were shown to produce elevated levels of NO compared to the controls. The proinflammatory cytokines: IFN- γ , IL-6, TNF- α , and IL-1 β stimulate NO production *in vitro* in PBMCs from patients with CD and UC, suggesting that human PBMCs may constitute another cellular source of NO in IBD^[16,17]. Interestingly, this work reported a positive correlation between Th17 cytokines including IL-6, IL-23, IL-17A, and NO production in the plasma of patients with IBD. Moreover, the mucosal alterations were strongly correlated with high NOS2 and pSTAT3 expression in the colonic mucosa of patients with active IBD. These observations suggest that IL-17 may be a potent inducer of iNOS expression in the inflamed mucosa of IBD patients leading to the exacerbation of the tissue damage. The mechanism by which IL-17 induces NO production is likely dependent on the expression of NF- κ B. In this context, *in vitro* studies using osteoclast cells showed that IL-17 induced the high expression of the mRNA of the NF- κ B isoform RelA et p50^[73].

The negative regulation of iNOS could be achieved by Th2 derived cytokines (e.g., IL-13, IL-4). The inhi-

bitory effect of this cytokine on iNOS protein and mRNA expression has been demonstrated in the HT-29 epithelial cell line induced by IL-1 α /TNF- α /IFN- γ . Interestingly, at low levels and in the presence of TNF- α , these cytokines exert an inhibitory effect on iNOS expression and activation. While a high level of these cytokines could inhibit iNOS mRNA induction in the absence of TNF- α ^[74]. The mechanism of the inhibitory effect of IL-13 on iNOS expression in epithelial cells is dependent on the activation of the PtdIns 3-kinase pathway^[75].

In the same way, it has been shown that the immunosuppressive cytokine IL-10 down-regulates iNOS expression depending on the cell type. Indeed, unlike IL-13, IL-10 had no effect on iNOS expression in colonic epithelial cells but was able to inhibit NO production in mouse activated macrophages^[6,74]. Recently, it has been reported the inhibition of NO and reactive oxygen species (ROS) levels in a mouse carrying a selective deletion of IL-10Ra in macrophages, had less severe colitis than wild-type mice. These data suggest that the protective effect of IL-10 is mainly mediated through the down-regulation of NO and ROS production by macrophages^[76].

Globally, these observations and others suggest that cytokines present in the mucosa of patients with IBD modulate the iNOS expression and activity in the colonic epithelium and could play a homeostatic or inflammatory role in gut inflammation through iNOS modulation.

Many teams have shown that NO can, in turn, modulate the immune response by suppressing IL-12 production from dendritic cells and macrophages. In this manner, NO may control the generation of the Th1 response^[77]. More recently, a study reported that the expression of iNOS in macrophages and dendritic cells can modulate inflammatory cytokine expression including, TNF- α , IL-6, IL-12p70, and IL-23. Growing evidence supports this notion and suggests that NO may control T helper cell differentiation^[34,78]. Indeed, works conducted in an experimental model of colitis showed that an iNOS deficiency aggravated inflammation repetition and increased the percentage of Th17 cells. However, an NO donor molecule suppressed the IL-17 production in T cell-deficient NOS cultures and reduced the percentage of IL-17-producing CD4⁺ T cells. NO has been found to regulate IL-17 expression at the transcriptional level through the nitration of tyrosine residues in ROR γ t, inhibiting its binding to the promoter region of the *IL-17* gene^[34].

CONCLUSION

Cytokines play a crucial role in the pathogenesis of CD and UC as they orchestrate many aspects of intestinal inflammation. A disturbed balance between proinflammatory and immunoregulatory cytokines has been reported in IBD. High levels of proinflammatory cytokines detected in the mucosa of patients with IBD induce a decrease in NO-derived iNOS production.

A decrease in NO and iNOS activity has been closely associated with the initiation and maintenance of inflammation in human and experimental IBD. Evidence suggests that immunoregulatory and anti-inflammatory cytokines (e.g., IL-10, IL-13, and TGF- β) modulate the pro-inflammatory cytokine-derived iNOS expression and activity in intestinal inflammation, thus contributing to the maintenance of homeostasis in gut inflammation. In this context, several studies suggest that pro-inflammatory cytokines might be an important target for the modulation of intestinal inflammation. Moreover, studies using experimental models of IBD have led to a better understanding of cytokine involvement in the pathogenesis of IBD and have opened new lines of research based on their therapeutic relevance. To date, anti-TNF α is one of the most effective cytokine-based therapies for IBD. Nevertheless, several data have shown that the existence of a network of cytokines with multi-layered responses are involved in the perpetuation of the diseases and tissue injury. Therefore, it becomes rational to consider the possibility of simultaneous neutralization of more than one cytokine to provide long-term control of inflammation.

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2016 Inflammatory Bowel Disease: Global view

Infertility in men with inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) predominantly affects young adults. Fertility-related issues are therefore

important in the management of patients with IBD. However, relatively modest attention has been paid to reproductive issues faced by men with IBD. To investigate the effects of IBD and its treatment on male fertility, we reviewed the current literature using a systematic search for published studies. A PubMed search were performed using the main search terms "IBD AND male infertility", "Crohn's disease AND male infertility", "ulcerative colitis AND male infertility". References in review articles were used if relevant. We noted that active inflammation, poor nutrition, alcohol use, smoking, medications, and surgery may cause infertility in men with IBD. In surgery such as proctocolectomy with ileal pouch-anal anastomosis, rectal incision seems to be associated with sexual dysfunction. Of the medications used for IBD, sulfasalazine reversibly reduces male fertility. No other medications appear to affect male fertility significantly, although small studies suggested some adverse effects. There are limited data on the effects of drugs for IBD on male fertility and pregnancy outcomes; however, patients should be informed of the possible effects of paternal drug exposure. This review provides information on fertility-related issues in men with IBD and discusses treatment options.

Key words: Crohn's disease; Infertility; Inflammatory bowel disease; Male; Ulcerative colitis

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Core tip: In men with inflammatory bowel disease (IBD), factors such as surgery, medications, disease activity, and poor nutritional status are thought to contribute to infertility. Surgery with rectal incision is associated with sexual dysfunction (*e.g.*, erectile dysfunction, anejaculation, and retrograde ejaculation). Among medications, sulfasalazine causes reversible qualitative and quantitative semen abnormalities. No other medications seem to affect male fertility significantly. There are limited data on the effects of paternal exposure to IBD medications on pregnancy outcomes, but no significant increase in fetal risk has been noted except for thiopurines. Patients should be

appropriately informed of possible effects of paternal drug exposure.

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INTRODUCTION

Inflammatory bowel disease (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) is a chronic intestinal disorder usually diagnosed in early adulthood. The incidence of IBD has been found to be the highest between the second and fourth decade of life^[1], and fertility-related issues are therefore important clinical considerations.

Infertility is defined as a disease of the reproductive system characterized by failure to achieve a clinical pregnancy after ≥ 12 mo of regular unprotected sexual intercourse^[2]. Much attention has been focused on issues related to fertility in women with IBD, but relatively little attention has been paid to the reproductive issues faced by men with IBD. Male infertility is thought to be more prevalent in IBD patients than in the general population^[3]. From a case control study, Moody *et al*^[4] showed that the number of children born to men with CD is significantly lower in comparison to men with UC and the general population, but found no difference in the number of children between men with UC and the general population. Notably, the fecundability of the three groups did not differ significantly^[5], and the frequency of sexual intercourse was not significantly different between the patients with IBD and the matched controls^[6]. Heetun *et al*^[7] suggested that the smaller family size might be due to a fear of passing on the disease to offspring or a decision to limit family size rather than a physical effect of the disease. A recent systematic review of non-surgically treated men with CD revealed a 18%-50% reduction in fertility with no difference in reproductive capacity^[8].

Even if overall IBD itself does not seem to affect fertility in men, medications used to treat the disease, surgery, and malnutrition resulting from IBD may cause male infertility, including sexual dysfunction. Table 1 shows the possible causes of infertility in men with IBD. This article summarizes sexual and reproductive issues associated with male IBD patients.

SURGERY CAUSING MALE INFERTILITY

It is estimated that approximately 25%-35% of UC patients will ultimately require surgery for either a complication of the disease or inadequate control of symptoms, and 70%-90% of CD patients will need a surgical intervention at some point in the course of their disease^[9-11]. Surgery is required in cases of

Table 1 Possible causes of infertility in men with inflammatory bowel disease

Causes of infertility in men with IBD	Ref.
Surgery	[17,19-25]
Medications	[4,5,7,15,16,28-32,42,43]
Active disease	[15,16,76]
Poor nutrition	[15,77]
Alcohol use	[15,81-83]
Tobacco use	[15,83,86,87]
Psychological factor	[7,88,89]

IBD: Inflammatory bowel disease.

failure of medical management, risk of malignancy, intestinal obstruction and toxic megacolon. Especially in patients with CD, complications such as perianal abscesses, fistulas, and stenosis can occur during the course of the disease, and surgery is often indicated in these cases^[12,13]. Surgical treatment of perianal fistulas ranges from minimal surgery like seton and fistulotomy to definitive surgery with closure of the fistula tract or proctectomy and fecal diversion^[13]. Currently, the most frequently performed surgical procedure for UC is restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA), while intestinal resection is the most commonly performed surgical procedure for CD^[14].

Proctocolectomy with IPAA seems to be associated with sexual dysfunction in men^[15,16]. The sexual disturbances after proctocolectomy are usually due to damage to parasympathetic and sympathetic nerves during surgery, but sometimes due to anatomical alterations, fibrosis, or psychological factors^[17].

Sexual dysfunction is one of the etiologies of male infertility, and it includes erectile dysfunction and ejaculatory dysfunction such as retrograde ejaculation and anejaculation (no ejaculation). A meta-analysis found that the pooled incidence of sexual dysfunction from 21 studies comprising 5112 patients was 3.6%^[18], but this meta-analysis included both men and women. When focusing on only men, Berndtsson *et al*^[19] found that 12% of male patients with UC had ejaculatory dysfunction after IPAA. In a retrospective study, Huetting *et al*^[20] showed the incidence of erectile dysfunction or retrograde ejaculation in such patients to be 25.7%. A study of 122 men who underwent IPAA found that the prevalence of retrograde ejaculation increased from 1.6% preoperatively to 8.2% postoperatively, but the prevalence of erectile dysfunction was similar before and after IPAA^[21]. On the other hand, a large study by Farouk *et al*^[22] found sexual dysfunction in 1% of male patients ($n = 762$) at 1 year after IPAA, and in 2% ($n = 215$) at 12 years after IPAA. Table 2 shows an overview of past studies of sexual function after proctocolectomy in men^[17,19-25]. Regarding the treatment of sexual dysfunction due to rectal excision, there has been one randomized placebo-controlled trial for sildenafil for erectile dysfunction^[26]. This study showed a successful

Table 2 Studies of sexual function after proctocolectomy in men

Ref.	Year	No. of patients	Disease	Time since surgery	Sexual dysfunction after surgery	
					ED	EjD
Michelassi <i>et al</i> ^[23]	1993	24	UC	1.5 yr (median)	0%	19%
Damgaard <i>et al</i> ^[24]	1995	26	UC	2.8 yr (median)	3.80%	3.80%
Farouk <i>et al</i> ^[22]	2000	762	UC	1 yr	1%	
		215	UC	12 yr	2%	
Slors <i>et al</i> ^[17]	2000	40	Benign disease	2.8 yr (median)	10%	12.50%
Lindsey <i>et al</i> ^[25]	2001	156	CD, UC	6.2 yr (median)	14%	0%
Berndtsson <i>et al</i> ^[19]	2004	25	UC	1 yr	0%	12%
Huetting <i>et al</i> ^[20]	2004	35	CD, UC	3.5 yr (median)	25.70%	
Gorgun <i>et al</i> ^[21]	2005	122	CD, UC, others	3.6 yr (median)	12%	8.20%

CD: Crohn's disease; ED: Erectile dysfunction; EjD: Ejaculatory dysfunction; UC: Ulcerative colitis.

response in 79% of patients in the sildenafil-treated group. Given these data, Feagins *et al*^[15] suggested that they could reassure male patients that the occurrence of postoperative sexual dysfunction after IPAA for IBD is low and, when it does occur, it can be successfully treated with sildenafil in most cases. However, sperm banking should be offered before surgery considering that some patients with erectile dysfunction after IPAA fail to respond to medications and some patients may develop ejaculatory dysfunction after surgery, although they are few in number.

There are other surgical options for IBD apart from IPAA, but the data on postoperative fertility (or sexual function) remain limited. One report by Hultén suggested that ileo-rectal anastomosis has the advantage of avoiding rectal dissection and the associated risks of sexual disturbance, but increases the risk of cancer in the rectal stump^[27]. Good results in colectomy with ileo-rectal anastomosis require appropriate patient selection, good rectal distensibility criteria, and accurate endoscopic and histological surveillance for prompt treatment of any recurrence of pouchitis or onset of premalignant changes^[27].

MEDICATIONS CAUSING MALE INFERTILITY

Table 3 summarizes the effect of IBD medications on male fertility and the partner's pregnancy outcomes. It also notes recommendations for discontinuation of medications before attempting to conceive.

Sulfasalazine and 5-aminosalicylates

Sulfasalazine and 5-aminosalicylates (5-ASAs) have been used for the initial treatment of IBD and for long-term maintenance of disease remission^[28]. These drugs have anti-inflammatory activity.

Levi *et al*^[29] first reported 4 cases of male infertility associated with sulfasalazine in 1979. In all 4 cases, discontinuation of sulfasalazine led to successful conception. Subsequent studies showed that this medication causes reversible non-dose-dependent quantitative

and qualitative abnormalities of sperm in > 80% of men^[28,30,31]. Birnie *et al*^[32] examined 21 men with CD who received sulfasalazine and found that 18 of them had abnormal semen analysis results and 15 had oligozoospermia. Another study by Moody *et al*^[4] showed that 25% of men with IBD had no children, compared with 15% of men in the general population. They also found that 60% of male IBD patients who had no children were taking sulfasalazine. Sulfasalazine is a molecule that has two components: 5-ASA and sulfapyridine. The sulfapyridine metabolite is thought to be responsible for adverse effects on sperm, causing impaired sperm maturation or oxidative stress production^[33-36]. However, Wu *et al*^[37] found no correlation between reactive oxygen species production and sperm density, sperm motility, or hamster oocyte penetration capacity. The adverse effects of sulfasalazine on sperm have been shown to be fully reversible after discontinuation^[29,31,33,36,38]. Restoration of semen quality and fertility has also been shown after switching to a different 5-ASA compound without the sulfapyridine component, such as mesalazine (also called mesalamine)^[39,40]. Zelissen *et al*^[41] evaluated semen quality in 11 patients with IBD during sulfasalazine treatment and 4 mo after replacing sulfasalazine with an oral slow-release preparation of 5-ASA, and observed significant improvements in sperm count, morphology, and motility during 5-ASA treatment in comparison with sulfasalazine treatment. Notably, 3 pregnancies occurred during the study period.

On the other hand, there is a case report of mesalazine-induced oligozoospermia in a young man with UC. In that case, semen analysis results returned to near normal and pregnancy occurred after mesalazine treatment was stopped, but the patient's semen parameters worsened after resuming mesalazine^[42]. Moreover, we have reported a retrospective study of the negative influence of mesalazine on fertility in men with IBD^[43]. In this study, 7 of 1225 male subfertile patients had received mesalazine. In 6 of them, mesalazine was discontinued and sperm motility and total motile sperm count were significantly improved. After discontinuation of mesalazine, 4 of the 6 patients achieved pregnancy with their partners.

Table 3 Effects of medications used for inflammatory bowel disease on male fertility

	Infertility	Pregnancy complications	Recommendations
Sulfasalazine	Reversible	One study	Switch to a different 5-ASA
Mesalazine	One study	None reported	Discontinue only in stable disease
Corticosteroids	No	None reported	Only use short periods
Thiopurines	No	Controversial	No recommendation
Methotrexate	Unclear	None reported	Discontinue in the case of erectile dysfunction
Cyclosporine	No	None reported	No recommendation
Infliximab	Unclear	None reported	No recommendation

5-ASA: 5-Aminosalicylate.

However, mesalazine should be discontinued in only patients with stable disease, and it is possible that low IBD activity itself might have contributed to the improved semen analysis results in the patients who discontinued mesalazine.

With respect to pregnancy complications, Moody *et al*^[41] suggested an increased risk of congenital malformations in children born to men on sulfasalazine, but a meta-analysis examining the risk of adverse pregnancy outcomes in women with IBD after exposure to 5-ASAs including sulfasalazine showed no significant increase in congenital abnormalities, stillbirths, spontaneous abortions, preterm deliveries, or low birth weight^[44].

From the evidence accumulated to date, discontinuation of sulfasalazine is recommended for prospective fathers, but not discontinuation of 5-ASA compounds lacking the sulfapyridine moiety.

Corticosteroids

Corticosteroids are potent anti-inflammatory agents used for moderate to severe relapses of both CD and UC, but they have no role in maintenance therapy. Corticosteroids inhibit several inflammatory pathways by suppression of interleukin transcription; induction of I-kappa B, which stabilizes the nuclear factor kappa B complex; suppression of arachidonic acid metabolism; and stimulation of apoptosis of lymphocytes within the lamina propria of the gut^[45].

Limited data are available on the effects of corticosteroids therapy on fertility for men with IBD. Lerman *et al*^[46] found a reversible reduction in fertility in rats exposed to corticosteroids in spite of no changes in sperm count and motility. In a study of 5 endurance-trained men, Roberts *et al*^[47] showed that an increase in endogenous steroids might be correlated with a subsequent decrease in sperm concentration 74 d later. In contrast, in a study of 70 men with CD and a group of age-matched controls, Burnell *et al*^[48] found no correlation between male infertility and steroid use. In a study of IBD patients undergoing azathioprine (AZA) treatment, the additional administration of corticosteroids had no negative influence on seminogram findings^[49]. Definite conclusions regarding the effects of corticosteroids on male fertility cannot be drawn at present because of insufficient data.

Thiopurines

AZA and its active metabolite 6-mercaptopurine (6-MP) are widely used as adjunctive therapy in IBD and as corticosteroid-sparing therapies although they are unapproved therapies for IBD^[47].

In a study of 18 men with IBD who received AZA, no worsening of semen analysis results was found, and 6 of the men fathered children during the study period^[49]. In a survey of 164 male renal transplant recipients, Xu *et al*^[50] concluded that long-term treatment with cyclosporine, AZA, and corticosteroid had no obvious effect on fertility.

A study of male mice exposed to 6-MP showed no reduction in sperm quantity or quality, but a significantly increased incidence of abortion was noted. The authors suggested that this indicated occult sperm damage^[51]. In a study of male patients with IBD who were treated with 6-MP, Rajapakse *et al*^[52] revealed that the incidence of pregnancy-related complications was significantly increased when the father had used 6-MP within 3 mo of conception. Another study showed that paternal use of AZA or 6-MP before conception was associated with an increased, but not statistically significant, risk of congenital abnormalities^[16,53]. Conversely, Francella *et al*^[54] found no significant difference in pregnancy outcomes for both men and women taking 6-MP as compared with controls. Teruel *et al*^[55] evaluated the outcomes of pregnancies in which the father was exposed to thiopurines at the time of conception, and found no significant difference in unsuccessful pregnancies, namely, spontaneous abortions, ectopic pregnancies, anembryonic pregnancies, or fetal deaths. They concluded that routine alteration of treatment regimens was not recommended for men taking thiopurines when attempting to conceive. According to a review by Akbari *et al*^[56] concerning the effects of thiopurines on birth outcomes, thiopurine exposure in men with IBD at the time of conception was not associated with congenital abnormalities^[28].

In summary, thiopurines do not appear to deteriorate semen quality. Some studies have suggested that paternal thiopurine treatment is associated with an increased risk of pregnancy complications, but in most past studies, paternal thiopurine exposure was not related to congenital

abnormalities. Regarding the use of thiopurines in male IBD patients who wish to conceive, Sands *et al.*^[28] proposed that health care providers should inform them that there is a possibility of an increased risk of congenital defects and pregnancy complications although fertility does not seem to be affected.

Methotrexate

Methotrexate (MTX) is positioned as a second-line immunosuppressive agent used in patients resistant or intolerant to AZA or 6-MP. Polyglutamated metabolites of MTX act through the inhibition of dihydrofolate reductase, and the inhibition of cytokine and eicosanoid synthesis are thought to play a role^[45].

MTX is known to have teratogenic effects in women, and it is classified by the American Food and Drug Administration under Pregnancy Category X, which means that it is contraindicated during pregnancy^[7]. However, data are scarce on the effect of MTX on male fertility. Studies of animals exposed to MTX showed altered spermatogenesis, cytotoxicity, and degeneration of spermatocytes, Sertoli cells, and Leydig cells^[15,28,57,58]. In 1980, Sussman *et al.*^[59] reported severe oligozoospermia after MTX administration but a return to normal sperm concentrations after discontinuation of MTX. The antifolate mechanism of MTX, which results in decreased DNA synthesis rates and subsequent inhibition of cellular proliferation, likely causes reversible oligozoospermia^[28]. El-Beheiry *et al.*^[60] investigated the effects of MTX on fertility potential in 26 male psoriatic patients. They showed no abnormalities in semen analysis, testicular histology, or spermatogenic function observed using radioactive phosphorus, although a longer follow-up was required to rule out the possible teratogenic effects of the drug.

There have been no reports of MTX-induced adverse pregnancy outcomes in men exposed to the drug. Recently, Weber-Schoendorfer *et al.*^[61] performed a prospective observational cohort study involving 113 pregnancies where the father was treated with low-dose MTX around the time of conception. As compared with 412 pregnancies without MTX exposure, no increase was observed in the rate of major birth defects or the risk of spontaneous abortion. Further, gestational age at delivery and birth weights did not differ significantly between the groups. Given these results, they concluded that it seems reasonable not to postpone family planning in the case of unavoidable paternal MTX therapy.

However, the active metabolites of MTX could remain in cells or tissues for several months after discontinuation^[16]. Furthermore, MTX seems to be associated with erectile dysfunction^[62-64]. In most of the literature reviews, discontinuation of MTX was recommended at least 3-4 mo before a planned conception for men with IBD^[7,15,16].

Ciclosporin (cyclosporine)/tacrolimus

Ciclosporin (CsA) is a calcineurin inhibitor used for treating severe IBD. It prevents clonal expansion of T cell subsets with a rapid onset of action. Tacrolimus is

another calcineurin inhibitor, and is often preferred in transplant recipients^[45]. CsA and tacrolimus differ in their chemical structure: Tacrolimus is a macrocyclic lactone, while CsA is a cyclic endecapeptide. However, they act in a similar manner as calcineurin inhibitors.

In a review, Sands *et al.*^[28] introduced one study using male mice exposed to CsA, and remarked on the presence of abnormal sperm, oligozoospermia, decreased motility, decreased testicular weight, and decreased testosterone concentrations. A study in rats found that CsA had a deleterious effect on spermiogenesis by directly impairing spermiogenic cell development and by impeding Sertoli cell function^[65]. In humans, small studies have not found an association between CsA use and male fertility^[16,66-68]. There have been no reports of adverse pregnancy outcomes in partners of men receiving CsA.

Monoclonal antibodies against tumor necrosis factor- α

Three biological agents are used for the treatment of IBD, namely, infliximab (IFX), adalimumab, and certolizumab. All agents are monoclonal antibodies against tumor necrosis factor- α (anti-TNF). IFX is a chimeric anti-TNF antibody, consisting of 75% human IgG and 25% murine component. Adalimumab and certolizumab are humanized anti-TNF antibodies. These agents are indicated in CD resistant to standard immunosuppression therapy. IFX is also indicated in UC and fistulating CD^[45].

Few studies have examined the effects of anti-TNF on male fertility. IFX is the most studied of the three agents^[28]. One animal study using analogous anti-TNF agents revealed no adverse effect on male fertility^[7,69]. In a study of 10 men (8 with IBD, 2 with indeterminate colitis), Mahadevan *et al.*^[70] showed a significant increase in semen volume one week after IFX infusion and a trend toward decreased sperm motility. In contrast, in a study of 26 men with spondyloarthritis, Villiger *et al.*^[71] showed no statistically significant difference in sperm quality between healthy controls and patients treated with anti-TNF. They recommended the continuation of anti-TNF treatment when fatherhood was planned. Further, in a prospective study of 10 men with spondyloarthritis and 20 healthy male controls, Ramonda *et al.*^[72] found a statistically significant decrease in sperm aneuploidies and normal hormone levels after a 12-mo anti-TNF regimen and concluded that anti-TNF agents appeared to be safe for testicular function and male fertility.

Exposure to anti-TNF agents in men prior to a planned conception does not seem to cause embryo toxicity. One study that investigated medical records of men with ankylosing spondylitis reported that 4 patients had fathered 6 healthy children during IFX treatment^[73]. A systematic review by Puchner *et al.*^[74] did not find any documentation of miscarriages or physical abnormalities associated with anti-TNF treatment and paternity. Instead, an improvement in sperm motility and vitality during anti-TNF treatment was shown in that review. The

authors suggested that the improvement might be due to a decrease in disease activity.

OTHER FACTORS CAUSING MALE INFERTILITY

Disease activity

Active disease seems to affect male reproductive and sexual function^[15,16]. The presence of pro-inflammatory cytokines, including TNF, in the male urogenital tract could lead to cytokine-mediated antifertility effects. Furthermore, inflammation is associated with elevated levels of reactive oxygen species and oxidative stress, both of which have a negative effect on male fertility^[75]. Regarding sexual function, Timmer *et al.*^[76] showed that men with IBD in remission or with mild disease activity had similar rates of erectile dysfunction as compared with controls, whereas men with severe IBD activity had higher rates. Thus, control of IBD activity is recommended for men planning to conceive.

Nutrition

Poor nutritional status in men with IBD might cause infertility. El-Tawil suggested a possible relation between decreased testicular function and zinc deficiency, which has been found in up to 70% of patients with CD^[77]. To date, no other studies have specifically addressed the contribution of nutritional status to male infertility in IBD, but Feagins *et al.*^[15] proposed that optimizing nutritional status is important for men with IBD who are attempting to father children.

Alcohol use

There are several studies that implicate a negative effect of alcohol consumption on the course of IBD^[78]. Swanson *et al.*^[79] showed that alcohol resulted in exacerbation of gastrointestinal symptoms in patients with non-active UC and CD. Jowett *et al.*^[80] indicated that alcohol consumption increased the risk of disease exacerbation in patients with UC. Thus, alcohol use could activate the disease in the patients with IBD. Moreover, past studies implicated alcohol use in decreasing sperm quality and fertility in men^[15,81-83]. Alcohol is considered as one of factors that might be contributing to male infertility in men with IBD.

Tobacco use

Smoking is the most researched environmental factor associated with IBD. It has been observed that smoking has a varying impact on CD and UC, contributing to an increased risk for individuals with CD and a protective role in individuals with UC^[1]. The mechanism of these paradoxical effects of smoking on CD and UC is not well understood. It is hypothesized that nicotine and oxidative stress play some role^[1,84].

Even if smoking protects against UC, smoking itself impairs fertilization capacity^[83]. Tobacco combustion

produces many chemical compounds with potential deleterious effects on male germ cells^[85]. The toxins originating from cigarette smoke can decrease sperm mitochondrial activity and damage the chromatin structure in human sperm^[83]. From a recent meta-analysis of 20 studies with 5865 participants, smoking was found to be a significant risk factor for decreased semen parameters in men^[86]. Therefore, smoking cessation is expected to have a positive influence on semen quality and consequently male fertility.

Psychological factor

Past studies showed lower birth rates to men after IBD diagnosis than before diagnosis compared with controls^[5,48]. These results meant that IBD men might consider voluntary childlessness apart from physiological factors that could reduce fertility^[87]. This voluntary childlessness appears to result from concerns about adverse reproductive outcomes that may not be justified, or patients' fear of transmitting the disease^[7,88]. In a questionnaire survey, Mountfield *et al.*^[88] concluded that patients require accurate counseling addressing fertility and pregnancy outcomes in IBD to assist in their decision making.

TREATMENT OF INFERTILITY IN MEN WITH IBD

Active inflammation, lifestyle factors (alcohol use, tobacco use), medications, poor nutritional status, and rectal incision seem to affect fertility in male IBD patients^[15]. First of all, it is important to control IBD activity. If the patient shows poor nutritional status, optimizing their nutritional status is recommended. Tobacco cessation is strongly recommended when the patient is a smoker. If possible, discontinuation of medications associated with male infertility is recommended for prospective fathers. Table 3 shows the recommendations for each drug. In patients taking sulfasalazine, switching to a different 5-ASA is advised at least 4 mo prior to attempting to conceive^[28]. In patients with stable IBD who are receiving mesalazine, discontinuation of the drug might restore fertility^[43]. To avoid any potential adverse events, corticosteroids should be used for short periods to control active disease^[28]. Although discontinuation of MTX is recommended 3-4 mo before attempting to conceive in most of the past reviews, there is insufficient evidence for males to support this recommendation. The risks of MTX discontinuation might outweigh the unsubstantiated hypothetical benefits. Discontinuation should be considered only in the case of erectile dysfunction. At present, there is insufficient evidence to recommend discontinuation of thiopurines, CsA, and anti-TNF agents such as IFX. Sperm banking should be offered to patients who plan to undergo proctocolectomy, because post-operative anejaculation, despite its low incidence, is a potential irreversible complication.

CONCLUSION

This review aimed to provide further insights into relationship between IBD and male fertility, a topic that has received relatively little attention in the literature. Rectal incision can potentially lead to sexual dysfunction after surgery, and sexual dysfunction may cause male infertility. Of the medications used for IBD, sulfasalazine causes reversible oligoasthenoteratozoospermia. No other medications seem to significantly affect fertility in men although small studies suggested some adverse effects. In the case of erectile dysfunction, discontinuation of MTX should be considered because MTX appears to be associated with erectile dysfunction. There are limited data about the effects of other drugs on male fertility and pregnancy outcomes; however, patients should be appropriately informed of the possible effects of paternal drug exposure. Considering that IBD predominantly affects young adults of reproductive age, gastroenterologists treating IBD patients should pay more attention to fertility-related issues. Sperm banking is an option for fertility preservation before surgery or initiation of a potentially gonadotoxic medication.

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2016 Pancreatic Cancer: Global view

Management of pain in chronic pancreatitis with emphasis on exogenous pancreatic enzymes

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Abstract

One of the most challenging issues arising in patients with chronic pancreatitis is the management of abdominal pain. Many competing theories exist to explain pancreatic pain including ductal hypertension from strictures and stones, increased interstitial pressure from glandular fibrosis, pancreatic neuritis, and ischemia. This clinical problem is superimposed on a background of reduced enzyme secretion and altered feedback mechanisms. Throughout history, investigators have used these theories to devise methods to combat chronic pancreatic pain including: Lifestyle measures, antioxidants, analgesics, administration of exogenous pancreatic enzymes, endoscopic drainage procedures, and surgical drainage and resection procedures. While the value of each modality has been debated over the years, pancreatic enzyme therapy remains a viable option. Enzyme therapy restores active enzymes to the small bowel and targets the altered feedback mechanism that lead to increased pancreatic ductal and tissue pressures, ischemia, and pain. Here, we review the mechanisms and treatments for chronic pancreatic pain with a specific focus on pancreatic enzyme replacement therapy. We also discuss different approaches to overcoming a lack of clinical response update ideas for studies needed to improve the clinical use of pancreatic enzymes to ameliorate pancreatic pain.

Key words: Pancreatic enzyme replacement therapy; Chronic pancreatitis; Pancreatic insufficiency; Protease; Clinical trials; Trypsin; Fat malabsorption; Pain

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Core tip: Pancreatic enzyme replacement therapy

has long been used as a non-invasive treatment for chronic pancreatic pain. Enzyme therapy aims to restore feedback inhibition of pancreatic secretion to lessen pain caused by pancreatic ductal hypertension, increased pancreatic interstitial pressure, and pancreatic ischemia. Although enzyme therapy may play a role the key is individualization of therapy based on disease etiology and severity. Here we review the literature regarding the efficacy of enzyme therapy and the evidence gathered for an entero-pancreatic feedback loop. We also describe alternative strategies for improving pain therapy including using uncoated enzymes with gastric acid suppression.

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INTRODUCTION

Although the pancreas was known to ancient Greeks, its role in health and disease remained obscure until recent times. One of the earliest cases of chronic calcific pancreatitis is described in *History of the Pancreas* by Howard and Hess^[1] in which they relate that in 1678 de Graaf reported the case history of a patient seen by a Dr. Gajea. The patient, a nobleman, was "seized by vomiting and diarrhea because of an uncontrolled use of wine and seafood"^[1]. At autopsy, seven or eight stones the size of a chick pea were found blocking the pancreatic duct^[1]. Later, diabetes was recognized as a complication of chronic pancreatitis^[2]. Despite a plethora of autopsy cases, case reports, and reviews^[1,3], a clear understanding of the manifestations of chronic pancreatitis had to await medicine's advance to when surgeons could safely enter the abdomen as well as the development of laboratory testing and radiographic imaging.

Pancreatitis can be classified broadly as acute or chronic^[4]. In acute pancreatitis, the glands undergo varying degrees of edema, inflammation, and possibly necrosis^[4-6]. Although a majority of the glands may be injured, most recover^[7]. Chronic pancreatitis is thought to be the end result of a long-term inflammatory process that results in both morphological and structural changes^[7]. This has been proposed as a two-step process in which functional and structural impairment to pancreatic secretion eventually leads to activation of zymogens resulting in local destruction of glandular tissue eventuating in fibrosis^[7]. This may also result in marked pancreatic structural alterations including formation of pseudocysts and ductal strictures and repeated cycles of increasing damage and inflammation ultimately resulting in both exocrine and endocrine insufficiency^[4,6,7]. Ductal dilatation and intraductal calcifications are common in chronic pancreatitis^[4-6] and such architectural changes

allow one to reliably distinguish between acute and chronic pancreatitis. However, chronic pancreatitis can occur without gross changes and still be diagnosed based on the presence of abnormal structure and function^[7].

Histology is the diagnostic gold standard for chronic pancreatitis, but pancreatic biopsy is potentially dangerous and not routinely performed^[8]. Instead, there are a myriad of functional tests available such as the cholecystokinin-secretin test^[8]. A comparison of the cholecystokinin-secretin test to pancreatic tissue biopsy reported a significant correlation between histology and peak bicarbonate concentration (sensitivity of 67% and specificity of 90%)^[9]. In fact, the functional tests are even more sensitive than endoscopic retrograde cholangiopancreatography (ERCP)^[9]. Overall, the secretin stimulation test is considered the most sensitive test for diagnosing chronic pancreatitis but is not widely available^[7,10]. Imaging studies including radiographs, ultrasound, computed tomography, and magnetic resonance imaging identify abnormal pancreatic structure. ERCP and endoscopic ultrasound are the most widely used to diagnose chronic pancreatitis^[8]. A number of classification schemes have been proposed such as the Cambridge and Rosemont Classifications. The Cambridge classification uses findings seen on ERCP, ultrasound, and CT^[5], whereas the Rosemont classification diagnoses chronic pancreatitis based on major and minor features present on endoscopic ultrasound^[11]. Chronic pancreatitis is also classified for therapeutic studies as large or small duct disease because the two variants differ in natural course and treatment responses^[7]. For example, patients with small duct disease tend to have better pain response to pancreatic enzyme supplementation compared to those with large duct disease^[7].

ETIOLOGY OF CHRONIC PANCREATITIS

Worldwide, alcohol use is the most common cause of chronic pancreatitis in adults and in most series accounts for approximately 70% of cases (Table 1). A wide variety of other etiologies (cystic fibrosis, hypertriglyceridemia, tumor, pancreatic resection, familial, congenital abnormalities, tropical, autoimmune, genetic) account for approximately 10%, and the remaining 20% are currently considered idiopathic^[12]. The focus of this review is on the medical management of patients with chronic pancreatitis presenting with chronic abdominal pain.

PANCREATIC PAIN

Although the proportion of patients with chronic pancreatitis and pain is unclear, many, if not most, patients are originally identified because they seek medical help due to abdominal pain^[13]. Other presentations include signs of endocrine or exocrine dysfunction without pain^[14]. It has been estimated that overall 5% to 10% of patients with chronic pancreatitis, especially those with late-onset idiopathic disease, do not suffer from abdominal pain^[13]. Episodic pain is a defining symptom of chronic

Table 1 Common causes of chronic pancreatitis

Toxic metabolic
Xenobiotics
Alcohol
Cigarette smoking ^[12]
Genetic mutations
CFTR mutation (Cystic Fibrosis Transmembrane Conductance Regulator) PRSS1 mutation (Protease, Serine 1)
SPINK1 mutation (Serine Peptidase Inhibitor, Kazal type 1)
CTRC (chymotrypsin C)
Chronic Obstruction of main pancreatic duct
Cancer
Post-duct destruction in severe attack
Recurrent acute pancreatitis
Autoimmune
Idiopathic
Early or late onset
Tropical

pancreatitis and is classically described as constant pain in the epigastric area with radiation to the back^[13]. Painful episodes last roughly a week and are often accompanied by fatigue, nausea, vomiting, food avoidance, and weight loss^[15]. Pain is typically worsened with food intake and may be ameliorated in part by leaning forward, sitting up, food avoidance, or use of heating pads to the back or abdomen^[7,15]. The pain can be severe but varies widely among patients and even in the same individual^[13]. This variation complicates interpretation of therapy during pain-free intervals between exacerbations^[13]. A thorough history often reveals multiple similar prior episodes, alcohol abuse, and symptoms of weight loss, diarrhea and steatorrhea^[7]. While alcohol use is often described as the most common trigger for symptoms (*e.g.*, pain occurring twelve to forty-eight hours after alcohol use)^[16], many report no consistent association between alcohol use and pain^[13,17-21]. Physical examination is typically negative with the exception that pain in the epigastric region may worsen with palpation.

Disease progression may be associated with a change in the characteristics of pain. Early in the disease, pain tends to be periodic which may then progress to constant debilitating pain^[22]. Pain resolves in some patients as the glands are destroyed and the disease “burns out”. However, this may require more than 18 years^[13,19,20]. While it has been suggested that viable pancreatic tissue may be required for pancreatic pain^[15], the natural course of pain in chronic pancreatitis is notoriously difficult to predict. For example, a longitudinal study with 113 patients noted that the pain decreased in 42%, did not change in 32%, and increased in 26% over a 4 year observation period^[23]. In contrast, another study reported 85% of patients achieved pain relief at a median of 4.5 years^[19]. Patients achieving pain relief were most often those with increased pancreatic calcifications and dysfunction^[19].

A large multi-center study evaluated the frequency of different pain patterns among 540 patients with chronic pancreatitis^[24]. Their characterization focused on

Table 2 Pattern of pancreatic pain

Episodic mild to moderate pain
Constant mild to moderate pain
Typically pain free between episodes of severe pain
Constant mild pain with episodes of severe pain
Constant pain

frequency (intermittent vs constant) and severity (mild, moderate, or severe); only approximately 20% of patients were unable to self-characterize their pain pattern. Pain patterns (Table 2) were originally scored into one of 5 patterns based on the American Gastrointestinal Association's technical review^[25]. The most common pain patterns were constant mild pain with episodic severe pain (56%) and typically pain free with episodic severe pain (31%). Overall, constant pain was more common than intermittent pain (52% vs 45%). Patients with intermittent pain tended to be older while those with constant pain were current smokers and had alcohol as the primary etiology of their chronic pancreatitis. As might be expected, those with constant pain and those with severe pain were more likely to be disabled, have poor quality of life, and to utilize health care resources. However, it has been estimated that 30% to 50% of patients with chronic pancreatitis will eventually become pain free^[13].

Ammann *et al.*^[20] study of pain in chronic alcoholic pancreatitis provided data on 207 patients. None were addicted to narcotics or had an inflammatory mass as the potential cause of pain. Two pain patterns were common. In the first pattern, patients experienced short episodes of pain separated by pain-free periods lasting from months to years. Patients with the second pain pattern had persistent daily pain or clusters of severe pain typically occurring 2 or more days per week for at least 2 mo. Among those with intermittent pain and not requiring surgery, 50% had pain relief within 6 years increasing to more than 80% at 10 years. All of those with persistent pain underwent surgery because of the presence of a pseudocyst (most common), presumed high ductal pressure (large duct disease with or without ductal stones and minimal or no exocrine insufficiency), or biliary obstruction. Overall, the response to pain and the proportion developing pancreatic insufficiency in the two groups were similar. In the total series, the most common association with chronic pain was narcotic addiction, and these patients were few in number and were excluded. However, in many series the management of pain in patients with chronic pancreatitis is complicated by narcotic and alcohol dependencies^[13].

PATHOGENESIS OF PANCREATIC PAIN

The pathogenesis of chronic pancreatic pain is poorly understood. In the 19th century, thought centered on ductal obstruction and the passage of a stone similar to what occurs with salivary gland or biliary stones,

Table 3 Mechanisms of pain in chronic pancreatitis

Increased intraductal pressure
Ductal obstruction from strictures/stones
Increased intrapancreatic pressure (compartment-like syndrome)
Fibrosis causing lack of distensibility
Neuropathic
Entrapment of nerves
Damage of nerves by enzymes
Increased nerve tissue
Pancreatic ischemia
Worsened during increased enzyme secretion

as well as pressure or other damage to the celiac axis (e.g., neuralgia coeliaca)^[3]. Currently, the major theories focus on increased pancreatic pressure (e.g., intraductal pressure, pancreatic interstitial hypertension, or ischemia) and neurogenic causes (Table 3).

DUCTAL HYPERTENSION

Ductal hypertension is often considered “the most important cause of pain”^[13] based on the concept that ductal strictures and calculi can cause ductal obstruction which leads to increased ductal pressure, and thus pain, during pancreatic secretion^[7,26]. It has been suggested that one role of alcohol in pancreatitis is to promote stone formation in pancreatic secretions^[27]. The presence of these stones promotes inflammation leading to scarring and strictures which then elevate intraluminal pressures^[27]. Clinical studies have indeed confirmed elevated pancreatic ductal pressures in patients with chronic pancreatitis. For example, normal pancreatic ductal pressure ranges from 7 to 15 mmHg while ductal pressures ranging from 20 to 80 mmHg have been measured in patients with chronic pancreatitis^[28–30]. A direct relationship between the reduction in ductal pressure and relief of pancreatic pain has also been reported^[31]. For example, there are numerous studies demonstrating pain reduction or relief following decompression of a dilated duct or pseudocyst using drugs, endoscopic stents, by disintegration of pancreatic stones *via* extracorporeal shock waves, surgical drainage procedures, or pancreatic resections^[28,32].

While clinical data suggests that pancreatic pain can be reduced by eliminating ductal strictures and obstructions, decreasing pancreatic secretion, or both, significant obstruction is not universally apparent in painful chronic pancreatitis^[26] and ductal surgery does not uniformly relieve pain^[16]. For example, the prevalence of major duct strictures was reported to be similar (e.g., about 60%) in patients with painful and painless chronic pancreatitis^[21]. However, ductal pressures were not measured^[21,26].

INTERSTITIAL HYPERTENSION

A related theory focuses on increased pancreatic interstitial hypertension which has been reported to be higher

in patients with painful chronic pancreatitis than in painless chronic pancreatitis (e.g., a median of 7 mmHg vs 22.5 mmHg)^[33]. In those patients with pain, drainage procedures involving the main duct or a communicating pseudocyst often result in both pain relief and a reduction in interstitial pressures to normal levels^[33]. An extensive study of the relation of ductal and interstitial pressure in chronic pancreatitis was performed using a cat model^[34]. Perfusion of the normal main duct at physiologic flow rates resulted in an increase in ductal pressures but no significant change in interstitial pressure. Perfusion following partial obstruction of the main pancreatic duct at the neck of the pancreas resulted in a further increase in ductal pressure but again without an increase in interstitial pressure. These data suggest that the normal pancreas has sufficient distensibility to dissipate the increase in ductal pressure. Following encasement of the pancreas in latex to decrease its ability to expand, perfusion of the pancreas resulted in significant increases in both ductal and interstitial pressures. Finally, to simulate chronic pancreatitis, the main pancreatic duct was obstructed for 5 wk resulting in histological changes similar to chronic pancreatitis in humans. Perfusion of the duct then resulted in an increase in both ductal and interstitial pressures leading the authors to conclude that the loss of distensibility in chronic pancreatitis likely results in a compartment-like syndrome in which secretion produces increased ductal and interstitial pressures both of which can be partially or completely relieved by pancreatic surgery^[26]. They also showed reduced pancreatic blood flow in the cat model^[34,35] suggesting possible pancreatic ischemia.

PANCREATIC ISCHEMIA

The ischemia hypothesis is based on the concept that increased interstitial pressures and surrounding fibrosis could increase vascular resistance leading to decreased perfusion of pancreatic tissues^[34–38]. As noted above, in the cat chronic pancreatitis model, basal blood flow was reduced by 40% compared to the normal pancreas^[34]. In addition, pancreatic secretagogues increased normal blood flow by 27% but decreased blood flow by 14% in animals with chronic pancreatitis. Decompression of the obstructed pancreatic duct resulted in both an increase in basal flow and return to the normal increase following stimulation of secretion consistent with the notion that parenchymal damage and pancreatic pain could be secondary to ischemia (*i.e.*, a compartment-like syndrome).

PANCREATIC NEURITIS

A final, but related, pain theory is based on the concept that altered pancreatic architecture results in inflammation of nerves and altered feedback mechanisms^[13,39]. It has been proposed that chronic inflammation of peripancreatic nerves may increase nerve tissue through up-regulation of neuropeptides^[28]. The fact that mean diameter of

peripancreatic nerves in chronic pancreatitis patients was significantly greater than controls led to the suggestion that the increased nerve diameter was caused by a fibrotic process that strangulated the nerves^[13]. Microscopic analyses have also shown disruptions in perineural structure which could theoretically expose nerves to damaging inflammation, enzymes, and inflammatory cells^[28]. Specifically, the number of eosinophils present in pancreatic perineural tissue was shown to correlate with pain and alcoholism scores^[40]. The pancreas is highly innervated, and it has been suggested that pain reduction following surgical removal of the head of the pancreas is related to removal of the most highly innervated region^[27,41]. The pain relief obtained by removal of inflammatory pancreatic masses is thought to possibly relate to removal of damaged nervous tissue^[39]. In the last decade, research has focused on histologic and biochemical features in the involved pancreas, as well as changes in cortical reorganization and electroencephalographic findings and the similarities to patients with neuropathic pain (e.g., reviewed in^[39,42-44]). Similar findings have been described in humans and experimental animals with chronic pancreatitis. It is however important to note that the neurogenic theory cannot, by itself, explain pain relief in pancreatic "burn out" or after reduction of intraductal pressures through procedures^[13].

TREATMENT OF PAIN IN CHRONIC PANCREATITIS

Pain in patients with chronic pancreatitis is often extremely difficult to manage in that patients frequently receive narcotics and a significant proportion of patients develop dependency on both narcotics and alcohol. Severe constant pain often indicates the presence of a complication such as a pancreatic pseudocyst and should prompt targeted investigations^[45,46]. One of the first goals of the clinician is to ensure that the pain is related to chronic pancreatitis and not to some another condition^[32]. Patients with chronic pancreatitis may also have malabsorption resulting in flooding of the colon with nutrients leading to meteorism or other symptoms of malabsorption^[47-49]. One topic heading in Howard and Hess's *History of the Pancreas* is entitled "Treat the pain, not the disease"^[1] (page 291), emphasizing that patients with chronic pancreatitis often have multiple overlapping issues and correct diagnoses and a multidisciplinary approach is essential for successful treatment^[13]. Pain remains the most common primary indication for surgical or endoscopic intervention. Treatment failure or only partial success is common^[13]. The focus of this paper is on medical treatment of pain in chronic pancreatitis. Nonetheless, medical, endoscopic, and surgical treatments may all be required for a successful outcome.

LIFESTYLE CHANGES

Cessation of alcohol

Although alcohol is involved in a large percentage of cases of painful chronic pancreatitis, it remains unclear why only a small percentage of those who abuse alcohol develop chronic pancreatitis. Chronic pancreatitis is more common among those who also smoke^[12]. It is likely that there is a genetic predisposition that associates alcohol or alcohol and smoking with pancreatitis, but no single genetic association has yet been discovered^[12]. Because alcohol-induced chronic pancreatitis is a progressive disease leading to structural and functional pancreatic changes, theoretically abstinence from alcohol could result in a reduction or elimination of pain, decrease the degree of pancreatic dysfunction, reduce mortality, and promote a return to normal activity^[13]. It has repeatedly been suggested that cessation of alcohol improves the course of the disease^[13,50]. For example, in one large study of the natural history of alcoholic chronic pancreatitis, 75% of patients continued to drink and in those patients the death rate and level of physical impairment were three times higher^[19]. All agree that one focus should be on promoting cessation of alcohol and tobacco use. However, bouts of pain in alcohol-induced chronic pancreatitis still occur after the cessation of alcohol^[18]. The benefits in terms of prevention of flares may in part depend on the stage of the disease in that alcohol, as a secretagogue, may have minimal effect on patients with little or no remaining exocrine function^[13].

DIET

Patients and their families often inquire about diet therapy. It has been recommended that meals be low in fat and that large meals be avoided to possibly minimize hyperstimulation of the pancreas^[13,27]. However, few of these dietary recommendations are evidence-based. Many patients with chronic pancreatitis will have clinical or subclinical deficiencies in vitamins and micronutrients^[51,52]. Testing for retinol-binding protein, prealbumin, magnesium and transferrin has been recommended^[8,51,53]. Because smoking is a risk factor for chronic pancreatitis, the formation of stones, and also calcifications, cigarette smoking should be strongly discouraged^[15,54].

ANTIOXIDANTS

An intriguing aspect of dietary therapy in chronic pancreatitis is the emerging possible role of antioxidants. For example, Rose *et al.*^[55] reported deficiencies in selenium, vitamins A, C, and E, and riboflavin compared to healthy controls and patients with recurrent acute pancreatitis. Other studies have reported decreased intake of micronutrients in chronic pancreatitis patients^[56]. These findings fueled the hypothesis that a reduction in these micronutrients could enhance oxidative stress and link to

the development of chronic pancreatitis^[27,52]. Allopurinol can theoretically decrease toxic free radicals *via* its action on xanthine oxidase^[27] leading to trials seeking to alleviate pancreatic pain with allopurinol. However, small studies reported no significant effects^[57]. In contrast, a randomized trial of antioxidant supplementation with selenium, ascorbic acid, B-carotene, α -tocopherol, and methionine reported a significant reduction in the number of painful days per month^[58]. A meta-analysis of antioxidants in chronic pancreatitis reported a small but significant reduction in visual analog scale pain scores (0.33 out of 10) along with an adverse effect rate of 16% of "mostly mild" symptoms^[30]. Finally, a Cochrane review concluded that antioxidant therapy provides slight benefits and also reported adverse events in about 17%^[59]. The role of antioxidant therapy in pain in chronic pancreatitis remains unclear and further investigation is warranted^[60].

ENDOSCOPIC THERAPY

Endoscopic therapy has continued to play a role in the diagnosis and treatment of chronic pancreatic pain. Recent Cochrane reviews concluded that endoscopic therapy is not as effective as surgical intervention for pain relief, but endoscopy remains a viable option because of its availability and relative safety^[59]. The Cochrane reviews were not able to clearly delineate differences between endoscopy and surgery regarding mortality and morbidity and recommended that options be presented to the patient and a joint decision be made^[59]. Most endoscopic therapy is utilized for patients with intractable pain or nutritional deficiencies after more conservative therapy has failed^[27]. Despite the lack of clear definitions of significant obstruction or methods to reliably identify patients amenable to endoscopic treatment, endoscopy has proven to be useful in relieving duct obstruction secondary to strictures, stones, or ampullary stenosis^[27]. An alternative method is the combination of extracorporeal shock-wave lithotripsy followed by endoscopic removal of remaining debris and stones^[27]. It has been reported that 80% of stones can be removed with approximately half of the patients reporting long-term pain relief^[61]. A comparison of extracorporeal shock wave lithotripsy and extracorporeal shock wave lithotripsy plus endoscopic drainage in painful chronic pancreatitis found no significant difference after 2 years (*i.e.*, 38% of patients with extracorporeal shock wave lithotripsy alone reported pain relapse vs 45% of those with combined therapy)^[62]. However, both groups experienced a significant reduction in pain episodes per year. Importantly, there was no placebo group and the cost of the combined treatment was three times greater^[62].

Another alternative is placement of pancreatic ductal stents. In one study, 94% of 75 patients receiving pancreatic duct stents and dilation of duct strictures initially reported improved symptoms and, after a mean follow-up of three years, 53% remained symptom free^[63]. Another study reported symptomatic improvement in 57% of

61 patients over a mean of 19 mo^[64]. Anecdotally, pain relief appears to correlate with stone removal resulting in a decrease in main duct diameter^[27]. Although stent placement is associated with stent migration, occlusion, aggravation of chronic pancreatitis and further duct changes, the availability and relatively low invasiveness compared to surgery makes endoscopic therapy a first-line consideration for treatment of ductal strictures and obstruction in the management of pancreatic pain^[27].

SURGERY

Prior to the advent of endoscopic therapy for pancreatic ductal disease, the primary approach involved surgical interventions. A variety of surgical options were developed and the surgical approach continues to evolve. Nonetheless, no surgical intervention has proved to be one hundred percent effective. The role of surgical therapy is to deal with and prevent complications, as well as attempt to achieve pain control^[61]. Indications for surgery include non-resolving ductal or common bile duct stenosis, intractable pain, internal pancreatic fistulas unresponsive to less invasive therapy, vascular erosions, or uncontrollable pancreatic pseudocysts^[61]. Traditional surgical options for chronic pancreatitis can be divided into procedures that focus on resection of pancreatic tissue and procedures that focus on drainage of pancreatic ducts^[61]. Resection-based procedures such as the Whipple operation, distal and total pancreatectomies, and the pylorus-preserving pancreatoduodenectomy were developed in part to relieve obstruction and because of the belief that chronic pancreatic pain also stemmed from perineural pancreatic inflammation. Drainage-based procedures such as the Frey procedure, Beger procedure, sphincterotomies, and pancreaticojejunostomies were designed to relieve ductal obstructions and ductal hypertension^[61]. No gold standard exists, and the surgical procedures used to control pancreatic pain are individualized based on the anatomy, the condition of the patient, and the skill and experience of the surgeon.

In addition to more traditional options, pancreatic autotransplantation and a resurgence in neuroablation are emerging therapies. Endoscopic or even surgical neurolysis of the celiac ganglion remains an option in high-risk surgical patients or in patients who need additional therapy post-operatively^[65]. Although less-invasive than traditional surgery, only 10% of neurolysis patients showed a benefit at 24 wk and two-thirds of patients required additional surgery^[65]. Pancreatic autotransplantation can supplement resection-based surgery to preserve islet cell function and stave off endocrine insufficiency^[65]. For example, the Mirkowitch technique uses a pellet of purified islet cells and segmental transplantation with resected pancreatic tissue that is implanted into the thigh^[61].

There are significant limitations to surgical options for treating chronic pancreatic pain in that while pain relief and quality of life can be improved, exocrine and endocrine insufficiency frequently accompany respective options^[66,67]. Patients undergoing surgery for chronic

pancreatitis have substantial hospital readmission rates. One recent study found that 31.5% of patients were readmitted in the first 30 d postoperatively and 42.3% were admitted in the first 90 d^[68]. These substantial readmission rates are a significant problem especially since reimbursement rates are being more closely tied to outcomes such as rehospitalization. Factors that have been suggested to possibly help maximize surgical outcomes include early surgical intervention, alcohol cessation, retention of duodenal tissue, and concurrent medical therapy^[68,69]. One reason given for poor pain control following surgical therapy is that some patients have altered central pain processing^[70]. Methods are needed to be able to better select those patients who are destined to have a poor response as post-operative pain remains a significant problem.

The most recent Cochrane review of surgical intervention for obstructive chronic pancreatitis showed that early surgical intervention seemed, but was not definitely shown, to have potential benefits as compared to conservative therapy^[59]. More importantly, the review concluded that surgical intervention produced better pain relief scores over a two and five year period (relative effect 1.62, 1.65) with a lower chance of resultant exocrine pancreatic insufficiency compared to endoscopic therapy^[59].

PANCREATIC ENZYMES AND THE NEGATIVE FEEDBACK THEORY

The observation that pancreatic enzyme therapy in some patients with chronic pancreatitis results in a reduction in pain has lead to studies that attempt to understand the phenomenon and achieve more reliable results. The theories of ductal and interstitial hypertension and decreased distensibility of the damaged parenchyma note that pancreatic secretion is associated with a further increase in pressure and likely involved in the pathogenesis of pain. Surgical and endoscopic therapies are primarily aimed at altering pancreatic anatomy to facilitate passage of pancreatic juice. Theoretically, replacing endogenous secretion with exogenous pancreatic enzymes will reduce endogenous secretion in response to meals, blunt the increase in ductal and parenchymal pressure, and reduce pain.

NEGATIVE FEEDBACK INHIBITION OF PANCREATIC SECRETION

The normal human pancreas secretes continuously at a low rate. When food enters the duodenum, the hormones cholecystokinin (CCK) and secretin are secreted to deliver pancreatic enzymes (CCK) and bicarbonate (secretin) into the duodenum^[32,71,72]. While much is known about the initiation of pancreatic enzyme release, less is known about how the process is stopped. However, there is evidence of negative feedback inhibition related to the presence of proteases in the

duodenum. This was first shown in rats by Green and Lyman^[73] and subsequently confirmed by a number of other investigators^[74-77]. Feedback inhibition is known to occur in the rat, chicken^[78] and pig^[79]. In rats, one mediator of secretion is CCK^[75]. For example, diversion of pancreaticobiliary secretions from the duodenal lumen resulted in a threefold increase in pancreaticobiliary protein secretion^[75,80,81]. Pancreatic secretion was also associated with a significant rise of plasma CCK in diverted rats compared to basal levels (16 ± 4 pmol/L from 0.5 ± 0.8 pmol/L respectively)^[75]. More specifically, perfusing the duodenum with pancreaticobiliary secretions or trypsin alone (*via* cannulation near the ampullary site) resulted in a decrease in pancreatic protein secretion and plasma CCK to near basal levels and essentially abolished the stimulatory effect of pancreaticobiliary secretion diversion on pancreatic secretion^[75,77]. An alternate approach was to add a trypsin inhibitor to the pancreaticobiliary secretions to functionally remove trypsin which resulted in an increase in pancreaticobiliary protein output similar to pancreaticobiliary secretion diversion alone^[75]. When the proteinase inhibitor, FOY-305, was given to rats by orogastric tube^[76] there was a 15-fold increase in peak serum CCK levels and an increase in pancreatic protein and enzyme secretion^[76].

In the rat, the negative feedback mechanism appears to be protease-specific as perfusing the duodenum with amylase does not affect protein output^[75,82,83]. The role of CCK was confirmed by showing that the intravenous infusion of the CCK antagonist proglumide before and after pancreaticobiliary secretion diversion reduced protein outputs to near basal levels^[75]. Discontinuation of the proglumide infusion removed the inhibition of pancreatic secretion^[75]. The feedback mechanism appeared localized to the proximal intestine as ileal perfusion of trypsin did not affect pancreatic output^[75]. Subsequent studies have been based on the hypothesis that the presence of trypsin in the duodenum down regulates CCK release resulting in a decrease in pancreatic protein output. The molecular mechanism of the interaction remains unclear. It has been suggested that a protease-sensitive mediator that controls CCK release is present in the duodenal mucosa, or alternatively, is secreted within the pancreatic juice^[27,84,85]. Other data suggest that the feedback loop is not confined to the interactions between trypsin and CCK as neural pathways mediated by acetylcholine also appear to play a role^[75]. For example, the intravenous infusion of acetylcholine, intraarterial infusion of tetrodotoxin, and intraluminal addition of lidocaine all abolished the rise in CCK and pancreatic output in pancreaticobiliary secretion diverted rats^[75,86,87]. The mechanism for the cholinergic pathway remains unclear, but it has been suggested to possibly mediate secretion of the protease-sensitive proteins or be important to their action^[75].

NEGATIVE FEEDBACK - EXPERIMENTAL STUDIES IN HUMANS

Owyang *et al.*^[88] attempted to demonstrate dose-de-

pendent pancreatic enzyme output suppression following intraduodenal infusion of proteases in healthy subjects. Exocrine pancreatic enzyme suppression required a minimum infusion of 0.5 mg/mL of trypsin, with maximal suppression with 1.0 mg/mL. Suppression was not seen with infusions of amylase and lipase. Suppression also correlated with a decline in CCK levels^[88]. Interestingly, while a postprandial increase in plasma CCK was not seen in the presence of duodenal infusions of trypsin, a small increase in pancreatic enzyme secretion was observed. The authors hypothesized this was evidence of a separate pancreatic control mechanism, perhaps cholinergic^[88]. A subsequent investigation examined the possibility of two distinct feedback mechanisms by stimulating duodenal volume and osmoreceptors by infusing normal saline at increasing rates and increasing osmolality^[89]. They noted a dose-related increase in pancreatic output without an effect on plasma CCK levels^[89]. Prior studies in rats had also shown a decrease in pancreatic output with anticholinergic agents, but plasma CCK was also affected^[75]. The effect on pancreatic output was reversed by intraduodenal atropine but not by intraduodenal proteases^[89]. However, the addition of a phenylalanine solution dramatically increased CCK levels and enzyme output. The effect was reduced with the intraduodenal infusion of proteases. The addition of both atropine and proteases completely abolished the pancreatic enzymatic response to intraduodenal phenylalanine^[89].

While negative feedback mechanisms in humans have been clearly demonstrated, not all studies have been consistent^[90], and many studies used super-physiologic amounts of trypsin^[49,91]. The earliest example measured pancreatic secretory output after intraduodenal infusion in a man with carcinoma of the ampulla of Vater which completely blocked biliary and pancreatic secretions from the small intestine^[92]. Pancreatic secretory output was measured *via* a percutaneous transhepatic cholangiography catheter. Intraduodenal infusion of the patient's pancreaticobiliary secretions reduced pancreatic secretions and the effect was reversed by a trypsin inhibitor (soy bean trypsin inhibitor which is relatively trypsin-specific)^[92]. A similar experiment was done after pancreatoduodenectomy with similar results except that the proximal duodenum had been removed suggesting that the site for stimulation extends beyond the periampullary region^[93].

The most detailed study infused an essential amino acid solution into the duodenum and compared pancreatic outputs in patients with differing severity of chronic pancreatitis and healthy controls^[94]. The addition of trypsin, 10 mg/mL, resulted in an approximately 32% decrease in pancreatic secretions in patients with reduced pancreatic output and a 74% decrease in those with normal pancreatic secretion. No inhibition was seen in patients with low pancreatic bicarbonate secretion and steatorrhea^[94]. Chronic pancreatic enzyme therapy was also associated with a 27% decrease in basal pancreatic

secretion and a 46% decrease in amino acid stimulated secretion. A dose-response of trypsin inhibition of exocrine secretion was evaluated in one patient during amino acid infusion. The minimum concentration of trypsin required to inhibit pancreatic exocrine secretion was 0.9 mg/mL and maximum suppression required a trypsin concentration of at least 2.5 mg/mL. Perfusion experiments with amino acids plus trypsin and the relatively trypsin-specific inhibitor, ovomucoid, was still associated with an increase in chymotrypsin secretion. Chymotrypsin (10 mg/mL) also decreased amino acid-stimulated trypsin output whereas protease-free lipase and amylase did not, confirming that only trypsin, chymotrypsin, and pancreaticobiliary secretions suppress pancreatic enzyme secretion in humans. However, the effect was minimal to absent in patients with advanced pancreatic exocrine insufficiency^[94]. In addition, patients with advanced insufficiency did not experience pain relief with enzyme supplementation^[94].

Studies using pancreaticocystostomies following simultaneous kidney and segmental pancreatic transplantations have also demonstrated feedback inhibition^[95,96]. For example, pancreatic exocrine secretions were collected from pancreaticocystostomies after administration of a Lundh test meal orally with or without addition of 6 pancrelipase capsules orally. Total amylase decreased by more than a third, and peak amylase fell 63% with supplemental enzymes. The pancrelipase capsules reduced amylase secretion 16% below basal secretion, and within 1.5 h two of the patients experienced cessation of all graft secretion^[96]. Importantly, inhibition of pancreatic exocrine secretion occurred despite denervation of pancreatic tissue consistent with the presence of a hormonally mediated feedback mechanism.

Overall, the results in humans were consistent with the presence of several distinct feedback pathways, one being under hormonal control mediated by proteases (e.g., trypsin/chymotrypsin)^[31,97-99], and another by neural control mediated by acetylcholine^[89]. However, not all studies have been positive. For example, intrajejunal infusion of normal saline, pancreaticobiliary secretions, and pancreaticobiliary secretions inactivated by heat into normal healthy humans found no significant difference suggesting the absence of a jejunal-pancreatic feedback mechanism^[100]. However, this failure can likely be explained by the inhibitory effect being localized to the duodenum, which was not perfused. Studies that infused an active or an inactivated trypsin inhibitor (aprotinin, which is relatively trypsin-specific) into the duodenum of healthy subjects have also reported no significant difference in pancreatic output between the infusates^[101,102]. However, as shown previously, in humans both trypsin and chymotrypsin are effective in activating the feedback pathway whereas in rodents the effect appears to be more specific to trypsin. Thus, the use of trypsin-specific inhibitors did not reduce the effect of chymotrypsin present.

USING ENZYMES FOR PAIN - CLINICAL TRIALS AND META ANALYSES

The use of pancreatic enzymes in the therapy of gastrointestinal disease has a long history^[103,104]. As noted previously, some, but not all, patients with pancreatic pain respond^[49]. The potential mechanisms include feedback inhibition of pancreatic secretion, improvement in digestion that reduces or eliminates symptoms attributable to malabsorption, or altered nutrient-microbiome interactions. As with any medical treatment, for effectiveness one first looks for the results of randomized placebo-controlled studies with well-matched and well-described patient populations and for head-to-head comparisons of different formulations. With pancreatic enzymes, the search leads to more disappointments than enlightenments. Investigators have generally studied what was readily available to them in terms of products and patients. Ideally, a study of pancreatic enzymes for feedback inhibition of enzyme secretion would utilize formulations that reliably produce high intraduodenal concentrations of trypsin and chymotrypsin. For maldigestion, one would choose a preparation that reliably delivered high concentrations of active lipase into the proximal intestine^[105]. The choice of formulation has been complicated by the recent removal of traditional products and the substitution of products primarily available as enteric-coated enzymes that fail to reliably release their contents in the duodenum^[105].

Despite these problems, it is worthwhile to review the available data which includes several meta-analyses such as one in 1997 by Brown *et al.*^[106] and another in 2010 by Shafiq *et al.*^[107]. Brown *et al.*^[106] included six randomized, placebo-controlled, double blind, prospective studies containing 189 patients with confirmed chronic pancreatitis^[94,108-112]. The primary outcome measure was the percentage of patients preferring enzymes to placebo^[106]. In only one study was there a greater than 50% preference for enzymes as compared to placebo (*i.e.*, 85%)^[108]. Only that result was statistically significant^[108] and the authors concluded that the available studies did not support the hypothesis that pancreatic enzyme supplementation was useful to treat abdominal pain associated with chronic pancreatitis^[106]. However, it is important to note that the pancreatic enzyme products used differed among studies not only in formulation but also in dosage and timing^[106]. The studies also differed in relation of method of diagnosing chronic pancreatitis, length of treatment, and scoring of pain, as well as etiology of pancreatitis, disease severity, and degree of exocrine dysfunction. Two of the studies used non-enteric-coated preparations^[94,108] and four used enteric-coated formulations^[109-112]. The study using non-enteric-coated enzymes was the only study that demonstrated a significant patient preference of enzymes over placebo^[108]. The second meta-analysis set out to address the effect of enzymes on weight loss, steatorrhea, fecal fat, quality of life, and pain in patients

with chronic pancreatitis. They also addressed the role of enteric-coated vs non-enteric-coated formulations and dosage schedules. They specifically reported on the frequency of abdominal pain, duration of pain episodes, intensity of pain, and analgesic use^[107]. Ten studies were included with a total of 361 patients^[94,108-116]. The analysis included five of the six studies in Brown *et al.*^[106] review. Heterogeneity and overall poor data continued to be a hindrance. There were many issues with regard to understanding the effect of pancreatic enzyme supplementation on pain intensity. Although five studies specifically addressed pain, only two studies^[111,112] provided mean pain scores and standard deviations. However, the two studies used different pain scores (*i.e.*, 0 to 5 vs 0 to 3). Mössner *et al.*^[111] reported a nonsignificant improvement of pain with enteric-coated enzymes as compared with placebo (1.26 ± 0.8 vs 1.08 ± 0.8 , respectively). Conversely, Larvin *et al.*^[112] reported a significant improvement with enteric-coated enzymes vs placebo (1.93 ± 1.04 vs 2.05 ± 0.8 , respectively). The remaining 3 studies either did not report standard deviations or reported pain scores differently such as a mean, median, or sum. This mix of results precluded data pooling. Four studies examined the effect of enzymes on analgesic use but did not report standard deviations. Specifically, Isaakson *et al.*^[108] reported a small nonsignificant decrease in analgesic consumption (7.8 tablets with enzymes vs 8.9 with placebo) whereas Halgreen *et al.*^[110] reported a nonsignificant decrease in analgesic consumption scores with enzymes as compared to placebo in patients with steatorrhea (49 vs 58 respectively) and a nonsignificant increase in patients without steatorrhea (57 vs 48). Larvin *et al.*^[112] also reported a nonsignificant decrease in analgesic consumption, reported as mean daily analgesic use with enzymes as compared to placebo (*e.g.*, 45 mg vs 51 mg, respectively). Finally, Malesci *et al.*^[109] also reported a nonsignificant increase in median analgesic consumption score in enzymes vs placebo (12 with range of 0 to 34 vs 0 with range of 0 to 44, respectively). The frequency of abdominal pain and duration of pain episodes were not addressed in any of the included studies^[107]. The meta-analysis also included one study of enzyme dosing schedules on effectiveness in improving malabsorption but not on reducing pain intensity, pain duration, or use of analgesics^[115].

Only one study met the criteria for assessment of quality of life, perhaps an indirect measure of pain control^[107]. The double-blind, two week study used the Clinical Global Impression of Disease Symptoms Scale to evaluate quality of life after only two weeks of enzyme supplementation or placebo^[113]. The use of enzymes resulted in an improvement in quality of life which approached statistical significance ($P = 0.063$)^[113]. The final study in the meta-analysis compared non-enteric-coated with enteric-coated enzymes and focused on changes in steatorrhea^[114]. In that study, patients receiving uncoated enzymes plus cimetidine or uncoated

enzymes alone improved steatorrhea better than those receiving enteric-coated enzymes^[114].

A recent review of clinical trials using enzymes for painful chronic pancreatitis^[117] included three studies not previously discussed in the meta-analyses. One of them, a study by Czako *et al.*^[118], was a multi-center prospective observational study of pancreatic enzyme supplementation on quality of life and abdominal pain in 70 patients divided into supplemental enzyme naïve patients with a new diagnosis of chronic pancreatitis and patients previously diagnosed and treated with oral enzymes. Patients received enteric-coated microspheres with the dosage based on the severity of exocrine insufficiency^[118] along with an H₂ receptor blocker. Thirty-five percent of patients in the new diagnosis group had severe degree pancreatic exocrine insufficiency compared to 64% in the previously diagnosed group. Analgesics were given if requested but the type, dosage, and frequency were not recorded and no control group was included. Outcome was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 modified by adding a disease-specific symptom scale including questions about steatorrhea and abdominal pain^[118]. The duration of the study was 4 wk. Overall, they reported a small but significant increase in mean body weight, decreases in defecations per week, and decreases in mean pain scores in both groups (pain score 47.1 to 35.9 in the mild steatorrhea group and 37.8 to 29.4 in the severe steatorrhea group)^[118] as well as significant increases in global quality of life^[118]. As promising as these results may seem in regards to improving symptoms of pancreatic exocrine insufficiency and relieving abdominal pain, no control group was included and the effect of analgesic use was not reported.

A recent study observed 294 patients with chronic pancreatitis and exocrine pancreatic insufficiency on pancreatic enzyme replacement for a year. The patients were divided into those currently taking enzymes and those with a new diagnosis of exocrine pancreatic insufficiency who were enzyme replacement naïve^[119]. Patients were given daily doses of an enteric-coated mini-microsphere preparation, Creon, and the presence of recurring pain and changes in quality of life were assessed. At the end of the study, a significant portion of patients reported a decrease in recurrent abdominal pain (66.3% with recurrent abdominal pain before treatment vs 34.3% after, $P < 0.001$)^[119]. The percent decrease between cohorts was comparable. Similarly, after 12 mo of treatment, the mean total gastrointestinal quality of life index score improved significantly for the entire patient pool, as well as for individual cohorts^[119]. Physical function and emotion subcategories also improved significantly^[119]. However, despite the impressive results, the lack of a placebo makes it impossible to distinguish between the natural history of the disease and a specific effect of enzyme therapy. Actual dosages were not recorded which made analysis of optimal dosing

impossible. The improvement in recurrent abdominal pain in the group previously treated with pancreatin could be related to improved compliance or a more effective treatment regimen. It would have been interesting to include a group randomized to continuing their previous regimen. Only 31% of the patient population had chronic pancreatitis due to alcohol abuse, which may represent a more difficult to treat group.

In conclusion, the heterogeneity in terms of patient characteristics (*e.g.*, presence, absence and severity of exocrine insufficiency, etiology of pancreatitis, reason for presentation, use of narcotics, formulation, dosage, and administration of enzymes in relation to meals, *etc.*) greatly affects the outcome of studies attempting to evaluate pain relief in chronic pancreatitis. Heterogeneity makes meta-analysis a very blunt instrument for evaluation of the effectiveness of therapy or for helping to decide which therapy is ideal for an individual patient. Clearly some patients respond. Current enteric-coated enzyme products are unlikely to be highly effective either in terms of providing sufficient intraduodenal trypsin activity to engage the feedback mechanism or to fully correct steatorrhea. Future studies should either focus on trying to understand why those patients respond or to carefully select parameters thought to be important, such as providing a critical amount of trypsin or chymotrypsin activity into the duodenum. One can reasonably conclude that patients with exocrine pancreatic insufficiency benefit from correction of malabsorption and the ensuing nutritional deficiencies as well as improvement of gastrointestinal symptoms including pain associated with malabsorption. Reviews of the issues with providing adequate delivery of pancreatic enzymes for treatment of malabsorption are recommended for those wanting additional details regarding use of pancreatic enzymes for malabsorption^[105].

USE OF PANCREATIC ENZYMES IN CHRONIC PANCREATITIS

Administration of exogenous pancreatic enzymes has long been used as an adjuvant to the treatment of patients with pancreatic pain largely based on the premise that replacement of lost enzymes might rest the pancreas. The current rationale is that feedback inhibition of pancreatic secretion reduces CCK release and prevents pancreatic hyperstimulation and pain^[27]. However, achieving this goal requires the ability to provide sufficient active trypsin/chymotrypsin to the proximal intestine. An alternative or complimentary use of enzymes in chronic pancreatitis is to treat overt or occult nutritional deficiencies. For example, low serum magnesium, hemoglobin, albumin, prealbumin, and retinol binding protein levels (a surrogate for fat soluble vitamins) along with a hemoglobin A1C above normal limits are all highly associated with exocrine pancreatic insufficiency^[53]. Specifically, vitamin A (3%), D (53%), E (10%), and K (63%) deficiencies are often present in

patients without clinically apparent malabsorption^[51,120]. The long term use of enzyme therapy for those with enzyme insufficiency is associated with improvements in stool frequency, fecal fat loss, stool consistency, and both clinician and patient assessment of symptoms^[113,121]. However, past and current formulations of pancreatic enzymes are not ideal for achieving feedback inhibition or relief of exocrine pancreatic insufficiency^[105,122].

The majority of currently available supplemental pancreatic enzymes are available as enteric-coated microspheres formulated as capsules or tablets. However, none of these preparations will reliably release their contents within the critical zone of the duodenum-proximal small bowel^[105]. Uncoated enzymes are also available both from pharmaceutical companies and from health food stores^[105,123-125]. Lipase is irreversibly inactivated when the pH falls below 4, whereas proteases are much more pH resistant and are more likely to survive transport through the stomach. However, they can both be destroyed by pepsin. The transplant studies used pancrelipase, specifically enteric-coated Pancrease^[96]. Slaff *et al*^[94] also clearly demonstrated feedback inhibition in 3 chronic pancreatitis patients without steatorrhea by using 30 d of non-coated Viokase, 8 tablets q.i.d. The high dose, currently available non-enteric enzyme, Viokase, (*i.e.*, with 20880 USP units of lipase) contains 78300 USP units of protease/tablet. If all the protease activity was from trypsin (which it is not) each tablet would contain only approximately 3 mg of trypsin. The dose-response experiments in man suggested at least 1 mg/mL was required for feedback inhibition. It would therefore be very unlikely that this minimum level would be achieved *in vivo* using Viokase even if all the protease activity survived transport through the stomach. Acid-stable proteases are available as over the counter medications, but to our knowledge the ability of the drug to initiate feedback regulation of pancreatic secretions or its resistance to acid-pepsin has not been tested in man. One such inexpensive, over the counter product, "Essential Enzymes 500", has been used successfully in irritable bowel syndrome. It contains 12 mg of acid stable proteases/capsule^[124]. Studies are still needed using acid stable proteases for their ability to initiate feedback inhibition of pancreatic secretion.

GENERAL RECOMMENDATIONS FOR ENZYME USE AND TREATING CHRONIC PANCREATIC PAIN

Unfortunately, there are very little long-term data exploring the efficacy of treating chronic pancreatic pain with enzyme supplementation. One recent study included daily treatment with enteric-coated pancreatin for one year and noted a significantly positive impact on pain, quality of life, and emotional and physical well-being in both chronically treated and treatment naïve patients^[119]. No placebo group was included which is important considering the high placebo effect reported in prior studies^[32,109-111]. Another

recent study compared pancreatin alone, pancreatin plus a proton pump inhibitor, or pancreatin plus a proton pump inhibitor and the NSAID aceclofenac^[126]. All three regimens produced significant improvements in pain compared to no pretreatment, but the lack of a placebo questions whether the effect was due to the enzymes.

Evaluation of a new patient with suspected chronic pancreatitis requires careful consideration of multiple factors and includes a search for potentially correctable conditions (Table 3). One must also be aware of the possibility of an occult malignancy. It is important to attempt to identify and treat any nutritional deficiencies present and to strongly discourage alcohol use and smoking. This review focuses on pancreatic pain, a condition where treatment typically requires a variety of expertise often including experts in pain management. Severe pain will likely require narcotics which may eventuate in narcotic addiction. One should try to use non-narcotic drugs whenever possible (*e.g.*, nonsteroidal anti-inflammatory drugs, acetaminophen, and tramadol) and avoid opiates with a higher predilection to abuse such as *Dilaudid* (hydromorphone) and oxycodone.

Until recently the mainstay of chronic pancreatitis pain management has been opioid-based. However, as the risks of long-term opioid therapy have crystalized, clinicians are increasingly looking for alternatives. Prescription opioid overdoses have quadrupled in the last 15 years, and deaths from drug overdose are now more common than automobile collision fatalities^[127]. In an effort to educate healthcare providers and curb these growing statistics, the United States Center for Disease Control issued a statement in March 2016 with guidelines regarding the prescription of opioids^[127], which includes recommendations about the preferred use of non-opioid pharmacologic and non-pharmacologic therapy. They also recommend consistent reevaluation of risks and benefits of opioid therapy, using the lowest effective dosage, the avoidance of extended-release tablets and a warning against the use of opioids with benzodiazepine therapy. Clinicians providing care to chronic pancreatitis patients with high levels of pain may find these guidelines helpful in their efforts to help with pain control.

Opioid therapy can be quite effective for the short-term management of acute pain, but the long-term benefits of opioid therapy are murky as the majority of studies are of short duration^[127]. Long-term comparative studies are rare and often show those who receive opioid therapy to have poorer function, are less likely to return to work, and are less likely to have good pain control^[128,129]. Opioid therapy also affects smooth muscle tone leading to gastrointestinal motility disturbances and abdominal pain^[130-134]. While morphine is effective in reducing pain in chronic pancreatitis, a double-blinded comparison with tramadol reported that patients with chronic pancreatitis preferred tramadol to morphine for anesthesia^[130]. In addition, tramadol does not increase smooth muscle tone in the sphincter of Oddi^[130].

The first choice for chronic pain should likely be non-steroidal anti-inflammatory drugs (NSAIDs) and/

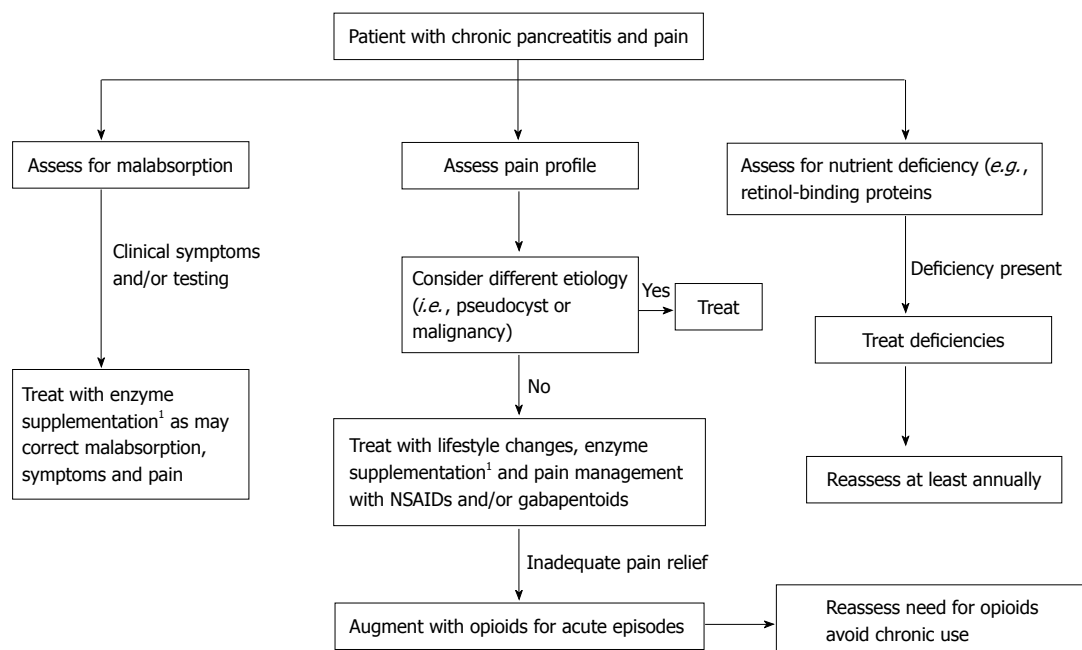


Figure 1 Flow chart demonstrating recommendations for using pancreatic enzyme replacement therapy in a patient with abdominal pain and chronic pancreatitis. ¹Start with non-enteric coated products such as Viokace along with a PPI. The figure suggests approaching the patient with a three-pronged method. First, one should assess the patient's pain profile and investigate whether the pain is from chronic pancreatitis alone or from other etiologies, *i.e.*, a developing pseudocyst or malignancy. Next, pain control should be attempted first with conservative measures such as lifestyle changes, enzyme supplementation, NSAIDs, and/or gabapentoids before moving to treat with opioids. If opioids are deemed appropriate for pain control, the decision should be consistently reassessed as to avoid dependency and addiction. Second, one should assess the patient for malabsorption, and if present, the patient should be treated with exogenous enzymes as that may improve absorption and pain symptoms. Lastly, the physician should assess the patient's nutritional status and correct deficiencies, if present. A non-enteric-coated enzyme such as Viokace along with a proton pump inhibitor is recommend for first-line enzymatic treatment. Alternatively, can use combination of non-enteric-coated and enteric-coated formulations. NSAIDs: Nonsteroidal antiinflammatory drugs; PPI: Proton pump inhibitors.

or gabapentoids to treat neuropathic pain. Generally, NSAIDs are used for analgesia and full anti-inflammatory doses are neither required nor indicated. Primarily analgesic NSAIDs include low dose naproxen, ibuprofen, nabumetome and etodolac^[135]. Higher doses typically do not increase analgesia but increase risk of side effects. Co-therapy with a proton pump inhibitor should be considered. Gabapentoids such as pregabalin are often used as adjuvant therapy due to possible similarities between chronic pancreatic pain and neuropathic pain^[136]. A study of pregabalin enrolled patients who were concurrently undergoing opioid therapy and reported success suggesting a role for pregabalin in chronic pancreatitis pain^[136]. However, none of these approaches are without accompanying side effects and long-term studies are needed.

The natural history of pain in any particular patient is impossible to predict^[23]. In general, those with constant pain have a worse prognosis than those with intermittent pain^[24]. While pancreatic enzyme therapy is a mainstay in the therapy of exocrine pancreatic insufficiency, it can also be used in an attempt to produce feedback inhibition of enzyme secretion although this is likely only useful for those who retain exocrine function.

Prior studies have suggested that feedback inhibition was only effective in those without steatorrhea^[94]. Indeed, longer term studies in pancreatic pain have confirmed that those with pain and pancreatic insufficiency generally are

the most difficult to treat^[17,94]. However, a reduction in malabsorption can also lead to reduced symptoms^[47-49]. We recommend that all patients with chronic pancreatitis should be screened for nutritional deficiencies which includes measuring serum magnesium, hemoglobin, albumin, prealbumin, retinol binding protein levels, hemoglobin A1C, and body mass index. For those with low retinal binding protein, one should consider that fat-soluble vitamin deficiencies are likely present. For initial therapy for patients with pancreatic pain, we recommend that the focus be on correcting nutritional deficiencies and malabsorption. Treatment of fat malabsorption requires at least 20000-30000 USP units of lipase/meal^[105]. One might start with the non-enteric-coated 10000 lipase unit Viokace formulation (*i.e.*, one tablet at the beginning of the meal or just before the meal, and 2 or 3 more tablets spread throughout the meal plus one per snack). The use of a proton pump inhibitor is recommended, possibly as a double dose such as 40 mg of esomeprazole twice a day, to reduce destruction of lipase during transit through the stomach. Potassium competing acid blockers should simplify therapy when they become available in that they provide reliable pH control. Alternatively, one could use a combination of non-enteric-coated and enteric-coated formulations^[105]. Improved nutritional status should be assessed at least once a year and include measuring serum magnesium, hemoglobin, albumin, prealbumin, retinol binding protein levels (a surrogate for fat soluble

vitamins), and body mass index (Figure 1).

However, enzyme therapy is not without its pitfalls as properly mimicking the normal physiology of nutrient digestion and absorption is difficult^[47,105]. Enteric-coated enzymes must mix properly in the stomach, not separate from the meal, and dissolve and remain active in the duodenum as to allow proper metabolism, digestion, and absorption for the completion of the feedback loop and resting of the pancreas. With current formulations, delivery of sufficient protease to the duodenum is impossible with enteric-coated products and difficult with non-enteric-coated ones.

CONCLUSION

Whether pancreatic enzyme administration for chronic pancreatic pain is effective remains a debated subject. Although reviews on the topic suggest there is little evidence for benefit, the conclusions are based on a potpourri of studies which vary dramatically in design and execution. Enzyme preparations also differ greatly in size, composition, and action but are generally treated as equal despite a lack of information and formal studies. A majority of experiments have used only enteric-coated preparations which have been shown to separate from meals and dissolve distal to the duodenum. Until formal studies including head to head comparisons are performed and characteristics such as mixing and dispersion properties are known, we are left without the crucial information needed. Uncoated enzymes, while largely being replaced by more modern preparations, have shown promise in treating pain and need to be explored further. Use of acid resistant proteases are needed as well as better strategies to overcome the gastric and intestinal pH barriers to maximize proper enzyme delivery to the duodenum for both uncoated and enteric-coated preparations. Future studies evaluating the use of enzymes with concurrent antacid and/or anti-secretory therapies, especially with potassium competing acid blockers, are needed. Furthermore, clinical trials will ideally include long-term treatment arms and large treatment groups to allow for more reliable data gathering. Patients should also be subcategorized based on etiology and severity in order to specifically study response to treatment. Most importantly, the complexity of data gathered here should serve to help individualize enzyme replacement therapy. For example, clinical trials could be done to confirm suggestions that patients with idiopathic chronic pancreatitis and mild to moderate exocrine insufficiency respond better to enzyme therapy than those of alcoholic origin and severe exocrine impairments. Pain scores must be standardized and validated. Exogenous enzyme therapy may decrease secretion, is noninvasive, has relatively no adverse effects, and improves malabsorption in those with exocrine insufficiency. There is little to be lost and potentially much to be gained by trying enzyme therapy, but more studies are needed before they can be used in evidence-based medicine.

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What is the best way to manage screening for infections and vaccination of inflammatory bowel disease patients?

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increased risk of opportunistic infections, in particular of viral or bacterial etiology. Despite the existence of international guidelines, many gastroenterologists have not adopted routine screening and vaccination in those patients with IBD, which are candidate for biologic therapy. Available strategies to screen, diagnose and prevent bacterial and viral infections in patients with IBD prior to start biological therapy are discussed in this review.

Key words: Inflammatory bowel disease; Opportunistic infections; Immunomodulators; Corticosteroids; Anti-tumor necrosis factor agents

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Core tip: The increasing use of biologics as a mainstay of therapy in inflammatory bowel disease (IBD) is associated with an increased risk for a variety of infections, many of which are preventable by prior screening and vaccination. While immunocompetent IBD patients can be vaccinated using standard vaccination schedule, special guidelines need to be followed for IBD patients getting immunosuppressive therapy (IST). This article provides a review of the issues surrounding immunizations in the IBD patient and a practical guide for clinicians regarding the appropriate screening for infections and vaccinations to administer both before and during IST.

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Abstract

The use of biological agents and immunomodulators for inflammatory bowel disease (IBD) is associated with an

INTRODUCTION

Biological agents have represented a breakthrough in the therapy of inflammatory bowel disease (IBD) in the last

20 years: Tumor necrosis factor alpha inhibitors (anti-TNF) and other monoclonal antibodies targeting interleukin 12 (IL-12), IL-23, and cellular adhesion molecule ligands $\alpha 4$ integrin and $\alpha 4\beta 7$ integrin. The European Crohn's and Colitis Foundation (ECCO) outlines that IBD patients treated with corticosteroids (prednisone 20 mg/d equivalent for 2 wk or more), immunomodulators (6-mercaptopurine, Azathioprine, Methotrexate), and biological agents should be considered immunocompromised and at risk for opportunistic infections^[1]. This has been confirmed by several studies, highlighting the increased incidence of severe infections in patients with IBD on biologics^[2,3]. A pivotal study in the field^[4] has evaluated the independent predisposing factors to severe infections with a case-control designed study. The results underlined how immunosuppressive therapy (steroids, thiopurines, and anti-TNF) were associated with an increased risk of severe infections (OR: 2.9, 95%CI: 1.5-5), and that the risk was greatly increased when two or more drugs were combined (OR: 14.5, 95%CI: 4.9-43). The TREAT Registry (Crohn's Therapy, Resource, Evaluation, and Assessment Tool) has individuated prednisone, infliximab, disease activity (moderate to severe), and narcotic analgesic treatment as independent factors associated with serious infections^[3]. A recent Cochrane review, with a meta-analysis of randomized controlled trials, controlled clinical trials, and open-label extension studies of biologics for several indications, reported an OR of 1.28 (95%CI: 1.09-1.50) for serious infections for patients on any biologic^[5]. However, a subgroup analysis of patients included in IBD trials did not show a significantly increased risk of infection (OR: 1.28, 95%CI: 0.67-2.44)^[4]. In this review, we aim to outline the most relevant opportunistic infections in IBD with focus on the discussion of the screening and prevention strategies through vaccination or chemoprophylaxis in IBD patients prior to start biological therapy.

BACTERIAL DISEASES

Mycobacterium tuberculosis

The worldwide incidence of tuberculosis (TB) has been estimated by the World Health Organization in 9.6 million cases with 1.5 million deaths in 2014^[6]. The risk of reactivation of latent TB (LTB) is 5-fold increased in the first 52 wk. after initiation of anti-TNF therapy^[7-9].

TNF has a central role in the immune response to *Mycobacterium tuberculosis*. It is fundamental for macrophage activation and in the formation and maintenance of granuloma where mycobacteria are sequestered^[7]. This is a main reason why therapy with anti-TNF agents can reactivate latent TB. Generally these cases are extra pulmonary or disseminated TB^[10]. The American College of Gastroenterology and the American Gastroenterological Association, as well as the ECCO recommend screening for LTB before starting biological therapy^[11-14]. The most commonly employed screening tests are tuberculin skin test (TST), QuantiFERON TB-Gold (QFT-G) and chest radiography. *In vitro* assays

based on interferon-gamma release (IGRA), such as the QFT-G and T-SPOT.TB, have been recently claimed to be more specific and sensitive than TST, particularly in the previously vaccinated and immunosuppressed population^[9,15]. IGRAs employ antigens specific for *Mycobacterium tuberculosis*, not cross-reactive with Bacillus Calmette-Guérin (BCG). A meta-analysis^[16] has calculated the specificity of QFT-G and TST for LTB screening. In subjects who had not been vaccinated with BCG, the specificity of QFT-G was 99% (95%CI: 98%-100%) and that of TST was 97% (95%CI: 95%-99%). However, in subjects vaccinated with BCG, the specificity of QFT-G was 96% (95%CI: 94%-98%) while that of TST was only 59% (95%CI: 46%-73%). A Swiss study has compared TST and QFT-G performances in IBD patients^[17]. The studied population comprised 114 patients with Crohn's disease (CD), 44 with ulcerative colitis (UC), 10 with indeterminate colitis and 44 control subjects. In this study the prevalence of BCG vaccination was 71%, while 81% of the IBD patients were treated with immunosuppressive therapy (IST). Less patients treated with IST were TST positive compared to those not treated with IST (14% vs 34%, $P = 0.007$), while no difference was evident for the interferon-based test QFT-G (9% vs 6%). The correlation of TST and QFT-G in IBD patients was negative in this study ($k = -0.0297$, -0.0314 in vaccinated and -0.0538 in non-vaccinated patients). However the two tests showed a better agreement in control subjects ($k = 0.13$), and particularly in non-vaccinated controls ($k = 0.62$).

These results were confirmed by a study by Andrisani *et al.*^[18] performed on 92 Italian IBD patients who underwent infectious disease screening before starting therapy with anti-TNF (only one of them was vaccinated with BCG). A discordant result between QFT-G and TST was found in 10.8% IBD patients ($k = 0.508$). Patients treated with IST had higher degree of disagreement (14.3%, $k = 0.39$), while the patients not treated with IST had a 100% conformity of the two tests. A systematic review and meta-analysis has evaluated the findings of IGRA tests^[19] in IBD patients. In the nine selected studies, different results were found for the agreement between skin test and the different IGRAs. TST and QFT-TB Gold/QFT-TB Gold In-Tube had a rate of uniformity of 85% (95%CI: 77-90), while the conformity of TST and T-SPOT.TB was 72% (95%CI: 64-78). A relevant problem in interpreting these results is the occurrence of indeterminate test. In this meta-analysis it was 5% (95%CI: 2-9) for all QFT-tests. IST therapy affected both QFT-G scores (OR: 0.37, 95%CI: 0.16-0.87) and TST outcomes (OR: 0.28, 95%CI: 0.10-0.80) in these studies ($P = 0.02$). Patients with LTB infection should be treated with a 9 mo. course of isoniazid. This prophylaxis should preferably be conducted in strict cooperation with infectious disease specialists and/or pneumologists. The usual isoniazid protocol is generally well tolerated. Although IBD patients may already be on pharmacological treatment, there is no evidence of an increased risk of liver toxicity related

to isoniazid^[20]. Even if not formally assessed in clinical studies, there is general agreement that a minimum of 2 mo should be waited after start of chemoprophylaxis for LTb before anti-TNF therapy is initiated^[7,15], if the clinical condition of the patient allow this delay. However, chemoprophylaxis does not guarantee that LTb will not reactivate during anti-TNF therapy: A reactivation rate of 19% has been described in a retrospective study, indicating that routine TB surveillance during and after anti-TNF drugs treatment must be performed^[21].

Clostridium difficile

Clostridium difficile infection (CDI) manifests with laboratory signs and symptoms that may be confused with a relapse of inflammatory activity in an IBD patient^[22]. For this reason, it is mandatory to perform specific diagnostic tests for CDI in IBD relapses characterized by profuse diarrhea, with or without the presence of blood, by signs of dehydration and leukocytosis. The most common tests employed for CDI diagnosis are enzyme-linked immunosorbent assay (ELISA) for toxin A and B^[23] and polymerase chain reaction (PCR) assays (which have greater specificity and sensitivity). Although toxigenic culture can be considered as the "gold standard" technique for this diagnosis, it is infrequently performed^[23]. According to the Infectious Disease Society of America (IDSA), a 2-step method should be used. As a first step, an ELISA for the *Clostridium difficile* common antigen, glutamate dehydrogenase is performed. If positive, the presence of pathogenic strains can be confirmed by other techniques as cell cytotoxicity assay or toxigenic culture^[24]. Treatment includes initially oral metronidazole and oral vancomycin, or in severe cases simultaneous administration of intravenous metronidazole and oral vancomycin^[25]. Fidaxomicin has been recently approved for CDI^[26,27]. In recent years, a innovative methodology has demonstrated its efficacy for treatment of recurrent CDI: Fecal microbiota transplantation^[28]. Although the donor selection criteria and the optimal condition for fecal instillation are still not clearly defined, the method is widely and successfully employed^[29]. FMT has been employed also for IBD patients with CDI in a recent study^[30] using standardized frozen preparation, showing efficacy in treating the infection.

Streptococcus pneumoniae

Pneumococcus may cause, besides lung infection, also invasive disease as bacteremia and meningitis. Immunocompromised hosts are at risk for these complications, and cases have been described in IBD patients treated with infliximab^[31]. Vaccination is recommended for prevention of pneumococcal infections in special at risk populations. The main risk categories applicable to IBD patients are age 65 years and older, smoking and use of immunosuppressive agents. Two vaccines have been approved against pneumococcal infections: A 23-valent-polysaccharide vaccine (PPSV23) and a 13-valent conjugate vaccines (PCV13). The coverage

of the two vaccines is only partly overlapping. The Advisory Committee on Immunization Practices (ACIP) guideline have been released with differential indications for different age and disease groups. In particular, ACIP suggests the following vaccination scheme for immunocompromised adults aged 19 years or older: If naïve to pneumococcal vaccine, they should receive first PCV13 and, at least 8 wk later, a shot of PPSV23^[32]. Those subjects who had previously been vaccinated with PPSV23 should receive, at least one year later, an injection of PCV13^[33]. While data concerning the need for revaccination with conjugated vaccine are scant, PPSV23 revaccination after 5 years is recommended for immunocompromised patients^[34]. However, the response to *Streptococcus pneumoniae* vaccinations may be impaired in IBD patients treated with immunomodulators, particularly when they are used in combination^[35,36]. For this reason, it would be advisable to perform vaccinations for pneumococcal infections before starting immunosuppressive drugs. Pneumococcal infections can usually be diagnosed by cultures or by search for urine antigens of *Streptococcus pneumoniae*. While pneumonia is generally treated with success with fluorquinolones, treatment of meningitis should rely on isolation of the organism and *in vitro* susceptibility testing^[37].

VIRAL DISEASES

Hepatitis B virus

The prevalence of hepatitis B virus (HBV) in patients with IBD is similar to that of the general population^[38]. The risk for hepatitis B reactivation has been clarified in a multicenter study^[38] of 2076 Spanish IBD patients. This study has shown a lower prevalence of HBV antigens and/or antibodies than previously reported, and not different from control population. The HBV surface antigen (HBsAg) was present in no more than 1% of IBD patients, while the positivity rates for anti antibodies against the HBV core antigen (HBcAb) were 7.1% for CD and 8% for UC. A French study^[39] showed similar results, with a prevalence of HBcAb of 2.78% in CD patients and of 1.59% in UC patients, not different from those detected in the control unselected population. Other studies^[40-42] have shown in IBD patients a higher prevalence of HBV infection. Two Italian studies have reported somehow different results: Biancone *et al*^[41] described a higher prevalence of HBcAb in CD and UC patients (10.9% and 11.5%, respectively), when compared to controls (5.1%, $P < 0.02$). Papa *et al*^[43] reported that only one patient out of 301 (0.3%) was an HBsAg carrier, while 22 (7.3%) were anti-HBc positive.

TNF- α is important in regulating hepatitis B replication^[44] and cases of reactivation of the virus under TNF inhibitors have been published^[45,46]. All IBD patients should be tested for HBV infection (HBsAg, anti-HBs, anti-HBc) to assess infection or vaccination status. It is important to check also for anti core antibodies, as

they could represent the only positive test in particular situations, such as the case of immunosuppressed patients or hepatitis C virus (HCV)/human immunodeficiency virus (HIV) co-infections^[47]. However, a low rate of false positivity has been described. In patients that show positive findings of HBV infection, the search of HBeAg, anti HBe, and HBV DNA should also be performed.

Cases of reactivation on Infliximab therapy have been described not only in hepatitis B surface antigen (HBsAg)-positive patients but also in HBsAg-negative/anti-HBc (hepatitis B core antigen)-positive patients^[48]. Hepatitis B reactivation is associated with significant morbidity and mortality due to hepatic failure^[49].

During anti-TNF therapy, "occult" HBV carriers (those who are anti-HBc+), need a frequent check of tests of liver function and of HBV markers: The appearance of HBV-DNA or HBsAg positivity indicates reactivation of the infection^[1]. In chronic HBsAg-positive carriers, antiviral prophylaxis is recommended before administering immunosuppressive agents. If IST is anticipated to be conducted for a period of more than one year (as frequently happens in IBD), prophylaxis of HBV reactivation should be performed with nucleotide/nucleoside analogues rather than with lamivudine due to the lower incidence of mutations that generate resistance to the drug^[1]. The American Association for the Study of Liver Diseases (AASLD)^[50] and the European Association for the Study of the Liver recommend the early introduction of nucleoside/nucleotide analogues (NAs) for all HBsAg-positive patients requiring IST. Prophylaxis of HBV reactivation must be started at least one week before IST and it should last for 6 mo to 1 year after its accomplishment, because the reactivation of HBV may happen even after immunosuppression is withdrawn^[50,51].

Patients with high levels of HBV DNA (> 2000 IU/mL) at baseline should carry on the antiviral therapy until the same end points as for non-immunosuppressed patients are reached.

All seronegative (negative or low-titer HBsAb) patients should be vaccinated at diagnosis; however, this occurs in less than half of the patients^[52]. It is safe to administer the standard vaccination protocol to patients with IBD on immunosuppressive medications, but the response may be significantly reduced, and an intensified vaccination protocol may be required. Post-vaccination HBsAb titers should be monitored, and, if non-protective (< 10 mU/mL), a booster dose or revaccination should be administered^[47]. HBV vaccination seems not to be very common in IBD patients, according to four studies exploring the topic. Positive anti-HBs and negative HBcAb, as indication of efficacious vaccination was detected in only 12%, 48.9%, 24% and 21.7% of the four patients cohorts from Spain, Italy, France and China, respectively^[38,43,53,54]. Vaccination programs are significantly different across Europe for what concerns period of initiation of the programs age and target population (newborns, adolescent and pre-adolescent

subjects, only for high-risk groups, etc.)^[55]. For these reasons, it is recommended to determine of the infectious or vaccination status at the time of the first diagnosis of IBD. If possible, seronegative subjects (HBsAg, HBcAb and HBsAb negative) should be vaccinated as soon as possible in order to reduce future problems in management.

HCV

The prevalence of HCV in patients with IBD is similar to that of the general population^[38]. There are no data to suggest that biologics are associated with reactivation or exacerbation of the course of HCV^[56,57]. Anti-TNF medications are generally considered safe in patients with HCV^[43,58]. The prevalence of HCV infections in IBD patients has been recently evaluated in studies performed in Italy^[41,43], France^[53], Spain^[38], and China^[54]. From these reports, the prevalence of hepatitis C infection in IBD patients seems to be not different from the general population. Biancone *et al*^[41] reported that the prevalence of anti-HCV antibody positive individuals was 7.4% in CD patients, 0.6% in UC patients and 5.1% in the controls. The ECCO guideline^[1] suggest to perform HCV screening before starting treatment with immunosuppressive drugs for IBD, although the positivity of HCV testing is not a contra-indication for IST. Testing should be performed by search for anti-HCV antibodies and, if antibodies are positive, by HCV-RNA. In case of positivity, these tests should be repeated periodically during immunosuppressive treatment. Prophylactic treatment is currently not available to prevent reactivation of HCV infection. Interferon, which was the milestone of antiviral therapy for HCV until the advent of direct-acting antiviral agents, is contraindicated in IBD forms that require IST.

Cytomegalovirus

Cytomegalovirus (CMV) infection or reactivation can occur in patients with immunosuppressive conditions. CMV may produce retinitis, pneumonia, encephalitis, and other invasive infections^[59]. A number of studies have described an association between severe steroid-refractory IBD and CMV infection^[60,61]. Colonic CMV disease was observed in steroid-refractory UC (active), with a prevalence of 32%^[60] in a prospective case-control report. There is no CMV vaccine available. Histopathology combined with immunohistochemistry (IHC) is specific and sensitive for detecting CMV infection in tissue or biopsies. PCR for CMV DNA is commonly employed both in blood and in biopsies to confirm the diagnosis. Screening for CMV infection is not necessary before starting immunomodulator therapy^[14]. When CMV is detected in the intestinal mucosa of patients with severe steroid-resistant colitis treated with immunomodulators therapy, antiviral therapy should be initiated. The discontinuation of immunomodulators should be considered until symptoms of colitis ameliorate or in case of systemic CMV disease.

Varicella zoster virus

Varicella zoster virus (VZV) can be associated with a significant morbidity and mortality in immunocompromised patients. VZV is an herpes viruses that persists after acute infection in a latent state in autonomic ganglia, dorsal nerve roots, and cranial nerves^[62]. Later in life it can reactivate as zoster. In addition to clinical signs, that are generally typical, PCR for VZV or fluorescence testing can be performed on biological material such as vesicular fluid, sputum, and cerebrospinal fluid. A four-fold or greater rise in VZV antibody titer in acute and late serum specimens is diagnostic of VZV infection^[63]. The increased risk of VZV reactivation is not specific only to biologics. In a recent large cohort study^[64] including more than 33000 patients treated with anti-TNF medications and 27000 control individuals treated with non-biological anti-inflammatory medications for various indications (3850 patients with IBD), the risk of herpes zoster was similar in patients with IBD treated with anti-TNF agents and with thiopurines.

VZV-related complications can be easily prevented by vaccination. However, live vaccine for varicella must not be administered to patients on immunosuppressive therapies^[65], including azathioprine, methotrexate, 6-mercaptopurine, and infliximab^[66]. In this regard, it should be noted that Lu *et al*^[67] have described good tolerance for VZV vaccine in six patients with IBD receiving immunosuppressive drugs (6-MP or infliximab). Prospective studies are needed to delineate the risks and benefits of live varicella vaccine in patients with IBD. Probably, the better behavior should be to test for VZV patients as early as possible after diagnosis and to vaccinate those previously unexposed before prescribing immunosuppressive treatments. Recently, the use of a zoster vaccine has been suggested for patients who are VZV positive and at risk of developing herpes zoster (*e.g.*, the elderly). Currently, guidelines suggest a lag time before the varicella and zoster vaccine and the start of immunosuppression of 14 d to 1 mo^[68,69]. The vaccine should not be administered for at least 1 mo. after the cessation of immunosuppression^[68,69]. A study of zoster vaccine given to patients on biologics has detected, however, no association with short-term increase in herpes zoster incidence. In the meantime, it was associated with a lower herpes zoster incidence at a follow-up of two years (6.7 vs 11.6 cases per 1000 person-years; $P < 0.001$)^[70]. For those patients with IBD which are VZV seronegative and treated with immunosuppressive drugs, who experience exposure to subjects with active VZV infection, passive immunization with high-dose VZV IgG^[69] should be considered.

HIV

All IBD patients undergoing IST should receive testing for HIV infection (by search of HIV p24 antigen and antibody, and, if acute infection is suspected, by PCR) to exclude unidentified infection. This should be done in order to avoid possible adverse outcomes of immunosuppressive

drugs in HIV infected subjects^[1]. Several case series and case reports describing patients who are infected with HIV and were treated with anti-TNF medications for various indications have been published and all the patients who were submitted to therapy had a satisfactory CD4 cells count, no co-infection, and low HIV viral load^[71]. However, because there are limited data on the effect of treatment with HAART on the course of concomitant HIV and IBD, no recommendations are available^[1]. Nevertheless, HIV infection is not to be considered a contra-indication to anti-TNF therapy.

Human papillomavirus

Human papillomavirus (HPV) is a sexually transmitted infection. It is a common infection and is the causative agent for cervical cancer and premalignant conditions^[72,73]. The American College of Obstetricians and Gynecologists guideline requires to initiate screening for cervical cancer at 21 years of age, independently of the age of beginning of sexual activity^[74]. There are some studies that have suggested how women with IBD could have a higher incidence of cervical dysplasia^[75,76]. There is an increased incidence of HPV-associated warts or condylomata in patients taking immunosuppressants; however, no data suggesting a specific association with biologics are available^[77]. Women affected by IBD should have cervical smears and HPV vaccination according to the general population guidelines^[74]. The available vaccine is quadrivalent, and it is given as three doses during a period of 6 mo. The vaccine is indicated for women of the age of 9 to 26 years, both before and after initiation of sexual activity^[75]. HPV vaccine is also recommended for young males, with vaccination at the age of 11 to 12 years, and catch-up for those aged 13 to 21 years. However, vaccination policies are diverse in different countries. Therapy of eventual abnormal findings at cervical smears includes colposcopic examination, and surgical excision.

Herpes simplex virus

In immunocompromised patients, herpes simplex virus (HSV) infection may cause severe disseminate infection of different organs (including encephalitis, meningitis, pneumonia, gastrointestinal infection, and hepatitis)^[78,79]. Diagnosis of HSV infection is generally suspected based on clinical findings. It can be confirmed by cytology, by PCR, and by search for specific circulating immunoglobulin G (IgG) and IgM. IBD guidelines from the ECCO dissuade to start IST during when an HSV infection is ongoing^[1]. Only those immunosuppressed patients who manifest recurrent infection from HSV type 1 or 2 should receive specific chemoprophylaxis^[80].

Epstein-Barr virus

Epstein-Barr virus (EBV) is a common B-cell lymphotropic gamma-herpes virus infection in humans. Most of the severe EBV diseases, as hemophagocytic lymphohistiocytosis, occur when primary infection happens in immunosuppressed patients; for this reason it is

Table 1 Screening and vaccinations for inflammatory bowel disease patients prior to start immunosuppressive including anti-tumor necrosis factor therapy

Infection	Tests	Recommended screening	Vaccine
TB	LTB should be tested by a combination of patient history, chest X-ray, TST and QFT-G	Yes	Always contraindicated during immunosuppressive therapy and in children exposed in utero to anti-TNF, up to 6 mo of age, like any other live vaccine
<i>Clostridium difficile</i>	Enzyme immunoassay Against toxin A and B and PCR assays	Not necessary	Not available
<i>S. Pneumonia</i>	Culture of relevant clinical samples (blood, CSF, good respiratory sample), urine	Not necessary	Yes
HBV	Blood test for HBsAg, anti-HBsAb and HBcAb to determine HBV status. In patients with positive HBsAg, viremia HBV-DNA should also be quantified	Yes	Recommended standard or double dose schedule
HCV	HCV serology	Yes	Not available
CMV	CMV serology	No	Not available
HIV	Blood test for HIV serology	Yes	Not available
VZV	VZV serology	Yes	Vaccine available, vaccinate before starting immune suppressants
HPV	Cervical cytology	Yes	Recommended
HSV	HSV serology	Not necessary	Not available
EBV	EBV serology	Advisable	Not available
Influenza virus	clinical signs and laboratory evaluation	Not necessary	Recommended

TNF: Tumor necrosis factor; TB: Tuberculosis; LTB: Latent tuberculosis; TST: Tuberculin skin test; QFT-G: Quanti FERON TB-Gold; PCR: Polymerase chain reaction; CSF: Cerebrospinal fluid; HBV: Hepatitis B virus; HCV: Hepatitis C virus; CMV: Cytomegalovirus; HIV: Human immunodeficiency virus; VZV: Varicella zoster virus; HPV: Human papillomavirus; HSV: Herpes simplex virus; EBV: Epstein-Barr virus.

Table 2 Vaccination of inflammatory bowel disease patients on immunosuppressive therapy

Vaccine	Dose	Safety
Inactivated vaccines		
HAV	2 doses	Yes
HBV	3 doses	Yes
HAV and HBV	3 doses	Yes
HPV	3 doses	Yes
Influenza (trivalent)	Annually	Yes
Meningococcal	≥ 1 dose	Yes
Pneumococcal	1 dose and 1 booster in 5 yr	yes
Tetanus and diphtheria	Every 10 yr	Yes
Live attenuated vaccines		
BCG	1 dose	Contraindicated
MMR	1 or 2 doses	Contraindicated
Varicella	2 doses	Contraindicated
Zoster	1 doses	Contraindicated

HAV: Hepatitis A virus; HBV: Hepatitis B virus; HPV: Human papillomavirus; BCG: Bacillus Calmette Guérin; MMR: Measles, mumps, and rubella.

advisable to test IBD patients for EBV serology before start biological or immunosuppressive therapy^[81]. EBV-associated lymphomas have been described in patients with CD treated with 6-MP or azathioprine^[82,83]. An observational cohort study was conducted in France, the CESAME (Cancers et Surrisque Associe aux Maladies inflammatoires intestinales En France) study. In this IBD cohort the incidence of lymphoproliferative diseases was evaluated according to the treatment with thiopurines during a period of 3 years. This research described how

the risk of lymphoproliferative diseases is increased in thiopurine users with a hazard ratio of 5.28 (95%CI: 2.01-13.9, $P = 0.001$)^[84]. Two types of thiopurine-induced lymphoma in IBD are EBV-related: the post-transplant-like lymphoma that develops in adult patients seropositive for EBV and a fatal early post-mononucleosis lymphoproliferation that may develop in young men (< 35 years) seronegative for EBV^[85,86]. While antiviral drugs have no beneficial effect on EBV-induced B-cell proliferation, rituximab is the drug of choice for treating established B-cell lymphoma^[87]. Screening for EBV infection before initiation of immunomodulator therapy should be considered. Anti-TNF monotherapy could be used in preference to thiopurines in EBV seronegative patients at the clinician's discretion^[61]. No EBV vaccine is available.

Influenza virus

Influenza viruses A and B cause seasonal epidemics. In healthy subjects who are immunocompetent, influenza usually behaves as an acute, self-limiting illness of upper respiratory tract. Patients on IST, including patients with IBD on IST, are considered to be at high risk for complications: Viral and bacterial pneumonia, acute respiratory distress syndrome, encephalopathy, myocarditis, pericarditis, and myositis^[1]. The diagnosis of influenza is made a combination of typical clinical signs and of laboratory tests. The gold standard for diagnosis is PCR testing from respiratory specimens^[88]. The most effective way to prevent influenza and its complications is vaccination. The vaccine approved for use in individuals older than 6 mo of age, including immunosuppressed

patients is the injectable inactivated trivalent vaccine^[89]. Vaccination against influenza with inactivated vaccines is recommended for (IBD) patients according to published guidelines both in the US and Europe. Some studies have suggested quantitatively reduced response to influenza vaccine in IBD patients on combined immunosuppression^[90]. However, due to the lack of specific data, there is not a current recommendation for a repeated dose of vaccine or for checking serological response after vaccination in these patients^[1]. Based on the currently available data, influenza vaccine is safe and well tolerated in IBD patients^[91].

CONCLUSION

It is crucial that physicians involved in IBD care perform a careful investigation for infectious disease before starting immunomodulation. The development of new biological drugs and the increase in their use now and in the future involves a thorough selection of patients with IBD before starting therapy. A careful screening allows the doctor to avoid having to suspend a biological therapy due to the appearance of infections with the risk of reactivation of the underlying disease (Table 1). Although, it is necessary for the IBD community to obtain data on new biomarkers with predictive value on the development of opportunistic infections, in order to set up the necessary preventive measures and to choose the better therapeutic strategies for those high-risk patients. Particular attention must be paid to specific populations, like children and elderly patients, which might deserve peculiar clinical approaches to obtain the maximum clinical benefit and minimize the risks. Routine vaccination schedules are recommended for most IBD patients, following the standard guidelines applicable to general population. However, live vaccinations are contraindicated in immunocompromised patients (Table 2). Patients who are frequent travelers (both for job or recreation) particularly to geographic regions affected with endemic infections also warrant a specific consideration by the IBD specialist. A helpful aid for the clinician is the use of a specific checklist for infectious disease screening and vaccination^[1]. A strict cooperation with infectious disease specialists is advisable for the correct prevention of opportunistic infections in IBD patients treated with biological therapies.

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Intestinal neuronal dysplasia type B: A still little known diagnosis for organic causes of intestinal chronic constipation

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Abstract

Intestinal neuronal dysplasia type B (IND-B) is a controversial entity among the gastrointestinal neuromuscular disorders. It may occur alone or associated with other neuropathies, such as Hirschsprung's disease (HD). Chronic constipation is the most common clinical manifestation of patients. IND-B primarily affects young children and mimics HD, but has its own histopathologic features characterized mainly by hyperplasia of the submucosal nerve plexus. Thus, IND-B should be included in the differential diagnoses of organic causes of constipation. In recent years, an increasing number of cases of IND-B in adults have also been described, some presenting severe constipation since childhood and others with the onset of symptoms at adulthood. Despite the intense scientific research in the last decades, there are still knowledge gaps regarding definition, pathogenesis, diagnostic criteria and therapeutic possibilities for IND-B. However, in medical practice, we continue to encounter patients with severe constipation or intestinal obstruction who undergo to diagnostic investigation for HD and their rectal biopsies present hyperganglionosis in the submucosal nerve plexus and other features, consistent with the diagnosis of IND-B. This review critically discusses aspects related to the disease definitions, pathophysiology and genetics, epidemiology distribution, clinical presentation, diagnostic criteria and therapeutic possibilities of this still little-known organic cause of intestinal chronic constipation.

Key words: Intestinal neuronal dysplasia type B; Hyperplasia of the submucosal nerve plexus; Intestinal chronic constipation; Gastrointestinal neuromuscular diseases; Dysganglionosis

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Core tip: Intestinal neuronal dysplasia type B (IND-B) is a controversial entity among the gastrointestinal neuromuscular disorders. Chronic constipation is the most common clinical manifestation of patients. IND-B primarily affects young children and mimics Hirschsprung's disease, but has its own histopathologic features characterized mainly by hyperplasia of the submucosal nerve plexus. Despite the intense scientific research in the last decades, there are still knowledge gaps regarding IND-B. This review critically discusses aspects related to the disease definitions, pathophysiology and genetics, epidemiology distribution, clinical presentation, diagnostic criteria and therapeutic possibilities of this still little-known organic cause of intestinal chronic constipation.

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INTRODUCTION

Intestinal neuronal dysplasia (IND) is a pathological condition that affects the intestinal submucosal nerve plexuses and may occur alone or associated with other neuropathies, such as Hirschsprung's disease (HD). IND belongs to the group of the gastrointestinal neuromuscular diseases and was most recently included in the classification for this heterogeneous group of complex changes in the enteric nervous system^[1,2].

More than 40 years after its original description^[3], the pathology of IND remains incompletely elucidated. Commonly, IND is associated with clinical symptoms of intestinal chronic constipation that affect children in their first years of life, similar to those of HD, but IND has its own histopathologic features characterized by hyperplasia of the submucosal nerve plexus^[4].

Despite the intense scientific research performed in the last decades which includes more than 250 published scientific articles, there are still gaps in the knowledge on IND's definition, pathogenesis, diagnostic criteria and therapeutic possibilities^[2,5,6].

HISTORICAL ASPECTS

The term IND was first used by Nezelof *et al.*^[7] in 1970 to describe three cases of congenital megacolon associated

with hyperplasia of the myenteric nerve plexus. One year later, Meier-Ruge presented the first formal description of IND as a condition that is typically associated with low intestinal obstruction and that could resemble HD but with distinctive histopathological features, such as hyperplasia of the submucosal nerve plexuses and increased Acetylcholinesterase activity (AChE) in the parasympathetic nerve fibers in the lamina propria of the mucosa^[3].

In 1977, Puri *et al.*^[8] described one case of IND associated with HD with rectosigmoid aganglionosis of the nerve plexus and IND in the descending and transverse colon.

In 1983, Fadda *et al.*^[9] proposed the classification of two clinical and histopathological subtypes of IND: IND type A (IND-A), an extremely rare form, characterized by congenital hypoplasia of the adrenergic enteric nervous system; and IND type B (IND-B), characterized by malformation of the cholinergic submucosal plexus, accounting for more than 95% of all cases.

In 1990, a consensus meeting in Frankfurt, Germany, defined the morphological criteria for the diagnosis of IND-B^[10]. Since that time, these criteria have been widely used both in clinical practice, follow-up studies and genetic investigations^[5,11-15]. Furthermore, in the 90s, new criteria were proposed that gave greater importance to the need for the identification of giant ganglia in the submucosa for the diagnosis of IND-B^[16,17]. In the several published criteria, the giant ganglia are defined by the presence of a minimum number of ganglion cells ranging from 6 to more than 10 per ganglion^[6,18-21].

Given this lack of diagnostic standardization, in 2004, Meier-Ruge *et al.*^[5,22] proposed quantitative criteria for the histopathologic diagnosis of IND-B. They defined IND-B by the presence of at least 20% giant nerve ganglia in the submucosa, with more than 8 ganglion cells each, based on the examination of a minimum of 25 submucosal ganglia. Additionally, they used a histochemical panel in frozen sections for the analyses of lactate dehydrogenase, succinyl dehydrogenase and nitric oxide synthase^[5,22].

All of these changes in the proposed histopathological criteria for the diagnosis of IND-B have not only caused disparities in its definitions but also skepticism about its existence. The main unsolved problem highlighted in recent publications is if there is a causal relationship between the histological findings and clinical symptoms that would justify the characterization of IND-B as a specific entity^[2,4,6,23]. Regarding this situation, the current opinions converge on the need for further research to elucidate the many uncertainties about the clinical and morphological characterization of IND-B^[2,4,24].

CLASSIFICATION

Two forms of IND are recognized^[9]. IND-A is extremely rare and occurs in less than 5% of all IND cases. Patients with IND-A typically present in the neonatal period with

symptoms may vary from acute intestinal obstruction to diarrhea with hemorrhagic stools. IND-A is characterized by hypoplasia or aplasia of the adrenergic enteric nervous system^[6,25]. A moderate increase in the acetylcholinesterase activity of the parasympathetic nerves is the reason that such cases are termed IND. In 2005, Meier-Ruge and Bruder^[26] considered IND-A to be as a necrotizing enterocolitis caused by immaturity of the sympathetic nervous system of the distal colon. The sympathetic innervation is decreased to different degrees in these patients. The absence of sympathetic synapses within the ganglia of the myenteric plexus and the resultant increase in parasympathetic tone are considered to be responsible for the focal colon spasms. Disorders of blood flow and decreased mucus production seem to be the major factors in the pathogenesis of necrotizing enterocolitis. In the majority of cases, the sympathetic innervation is normal by the eighth month of age. Cases that do not present with this development until 10 mo old may be related to sympathetic aplasia^[26].

In contrast, IND-B represents more than 95% of all cases, which explains why this entity has been more frequently studied in the literature and why many authors consider IND to be a synonym for IND-B^[2]. IND-B is characterized by hyperplasia of the parasympathetic submucosal plexuses. Typical histological features of IND-B include hyperganglionosis, giant ganglia, ectopic ganglion cells and increased AChE activity in the lamina propria and around the submucosal blood vessels. The changes associated with IND-B are more common in the distal colon; however, they can affect any segment of the enteric nervous system and occur in different age groups ranging from newborns to adults and alone or in combination with HD^[5]. Subtype B can cause severe constipation in childhood, unresponsive to clinical management and can be associated with soiling and hemorrhagic stools, acute bowel obstruction or enterocolitis episodes. Occasionally, IND-B symptoms mimic those of HD, which is its main differential diagnosis. All IND cases associated with HD are of the B subtype^[1,4,6,26].

EPIDEMIOLOGY

In 2007, Granero Cendón *et al.*^[27] estimated the incidence of IND-B as approximately 1 per 7500 newborns. However, the frequency of IND-B varies widely, and the reported rates range from 0.3% to 40% of all rectal suction biopsies^[5,28-30]. This wide variation may be attributable to the lack of consensus on the diagnostic criteria^[6,31]. There is also an irregular geographical distribution; the highest rates of diagnosis are in European countries, which can be explained by the fact that the majority of the published research comes from this continent^[32].

The latest published series by Taguchi *et al.*^[33] (2014) involved a retrospective multicenter study of cases of IND-B in 167 centers in Japan from 2000 to 2009.

These authors reported 13 cases based on standardized morphologic criteria from all of the included centers^[33]. However, when the quantitative criteria of Meier-Ruge *et al.*^[5,22] were applied, only 4 of the 13 cases sustained the IND-B diagnosis.

IND proximal to a segment of aganglionosis is not uncommon and has been suggested to be a possible cause of persistent bowel problems after surgery for HD. This association may occur in 6% to 44% of HD patients^[5,28,34,35].

GENETIC ASPECTS

Recent studies have addressed the role of genetic and molecular commands in the migration and development of the neuroenteric cells^[36,37]. The proto-oncogene rearranged during transfection (RET) and RET protein act in the migration and proliferation of neuroblasts. Approximately 50% of patients with familial HD present RET proto-oncogenic mutations. This finding highlights the importance of this gene alteration in the pathogenesis of dysganglionosis^[36]. Over 20 different mutations have been described in this proto-oncogene, and some of the polymorphisms are associated with particular phenotypes, such as the extension of the aganglionic segment in HD^[37].

Similarly, the existence of a genetic component potentially responsible for IND-B has been investigated. The evidence for this component came from a study of monozygotic twins affected by the disease and reports of families in which several members had the histopathological diagnoses of IND-B over multiple generations^[14,38]. Because IND-B and HD are derived from the enteric nervous system, changes often occur simultaneously in the same patient, and common molecular pathways are likely to be involved in the genes of the two pathological conditions^[39]. However, mutations in genes considered to be most relevant to HD, such as RET, glial cell line-derived neurotrophic factor (GDNF), and other selected genes in patients with IND-B, have not yet been identified in patients with IND-B^[40-44]. Only some combinations of single nucleotide polymorphisms in the RET proto-oncogene have been identified in patients with IND-B^[45].

IND-B has been described in some families with other associated congenital anomalies of the gastrointestinal tract, such as intestinal malrotation and multiple endocrine neoplasia type 2^[15,30,46,47]. Recently, twins from a Turkish family who presented with IND-B associated with congenital short bowel syndrome were described^[48], which raises the possibility that mutations in the Cocksackie- and adenovirus receptor-like membrane protein (CLMP) gene could be related to IND-B because CLMP is essential for intestinal development, and its expression is related to molecular junctional adhesion^[46].

Different experimental studies in rats and mice have demonstrated that homozygous animals deficient in the *NCX/Hox11L.1* gene present with megacolon and hyperplasia of the myenteric nerve plexus^[49-52].

However, Costa *et al.*^[14] (2000) and Fava *et al.*^[42] (2002) failed to demonstrate the presence of mutations or molecular defects in the Hox11L.1 coding region in humans with IND-B.

Another possible genetic mechanism is related to endothelin receptor B^[31]. One of the endothelin receptors (END3) plays an important role in the development of the enteric nervous system of mice. Holland-Cunz *et al.*^[53] (2003) reported that mice presenting with a heterozygous deficiency in this receptor exhibit histopathological changes similar to IND-B, although they do not exhibit clinical signs of bowel dysmotility. These findings were also not reproducible in human research.

PATHOGENESIS

The pathogenesis is also a part of the array of uncertainties regarding IND-B. Several hypotheses have been discussed, although none are widely accepted^[6,24].

The histopathological changes that characterize IND-B may come from a genetically primary change that directly influences the embryological development of tissues derived from the neural crest^[6]. However, these findings have only been identified in experimental studies^[14,49-52]. This hypothesis is supported by the association with other intestinal and extra-intestinal congenital anomalies^[15,54,55].

Another research line conceives IND-B as an adaptive response of the enteric nervous system. IND-B has been considered to be secondary to acquired phenomena caused by congenital obstructions or inflammation occurred during pre-, peri- or post-natal periods in humans^[12,13,18,56,57]. Morphological findings suggestive of IND-B have been observed in intestinal segments proximal to areas of intestinal atresia, rectal mucosal prolapse and ileostomy, intestinal intussusception, imperforate anus and necrotizing enterocolitis^[56,58,59]. This secondary histopathologic response to a bowel obstruction has also been tested in experimental studies with conflicting results^[60-62]. Pickard *et al.*^[60] (1981) observed ganglionic hyperplasia in the dilated segment of the proximal jejunum in an experimental model of intestinal atresia in sheep fetuses. The same results were not reproduced by Moore *et al.*^[61] (1993) in a model of partial colon obstruction in adult rats. These authors observed a decrease in the number of ganglion cells in the myenteric nerve plexuses of rats submitted to partial intestinal obstruction. This decrease was explained by an increase in colonic diameter secondary to bowel obstruction^[61]. The most recent study on this subject was from Gálvez *et al.*^[62] (2004) who identified histopathological changes suggestive of IND-B in some adult rats in a model of chronic colonic obstruction.

An association between IND-B and HD has also been reported^[8,35,63-65]. In such cases, the segments proximal to the aganglionic obstructed segment present histological characteristics of IND-B^[6,54]. Thus, these morphological changes of the nerve plexuses of the proximal submucosa segment can be explained both by

a primary embryonic modification of the enteric nervous system that could be considered a neurocristopathy that shares a common origin with HD and by a minor change in response to a distal intestinal obstruction^[54,63-66].

There is also some evidence that the histopathological changes observed in IND-B can be part of the normal development of the enteric nervous system. As a patient gets older, there is an increase in the size of the ganglion cells and a decrease in their number in the submucosal nerve plexuses^[5,22,67-69].

Another conflicting issue is related to whether a cause-effect relationship exists between the histopathological findings of IND-B and the clinical symptoms. In most cases, the diagnosis of IND-B is based on histopathological examinations of rectal biopsies from patients who presented severe constipation^[6]. However, histopathological changes similar to those of IND-B have been found in the colon of 36 completely asymptomatic children^[69]. Other studies have failed to demonstrate correlations between the histopathological findings, clinical symptoms, radiological and manometric changes^[11,12,47,67]. These controversies support the authors who do not consider IND-B as a distinct entity but rather a histopathological alteration of the enteric nervous system that may or may not cause clinical manifestations^[6,23,24,64].

CLINICAL PRESENTATION

Intestinal chronic constipation has been reported as the commonest clinical presentation in IND-B case series^[6,57]. In addition to the decrease in bowel movement frequency, the presence of straining at stool, bulky and hardened stools, fecal overflow incontinence and rectal bleeding are usually present as signs and symptoms of chronic constipation^[70,71]. Therefore, IND-B must be part of the differential diagnosis of possible organic causes for constipation in childhood^[72].

In some cases, these symptoms may begin in the first years of life with delays in meconium passage, abdominal distension, vomiting and failure to thrive^[73,74]. A portion of patients continue to exhibit symptoms throughout life and frequently present with severe constipation unresponsive to several treatment modalities^[75-77]. These symptoms may improve after 4 years of age, which supports the hypothesis of maturation of the enteric nervous system early in life, since in these cases the histopathological findings of IND-B could disappear concurrently with the symptoms^[5].

Severe symptoms, such as enterocolitis episodes, bowel obstruction, volvulus and intussusceptions are rare complications described in different age groups^[78-81]. In recent years, an increasing number of cases of IND-B in adults have been described^[82-86]. Some of these cases have exhibited symptoms of severe constipation since childhood^[82,83], whereas others experienced the onset of symptoms at adulthood^[84]. Some patients develop serious complications, such as chronic intestinal pseudo-obstruction, acute bowel obstruction or intestinal infarction^[84-86]. The oldest reported patient received a

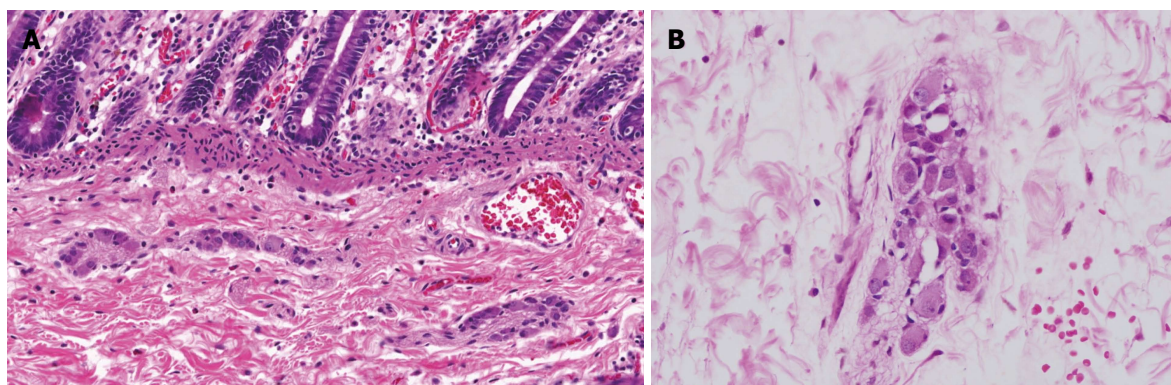


Figure 1 Histological findings of intestinal neuronal dysplasia. A: Giant ganglia in the submucous plexus with more than eight nerve cells (HE, 200 ×); B: High power view (HE, 400 ×).

confirmed diagnosis at 71 years of age^[83].

DIAGNOSIS

The diagnostic workup used in patients with IND-B must be the same routinely performed during investigation for organic causes of intestinal constipation, particularly focused to exclude HD, which is the most prevalent intestinal dysganglionosis^[6,57,72,87]. However, anorectal manometry and barium enema, which are established tests for HD screening, do not present specific results for IND-B^[88,89]. Barium enema frequently demonstrate an increased caliber of the rectosigmoid, which is a nonspecific finding typical of patients with constipation, but also may demonstrate conical transition zone, similar to HD^[31,89]. Anorectal manometry can reveal absence or presence of an anorectal inhibitory reflex, commonly with atypical morphology, which contributes little to the diagnostic investigation of IND-B^[5,31,89].

Thus, the diagnosis of IND-B essentially relies on histopathological analyses of rectal biopsies^[2,4]. The morphological criteria for its diagnosis have changed substantially over the years, leading to difficulties for clinical practice and comparisons between studies. Hyperplasia of the submucosal nerve plexuses is the morphological finding that defines IND-B but that is characterized in different manners according to the adopted criterion^[5]. Some authors emphasize the need for the presence of a minimum number of ganglion cells per ganglion or a minimum number of ganglia with these characteristics among the analyzed ganglia for a diagnosis of plexuses hyperplasia^[16,22,75,90] (Figure 1). Other morphological features, such as the presence of ectopic ganglion cells, increased acetylcholinesterase activity, ganglion cells with a “button” appearance and hypertrophy of the nerve trunks, are considered diagnostic criteria in some studies^[9,10,16,68,79,91].

The criteria described by Meier-Ruge *et al.*^[22] (2004) and slightly altered by Meier-Rouge *et al.*^[5] (2006) suggest a quantitative analysis of the number of ganglion cells in the nervous submucosal plexuses and the identification of at least 20% giant ganglia with at least 8 neurons each, in 25 analyzed nerve ganglia. Frozen 15-μm-thick

sections are mandatory and must be subjected to a panel of histochemical tests for lactate dehydrogenase, succinyl dehydrogenase and nitric oxide synthase^[5,22]. Although these criteria have been accepted by the scientific community, there are few reports of their use in large series of patients with IND-B^[33]. The requirement for fresh frozen sections and the fact that the specific histochemical stainings are not available in most pediatric pathology laboratories are limitations that must be considered. Moreover, it is uncertain whether the numerical criteria applied in these analyses can be applied to 5-μm-thick histological sections embedded in paraffin for standard histological analyses with hematoxylin and eosin or immunohistochemical methods^[2,5].

TREATMENT

Given the numerous uncertainties about the definition, pathogenesis and diagnosis of IND, the lack of consensus regarding its treatment is not surprising. Patients with IND have been subjected to different treatments modalities, that may vary from clinical management, to surgical procedures^[57].

Clinical management includes dietary changes, laxatives and enemas^[6,32]. Schimpl *et al.*^[32] (2004) reported satisfactory results in 80% of 105 patients treated with dietary changes, cisapride, laxatives and enemas, in a median follow up period of 7.2 years. Clinical management must follow the currently used guidelines for the treatment of intestinal chronic constipation in children, including fecal desimpaction and laxatives^[72].

Although there is not a well-established role to surgical treatment as in Hirschprung's disease, there are some reports of this modality of treatment in IND-B^[83]. Surgical treatment can be performed through different techniques^[11,46,63,92]. Schärli^[11] (1992) reported favorable results with a posterior sphincteromyotomy in 13 patients, after a limited 6 mo follow-up period. Some case series with a small number of patients showed symptoms improvement after a temporary colostomy^[6,46,63]. Several reports described a colonic resection in patients with

IND-B, commonly performed by an anal pull-through procedure. In most of the cases, there were improvement in the number of bowel movements and in the obstructive symptoms. However, the time of follow-up, the surgical techniques and the length of the resected bowel are quite variable^[5,77,92].

The results obtained with these different types of treatment are very discordant. Long-term follow-up studies are lacking and the available studies involve limited numbers of patients^[32,57,75]. Thus, the available data nowadays still remains too scarce to establish a therapeutic guideline for IND-B^[32,57]. On the other hand, there is a real disease, with its own clinical manifestations and can not be classified only as an histopathological entity^[75].

The several types of clinical manifestations directly influence in the treatment. Cases of mild intestinal constipation, without systemic complications or obstructive symptoms, tend to be treated with a conservative clinical management. Most of these cases may resolve spontaneously up to the age of 4 years, due to the maturation of the enteric nervous system^[93]. On the other hand, IND-B may present with severe intestinal constipation, with infectious and obstructive symptoms, what require a more invasive treatment^[77,91]. Therefore, there is a tendency to consider the conservative choice as a first line therapy in IND-B. The surgical treatment through intestinal resections should be reserved for the cases refractory to at least 6 mo of clinical management, or in the presence of obstructive complications^[5,6,31,32,76].

CONCLUSION

IND-B can be considered as a pathological entity characterized by anomalies of the submucous plexus, with a considerable increase in the number of ganglion cells, commonly associated with different degrees of constipation in childhood. IND-B remains surrounded by controversies related to its definition, etiopathogenesis, diagnostic criteria and therapeutic possibilities. However, in medical practice, we continue to encounter children with severe constipation or intestinal obstruction who undergo to diagnostic investigation for HD and rectal biopsies show hyperplastic submucosal ganglia consistent with the diagnosis of IND-B.

In this context, it is of utmost importance to maintain our efforts to clarify the pathophysiology, diagnosis and treatment of this still little-known organic cause of intestinal chronic constipation.

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Clinical significance and management of Barrett's esophagus with epithelial changes indefinite for dysplasia

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Abstract

Barrett's esophagus (BE) is defined as the extension of salmon-colored mucosa into the tubular esophagus \geq 1 cm proximal to the gastroesophageal junction with biopsy confirmation of intestinal metaplasia. Patients with BE are at increased risk of esophageal adenocarcinoma (EAC), and undergo endoscopic surveillance biopsies to detect dysplasia or early EAC. Dysplasia in BE is classified as no dysplasia, indefinite for dysplasia (IND), low grade dysplasia (LGD) or high grade dysplasia (HGD). Biopsies are diagnosed as IND when the epithelial abnormalities are not sufficient to diagnose dysplasia or the nature of the epithelial abnormalities is uncertain due to inflammation or technical issues. Specific diagnostic criteria for IND are not well established and its clinical significance and management has not been well studied. Previous studies have focused on HGD in BE and led to changes and improvement in the management of BE with HGD and early EAC. Only recently, IND and LGD in BE have become focus of intense study. This review summarizes the definition, neoplastic risk and clinical management of BE IND.

Key words: Barrett's esophagus; Dysplasia; Progression; Biomarkers; Esophageal adenocarcinoma; Indefinite for dysplasia

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Core tip: Barrett's esophagus (BE) with indefinite for dysplasia (IND) is diagnosed when the epithelial abnormalities are not sufficient to diagnose dysplasia or the nature of the epithelial abnormalities is uncertain due to inflammation. The risk of prevalent neoplasia in BE with IND varies between 1.9% and 15%. The progression to advanced neoplasia reported varies from 0.43 to 1.2 cases per 100 person-years at risk. Predictors such as the length of BE segment, multi-focality of BE IND, age > 60

years, abnormal p53 expression, active inflammation, and abnormal DNA content as detected by flow cytometry may help in risk-stratifying this patient population.

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INTRODUCTION

Barrett's esophagus (BE) is a complication of chronic esophageal injury from gastroesophageal reflux disease (GERD) and develops when reflux damaged esophageal squamous cells are replaced by mucous-secreting columnar cells. A definitive diagnosis of BE is established by the extension of salmon-colored mucosa into the tubular esophagus ≥ 1 cm proximal to the gastroesophageal junction (GEJ) with esophageal biopsy showing intestinal metaplasia, defined by the presence of goblet cells^[1]. Intestinal metaplasia in BE is a well-established marker of esophageal adenocarcinoma (EAC), and as such patients diagnosed with BE undergo regular endoscopic surveillance and biopsy to detect dysplasia or curable neoplasia. According to the published criteria by Reid *et al*^[2] the biopsies are classified based on five-tiered histologic classification of dysplasia as negative for dysplasia, indefinite for dysplasia (IND), low-grade dysplasia (LGD), highgrade dysplasia (HGD) and intra-mucosal adenocarcinoma (IMAC).

Dysplasia remains the best available clinical marker for cancer risk. Published guidelines have recommended endoscopic surveillance and treatment strategies based on the grade of dysplasia. The management of LGD and HGD in BE has been reviewed extensively and discussed in many published guidelines. Many studies have focused on the high end of neoplasia in BE, HGD and IMAC, leading to a much improved and less invasive management^[3-5]. However, there is a paucity of data to guide the management of BE patients with IND. Besides, due to lack of definitive criteria for diagnosis, and greatest inter-observer variability, and uncertain clinical significance, natural history of progression of BE with IND and management are not clear.

This paper discusses the current literature and examines available evidence for the histologic criteria for diagnosis, its clinical significance, prevalence and risk of progression to cancer, and also the clinicopathologic and biomarker predictors that are associated with dysplasia progression among patients diagnosed with BE with IND. PubMed search was performed for the term "Barrett's esophagus indefinite for dysplasia" as of November 1, 2015 and studies were reviewed for prevalence and incidence rates of HGD/EAC in BE IND as well as predictors for progression in IND. One study shared part of the same database and was excluded^[6].

DIAGNOSIS OF BE WITH IND

The diagnosis "indefinite for dysplasia" is used when the biopsy findings are too marked for being negative, but not absolutely sufficient for the presence of dysplasia. The background regenerative changes may be related to inflammation or ulceration and may overlap with LGD that often makes it difficult to differentiate from true dysplasia. Less commonly technical factors related to biopsy specimen handling such as biopsy crushing artifact, thick tissue sectioning, marked thermal artifact and tangential embedding and sectioning also prevents accurate diagnosis of dysplasia and are categorized as BE with IND; In certain circumstances pathologists unaccustomed to certain types of fixatives, for example, Hollande's and Bouin fixatives that results in vesicular nucleus and prominent nucleolus, may overinterpret the changes as indicative of BE with IND^[7]. Rarely, the diagnosis of IND may be due to the dysplasia like changes present only in the bases of the crypts, also called "basal crypt dysplasia-like atypia", where the surface epithelium may not be involved^[8].

BE IND is diagnostically challenging and it is clear that its diagnostic reproducibility is poor^[7,9,10]. Histologic criteria used to diagnose BE IND varied in different studies (Table 1) and even more so by pathologists in routine practice. For instance, the criteria for IND described by Reid *et al*^[2] included moderate architectural distortion, nuclear abnormalities less marked than those seen in dysplasia, frequent dystrophic goblet cells, more extensive nuclear stratification, diminished or absent mucus production, increased cytoplasmic basophilia, and increased mitoses (Figure 1A). The diagnosis of IND should be limited to cases in which the changes are worrisome but not sufficient for the diagnosis of dysplasia (Figure 1B). Using similar criteria, other groups performed intraobserver and interobserver reproducibility studies and found that BE IND has significant interobserver variability^[7,11]. In daily pathology practice, the BE IND category appears to expand, one such example being basal crypt dysplasia-like atypia. The concept of basal crypt dysplasia-like atypia remains controversial and is interpreted by some groups as IND while others believe that it truly represents dysplasia without surface involvement.

NEOPLASTIC RISK OF BE IND

Regardless of the definition, illustration, and intraobserver/interobserver variability, BE IND category is not uncommonly used in daily pathology practice. Several studies recently investigated the clinical significance of BE IND and the results are reviewed and summarized in Tables 2 and 3.

RISK OF PREVALENT NEOPLASIA IN BE IND

Only few studies investigated the risk of neoplasia in BE IND. Prevalent neoplasia risk, defined as LGD, HGD or EAC detected within 1 year of the diagnosis of BE IND,

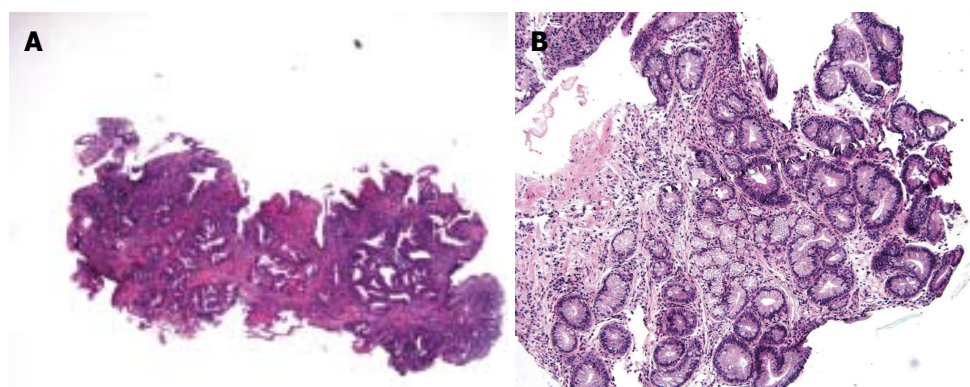


Figure 1 Examples of Barrett's esophagus with epithelial changes, indefinite for dysplasia. A: This esophageal biopsy shows inflamed BE with moderate architectural complexity, more extensive nuclear stratification, diminished or absent mucus production, increased cytoplasmic basophilia, resembling low-grade dysplasia, but there is presence of marked inflammation (HE stain, $\times 40$). This biopsy is best interpreted as indefinite for dysplasia; B: This tangentially sectioned esophageal biopsy shows foci of glands with enlarged and hyperchromatic nuclei (HE stain, $\times 100$). Because of the lack of surface epithelium as a result of tangential section, this biopsy is best interpreted as indefinite for dysplasia. BE: Barrett's esophagus.

Table 1 Histopathologic criteria for Barrett's esophagus with epithelial change indefinite for dysplasia

Ref.	Criteria
Reid <i>et al</i> ^[2] , 1988;	The architecture may be moderately distorted. Nuclear abnormalities are less marked than those seen in dysplasia. Other
Montgomery <i>et al</i> ^[7] , 2001	features that may lead to a diagnosis of IND include more numerous dystrophic goblet cells, more extensive nuclear stratification, diminished or absent mucus production, increased cytoplasmic basophilia, and increased mitoses
Sonwalkar <i>et al</i> ^[9] , 2010	Preserved gland architecture, mild crypt distortion, minimal nuclear stratification and slight nuclear atypia or enlargement
Kestens <i>et al</i> ^[15] , 2015	When a diagnosis of genuine dysplasia cannot be made. This is often due to the co-occurrence of inflammatory changes or when evaluation of surface maturation is not possible
Sinh <i>et al</i> ^[16] , 2015	Cytologic changes similar to those seen in LGD but with surface maturation or presence of inflammation
Duits <i>et al</i> ^[13] , 2015	Downgraded from BE LGD to BE IND by an expert pathology panel
Horvath <i>et al</i> ^[12] , 2015	The presence of architectural and cytologic atypia in small and mal-oriented biopsy specimen or those with inflammation or ulceration exceeding those expected for reactive changes. In some cases, it is due to basal dysplasia with surface maturation

BE: Barrett's esophagus; BE IND: Barrett's esophagus with epithelial change indefinite for dysplasia; LGD: Low-grade dysplasia.

Table 2 Risk of Prevalent neoplasia in patients with Barrett's esophagus with epithelial change indefinite for dysplasia

Ref.	Number of cases	Prevalent LGD, <i>n</i> (%)	Prevalent HGD, <i>n</i> (%)	Prevalent adenocarcinoma <i>n</i> (%)	Prevalent advanced neoplasia
Montgomery <i>et al</i> ^[11] , 2001	7	0 (0)	0 (0)	1 (15)	At least 1 (15)
Sonwalkar <i>et al</i> ^[9] , 2010	41	At least 1 (2.4)	0 (0)	At least 1 (2.4)	At least 1 (2.4)
Choi <i>et al</i> ^[14] , 2015	96	At least 14 (14.5)	Not known	Not known	At least 10 (10)
Horvath <i>et al</i> ^[12] , 2015	107	7 (8.2)	2 (2.35)	2 (2.35)	4 (4.7)
Kestens <i>et al</i> ^[15] , 2015	842	101 (12.1)	Not known	Not known	16 (1.9)
Sinh <i>et al</i> ^[16] , 2015	83	Not known	0 (0)	0 (0)	0 (0)

LGD: Low-grade dysplasia; HGD: High-grade dysplasia.

was reported in 3 studies and ranged from 12.9% to 25%. Prevalence of advanced neoplasia, *i.e.*, detection of HGD or EAC within 1 year of the diagnosis of BE IND, varied between 1.9% and 15%^[9,11,12,14,15]. When a 6-mo interval was used as a cut-off, the prevalence of LGD and advanced neoplasia in BE IND was at least 2.8%^[9]. In one case, the mucosal ulceration was associated with EAC^[11].

RISK OF INCIDENT NEOPLASIA IN BE IND

The incidence of neoplasia in BE IND is summarized

in Table 3. The incidence of all neoplasia in BE-IND is reported to be 4.5 cases per 100 person-years at risk. The progression to advanced neoplasia was 0.43 to 1.2 cases per 100 person-years at risk. The progression to EAC varied between 0.18 to 1.10 cases per 100 person-years at risk. In a study of 82 patients with BE IND, the mean length of BE segment was 6 cm in progressors vs 3 cm in non progressors ($P = 0.01$). The length of BE segment (HR = 1.2, 1.03-1.3) and multi-focality of BE IND (HR = 2.9, 1.09-7.6) were significantly associated with a higher risk of progression^[12]. One study examined the progression to advanced neoplasia

Table 3 Risk of Incident neoplasia in patients with Barrett's esophagus with epithelial change indefinite for dysplasia

Ref.	No. of cases	Follow up in months (range)	Incident LGD <i>n</i> (%)	Incident HGD <i>n</i> (%)	Incident adeno carcinoma <i>n</i> (%)	Incident advanced neoplasia (per 100 person-years)	Risk factors for progression to advanced neoplasia
Duits <i>et al</i> ^[13] , 2015	40	Median 31 (16-59)	0	1 (2.5)	0 (0)	0.9	Not done
Horvath <i>et al</i> ^[12] , 2015	82	Mean 59 (13-182)	14 (8.3)	3 (2.3)	2 (2.3)	1.2	p53 expression in >5% nuclei
Kestens <i>et al</i> ^[15] , 2015	631	Not known	No data	10 (1.6)	6 (1.0)	0.43	Older age
Sinh <i>et al</i> ^[16] , 2015	83	Mean 68.4 (SD: 37.2)	No data	3 (3.6)	1 (1.2)	0.86	Not done for BE IND group
Sonwalkar <i>et al</i> ^[9] , 2010	37	Median 38.7 (6-122)	3 (8.1)	0 (0)	3 (8.1)	Not done	Expression of AMACR in more than 1% of cells

BE IND: Barrett's esophagus with epithelial change indefinite for dysplasia; LGD: Low-grade dysplasia; HGD: High-grade dysplasia; SD: Standard deviation; AMACR: Alpha-methylacyl-CoA racemase.

Table 4 Guideline recommendations for the management of Barrett's esophagus with epithelial change indefinite for dysplasia

Guidelines	Diagnosis	Treatment and surveillance
ACG guidelines ^[1]		Acid suppressive medications for 3-6 mo A repeat endoscopy after optimization of should be performed If BE IND, surveillance in 12 mo
BSG guidelines ^[18]	Review by a second GI pathologist, and the reasons for use of the 'indefinite for dysplasia' category should be given in the histology report in order to aid patient management	Optimisation of antireflux medication Repeat endoscopy in 6 mo If no dysplasia is found, then the surveillance per non-dysplastic Barrett's oesophagus
ASGE ^[19]	Clarify presence and grade of dysplasia with expert GI pathologist	Increase antisecretory therapy to eliminate esophageal inflammation. Repeat EGD and biopsy to clarify dysplasia status
Australian Guidelines ^[20]	Confirm by a second pathologist, ideally an expert gastrointestinal pathologist.	Repeat endoscopy in 6 mo with Seattle protocol biopsies for suspected dysplasia (biopsy of any mucosal irregularity and quadrant biopsies every 1 cm) on maximal acid suppression If repeat shows no dysplasia, then follow as per non-dysplastic protocol If repeat shows low-grade or high-grade dysplasia or adenocarcinoma, then follow protocols for these respective conditions If repeat again shows confirmed indefinite for dysplasia, then repeat endoscopy in 6 mo with Seattle protocol biopsies for suspected dysplasia

BE IND: Barrett's esophagus with epithelial change indefinite for dysplasia.

in a cohort of BE IND ($n = 36$) which was downgraded from an original diagnosis of BE LGD and reported an advanced neoplasia incidence of 0.9 cases per 100 person-years at risk, similar to a rate of 0.6 cases per 100 person-years at risk in patients with BE negative for dysplasia ($n = 153$)^[13]. In contrast, BE LGD ($n = 75$) agreed upon by a panel of expert pathologists had an advanced neoplasia incidence of 9.1 cases per 100 person-years at risk^[13]. Using 6-mo follow-up as a cutoff, Sonwalkar *et al*^[9] (2010) reported that 8.1% of BE IND patients progressed to LGD and 8.1% BE IND progressed to EAC during a median follow up of 38.7 mo (range: 6-122). Interestingly, none of the 6 patients with BE IND progression had a consensus diagnosis of IND by all three reviewing pathologists.

Some studies did not distinguish between incident and prevalent dysplasia in BE IND. In a study by Montgomery *et al*^[11] the neoplasia detection rate among patients with BE IND during a median follow-up of 36 mo was 18% where 4 of 22 patients developed carcinoma. In another study, Choi *et al*^[14] reported 1-, 2-, and 3-year detection rates of HGD or EAC among patients with BE IND as

10%, 13% and 20%, respectively.

BIOMARKERS FOR RISK STRATIFICATION OF BE IND

Few studies evaluated the role of biomarkers to aid in predicting the progression of dysplasia and/or cancer. In a study of 96 BE IND patients, Choi *et al*^[14] identified active inflammation (by histology) and DNA flow cytometric abnormalities (either aneuploidy and/or increased 4N fractions greater than 6% of the nuclei) as significant risk factors associated with subsequent detection of dysplasia or neoplasia (hazard ratio for the combiner marker was 18.8, $P < 0.0001$). Sonwalkar *et al*^[9] reported that the expression of alpha-methylacyl-CoA racemase (AMACR) in more than 1% of cells correlated with progression in BE IND. However, this role of AMACR expression in risk stratifying BE IND was not seen in a subsequent study by Horvath *et al*^[17] and they instead showed that high expression of p53 (defined as intense staining in > 5% nuclei), later associated with prevalent advanced neoplasia and progression to advanced neoplasia in BE IND.

Clinical management of BE IND

The diagnosis of BE IND is challenging due to varying definitions and inter and intraobserver variability. Therefore, all biopsies should be reviewed by a second pathologist preferably a gastrointestinal pathologist. The patients are treated with aggressive acid suppression. Then, a surveillance endoscopy is performed within 6-12 mo. The biopsy protocol consists of four quadrant biopsies every 1 cm interval. If nondysplastic BE is found, then surveillance interval can be lengthened beyond one year. If LGD or HGD are found, then endoscopic eradication therapy should be considered after confirmation of the diagnosis. The guidelines for management of BE IND are presented by major societies^[1,18-20] and are summarized in Table 4.

CONCLUSION

In summary, the diagnosis of BE IND is difficult. Recent studies reveal that BE IND carries a significant risk of prevalent advanced neoplasia (at least 2.8%, 31 out of 1135 patients, ranging from 0% to 15%) (Table 2). In addition, the diagnosis of BE IND is associated with risk of progression to advanced neoplasia (0.43 to 1.2 cases person-years at risk) (Table 3). These figures are similar to the risk of LGD without histology review^[16], but much lower than the progression risk in consensus diagnosis of LGD^[13]. It is worth bearing in mind that 73% of cases with a diagnosis of BE LGD originally rendered by practicing pathologists were down-graded to BE IND or BE negative for dysplasia by an expert pathology panel^[13]. Therefore, cases with initial impression of BE IND or LGD should be reviewed by additional GI pathologists to confirm the diagnosis. Patients with a confirmed diagnosis of BE IND should be placed on intensive acid suppressive therapy and have a surveillance endoscopy with four quadrant biopsies every 1 cm interval in BE segment within one year. BE IND patients with follow-up biopsies which are negative for dysplasia have low risk of neoplasia progression and may be reverted to routine surveillance. The length of BE, multi-focality of BE IND, older age (> 60 years old), abnormal p53 expression, active inflammation, and abnormal DNA content as detected by flow cytometry are useful to risk-stratify this patient population. The role of these predictors in clinical management of patients with BE IND requires further scrutiny.

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Chinese *Helicobacter pylori* vaccine: Solution for an old challenge?

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Abstract

Helicobacter pylori (*H. pylori*) is an important cause for gastric cancer in high risk individuals. *H. pylori* colonizes more than 50% of the world's population and associated peptic ulcer disease and gastric malignancy have important public health implications. It has been classified as a class I carcinogen in 1994 by the World Health Organization. Clinicians are often prompted to eliminate the infection the moment it is detected. This also, unfortunately, led to reckless use of antibiotics and reports of increasing resistance are now worldwide. Each year, many of people die from gastric cancer; thus application of effective vaccine can reduce this relatively high mortality worldwide. *H. pylori* can be eliminated by antibiotics but efficacy is sharply decreasing. Moreover, current therapy is also expensive and with side effects. Vaccine may be the best solution to the above problem but there are many challenges in producing such an effective therapeutic vaccine. Recently, the Chinese group published in Lancet, a single-center, randomized, phase III study of an oral recombinant vaccine (Urease B subunit fused with heat-labile enterotoxin B derived from *Escherichia coli*) prescribed in the Chinese children (6-15 years) without a history of *H. pylori* infection. This review provides an insight into this new solution for an old challenge.

Key words: *Helicobacter pylori*; Resistance; Therapy; Vaccine; Antibiotics

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Core tip: *Helicobacter pylori* (*H. pylori*) remains the most prevalent gastric infection. One of the main questionable aspects of *H. pylori* is its high resistance to most of prescribed antibiotics and lack of useful vaccines. Vaccine may be the best solution to the above problem but there are many challenges in producing such an effective

therapeutic vaccine. That will be ideal that Chinese vaccine removes the need for bicarbonate administration because of its adverse side effects. Taking together, it is the first time that such a protective *H. pylori* vaccine is introduced to the world for high risk individuals.

Talebi Bezmin Abadi A, Lee YY. Chinese *Helicobacter pylori* vaccine: Solution for an old challenge? *World J Gastrointest Pharmacol Ther* 2016; 7(3): 412-415 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v7/i3/412.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v7.i3.412>

INTRODUCTION

It is time to stop Helicobacter pylori

Helicobacter pylori (*H. pylori*) infects over half of the world's population and associated peptic ulcer disease and gastric malignancy have important public health implications. Despite after two decades of antibiotics success, the primary problem still exists, and the reasons can be multifactorial^[1-3]. Some *in-vivo* conditions favor the persistence of *H. pylori* in the stomach but others oppose, and the clinical outcomes can be dependent on a delicate balance between a harmless inflammation and a more severe kind^[4]. Furthermore, we do not know the most effective eradication regime of *H. pylori*, as the following questions remained unsolved including the best duration of recommended regimens, best dosages and also the right combination of antibiotics^[5-8]. Although *H. pylori* infection can be efficiently eradicated using antibiotics, at least, in some patients, there are now reports of antibiotic resistance worldwide (Table 1). Finding an effective vaccine is the answer if resistance continues to increase^[9-12]. More than five international guidelines have been published that covers all aspects of *H. pylori* infection including diagnostic, treatment and also vaccine^[13,14]. Following years of continuous clinical experiments and trials, the promising goal for an effective vaccine now seems feasible. In September 2015, a report published in *Lancet* by the Chinese group brings high hope on a highly effective vaccine that we have been waiting for^[15]. If proven in further studies, then this groundbreaking finding will change management paradigm of *H. pylori* in the near future.

A *H. PYLORI* VACCINE THAT WORKS, FINALLY?

Effective vaccine should not just reduce the incidence but also global prevalence of *H. pylori*. Furthermore, to prove its efficacy we need a longer period of study observation and with a greater number of study participants to conclude its reliability before it can be recommended into any healthcare systems. There have been considerable interests to develop such an effective *H. pylori* vaccine for a long time but many obstacles had hampered the

development^[16]. Many of the *H. pylori* virulence factors and also secreted proteins such as urease were used as recombinant proteins to produce a protective vaccine, but because these factors only induced weak forms of immunity and also lack of safety, therefore many projects were abandoned^[13,17]. Therapeutic vaccines should be able to administer to both *H. pylori* positive children but also adults; although there is a potential risk for developing gastritis in susceptible patients^[18,19]. In these susceptible patients, however, we can still recommend therapeutic vaccine; since it can reduce: (1) risk of re-infection; and (2) decrease treatment duration. The disappointment in vaccine development may tip following the Chinese *H. pylori* vaccine published in *Lancet*^[15]. This was a single-center, randomized trial and a phase III study that examined an oral recombinant vaccine (based on Urease B subunit fused with heat-labile enterotoxin B derived from *Escherichia coli*) among the Chinese children (aged 6-15 years) without a prior history of *H. pylori* infection. In brief, after 12 mo of vaccination, 71% efficacy rate was observed, and this rate was around 55% after 3 years. Although seems effective in children, this study needs repeat among adults. Another limitation of this vaccine is that the authors found 20% of younger children were not protected from the infection. Notably, using better adjuvant in order to remove boosters for this vaccine may increase its popularity among clinicians for widespread prescription. Also it will be ideal that the Chinese vaccine removes the need for bicarbonate administration because of its adverse side effects. Taking together, it is the first time that such a protective *H. pylori* vaccine is introduced to the world for high risk individuals.

WHAT NOW AFTER THE CHINESE VACCINE?

While the published results for the Chinese vaccine seems promising, but there are still barriers before it gains wide acceptance. Besides the limitations mentioned in above section, the vaccine needs a proper Phase-III clinical trials for other populations. Besides the Chinese vaccine, there are other novel developments in the pipeline. Currently, there is a lack in knowledge on exact molecular mechanisms that contributed to cellular immunity against *H. pylori*. The urease enzyme was the first recombinant protein used to provide an effective vaccine for *H. pylori* in animal models^[20,21]. Recently, it has been established that regulatory T-cells are necessary to mount sufficient immune responses and this is important information for future development of a protective anti-*H. pylori* vaccine^[22]. *H. pylori*-immunogenic antigens such as catalase, vacuolating cytotoxin (VacA), urease, cytotoxin-associated gene A (CagA), heat shock proteins and also neutrophil-activating protein (NAP) had been examined to see if they are potential candidate antigens for vaccine^[23-27], but so far, the results have been inconclusive. Moreover, different mucosal routes such as

Table 1 Worldwide report of increasing *Helicobacter pylori* anti-biotic resistance

Year	Eradication rate	Ref.	Antibiotics
2001	97%	Asaka <i>et al</i> ^[9]	Clarithromycin Amoxicillin
2014	61%	Chen <i>et al</i> ^[10]	Clarithromycin Amoxicillin
2014	55%	Kutluk <i>et al</i> ^[32]	Clarithromycin Amoxicillin
2013	76%	Sardarian <i>et al</i> ^[11]	Clarithromycin Amoxicillin tinidazole
2013	80%	Zullo <i>et al</i> ^[33]	Clarithromycin Amoxicillin tinidazole
2014	69%	Nishida <i>et al</i> ^[7]	Clarithromycin Amoxicillin
2011	87%	Greenberg <i>et al</i> ^[6]	Clarithromycin Amoxicillin
2014	98%	Sugimoto <i>et al</i> ^[12]	Metronidazole Clarithromycin
2013	38%	Nishizawa <i>et al</i> ^[34]	Metronidazole Amoxicillin Clarithromycin

sublingual, rectal and intranasal were being evaluated but results were inconsistent^[27-30]. Recently, Chen *et al*^[31] examined *oipA* DNA construct carried by the bacterium, *Salmonella typhimurium* as a therapeutic vaccine. The authors concluded that *H. pylori* virulence factors including OipA and NAP may seem to be the better candidates to induce effective immunity, at least in the mouse models, and we shall await more results.

CONCLUSION

Due to the relatively high rate of antibiotic therapy failure in recent years, we have to investigate more about novel vaccines on *H. pylori*. At last, Chinese group proposed a useful formulation with less side effects which can inspire more hopes for clinicians to think actually about *H. pylori* mass eradication worldwide.

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Basic Study

Effects of aging on the architecture of the ileocecal junction in rats

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Author contributions: de Brito MC performed all of the experiments and analyzed the results for this work; Chopard RP planned experiments; Cury DP and Watanabe IS helped and analyzed the transmission and scan electron microscopy studies; Mendes CE helped edit the manuscript and figures; Castelucci P planned the immunohistochemistry study, wrote and edit the manuscript.

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Abstract

AIM: To evaluate the structural organization of the elastic and collagen fibers in the region of the ileocecal transition in 30 young and old male Wistar rats.

METHODS: Histology, immunohistochemistry (IHC), transmission electron microscopy and scanning electron microscopy were employed in this study. The results demonstrated that there was a demarcation of the ileocecal region between the ileum and the cecum in both groups.

RESULTS: The connective tissue fibers had different distribution patterns in the two groups. IHC revealed the presence of nitric oxide synthase, enteric neurons and smooth muscle fibers in the ileocecal junctions (ICJs) of both groups. Compared to the young group, the elderly group exhibited an increase in collagen type I fibers, a decrease in collagen type III fibers, a decreased linear density of oxytalan elastic fibers, and a greater linear density of elaunin and mature elastic fibers.

CONCLUSION: The results revealed changes in the patterns of distribution of collagen and elastic fibers that may lead to a possible decrease in ICJ functionality.

Key words: Ileocecal junction; Elastic fibers; Collagen fibers; Aging; Rats

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Core tip: The ileocolonic sphincter controls the forward and backward flow by integrating its motility with that of the distal ileum and proximal the ileocolonic sphincter controls the forward and backward flow by integrating its motility with that of the distal ileum and proxima. The ileocecal junction (ICJ) includes the muscle bundles in the terminal ileum, the intrinsic nerve plexus. The ICJ includes the muscle bundles in the terminal ileum, the intrinsic nerve plexus. Given the importance of knowing how the ICJ changes with age, the objective of this study was to characterize the morphological changes in the ICJ in rats aged 21 d and 2 years, using optical microscopy and electronic scanning and transmission methodologies. Additionally, the neurochemical characterization of the inhibitory neurons of the myenteric plexus, which are immunoreactive to the enzyme nitric oxide synthase, and the staining of the neuronal population were employed to identify immunoreactivity to HuC/D.

de Brito MC, Chopard RP, Cury DP, Watanabe IS, Mendes CE, Castelucci P. Effects of aging on the architecture of the ileocecal junction in rats. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 416-427 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v7/i3/416.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v7.i3.416>

INTRODUCTION

The ileocecal junction (ICJ) has two aspects: A wedge-shaped cavity that progressively narrows the orifice to form the ileum and is bordered by an upper lip and lower lip, joined by front and posterior commissures; and an invagination of the small intestine to the large intestine^[1-4]. Morphological differences between species can be related to the type of digestion, either partial or total, in the cecum^[5-8]. The smooth muscle cells of the ICJ maintain a high tone^[9]. The ileocolonic sphincter controls the forward and backward flow by integrating its motility with that of the distal ileum and proximal^[9]. The ICJ includes the muscle bundles in the terminal ileum, the intrinsic nerve plexus, and the presence or absence of the interstitial cells of Cajal (ICC). Many ICC associated with the myenteric plexus are observed in both the ileal and cecal sides of the valve^[10]. The neuronal density is lower in the cecum and ileal papilla compared to the terminal ileum. ICC exist within the myenteric plexus of the ICJ, and their density is similar to the adjacent bowel^[4]. Histochemistry for acetylcholinesterase (AChE) and NADPH-diaphorase (NADPH-d) histochemistry and immunohistochemistry for protein gene product 9.5 (PGP 9.5) and C-kit in the ICJ revealed two distinct coaxial

myenteric plexuses, together with superficial and deep submucosal plexuses. The C-kit immunostaining showed a continuous myenteric ICC network within the ICV^[6].

Additionally, the ICJ is accompanied by a framework of collagen and elastic fibers^[1-3,11]. The elastic fibers function to maintaining the elasticity of tissues throughout life^[12]. Changes to the collagen arrangement and its three-dimensional (3D) distribution may be related to the dissimilar biomechanical proprieties in the terminal ileum^[11]. Changes have been observed in the composition and architecture of the connective tissue with aging, resulting in the loss of elasticity and extensibility of different tissues^[12,13].

The loss of ICJ function is clinically important. The loss of ICJ function may cause fecal reflux, with the risk of bacterial colonization in the terminal ileum^[14]. Given the importance of knowing how the ICJ changes with age, the objective of this study was to characterize the morphological changes in the ICJ in rats aged 21 d and 2 years, using optical microscopy and electronic scanning and transmission methodologies. Additionally, the neurochemical characterization of the inhibitory neurons of the myenteric plexus, which are immunoreactive to the enzyme nitric oxide synthase (NOS), and the staining of the neuronal population were employed to identify immunoreactivity to HuC/D.

MATERIALS AND METHODS

We used 30 male Wistar rats (*Rattus norvegicus*) from the Central Animal Facility of the Institute of Biomedical Sciences, University of São Paulo in this study. The animals were housed in polypropylene cages that could hold up to three animals. The temperature was controlled at 21 °C ± 1 °C, and the humidity was approximately 60%. The animals were maintained on alternating cycles of 12 h of light and 12 h of dark, with a balanced diet and water provided *ad libitum*. All the procedures were approved by the Ethics Committee on Animal Experiments of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo. The animals were divided into two groups: (1) a young group, 21 d old ($n = 15$); and (2) an older group, 24 mo old ($n = 15$). Each group was analyzed using microscopy ($n = 7$), immunohistochemistry (IHC; $n = 2$), transmission electron microscopy (TEM; $n = 2$) and scanning electron microscopy (SEM; $n = 4$).

Histology methods

For light microscopy, the animals were euthanized with an overdose of xylazine (40 mg/kg) and ketamine (120 mg/kg) and then the ICJ was removed. The samples were fixed in 10% paraformaldehyde fixative for 24 h at room temperature before undergoing routine histological processing. Cuts were made in the longitudinal direction with a thickness of 5 µm using a Reichert Jung ultramicrotome. Samples were stained using hematoxylin and eosin (HE). Elastic fibers were revealed by staining with iron hematoxylin (Verhoeff), resulting in blue and

Table 1 Characteristics of the primary and secondary antibodies used in this study

Antigen	Host	Dilution	Source
Nitric oxide synthase	Sheep	1:1000	Chemicon
HuC/D	Mouse	1:100	Molecular probes
Anti-sheep IgG 488	Donkey	1:100	Molecular probes
Anti-mouse IgG 594	Donkey	1:200	Molecular probes

black tones^[15-17]. Staining with resorcin-fuchsin (Weigert), with and without oxone, showed mature elastic fibers as pink, and elaunin and oxytalan were stained in shades of purple and black^[16,17].

Picrosirius staining under polarized light allowed the observation of collagen birefringence, allowing the classification of the type or age of the collagen according to the color and light intensity of the refringence. Polarized light of picrosirius-stained samples evidenced yellow and red fibers (type I collagen) and green fibers (type III collagen). The stained slides were observed and digitized with a NIKON Eclipse E600 microscope and NIS-Elements AR software for documentation and further qualitative and quantitative analysis.

IHC fluorescence method

For IHC, the animals were euthanized with an overdose of xylazine (40 mg/kg) and ketamine (120 mg/kg). After collection, the samples were washed by immersion in phosphate-buffered saline solution (PBS; 1.15 mol/L NaCl and 0.01 mol/L sodium phosphate buffer, pH = 7.2), and then they were washed with PBS. After this procedure, the samples were sectioned by mesenteric margins and were subsequently fixed in wooden rafts with the mucosa facing down with the aid of pins. Subsequently, the sections were immersed in 4% paraformaldehyde fixative in 0.1 mol/L sodium phosphate buffer, pH 7.3 at 4 °C for 24 h. On the following day, the samples were removed from the fixative and washed in PBS three times with intervals of 10 min each. Then, some of the samples were stored in PBS containing sodium azide (0.1%) at 4 °C for preservation, and the others were transferred to PBS + 30% sucrose for 24 h at 4 °C. The next day, an exchange of substances in 50:50 PBS + 30% sucrose + Optimum Cutting Temperature Tissue Tek, Elkhart (OCT) was performed, and the samples were stored overnight. After this period, the switch was made at 100%. The samples were then stored at -80 °C to maintain their conservation. After the completion of all the procedures mentioned above, the samples were fixed on metal bases (stubs) in 10 µm slices, and cuts were made with an 1850 Leica cryostat at -25 °C. The sections were mounted on slides, which were stored at room temperature for 1 h and then immersed in 10% normal horse serum solution (NHS) and 1.5% Triton (Sigma) in PBS for 45 min at room temperature. Then, the samples were incubated with primary antibody (Table 1) for 48 h.

After 24 h, the samples were again subjected to

washes with PBS (three times for 10 min each) and were further incubated with a secondary antibody (Table 1). The tissues were immersed in 2.6-diamino-2-phenylindole dihydrochloride (DAPI) for five minutes to stain the nuclei of all the cells. Subsequently, the tissues were washed in PBS (3 times for 5 min each). Then, the slides were covered with a glycerol coverslip buffered in 0.5 mol/L calcium carbonate buffer (pH 8.6). Observations were performed with a Nikon 80i fluorescence microscope using the Nis Elements program. Sample preparations were also analyzed with a confocal scanning microscope (Olympus Fluorview FV10SW Laser).

TEM method

After pre-anesthesia, the animals were perfused with a modified Karnovsky fixative solution containing 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L sodium phosphate buffer at pH 7.4^[18]. After perfusion, samples were collected from the ICJ. The samples were post-fixed in 2.5% glutaraldehyde for 2 h at 4 °C. They were then washed in sodium phosphate buffer and post-fixed in 1% osmium tetroxide for 2 h at 4 °C. The samples were washed with brine, and then were immersed in 0.5% uranyl acetate overnight. On the following day, the samples were dehydrated in a series of increasing alcohol concentrations (from 2% to 70%) before baths in absolute alcohol and propylene oxide for 15 min each. The samples were then embedded in a mixture of Spurr® resin. The samples were placed in shallow molds of silicone, which were then placed in an oven at 60 °C for 48 h for polymerization of the resin. The blocks were then subjected to trimming, and thin sections from 0.5 µm to 1 µm were obtained with an ultra-microtome. The sections were then collected on glass slides, stained with toluidine blue and washed with 1% distilled water for observation by light microscopy for the delimitation of areas. Ultrathin sections approximately 70 nm thick were made and collected in 200 mesh copper screens. The sections were contrasted with 4% uranyl acetate solution for 10 min and were then washed with distilled water^[18] and 4% lead citrate for 10 min^[18]. All the cuts were made with an ultra-microtome (Leica Ultracut, Germany) in the multiuser laboratory of the Department of Biomedical Sciences Institute of Anatomy, University of São Paulo. The sections were analyzed by TEM (Fei Morgagni 265 and Jeol 1010 brand microscopes set to 80 kV).

SEM method

For SEM, the animals were anesthetized intraperitoneally with ketamine (60 mg/kg) and xylazine (20 mg/kg) and then were perfused with a fixative solution of modified Karnovsky containing 2.5% glutaraldehyde, 2% paraformaldehyde, and a 0.1 mol/L solution of sodium phosphate buffer at pH = 7.4^[18]; approximately 60 mL of solution was injected through the left ventricle of the heart. After perfusion, samples from the ICJ were then removed and immersed in fixative for 48 h at 4 °C. Then, the tissues were sectioned at the mesenteric margin.

Some of the samples were selected for SEM, and some were subjected to treatment with a 5% aqueous solution of sodium hydroxide (NaOH)^[19,20], which was changed daily for 4 d; the samples were then washed in distilled water for 2 d at 4 °C. After this step, all the samples (sectioned and macerated) were post-fixed in aqueous 1% osmium tetroxide for 2 h at 4 °C and dehydrated in a series of increasing alcohol concentrations and dried in a critical point apparatus (Balzers CPD-020) using liquid CO₂. After drying, the samples were mounted on aluminum metal bases and subjected to a metal cover with gold ions in the "sputtering" unit (Union Balzers SCD 040).

Quantitative analysis

Collagen fibers: The study of the collagen fiber system was performed by capturing random fields using the Imaging Software NIS program - Elements AR 3.1 for the observation of collagen types I and III. Six fields of each slide were captured with a $\times 20$ lens. From these images, collagen fiber type I (red) and type III (green) densities were quantified with a software tool that recognizes color variations in an image using the color channels of red, green and blue (RGB). This software allows the identification of one color at a time, yielding the average color intensity of the given area and the area of each field equivalent to 274252.78 pixels^[21].

Elastic fibers: The histomorphometrical examination was performed using linear density (Ld) estimation of the elastic system. The estimated elastic fiber length was derived from the formula $L = 2Q \times EV$, where L = length of the elastic fiber structure per unit volume, Q = number of cross-sectioned elastic fibers in the plane of the section and EV = unit volume. The fiber length per unit volume is directly proportional to the number of fiber intersections within the unit section area^[21-23]. The samples were analyzed with a 100X lens, immersion oil, and eye Kf 10 \times 18 compensation, with 117 points integration, showing 13 parallel lines vertically and 9 horizontally. The total area was 117000 μm^2 . The distance (L) between the points in this system was 10 μm according to the procedure reported^[24]. Histological sections of the ICJ were observed and documented under a microscope (Nikon Eclipse E600/NIS - Elements).

Statistical analysis

A statistical analysis was performed by comparing the linear density of the collagen and elastic fibers of the young and elderly groups. The data were analyzed statistically using Student's *t*-test with a significance level of $P < 0.05$.

RESULTS

Histological analyses

The HE staining showed that smooth muscle cells were distributed in the mucosa and submucosa of the intestine in both groups, and both regions showed an arrangement

in three different muscle layers: Two circular layers and one longitudinal muscle layer, with their cores in the central portion of the cells (Figure 1A-D). Spaces between smooth muscle cells were observed in the young group (Figure 1A, B); however, in the older group (Figure 1C, D), connective tissue fiber condensation between the smooth muscle cells was observed. The transition region had different cell characteristics. The ileum protruded into the cecum, while thickening occurred in the circular muscle layer. The cecum of both groups comprised a glandular epithelium without villi (Figure 1A-D).

Picrosirius staining under non-polarized light (Figure 1E and G) showed the general appearance of the ICJ. Picrosirius staining under polarized light showed the architecture of the Type I and Type III collagen fibers of the ICJ in both groups. These fibers were arranged around the smooth muscle cells and originated from both sides of the cecal ileum to form the transition surface. In addition, evaluating the structure of the connective framework revealed a clear predominance of type I collagen fibers, which was characteristic of mature tissue in the elderly group (Figure 1H), that were shorter, thicker and more numerous. Note that the type I collagen fibers in the young group (Figure 1F) were more elongated and thin and were less prevalent compared to the elderly group. Type III collagen fibers were more numerous in the young group (Figure 1F) and were thinner compared to the older group (Figure 1H).

Elastic fibers: Weigert staining with previous oxidation showed oxytalan fibers (Figure 2A and B). In the young group (Figure 2A), these fibers were arranged in parallel, were thinner compared to the elderly group (Figure 2B), and were thicker and curved. Weigert staining (Figure 2C) also showed elaunin fibers. These fibers were arranged in parallel and were straight and slender in the young group (Figure 2C), which was different from the observations in the elderly group (Figure 2D) in which these fibers were more curved and thick. With Verhoeff staining (Figure 2E and F), it was possible to identify mature elastic fibers in both groups. These showed more slender and straight fibers in the young group (Figure 2E). However, in the elderly group (Figure 2F) these fibers were thicker, shorter and more crooked.

IHC: Immunoreactive neurons and fibers were identified by HuC/D (Figure 3A and D) and NOS (Figure 3B, E) in the young and elderly groups. The nuclei of the smooth muscle cells were identified by DAPI staining (Figure 3C and F). Figure 3C and F shows the triple labeling of immunoreactive neurons with NOS, HuC/D and DAPI. There was a homogeneous distribution of cytoplasmic immunoreactivity for HuC/D and NOS in the myenteric neurons of both groups.

TEM: In the young group (21-d-old), the observation of the mucosa of the ileocecal transition region revealed the presence of smooth muscle cells sectioned transversely

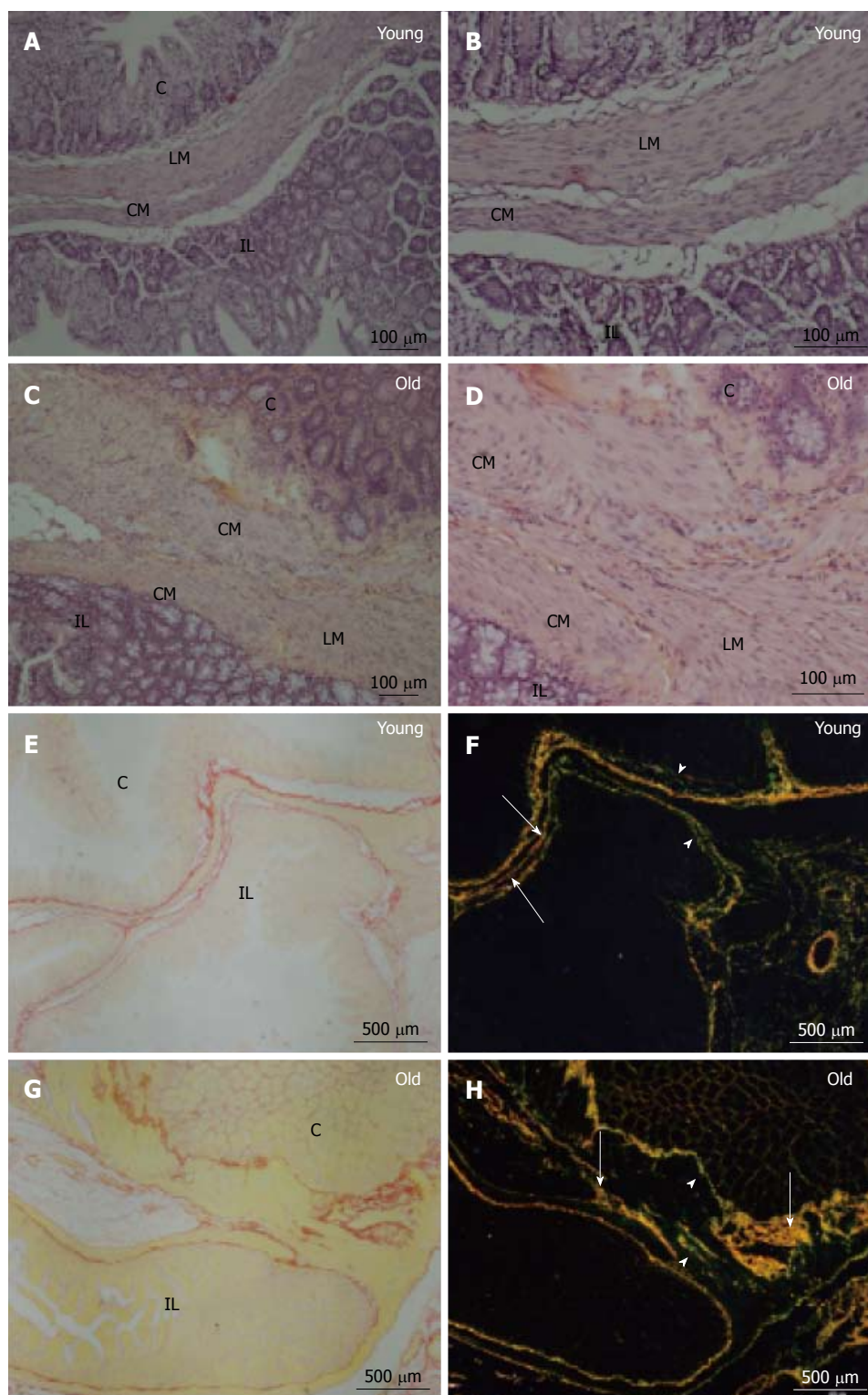


Figure 1 Histological sections of the ileocecal junctions in the young group and the elderly group. Hematoxylin-eosin staining (A-D) was performed to examine the ileum (IL), cecum (C), circular muscle layer (CM) and longitudinal muscle layer (ML). Picrosirius staining under non-polarized light (E and G) showed the transition region between the ileum (IL) and cecum (C). Picrosirius staining under polarized light (F and H) showed type I collagen fibers (yellow, orange and red) (arrows) and type III collagen fibers (green) (arrowhead).

(Figure 4A). The smooth muscle cells were surrounded by bundles of collagen fibers (Figure 4A and B). Between muscle cells, we observed numerous unmyelinated fibers (Figure 4B). The nerve fibers contained neurofilaments

and mitochondria (Figure 4B). In the elderly group (24-mo-old), longitudinal sections were observed (Figure 4C). Between muscle fibers in the elderly group, there was a larger amount of collagen fibers forming the

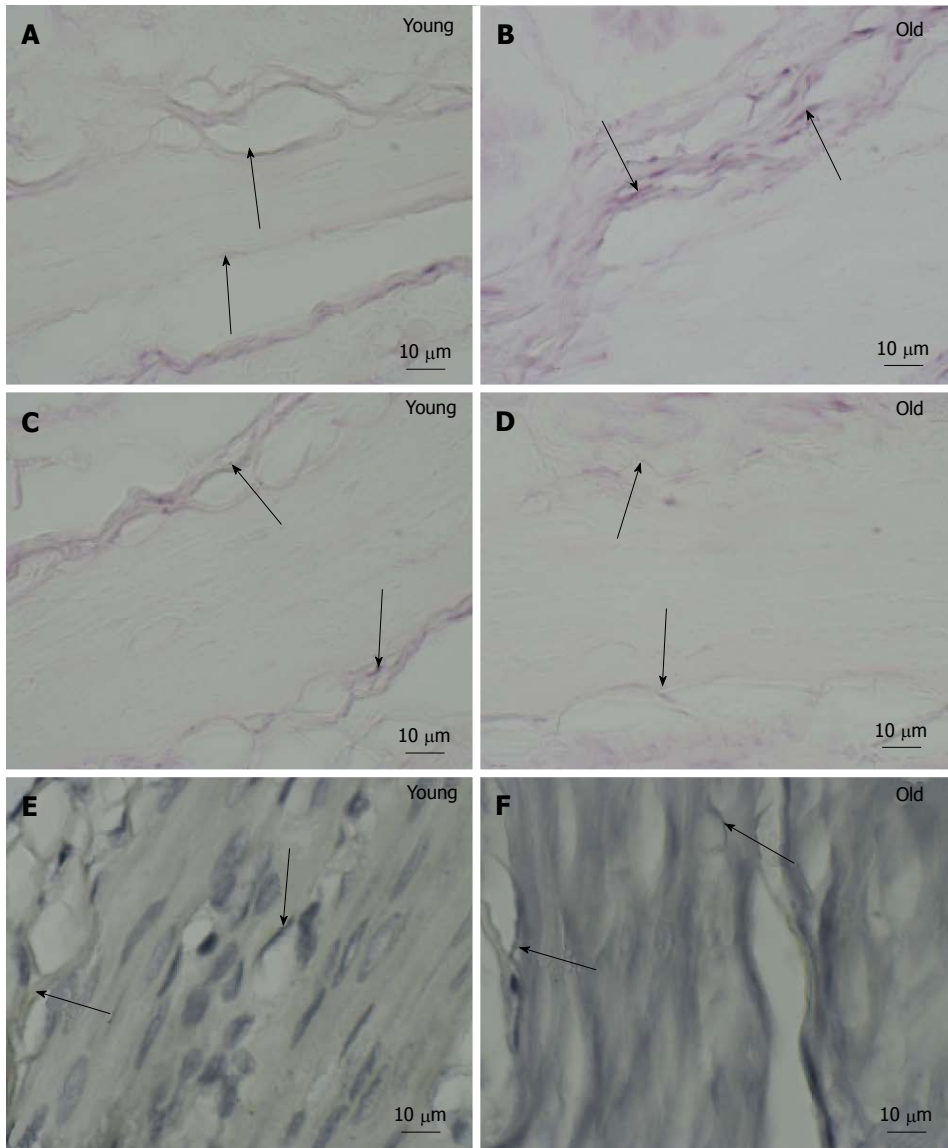


Figure 2 Histological sections of ileocecal junctions in the young group (A, C, E) and the elderly group (B, D, F) stained with Weigert with oxone (A, B), Weigert (C, D) and Verhoeff (E, F).

endomysium of the cells (Figure 4C). There were many unmyelinated nerve fibers of different diameters (Figure 4D).

SEM

SEM of ileocecal transition segment samples from the young and elderly groups revealed the transition region, with mucosal projections of the ileal and cecal regions (Figure 5). In the young group, the mucosal surface of the transition between the ileum and cecum showed a perfectly demarcated area where numerous elongated buds had formed on the microvilli in the ileum region (Figure 5A and B). After treatment with sodium hydroxide solution, the cell layers were completely removed and the ileocecal transition region was clearly identified, revealing numerous foramens in the cecum. There were foramens and laminar projections of collagen fibers in the ileum (Figure 5C and D); however, the cecal

region had only foramens without collagen (Figure 5D).

In the elderly group, the line delimiting the two regions was visible as a surface containing a groove (Figure 5E). A characteristic, normal-looking mucosa forming the microvilli was observed (Figure 5F). After treatment with a sodium hydroxide solution, the epithelial-tissue interface regions of the ileum and cecum showed that the ileum region had numerous laminar projections of collagen fibers interspersed with an essentially circular foramen (Figure 5G). In the region of the cecum, numerous circular and elongated foramens in a three-dimensional arrangement were observed (Figure 5H).

Quantitative analysis

An analysis of light intensity after picrosirius staining under polarized light showed that the value for the average linear type I collagen fibers in the young group

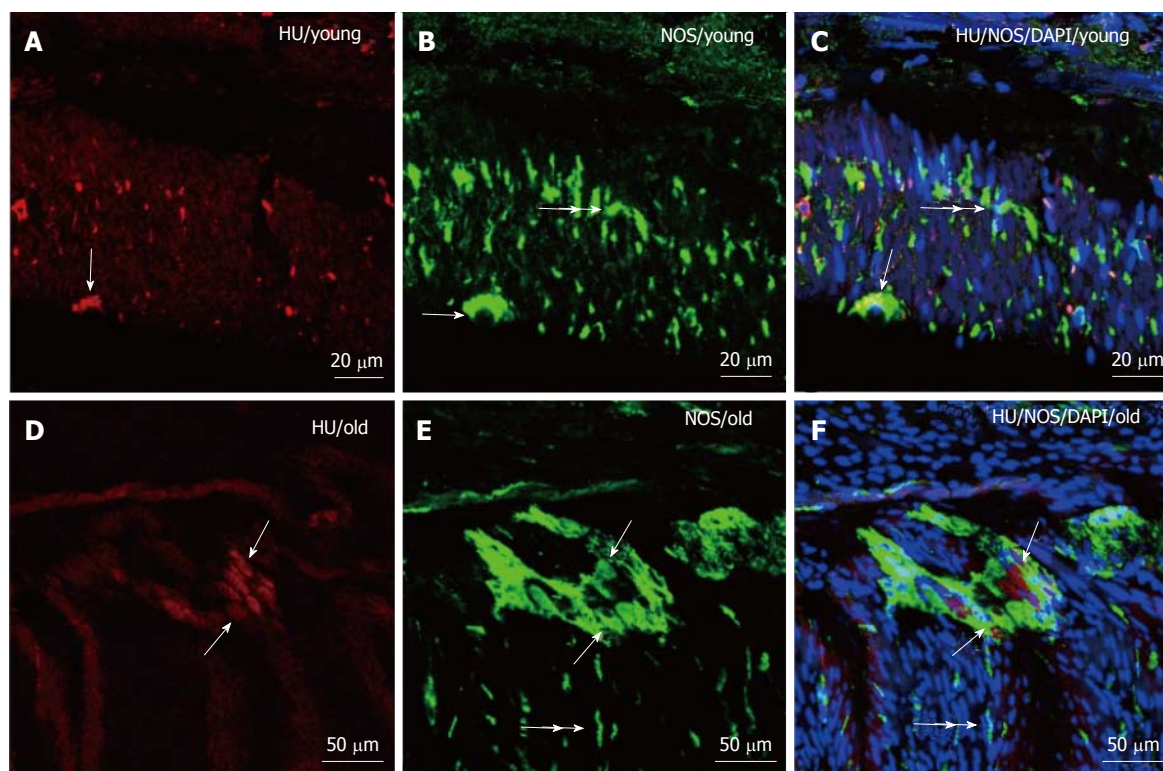


Figure 3 Immunohistochemistry misty final. HuC/D staining in immunoreactive neurons (A, D), NOS staining in immunoreactive neurons and nerve fibers (B, E), and DAPI staining of the nuclei of immunoreactive cells (C, F) of the ileocecal junction in young rats (A-C) and elderly rats. The co-localization of HuC/D, NOS and DAPI (C, F). The arrows indicate immunoreactive neurons stained for HuC/D (A, D), NOS (B, E), and triple colocalization with HuC/D, NOS and DAPI (C, F). The double arrows indicate immunoreactive fibers stained for NOS (B, E). The double arrows show muscle fiber nuclei stained with DAPI (C, F). NOS: Nitric oxide synthase; DAPI: 4',6-diamidino-2-phenylindole.

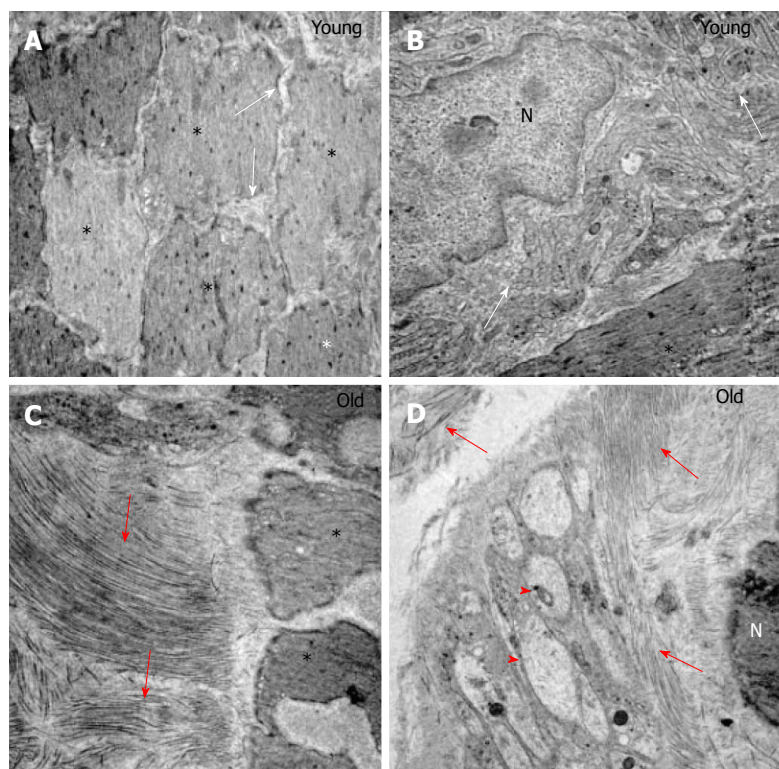


Figure 4 Transmission electron microscopy. A: A cross-section of the muscle layer showing the muscle fibers (*) and connective tissue (arrow) of the young group. Magnification: × 6000; B: A longitudinal section of the muscle layer showing muscle fibers (*), nuclei (N) and a set of unmyelinated fibers (arrows) of the young group. Magnification: × 20000; C: A longitudinal section of the muscular layer showing collagen fibers arranged in different directions (arrows) and muscle fibers of the elderly group. Magnification: × 10000; D: Nucleus (N) and collagen fibers arranged in different directions (arrows) and synaptic vesicles (arrowhead) of the elderly group. Magnification: × 10000.

was 10842.7 ± 212.6 pixels. The elderly group had a mean linear value of 16465.4 ± 184.4 pixels, a significant increase of 51.9% ($P < 0.001$) compared to that in the

young group (Figure 6A). The average linear value of type III collagen fibers in the young group was 21706.4 ± 47.9 pixels. The elderly group showed a mean linear

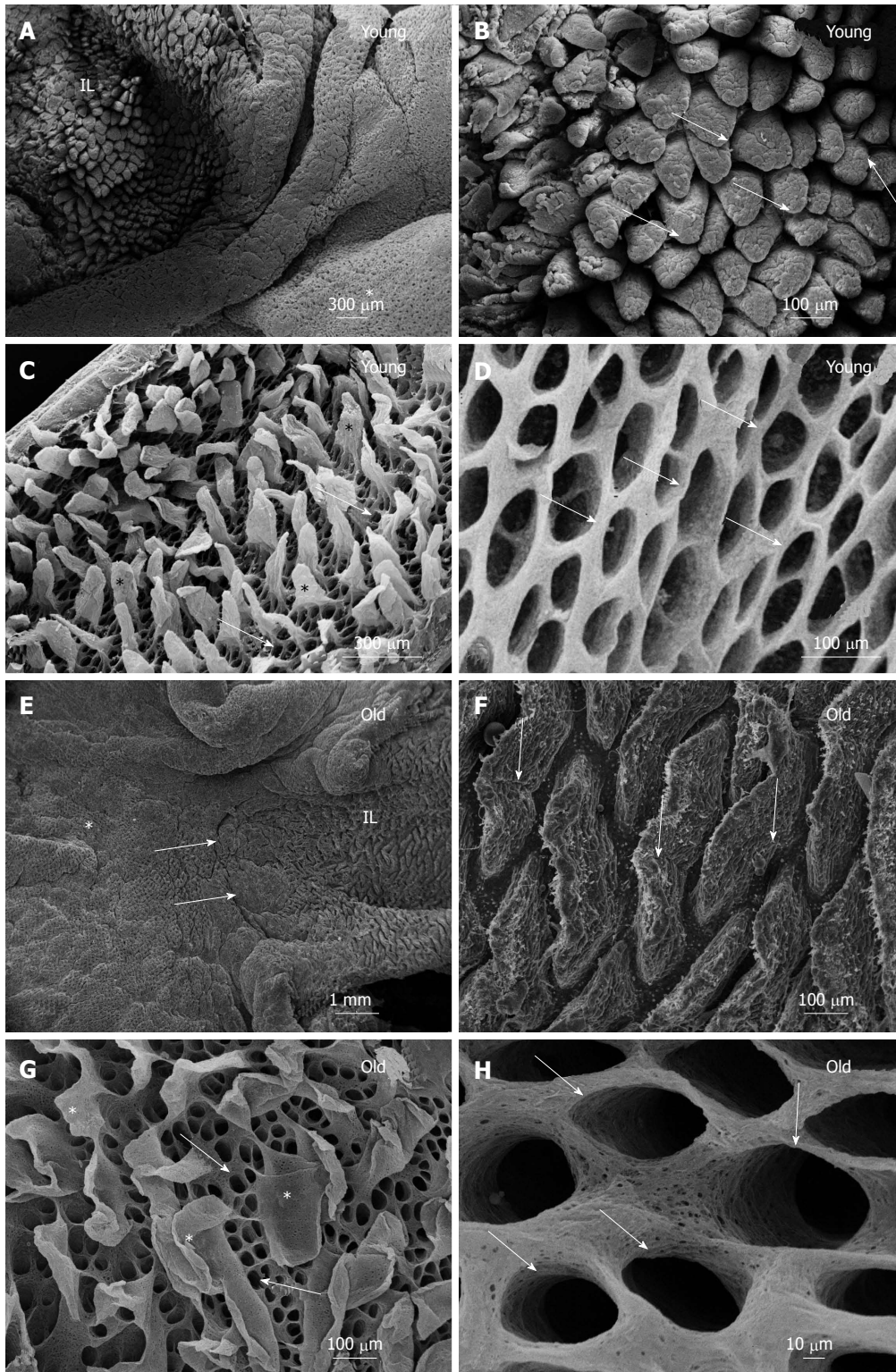


Figure 5 Normal mucosa of the rat ileocecal transition point of the young and elderly groups. A: Normal mucosa of the rat ileocecal transition point of the young showing the ileum (IL) and cecum (*); B: Villi with a normal appearance (arrows); C: Sample treated with NaOH solution. Image showing the connective tissue with different forms of blades (*) and numerous foramens (arrows) in a network of collagen fibers; D: Image showing the interphase cell surface tissue of the cecum, including the foramen; E: Ileum (IL), cecum (*) and ileocecal junction (arrows) of the elderly group; F: The highest increase in ileal villi (arrows); G: Sample treated with NaOH solution. Ileum tissue-shaped blade (*) and numerous foramens as a network of collagen fibrils (arrows). Image showing the foramen cecum surface (arrows).

value of 20876.9 ± 60.4 pixels, a significant decrease of 3.8% ($P < 0.001$) compared to the young group (Figure 6A).

The results for elaunin, which were obtained from samples stained with resorcin-fuchsin (Weigert), showed that the average linear density was $0.00692 \pm 0.00015/$

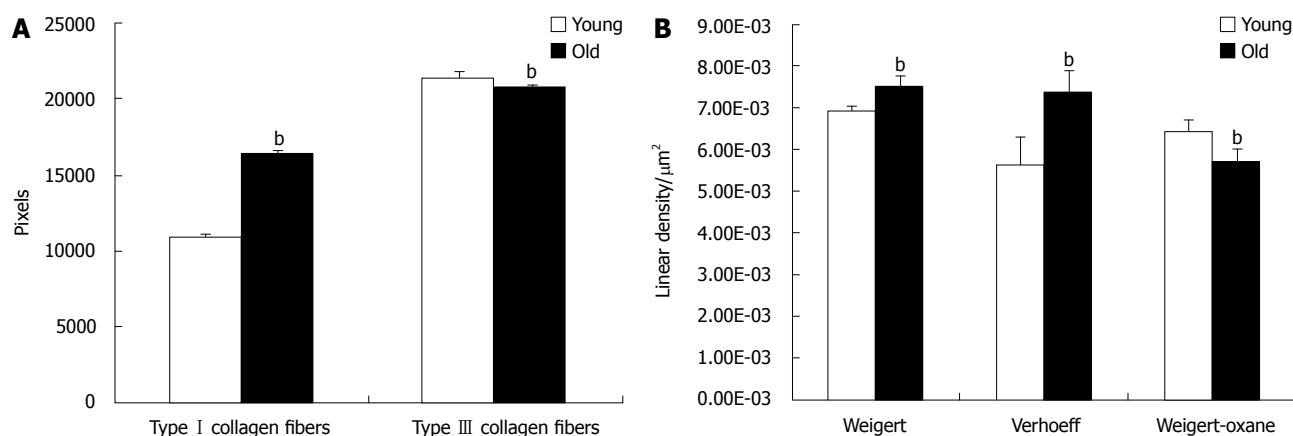


Figure 6 Quantitative analysis. Data related to the means \pm SD of the mean linear (pixels) of type I and type III collagen (A) and data indicating the linear density (LD) expressed as the mean \pm SD of oxytalan elastic fibers (Weigert) (μm^2), elauinin fibers (Verhoeff) (μm^2), and mature elastic fibers (Weigert-Oxane) (μm^2) (B) of the young and old groups. ^b $P < 0.001$.

μm^2 in the young group and $0.0075 \pm 0.00023/\mu\text{m}^2$ in the elderly group, a significant increase of 8.7% in the elderly group compared to the young group ($P < 0.001$; Figure 6B). Regarding the mature elastic fibers that underwent Verhoeff staining, the mean linear density was $0.00564 \pm 0.00067/\mu\text{m}^2$ in the young group and $0.0074 \pm 0.00049/\mu\text{m}^2$ in the elderly group, a significant increase of 31.2% ($P < 0.001$) in the elderly group compared to the young group (Figure 6B). After oxytalan, resorcin, and fuchsin staining (Weigert) and after oxidation with a 1% aqueous solution of oxone, the linear density of the fibers in the young group was $0.0064 \pm 0.0067 \mu\text{m}^2$, and that in the elderly group was $0.00575 \pm 0.00027/\mu\text{m}^2$. There was a significant decrease of 10.3% in the elderly group compared to that in the young group ($P = 0.001$; Figure 6B).

DISCUSSION

This study examined and compared the ICJ structures of young and aged rats. Histological analysis revealed that connective tissue fibers and smooth muscle cells were present in the ICJs of both groups. The ileum protruded into the cecum, as reported^[4], who used human ICJ samples and reported that the muscle layers of the ileum and large intestine extended into the ileal papilla. In this study, we observed a thickening and narrowing of the smooth muscle layer in both groups, as reported^[1-4,10].

Histologically, the elderly group exhibited a condensation of the connective tissue between smooth muscle cells in the transition; however, the young group displayed noticeable gaps between the smooth muscle fibers, which likely indicate limited development of collagen fibers. Similar results were described in colons of humans at different ages^[25], their data indicated that the connective tissue was more slender in the young group but had a larger amount of collagen and elastic fibers around the myenteric plexus in older individuals. Our results demonstrated that smooth muscle cells of the ICJ were distributed with three distinct muscle layers,

i.e., two circular muscle layers and a longitudinal layer, as also observed^[10]. In the transition region, there was a thickening of the circular muscle layer, as previously observed^[4].

The present data revealed the presence of NOS in the ICJ. Furness^[26] (2006) emphasized that NOS catalyzes the formation of the NO that is present in the myenteric plexus and neuronal processes in the gut, which acts to relax the muscle fiber function of the gastrointestinal musculature. Previous studies have shown the presence of NOS in the enteric ganglia and muscle fibers under various conditions, such as malnutrition and renutrition^[27,28], ischemia and reperfusion^[29-31] and obesity^[32,33]. The enteric neurons of the ICJ stain positive for NOS, PGP 9.5, and c-kit^[3,4,6,8]. Additionally, neuronal nitric oxide synthase (nNOS) is present in the myenteric, the submucosal ganglia and the IJC muscle layers in horse^[8]. In our study, we noticed the presence of NOS in the muscle layers and neurons in the ICJs of both the young and elderly groups, but we did not observe a difference in the intensity of the stain for HuC/D or NOS between groups. Major losses of enteric neurons occur during aging; these losses have been described in both the myenteric and submucous plexus ganglia of all regions of the gut in several mammalian species^[24,34]. Regarding significant changes in the number of myenteric neurons during aging in several species, including humans^[35,36], previous studies have suggested the possible involvement of the regulation of gastrointestinal functions. Moreover, Hoyle and Saffrey^[37] (2012) stressed that the thickening of the circular muscle layer is due to increased contractility during aging.

In our ultrastructural analysis, both groups had smooth muscle cells with elongated nuclei between these collagen fibers, forming an endomysium of smooth muscle cells. In the group of older animals, the collagen fibers were thicker. The mitochondria in both groups had different shapes and sizes. It should be noted that, in this study, the mitochondria were not quantified, but they appeared to be present in greater quantity in the elderly group. In agreement with observations obtained in this study,

the authors reported that in the submucosal plexus of the small intestine of rats at different ages, mitochondria were present in greater numbers in the elderly group and had a degenerative aspect^[38]. In the process of aging, changes in cell morphology occur that include the presence of pleomorphic mitochondria^[39].

The SEM results in both groups revealed a clear demarcation of the transition region, which was bounded by a line of flat connective tissue between the ileum and the cecum. In the ileum, numerous elongated buds were present, constituting microvilli. The villus morphology seemed to depend on age, exhibiting a sheet form, which has been predominantly reported in children. However, this form is present in adults as fingerlike projections^[40]. Collagen fibers were present on both sides of the cecal ileum, forming the transition surface. In the cecal portion, low laminar-form microvilli were observed, as were numerous forams of regular collagen fibers with interconnected formats.

Our results showed that the type I collagen fibers in the elderly group were more bulky and appeared thicker; they were classified as mature collagen. These fibers were resistant to traction and tension, giving strength to the tissue. In contrast, in the young group, we noticed a predominance of type III collagen fibers, which were thinner and were characterized as immature collagen, which produces flexibility in the tissue. In accordance with the results observed^[38], the elderly group exhibited a replacement of type III collagen with type I collagen around the submucosal plexus of the jejunum and ileum compared to the young group. This finding suggests that changes in the distribution of collagen fibers could damage the function of the submucosal ganglia. Also, authors reported an increase in the number of collagen fibers in the aorta of aged mice^[41]. In addition, authors showed that aging favored an increase in the diameter of collagen fibrils^[42], in agreement with our findings. Additionally, both collagen and elastic system fibers were more numerous in the enteric ganglia from the old subjects^[25]. Changes in the distribution pattern of collagen fibers in the ICJ can lead to intestinal disorders, such as decreased motility and changes in the retrograde return of feces, which consequently leads to the inflammation of the ileal mucosa. Therefore, the replacement of the collagen in the ICJ is not beneficial to the operating mechanism of the intestinal segment.

During aging, changes occur in the architecture of the collagen fibers, compromising the biomechanical and biochemical properties of tissues due to an accumulation of advanced glycation end-products (AGEs)^[43]. The authors also suggested that aging structurally changes the collagen monomer, which greatly affects both the fibrillogenesis process and the architecture of the collagen fibers.

Additionally, our results reveal that elastic fibers were identified by staining with Weigert oxone, Weigert and Verhoeff, indicating that three types of the elastic system fibers were present along the smooth muscles of the

ICJ. The linear density analysis revealed that oxytalan fibers were in greater quantity in the young group and were diminished in the elderly group. Similar results in the gastroduodenal junctions of young and old animals were reported^[21]. In the present work, the linear density of elaunin fibers and elastic fibers was increased in the elderly group compared to the young group, and oxytalan fibers was decreased in the elderly group compared to the young group. Similar results were described by^[44,45], who found that during aging, there was a decrease in oxytalan fibers and an increase mature elaunin and elastic fibers. Furthermore, elastin was thicker and more fragmented in older people, and there was a greater deposition of calcium in the amorphous material. In a study of aging cerebral meninges, reported decreases and increases in the oxytalan fiber contents of mature elaunin and elastic fibers, respectively^[46]. In studies on the vas deferens reinforced these findings, stating that there was an increase in elastin during aging^[47]. Moreover, study of the interspinous ligament during aging found the disappearance of oxytalan fibers^[48], which is in agreement with our results. In addition to our study, authors suggested that aging is accompanied by a significant and progressive reduction in oxytalan fibers and significant increases in mature elaunin and elastic fibers in the interfoveolar ligament^[44].

Elaunin fibers play an intermediary role between oxytalan fibers and mature elastic fibers, providing functional adaptation in different tissues. Based on the results, the function of the ICJ appears to change with aging, which is associated with changes in the patterns of distribution of collagen and elastic fibers, resulting in increased tensile strength and firmness, but decreased elasticity. With the decrease in the ICJ, oxytalan fibers can lose complacency, becoming less flexible, looser and less able to retreat. Moreover, increased amounts of mature elaunin and elastic fibers are present. We suggest that, with the gradual reduction of the elastic components and replacement of the collagen types in the fibers, ICJ function loss occurs due to the loss of elasticity and the resultant decreased distensibility.

Finally, we highlight the importance of the results of our morphoquantitative analysis of changes in the connective tissue of the ICJ in young and elderly groups, which revealed changes in the patterns of distribution of collagen and elastic fibers that may lead to a possible decrease in ICJ functionality, which may favor the occurrence of pathological processes. These results do not elucidate all the aspects of ICJ function; additional studies should be conducted in the future.

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COMMENTS

Background

The ileocecal junction (ICJ) has two aspects: A wedge-shaped cavity that progressively narrows the orifice to form the ileum and is bordered by an upper lip and lower lip, joined by front and posterior commissures; and an invagination of the small intestine to the large intestine.

Research frontiers

The neurochemical characterization of the inhibitory neurons of the myenteric plexus, which are immunoreactive to the enzyme nitric oxide synthase, and the staining of the neuronal population were employed to identify immunoreactivity to HuC/D.

Innovations and breakthroughs

The authors highlight the importance of the results of our morphoquantitative analysis of changes in the connective tissue of the ICJ in young and elderly groups, which revealed changes in the patterns of distribution of collagen and elastic fibers that may lead to a possible decrease in ICJ functionality, which may favor the occurrence of pathological processes.

Peer-review

The aim of the paper was to analyze the structural organization of the elastic and collagen fibers in the region of the ileocecal transition in 30 young and old male Wistar rats by using different updating techniques. The study is original, interesting and well conducted.

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Retrospective Cohort Study

Ethnic variations in ulcerative colitis: Experience of an international hospital in Thailand

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Abstract

AIM: To investigate the clinical characteristics, treatment, medication use, and treatment response in patients with ulcerative colitis (UC) across ethnic groups.

METHODS: This study retrospectively analyzed medical records of all 268465 patients who visited the Bumrungrad International Digestive Disease Center during 2005-2010. The demographics, clinical characteristics, medication use, results of investigations, and medical and surgical management for patients with UC were evaluated. Evaluation included sigmoidoscopy and colonoscopy performed in compliance with the American Society of Gastrointestinal Endoscopy practice guidelines. Patient ethnicities were categorized into seven groups: Thai, Oriental, South Asian (SA), Middle Eastern (ME), Caucasian, African, and Hispanic. UC pathological severity was classified into inactive, mild, moderate, and severe. Associations between categorical variables were analyzed using the χ^2 or Fischer's exact test. Associations between categorical and interval variables were analyzed using

Student's t-test and/or analysis of covariance.

RESULTS: UC was diagnosed in 371 of the 268465 patients: male 56.33%; ME 42%, Caucasian 23%, and Thai 19%. Annual incidence of UC was 82 cases per 100000 with wide ethnic variation, ranging from 29 to 206 cases per 100000 in Oriental and ME patients, respectively. Of the patients with UC, 16.71% had severe UC with highest incidence among the patients from ME (20.39%) and lowest among the Caucasian population (11.90%). ME had highest proportion of pancolitis (52.90%), followed by Caucasian (45.35%) and Asian (34.40%). Only 20.93% of Caucasian patients received steroid, compared with 26.40% and 27.10% of Asian and Middle Eastern, respectively ($P = 0.732$). Overall, 13.72% of UC patients did not respond to steroid therapy, with non-significantly higher proportions of non-responders among Asian and Middle Eastern patients (15.22% and 15.04%, respectively) ($P = 0.781$). On average, 5.93% underwent surgical management with ethnic variation, ranging from 0% in African to 18% in SA. Cancer was found in three (Thai, ME, and African) cases (0.82 institution-specific incidence).

CONCLUSION: Incidence, symptom duration, pathological severity, clinical manifestations, medication use, treatment response, need for surgical consultation, and cancer incidence of patients with UC potentially vary by ethnicity.

Key words: Ulcerative colitis; Ethnic groups; Anatomical pathological conditions; Medical tourism; Retrospective studies

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Core tip: Incidence and prevalence of ulcerative colitis have been shown to vary across geographical areas and ethnic groups. Patients from different ethnic origins and/or healthcare systems have been managed using the same guidelines for diagnosis and treatment of ulcerative colitis. In this study, comparative analysis of symptom duration, pathological severity, extra-intestinal manifestations, surgical consultation need, medication use, and cancer incidence across ethnic groups were presented. Understanding how these attributes vary by ethnicity is useful for service delivery design, especially in this facility that is responsible for the care of patients from diverse backgrounds.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory condition

of the colon in genetically susceptible individuals exposed to environmental risk factors^[1]; it is also an emerging global disease^[2]. Ethnicity has long been hypothesized as one of the determinants for developing UC based on the varying incidence and prevalence across geographical boundaries^[2-5]. In general, Asian and Middle Eastern populations have a lower incidence of UC than Caucasian individuals (6.3 vs 24.3 per 100000 person-years)^[2]. As patients in low-income countries have been diagnosed less frequently than those in richer countries, UC has been believed to be associated with industrialization of nations^[2].

Health care systems in each country may play significant roles in the diagnosis and management of UC. Although existing epidemiological data are useful for design of health service delivery within a country, these data might not be adequate for a healthcare institution that provides medical services in one setting to patients from various origins. Although developed countries have a higher incidence of UC, the effective health care systems have better clinical outcomes than that of less developed ones. In contrast, the epidemiologic findings are more likely to be affected by the genetics of the population than by the health care system.

Better understanding of the clinical course of UC can help to guide clinical decisions. Patients are symptomatic for varying lengths of time before the definitive diagnosis is made and their symptoms vary in severity at presentation. To accurately diagnose the disease properly and avoid disease progression due to delay in diagnosis, the patient should be evaluated by endoscopy with biopsy. Distribution of the lesions could guide medication choices and routes. A patient suffering pancolitis for longer period of time (*i.e.*, more than 10 years) has an increased risk of cancer and therefore should be re-assessed with colonoscopy at optimal time intervals. Recommended indications for surgical management have recently been updated but still rely on clinical judgment^[6].

As a large private hospital for medical tourism in Asia, Bumrungrad International Hospital (BIH) serves more than one million patients from at least 190 countries annually. This allows comparative analyses across ethnic groups. This study compared clinical characteristics including incidence and severity, medication use, treatment response, surgical consultation need, and cancer incidence across ethnic groups.

MATERIALS AND METHODS

This retrospective study analyzed demographics, clinical characteristics, results of investigations, as well as medical and surgical management information in medical records of all patients who visited the Digestive Disease Center (BIDDC) during 2005-2010. Colonoscopy and sigmoidoscopy were performed in compliance with the American Society of Gastrointestinal Endoscopy (ASGE) practice guidelines. Ulcerative colitis was diagnosed based on clinical grounds and supported by the appropriate findings on total colonoscopy, biopsy, and by negative

Table 1 Ethnicity distribution of ulcerative colitis (2008-2010) and total gastrointestinal patients (2005-2010)

Ethnicity	2005	2006	2007	2008	2009	2010	Total	%Total	UC	UC	Annual Incidence
Thai	18820	20093	20256	23423	24224	23456	130272	49%	69	19%	32
Oriental	5333	6737	7060	8237	8614	9614	45595	17%	23	6%	29
South Asian	2329	2206	2003	2344	2566	2935	14383	5%	33	9%	140
Middle Eastern	4567	5831	6418	7828	7726	9483	41853	16%	155	42%	206
Caucasian	4088	4712	5264	5209	5658	5572	30503	11%	86	23%	174
African	488	598	836	1050	1287	1193	5452	2%	5	1%	47
Hispanic	44	86	60	73	67	77	407	0%	0	0%	0
Total	35669	40263	41897	48164	50142	52330	268465		371		82 ¹

¹Overall annual incidence was calculated from UC cases identified from all patients during 2008-2010 and therefore was not equal to the column average annual incidence. UC: Ulcerative colitis.

Table 2 Duration of ulcerative colitis symptoms and severity at presentation by ethnicity

Ethnicity	Duration (mo)	95%CI	Inactive	Mild	Moderate	Severe	Severe%	Extra-intestinal	Surgery
Thai	6.34	2.67-10.00	2	41	16	10	14.49%	2.90%	5.80%
Oriental	13.44	-10.42-37.31	0	9	10	4	17.39%	0.00%	8.70%
South Asian	14.04	3.45-24.63	2	16	8	6	18.75%	15.15%	12.12%
Middle Eastern	18.46	10.63-26.29	8	55	58	31	20.39%	9.68%	3.87%
Caucasian	6.74	0.08-13.41	6	35	33	10	11.90%	9.30%	6.98%
African	34.67	-29.98-99.32	0	3	2	0	0.00%	40.00%	0.00%
Overall	13.06	9.05-17.07	18	159	127	61	16.71%	8.63%	5.93%

stool examination for infectious causes. All patients received total colonoscopy to confirm the distribution of the colitis. The Montreal classification was used to classify severity of the disease.

Patient ethnicities were arbitrarily categorized into seven groups: Thai, Oriental, South Asian, Middle Eastern, Caucasian, African, and Hispanic. With sample size limitation, some analyses were done using the patients in three major ethnic groups: Asian (Thai, Oriental, South Asian), Middle Eastern, and Caucasian. UC severity was classified based on pathological findings into inactive, mild, moderate, and severe using standard, well-accepted published criteria. The need for surgical consultation was based on the content of relevant operative note in medical record; only colon-related surgeries (*i.e.*, partial colectomy and total procto-colectomy, colostomy, and ileo-anal pouch) were included. A patient with clinical response to high-dose glucocorticoids (prednisone 40 to 60 mg/d or equivalent) within 30 d for oral therapy or 7 to 10 d for intravenous therapy was classified as steroid responsive. Steroid dependence was defined if glucocorticoids cannot be tapered to less than 10 mg/d within three months of starting steroids, without recurrent disease, or if relapse occurs within 3 mo of stopping glucocorticoids. A patient without a meaningful clinical response to glucocorticoids up to doses of prednisone 40 to 60 mg/d (or equivalent) within 30 d for oral therapy or 7 to 10 d for intravenous therapy was classified as steroid refractory.

Descriptive statistics were used where appropriate. Association between categorical variables was analyzed using χ^2 test or Fischer's exact test. Association between categorical and interval variables was analyzed using Student's *t*-test and/or analysis of covariance where

appropriate. The statistical analysis of this study was performed by the corresponding author who had formal biostatistics training as part of his doctoral education. This study was approved by Bumrungrad International Institutional Review Board (BI/IRB No.146-09-11).

RESULTS

Of 268465 individuals who visited BIDDG during 2005-2010, half were Thai (49%) (Table 1). The distribution of ethnicity of patients visiting the BIDDG was slightly different from hospital patient ethnic profiles (Thai: Non-Thai = 60:40). UC was diagnosed in 371 patients (Male 56.33%), 42% of which were Middle Eastern, 23% Caucasian and 19% Thai. Based on 2008-2010 data, overall annual facility-specific incidence of UC was estimated to be 82 cases per 100000 with wide ethnic variation, ranging from 29 to 206 cases per 100000 in Oriental and Middle Eastern patients, respectively.

Eighty-one percent of the patients presented with no more than one year of symptoms. Patients experienced UC symptoms for a mean of 13.06 mo (95%CI: 9.05-17.07) before their first visit to BIDDG (Table 2). Thai and Caucasian patients presented with a mean of 6.34 and 6.74 mo of UC symptoms, respectively. Middle Eastern patients had symptoms for more than 18 mo on average at presentation.

Overall, 16.71% of patients had severe UC with highest incidence among Middle Eastern patients (20.39%) and lowest among Caucasian (11.90%). Extra-intestinal manifestations were found in 8.63% of the patients with great ethnic variation (40% African vs 0% Oriental Non-Thai) (Tables 2 and 3). On average, 5.93% of the patients

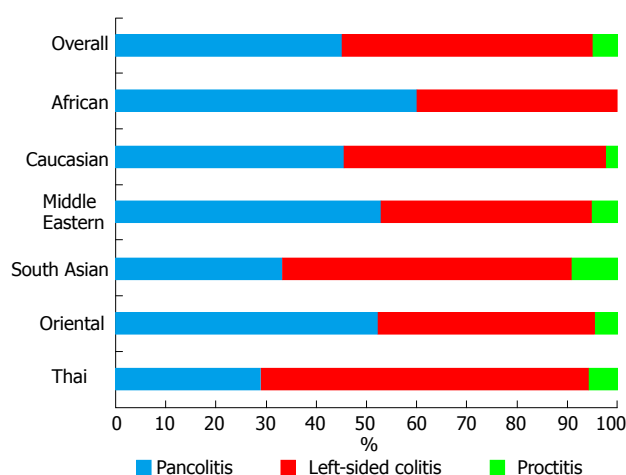


Figure 1 Distribution of ulcerative colitis across ethnic groups.

underwent surgical management with ethnic variation, ranging from 0% in African to 18% in South Asian patients (Table 2).

Pancolitis, left-sided colitis, and proctitis were identified in 45.02%, 50.13%, and 4.85%, respectively (Figure 1). Thai patients had significantly higher proportion of left-sided colitis (65.22%) than the other ethnic origins ($P = 0.005$). When compared across major ethnic groups, Middle Eastern patients had highest prevalence of pancolitis (52.90%), followed by Caucasian (45.35%) and Asian (34.40%) patients ($P = 0.021$).

Only 20.93% of Caucasian patients received steroid, compared with 26.40% and 27.10% of Asian and Middle Eastern, respectively ($P = 0.732$). Overall, 13.72% of UC patients did not respond to steroid therapy, with non-significantly higher proportions of non-responders among Asian and Middle Eastern patients (15.22% and 15.04%, respectively) ($P = 0.781$). Of 277 cases that received thiopurine, 8 patients were non-responders (19.05%), including 5 Middle Eastern patients. Caucasians were relatively the best responders to both steroids and thiopurine.

Cancer was found in three individuals (one Thai, one Middle Eastern, and one African) out of 366 cases, resulting in 0.82 institution-specific incidence. Low and high grade dysplasia was found in 2 and 1 cases, respectively.

DISCUSSION

Although existing incidence and prevalence of UC have been showed to vary across geographical areas^[2,4] and ethnic groups^[5], evidence from our study provided more data on the Middle Eastern patients and also suggested the potential variation of many other aspects (duration of UC symptoms, severity, distribution, and response to medications) across patients of differing ethnic origins. These attributes are useful for service delivery design, especially in this facility that is responsible for the care of patients from diverse backgrounds.

Based on the clinical findings, we hypothesized that there is potential association between symptom duration

Table 3 Extra-intestinal manifestations

Organs	Manifestations (No.)
Skin	Psoriasis (4)
	Erythema nodosum (1)
	Pyoderma gangrenosum (1)
	Dermatitis (1)
Musculoskeletal	Arthritis (4)
	Sacroilitis (1)
	Osteoporosis (4)
	Sclerosing cholangitis (4)
Liver	Oropharyngeal ulcer (2)
Miscellaneous	

and disease severity at presentation. UC has been a disease that progresses over time; hence, earlier and more aggressive management might be needed^[7]. As Middle Eastern patients had longer duration of symptoms and more severity at presentation than Thai and Caucasian, we observed that the “progressive” nature might be different across ethnicities and the degree of clinical management should therefore be different. Further study is still required to prove this concept, however.

Different initial anatomic locations of inflammation present with various clinical patterns that have different prognoses and different rates of complications which could lead to the need for surgery^[8]. The variation of anatomic distribution of the UC across ethnic groups suggested an association between anatomic location and patient ethnicity. We therefore propose that the association between anatomic location and clinical outcomes of UC could be confounded by patient ethnicity^[9].

Assuming comparably high socio-economic status of our patients, the Thai population had two times lower incidence of pancolitis than Oriental. This finding is useful for both clinicians and patients to choose “optimal” investigation when expense, invasiveness, and yield are of concern. That is, a Thai patient who had mild-to-moderate left-sided colitis from initial colonoscopy and prefers gentle procedure and/or has cost concern might be more likely to get sigmoidoscopy than a Japanese patient with similar conditions for follow-up visits. This is supported by our findings on different anatomic locations of UC across patient ethnic groups presented above. The dynamics of the clinical decision-making process would become more personalized, especially when a unified international standard of care for the procedure is not available.

Our institutional data revealed that Middle Eastern patients had almost twice the incidence of UC as that of Caucasian patients. Based on our informal customer interview, majority of the Middle Eastern patients either could not find a specialized center for inflammatory bowel disease or preferred to travel for care outside their countries. Our clinical practice has taken this into account by tailoring the initial investigations to meet the different needs. For example, although a Thai patient who presents with chronic diarrhea would receive stool examination and culture, a Middle Eastern patient with the exact same condition would also be tested for fecal

calprotectin^[10]. Ideally, tailored clinical services to patients from different origins should be based on the standard guidelines of the countries of origin. In reality, however, clinical practice guidelines are not readily available for all countries and the service delivery design, therefore, must be based on our institutional data.

Findings from our study might also be beneficial for modification of current international standard guidelines^[11,12]. Standard guidelines have been developed based on evidence from studies in specific populations therefore limited generalizability. We propose that each of the components in a guideline can be modified for optimal care for patients from each of the ethnic groups. For example, based on our data, 20.39% and 3.87% of Middle Eastern patients were severe UC and underwent surgical management, respectively. If a patient from this ethnic origin, were diagnosed as having severe UC and asked about his/her probability of surgical need, we would be able to calculate the conditional probability of 18%.

Some limitations of our study should be noted. First, generalization of our institutional incidence data was limited by potential selection bias. However, the main purpose of our study was to customize our medical services to meet relatively different needs of patients from various origins rather than to conclude about UC incidence of an ethnic origin. Second, anatomical change over time could be present in some patients but was not adjusted for in our analysis presented here. Some study limitations should be noted. First, the uses of institutional data may either under- or over-estimate the incidence. Although annual incidence of 1.2 to 20.3 cases per 100000 persons have been reported^[4], our institution-specific annual incidence of 82 per 100000 populations was much higher with great variation across ethnic origins. The main objective of this study was not to present population-based epidemiological data; the incidence data presented here therefore do not represent an ethnic group as a whole. Current literature on this topic has been dominated by Caucasian data whereas other ethnicities are less well represented. Our institution is a rare setting that serves patients from many geographic origins with significant ethnic variation. Although wide variation of the patients' country of origin existed, we do not have differential selection of patients.

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COMMENTS

Background

Ulcerative colitis (UC) is a chronic inflammatory condition of the colon in genetically susceptible individuals exposed to environmental risk factors. Incidence and prevalence of ulcerative colitis vary across geographical areas

and ethnic groups. In an era of globalization and medical tourism, a healthcare institution is more likely to provide care to patients of differing ethnic origins.

Research frontiers

In general, Asian and Middle Eastern populations have a lower incidence of UC than Caucasian individuals. Patients from different ethnic origins and/or healthcare systems have been managed using the same guidelines for diagnosis and treatment of UC.

Innovations and breakthroughs

In this study, comparative analysis of symptom duration, pathological severity, extra-intestinal manifestations, surgical consultation need, medication use, and cancer incidence across ethnic groups were presented.

Applications

Understanding how these attributes vary by ethnicity is useful for service delivery design, especially in this facility that is responsible for the care of patients from diverse backgrounds.

Peer-review

In this paper, the authors conducted a retrospective single-center study to investigate the clinical characteristics, treatment, medication use, and treatment response of patients with UC in Thailand. It is interesting that the results showed ethnic differences in severity, distribution, and response to treatments for UC.

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Retrospective Study

Incidence of leukopenia after intraperitoneal vs combined intravenous/intraperitoneal chemotherapy in pseudomyxoma peritonei

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Author contributions: Horvath P designed and performed the research and wrote the paper; Beckert S performed the statistical analysis and supervised the report; Struller F contributed to the research; Königsrainer A supervised the report; Königsrainer I interpreted the data, supervised the report and made the final revision of the paper.

Institutional review board statement: We confirm that retrospective data collection and dealing with personal data was conducted in accordance to the guidelines of the local ethics committee.

Informed consent statement: We confirm that all patients gave written or oral informed consent prior to their inclusion.

Conflict-of-interest statement: We declare that we have no conflict of interest.

Data sharing statement: No additional data are available.

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Abstract

AIM: To investigate the clinical impact of post-hyperthermic intraperitoneal chemotherapy (HIPEC) leukopenia, intraperitoneal and combined intravenous/intraperitoneal drug administrations were compared.

METHODS: Two patient cohorts were retrospectively analyzed regarding the incidence of postoperative leukopenia. The first cohort ($n = 32$) received Mitomycin C (MMC)-based HIPEC intraperitoneally (35 mg/m² for 90 min) and the second cohort ($n = 10$) received a bi-directional therapy consisting of oxaliplatin (OX) (300 mg/m² for 30 min) intraperitoneally and 5-fluorouracil (5-FU) 400 mg/m² plus folinic acid 20 mg/m² intravenously. The following data were collected retrospectively: Age, sex, length of operation, length of hospital stay, amount of resection including extent of peritonectomy, peritoneal cancer index, CC (completeness of cytoreduction)-status and leukocyte-count before cytoreductive surgery (CRS) and HIPEC, on days 3, 7 and 14 after CRS and HIPEC. HIPEC leukopenia was defined as < 4000 cells/m³.

RESULTS: Leukopenia occurred statistically more often in the MMC than in the OX/5-FU-group (10/32 vs 0/10; $P = 0.042$). Leukopenia set-on was on day 7 after CRS and MMC-HIPEC and lasted for two to three days. Three patients (33%) required medical treatment. Patients affected by leukopenia were predominantly female (7/10 patients) and older than 50 years (8/10 patients). The

length of hospital stay tended to be higher in the MMC-group without reaching statistical significance (22.5 ± 11 vs 16.5 ± 3.5 d). Length of operation ($08:54 \pm 01:44$ vs $09:48 \pm 02:28$ h) were comparable between patients with and without postoperative leukopenia. Prior history of systemic chemotherapy did not trigger post-HIPEC leukopenia. Occurrence of leukopenia did not trigger surgical site infections, intraabdominal abscess formations, hospital-acquired pneumonia or anastomotic insufficiencies.

CONCLUSION: Surgeons must be aware that there is a higher incidence of postoperative leukopenia in MMC-based HIPEC protocols primarily affecting females and older patients.

Key words: Pseudomyxoma peritonei; Mitomycin C; Oxaliplatin; Hyperthermic intraperitoneal chemotherapy; Postoperative leukopenia

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Core tip: Cyto-reductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) are considered the therapy of choice for patients with pseudomyxoma peritonei. Nevertheless this treatment is a major undertaking associated with elevated morbidity. The occurrence of postoperative leukopenia can deteriorate the patient's outcome by triggering complications like anastomotic insufficiencies or intraabdominal abscess formations so that surgeons must be aware that special patient subsets (primarily older patients and females) exist that are at a higher risk for developing post-HIPEC leukopenia.

Horvath P, Beckert S, Struller F, Königsrainer A, Königsrainer I. Incidence of leukopenia after intraperitoneal vs combined intravenous/intraperitoneal chemotherapy in pseudomyxoma peritonei. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 434-439 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v7/i3/434.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v7.i3.434>

INTRODUCTION

Pseudomyxoma peritonei (PMP) is a rare clinical condition generally associated with perforated appendiceal neoplasms. It is characterized by huge amounts of intra-abdominal gelatinous fluid collections accompanied by mucinous implants on the peritoneum. Preferred areas for these implants are areas of reduced peristaltic movement such as the ileocecal region, sigmoid colon and ligament of Treitz. Despite controversy regarding the pathological classification, PMP is nowadays classified as low-grade or high-grade disease.

Cyto-reductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) are the treatment of choice in patients with PMP, creating 5-year-survival

rates of 62% to 100% for low-grade, and 0% to 65% for high-grade PMP^[1]. Also for many other gastrointestinal and gynecological tumors this dual-approach was able to achieve a survival benefit^[1]. A part from its clinical benefits, morbidity rates after CRS and HIPEC remain high (up to 70%). Reasons are long operations, multivisceral resections including stripping of peritoneum thus creating large wound areas and the side-effects of HIPEC itself. Frequently used chemotherapeutic agents for HIPEC in PMP are Mitomycin C (MMC) und oxaliplatin (OX). These two combine beneficial properties for intraperitoneal administration. A high molecular weight resulting in decelerated systemic absorption and prolonged local toxicity makes these agents attractive for intraabdominal administration. Furthermore, enhanced toxicity is achieved by hyperthermia of the dialysate.

Systemic absorption of the chemotherapeutic agents and consecutive leukopenia can account for a variety of postoperative complications and might depend on the agent used and partly on the amount of stripped peritoneum, leading to a larger area of exposed sub-peritoneal veins, thus facilitating systemic absorption.

This study investigates the incidence of postoperative leukopenia in patients either treated with MMC only intraperitoneally or with OX/5-fluorouracil (5-FU) intravenously/intraperitoneally.

MATERIALS AND METHODS

From 2007 to 2015, 42 patients diagnosed with PMP originating from mucinous appendiceal tumors were included. All patients gave informed consent prior to their inclusion. Thirty-two patients were treated with an MMC-based HIPEC protocol (35 mg/m^2) for 90 min in an open-closed technique at 42.5°C as described elsewhere^[2]. Ten patients were treated according to an OX-based HIPEC protocol (300 mg/m^2) for 30 min in the same way and additionally 5-FU (400 mg/m^2) and folinic acid (20 mg/m^2) were administered intravenously prior to HIPEC.

The following data were collected retrospectively: age, sex, length of operation, length of hospital stay, amount of resection including extent of peritonectomy, peritoneal cancer index (PCI), completeness of cytoreduction (CC)-status and leukocyte-count before CRS and HIPEC, on days 3, 7 and 14 after CRS and HIPEC. HIPEC leukopenia was defined as $< 4000 \text{ cells/m}^3$.

All complications were graded using the Clavien-Dindo classification of surgical complications^[2].

Prior to CRS and HIPEC all patients underwent clinical examinations and blood tests to guarantee adequate performance status and computed tomography was performed to rule out extraabdominal disease.

CRS was conducted according to a standardized procedure consisting of midline laparotomy and screening of the abdomen for peritoneal tumor implants in order to define the PCI-score as described by Königsrainer *et al.*^[3]. After maximal cytoreduction, achieving a CC-0/1 status, HIPEC was administered. All anastomoses were

Table 1 Clinicopathological characteristics of patients with Mitomycin C and oxaliplatin/5-fluorouracil treatment

	MMC	OX/5-FU
No.	32	10
Gender		
Male (<i>n</i> = 20; 47%)	15	5
Female (<i>n</i> = 22; 53%)	17	5
Age (yr)	50 ± 14	54 ± 10
Length of operation (h)	09:41 ± 2:27	09:07 ± 1:44
CC-status		
CC-0	21	3
CC-1	11	7
PCI	20 ± 11	25 ± 10
Length of hospital stay (d)	22.5 ± 11	16.5 ± 3.5
Resections		
Total peritonectomy	30	10
Splenectomy	11	3
Omentectomy	25	3
Cholecystectomy	19	7
Colon	18	3
Small bowel	4	1
Anastomotic insufficiencies	0	0
Leucopenia	10	0
G-CSF treatment	3	0
HAP	2	0
SSI	1	1
PE	2	2
IAA	0	1
UTI	1	0

HAP: Hospital-acquired pneumonia; SSI: Surgical site infections; IAA: Intraabdominal abscess formation; PE: Pleural effusion; UTI: Urinary tract infection; G-CSF: Granulocyte-colony stimulating factor; MMC: Mitomycin C; OX/5-FU: Oxaliplatin/5-fluorouracil.

completed before HIPEC started. Total peritonectomy was defined as complete removal of the parietal peritoneum. In total six tubes were transcutaneously inserted into the abdomen to guarantee high-volume influx and efflux of the dialysate. Temperature was monitored to ensure an influx-temperature of 42.5 °C. After HIPEC and removal of all chemotherapy-containing fluid, the abdomen was lavaged again and then closed.

Statistical analysis

SPSS ver. 12.0 (SPSS Inc. Chicago, IL, United States) was used for statistical analysis and data are written as mean ± SD. A *P* < 0.05 was considered statistically significant when using the chi-square test and the *t*-test. The χ^2 test was used for nominal variables and the *t*-test for continuous variables.

RESULTS

In total 40 patients diagnosed with PMP underwent CRS and HIPEC. Complete data were available on all patients. Table 1 shows patients and treatment characteristics. Of the patients 53 (53%) were female. Mean age and PCI-score were comparable in the MMC- and OX/5-FU-groups without being statistically significant (50 ± 14 years vs 54 ± 10 years; PCI 20 ± 11 vs 25 ± 10). In 58% a CC-0 status and in 42% a

CC-1 status was achieved. Three patients in the MMC- and one patient in the OX/5-FU-group received systemic chemotherapy prior to CRS and HIPEC. These 4 patients underwent a FOLFOX regimen. Only one patient (MMC-group) with prior history of systemic chemotherapy developed postoperative leukopenia. The length of hospital stay tended to be greater in the MMC-group without reaching statistical significance (22.5 ± 11 d vs 16.5 ± 3.5 d). Total peritonectomy was conducted in 30 of 32 patients in the MMC-group and in all patients in the OX/5-FU-group. Splenectomy was necessary due to tumor involvement in 14 of 40 patients (12 in the MMC-group and 2 in the OX/5-FU-group). All anastomoses were performed prior to HIPEC and no anastomotic insufficiencies occurred. No statistically significant differences were observed between the MMC- and the OX/5-FU-group regarding occurrence of hospital-acquired pneumonia (HAP) (2 vs 0 patients), pleural effusion (PE) (2 vs 2 patients) surgical site infections (SSI) (one vs one patient), intraabdominal abscess formations (IAA) (0 vs 1 patient) and urinary tract infections (UTI) (1 vs 0 patient). Patients with HAP required antibiotic treatment (Clavien-Dindo grade II). Patients with SSI required bed-side wound treatment but no antibiotic treatment (Clavien-Dindo grade I). Two of four patients with PE needed pleural drainage (Clavien-Dindo grade IIIa). One patient with IAA required antibiotic treatment (Clavien-Dindo grade II) and one patient with a UTI also required antibiotic treatment (Clavien-Dindo grade II).

Leukopenia occurred in ten of 32 patients (31%) in the MMC-group. Of these ten patients with MMC-associated leukopenia seven were female. No patient in the OX/5-FU-group developed postoperative leukopenia. Figure 1 shows the postoperative course of the leukocyte count. Leukopenia occurred in all patients between day 6 and day 7 after CRS and HIPEC was administered for 2 to 3 d. Three patients required medical treatment with filgrastim till leukocyte counts were in normal range. Length of operation (08:54 ± 01:44 h vs 09:48 ± 02:28 h) and of hospital stay (25 ± 12 d vs 20 ± 10 d) were comparable and not statistically significant in patients with or without postoperative leukopenia (Table 2). Splenectomy was necessary in two (20%) of 10 patients with leukopenia and in twelve (40%) of 32 patients without leukopenia. Of 10 patients with leukopenia eight were older than 50 years which was statistically significant (60 ± 16 years vs 48.5 ± 11 years; *P* = 0.01).

Occurrence of postoperative leukopenia did not trigger surgical site infections, intraabdominal abscess formations, anastomotic insufficiencies or urinary tract infections.

DISCUSSION

PMP is a rare clinical condition arising in the vast majority of cases from ruptured appendiceal malignancies. In the past debulking surgery accompanied by systemic

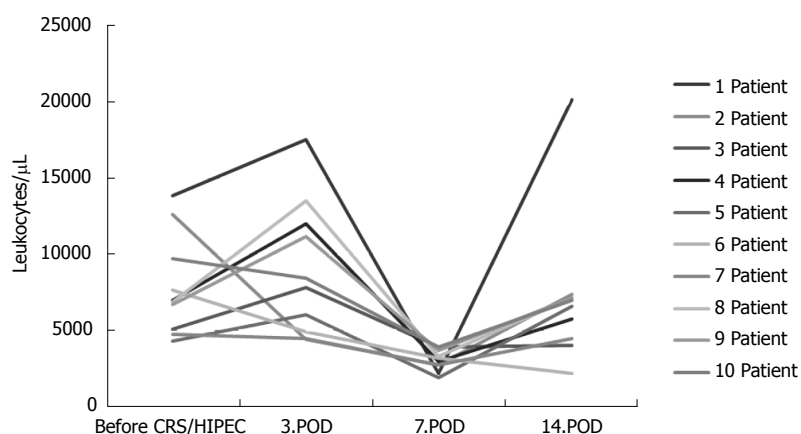


Figure 1 Course of leukocyte count/ μL . POD: Postoperative day; CRS: Cytoreductive surgery; HIPEC: Post-hyperthermic intraperitoneal chemotherapy.

Table 2 Clinicopathological characteristics of patients with and without leukopenia

	Leukopenia	No leukopenia
No.	10	32
Gender		
Male	3	16
Female	7	16
Age (yr)	60 ± 16	48.5 ± 11
MMC	10	22
OX/5-FU	0	10
Splenectomy	2	12
Hospital stay (d)	25 ± 12	20 ± 10
Length of operation (h)	$08:54 \pm 01:44$	$09:48 \pm 02:28$
HAP	2	0
SSI	1	1
PE	2	2
IAA	0	1
UTI	1	0

HAP: Hospital-acquired pneumonia; SSI: Surgical site infections; IAA: Intraabdominal abscess formation; PE: Pleural effusion; UTI: Urinary tract infection; MMC: Mitomycin C; OX/5-FU: Oxaliplatin/5-fluorouracil.

chemotherapy was applied. Due to the efforts of Sugarbaker PH this aforementioned strategy has been widely abandoned and superseded by a dual-approach therapy consisting of CRS and HIPEC, resulting in a 15-year survival rate of up to 60%^[1,4-8]. Furthermore Chua *et al.*^[1] were able to demonstrate an impressive median progression-free survival rate of 8.2 years after CRS and HIPEC thus once more emphasizing the efficacy of this dual-approach in disease control. Nevertheless, every surgeon dealing with CRS and HIPEC has to be aware that this therapy is a major undertaking accompanied by long operating times and multivisceral resections and is thus associated with high morbidity rates of up to 70% and mortality rates of up to 11%^[9-15]. MMC and OX are the most frequently used intraperitoneally administered drugs in PMP-patients. Both are alkylating chemotherapeutics, interfering with DNA and DNA-synthesis without being cell-cycle dependent^[16]. In order to potentiate the activity of the intraperitoneally administered OX, patients are given intravenous 5-FU and folinic acid 30 min prior to HIPEC. Because of a

pH incompatibility 5-FU cannot be mixed with OX for intraperitoneal use^[17,18]. Despite its advantageous pharmacokinetic properties MMC-induced leukopenia due to bone marrow toxicity after HIPEC is a known and frequently encountered side-effect of this treatment^[19]. In our study the incidence of MMP-induced leukopenia was 31% ($n = 10/32$ patients), which was lower than in other reports in the literature^[20,21]. None of the patients in the OX/5-FU-group developed postoperative leukopenia. This might be related to the different route of drug elimination. Oxaliplatin is predominantly excreted *via* urine, by tissue-binding and by renal elimination, whereas MMC undergoes hepatic metabolism which might contribute to systemic accumulation thus promoting occurrence of leukopenia. In accordance with other studies^[20,21], prior systemic chemotherapy was not associated with a higher risk of leukopenia. Only four patients (10%) in our study population were not chemo-naïve and one patient, having received MMC-HIPEC, developed postoperative leukopenia. Patients who received systemic chemotherapy prior to CRS and HIPEC and had a history of chemotherapy-associated leukopenia are at greater risk for further episodes of leukopenia. This is the main reason why some authors recommend a dose reduction in MMC-HIPEC in this special patient subgroup^[21]. In our analysis female sex and age (> 50 years) were associated with the occurrence of MMC-induced leukopenia. The phenomenon of female gender being associated with a greater risk for chemotherapy-induced leukopenia has been reported previously, but its reasons are still unknown. Bécouarn *et al.*^[17] provide an explanation for the association between leukopenia and female gender. The authors speculate that women harbor a relatively large surface area of the peritoneum combined with a smaller plasma volume as compared with men of equal weight^[22]. As it is a body-surface-area (BSA)-based MMC dose, women with equal weight and a smaller plasma volume have higher MMC-plasma concentrations than do males in the case of equal absorption, thus explaining a higher cytotoxic effect of MMC. Due to these circumstances some HIPEC-centers introduced lower doses of chemotherapeutics in HIPEC for female patients^[23].

Our data show that splenectomy was not associated

with a higher incidence of MMC-induced leukopenia. Only two (20%) of ten patients suffered from leukopenia after splenectomy whereas twelve (40%) of 30 patients without postoperative leukopenia were splenectomized. These data might suggest a protective effect of splenectomy on leukopenia due to post-splenectomy leukocytosis. As far as this is concerned the literature presents controversial results. Bécouarn *et al*^[17] found a higher, although not significant, incidence of neutropenia in the splenectomized patients after MMC-HIPEC, whereas Bidus *et al*^[24] reported also a potentially protective effect of splenectomy on leukopenia in patients receiving adjuvant systemic chemotherapy. The time when chemotherapy was administered could be the decisive variable for these conflicting results.

The role of peritonectomy in the pathophysiology of post-HIPEC leukopenia was negligible in our study because 38 out of 40 patients received total peritonectomy, so that we could not evaluate the definite effect of total peritonectomy on the incidence of post-HIPEC leukopenia.

In our study patients with post-HIPEC leukopenia tended to have a longer hospital stay (25 d vs 20 d) without reaching statistical significance. In accordance with the study by Hompes *et al*^[16] MMC-induced leukopenia neither elevated the risk of postoperative infections and anastomotic insufficiencies nor prolonged the patient's hospital stay. A larger study population might have found a higher global infection risks in patients with MMC-induced leukopenia. Nonetheless, this clinical condition should not be underestimated and especially in females, older patients and patients with a prior history of chemotherapy-induced leukopenia the possibility that a balance between optimal oncological treatment and systemic cytotoxicity, maybe achieved by a dose reduction in HIPEC, should be taken into account.

COMMENTS

Background

Hyperthermic intraperitoneal chemotherapy (HIPEC) followed by complete cytoreductive surgery is the therapy of choice for patients with pseudomyxoma peritonei (PMP). In the vast majority of cases ruptured appendiceal neoplasms are causal for PMP. HIPEC protocols for the treatment of PMP after complete cytoreduction include only intraperitoneal or concomitant intravenous/intraperitoneal drug administration. Mitomycin C (MMC) and oxaliplatin (OX)/fluorouracil (5-FU) are the most frequently used agents. The aim of the study was to find out the incidence of postoperative leukopenia depending on the chemotherapy regimen used.

Research frontiers

The bi-directional therapy consisting of HIPEC and complete cytoreduction is used in the vast majority of patients with PMP. Nevertheless this treatment is associated with high morbidity rates due to long operative times, multivisceral resections and by the HIPEC itself. HIPEC-associated leukopenia can further contribute to postoperative morbidity. The results show that especially in MMC-based HIPEC protocols and in female and in elderly patients post-HIPEC leukopenia can occur.

Innovations and breakthroughs

In this study the author demonstrated that MMC-HIPEC protocols provoke post-

HIPEC leukopenia between day six and seven after operation. Every surgeon dealing with this therapy should be aware of the fact and especially in women and older patients the incidence of post-HIPEC leukopenia is elevated, thus this special patient subset should be even more monitored in the postoperative course. Previous studies reported similar results and some of them also suggested a dose reduction in MMC-HIPEC in women and older patients.

Applications

This study suggests that in women and elderly patients a-priori a dose reduction should be taken into account in order to decreased the incidence of post-HIPEC leukopenia in MMC-protocols.

Terminology

HIPEC: Hyperthermic intraperitoneal chemotherapy: Combined with complete cytoreduction it is the treatment of choice for pseudomyxoma peritonei.

Peer-review

This paper presents an essential and interesting data. In my opinion this is a professional report of an important and currently still discussed in a lack number of papers problematic leukopenia incidence occurring after HIPEC. For me as a person who is working scientifically and clinically on HIPEC method this is a brief but very professional work which is very worthy. The properly presented data and good quality of English are a very strong plus points of this paper.

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Retrospective Study

Increase in colonic diverticular hemorrhage and confounding factors

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Informed consent statement: In this study, we do not necessarily need the individual agreement from a study subject. Because this study is retrospective study and based on the ethical guidelines for medical studies in which human body samples are not used informed consent procedure No.12 was applied. Information about the implementation of this research was presented on Fukuoka University Chikushi Hospital clinical research support center home page.

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Abstract

AIM: To classify changes over time in causes of lower gastrointestinal bleeding (LGIB) and to identify factors associated with changes in the incidence and characteristics of diverticular hemorrhage (DH).

METHODS: A total of 1803 patients underwent colonoscopy for overt LGIB at our hospital from 1995 to 2013. Patients were divided into an early group (EG, 1995-2006, $n = 828$) and a late group (LG, 2007-2013, $n = 975$), and specific diseases were compared between groups. In addition, antithrombotic drug (ATD) use and nonsteroidal anti-inflammatory drug (NSAID) use were compared

between patients with and without DH.

RESULTS: Older patients (≥ 70 years old) and those with colonic DH were more frequent in LG than in EG ($P < 0.01$). Patients using ATDs as well as NSAIDs, male sex, obesity (body mass index ≥ 25 kg/m²), smoking, alcohol drinking, and arteriosclerotic diseases were more frequent in patients with DH than in those without.

CONCLUSION: Incidence of colonic DH seems to increase with aging of the population, and factors involved include use of ATDs and NSAIDs, male sex, obesity, smoking, alcohol drinking, and arteriosclerotic disease. These factors are of value in handling DH patients.

Key words: Lower gastrointestinal bleeding; Colonic diverticular hemorrhage; Increase of incidence; Aging

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Core tip: Colonic diverticular hemorrhage (DH) is the most frequent cause of lower gastrointestinal bleeding. A rapid increase in the incidence of colonic DH has been seen with the aging population. One reason is the widespread adoption of antithrombotic drugs (ATDs) since the early 2000s, based on guidelines to prevent ischemic heart disease and ischemic cerebrovascular disease. DH is more likely in patients who are older, are men, obesity, use nonsteroidal anti-inflammatory drugs or ATDs, and have hypertension and diabetes associated with arteriosclerotic disease. These factors are of value in handling DH patients.

Kinjo K, Matsui T, Hisabe T, Ishihara H, Maki S, Chuman K, Koga A, Ohtsu K, Takatsu N, Hirai F, Yao K, Washio M. Increase in colonic diverticular hemorrhage and confounding factors. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 440-446 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v7/i3/440.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v7.i3.440>

INTRODUCTION

Lower gastrointestinal bleeding (LGIB) is often diagnosed in patients with overt bleeding, positive results for fecal occult blood, abdominal symptoms, and anemia. Colonoscopy is often the first test performed as an approach to the diagnosis and treatment of LGIB. In the United States, endoscopy is recommended "in the early evaluation of severe acute LGIB"^[1].

Use of oral antithrombotic drugs (ATDs) and non-steroidal anti-inflammatory drugs (NSAIDs) is increasing with the aging of the population, and the number of patients with diseases causing LGIB is increasing^[2,3]. In addition, the types of diseases being encountered are also changing. In particular, the prevalence of colonic diverticulum is increasing. The aging population in

Japan, as in Western countries, is showing an increase in diverticulosis^[4,5]. DH is also increasing in Japan. In fact, diverticular hemorrhage (DH) is now one of the most common causes of LGIB in Japan and Western countries^[2,3,6].

The present study examined changes over time in diseases causing LGIB that are associated with aging. In particular, this study sought to identify factors associated with changes in the incidence and patient characteristics of colonic DH.

MATERIALS AND METHODS

Among 42540 patients who underwent colonoscopy at our hospital during the 19-year period between January 1995 and December 2013, this retrospective study included those who underwent colonoscopy for overt LGIB. Our hospital is one of the emergency hospitals in Chikushino city, Fukuoka, Japan.

Our hospital also diagnoses and treats a large number of patients with inflammatory bowel disease (IBD). Patients with IBD (ulcerative colitis, Crohn's disease, Behçet's disease, intestinal tuberculosis) were excluded because of differences from other diseases in terms of diagnosis and treatment. We also excluded patients who developed bleeding after endoscopic treatment (*e.g.*, biopsy, polypectomy), or who experienced bleeding from the small bowel. As a result, this study included 1803 patients, all of whom were Japanese. This study was approved by the institutional review board of Fukuoka University Chikushi Hospital (R15-024) and was conducted in accordance with the Declaration of Helsinki. Also, since this was a retrospective study, the need to obtain informed consent to participate in the study was waived by the review board.

Factors evaluated included sex (male/female ratio) and age ≥ 70 years, Obesity [body mass index (BMI) ≥ 25 kg/m²]. Patients were categorized into 2 groups according to the use of oral ATDs [antiplatelet drugs ($n = 266$): Low-dose aspirin, ticlopidine, clopidogrel, cilostazol, limaprost alfadex, ethyl icosapentate, dipyridamole, sarpogrelate hydrochloride, beraprost sodium, diltazep; or anticoagulants ($n = 58$): Warfarin and dabigatran: No ATDs; ≥ 1 oral ATDs. Use of NSAIDs (loxoprofen, diclofenac, ibuprofen, etodolac, meloxicam, or celecoxib) was also examined. Two-group comparisons included the use or non-use of NSAIDs and the use or non-use of NSAIDs in combination with an antithrombotic drug.

Two-group comparisons for lifestyle factors included smoking or non-smoking and use or non-use of alcohol. We treated current and past smoking as positive for smoking, while only current drinkers were defined as positive for alcohol. Other factors examined other underlying diseases (cerebrovascular disease, ischemic heart disease, hypertension, hyperlipidemia, hyperuricemia, diabetes mellitus, chronic liver disease, and chronic kidney disease). Comorbidity was quantified using the

Table 1 Baseline characteristics of all patients undergoing colonoscopy for overt lower gastrointestinal bleeding

Age, sex and other factors	Overall <i>n</i> = 1803 <i>n</i> (%)
Sex	
Male	913 (50.6)
Female	890 (49.4)
Age, yr	
Mean	59.0 ± 18.6
≥ 70	582 (32.3)
< 70	1221 (67.7)
BMI, kg/m ²	
< 18.5	212 (11.8)
18.5 to < 25	1049 (58.2)
≥ 25	337 (18.7)
Unknown	205 (11.4)
Oral drugs	
ATDs	308 (17.1)
NSAIDs	115 (6.4)
ATDs + NSAIDs	26 (1.4)
Lifestyle habits	
Smoking (current/past)	551 (30.6)
Alcohol drinking (current)	625 (34.7)
Underlying disease	
Cerebrovascular disease	171 (9.5)
Ischemic heart disease	143 (7.9)
Hypertension	658 (36.5)
Hyperlipidemia	355 (19.7)
Hyperuricemia	91 (5.0)
Diabetes	207 (11.5)
Chronic liver disease	106 (5.9)
Chronic kidney disease	43 (2.4)
Charlson Risk Index	
≤ 1	1413 (78.4)
≥ 2	390 (21.6)
Blood transfusion	130 (7.2)

Each set of values represents mean ± SD or *n* (%). BMI: Body mass index; ATDs: Antithrombotic drugs; NSAIDs: Nonsteroidal anti-inflammatory drugs.

Charlson Risk Index (CRI)^[7]. Two groups were compared: CRI ≤ 1; and CRI ≥ 2. Transfusion requiring ≥ 2 units of blood was also examined as a factor.

Diagnosis

Colonoscopy was generally performed on the day of or the day after overt bleeding. Depending on the clinical symptoms and physical examination findings in each patient, colonoscopy was performed without pretreatment, or after pretreatment with an enema or bowel-cleansing agent. Olympus colonoscopes were used (PCF-240AZI, PCF-240AI, CF-240AZI, CF-240ZI, PCF-260AZI, PCF-PQ260I, and CF-260AI; Olympus, Tokyo, Japan). Colonic DH was defined as follows^[8].

Definite colonic DH (*n* = 207): (1) Active bleeding observed from a diverticulum; and (2) Presence of blood clots, erosions, or an exposed vessel near a diverticulum.

Either (1) or (2), absence of blood in the terminal ileum on total colonoscopy, and no other obvious cause of bleeding.

Suspected colonic DH (*n* = 66): Obvious bloody

stools, no blood in the terminal ileum on colonoscopy after bowel preparation, and the only likely cause of bleeding is DH.

Diseases causing LGIB were classified into hemorrhoids, ischemic colitis, DH, advanced colon cancer, early colon cancer/polyps/adenomas, infectious enteritis, angiodysplasia, drug-related enteritis, and no abnormal findings. Rectal ulcers, stercoral ulcers, rectal mucosal prolapse, pneumatosis cystoides intestinalis, enteric endometriosis, submucosal tumors, radiation enteritis, and nonspecific inflammation were categorized as "Others".

Study period

The 1803 patients who underwent colonoscopy for overt LGIB were divided into two groups by time period, with each consisting of about half of the patients. The early group (EG, *n* = 828) was treated from January 1995 to December 2006, and the late group (LG, *n* = 975) was treated from January 2007 to December 2013. Incidence of each disease during these two periods was compared.

Statistical analysis

All statistical analyses were conducted using the Statistical Analysis System package (SAS Institute, Cary, NC). The χ^2 test was used to compare categorical variables between the two groups. Values of *P* < 0.05 were considered statistically significant.

RESULTS

Colonoscopy

A total of 1803 patients underwent colonoscopy for overt LGIB at our hospital between 1995 and 2013. Table 1 summarizes the patient characteristics. Figure 1 shows the number of patients who underwent colonoscopy for overt LGIB each year, required hospitalization, required transfusions, and used oral ATDs and NSAIDs. The number of patients with overt LGIB tended to increase each year.

Comparison of specific diseases in EG and LG

Table 2 summarizes the specific diseases in the 1803 patients who underwent colonoscopy for overt LGIB. In EG, the most common cause of overt LGIB was hemorrhoids in 212 patients (25.6%), followed by ischemic colitis in 143 patients (17.3%), advanced cancer in 80 patients (9.7%), early cancer/adenomas/polyps in 53 patients (6.4%), and colonic DH in 49 patients (5.9%). In LG, the most common cause of overt LGIB was colonic DH in 224 patients (23.0%), followed by hemorrhoids in 220 patients (22.6%), ischemic colitis in 173 patients (17.7%), advanced cancer in 73 patients (7.5%), and early cancer/adenomas/polyps in 58 patients (5.9%).

Compared with LG, EG showed lower frequencies of patients ≥ 70 years old (*P* < 0.01) and bleeding from colonic diverticulum (*P* < 0.01). The number of patients with DH tended to increase each year, with a marked increase after 2003 (Figure 2).

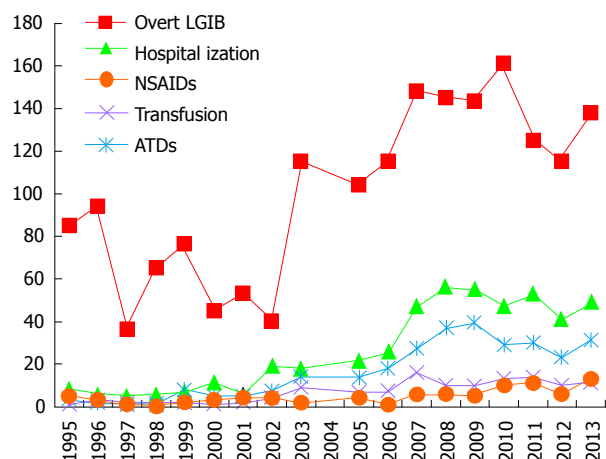


Figure 1 Annual totals and changes over time in number of patients undergoing colonoscopy for overt lower gastrointestinal bleeding, requiring hospitalization, requiring transfusions, and using antithrombotic drugs and nonsteroidal anti-inflammatory drugs (1995 to 2013). The incidence of lower gastrointestinal bleeding (LGIB) started to increase rapidly in 2002-2003, associated with increases in the number of patients hospitalized, receiving blood transfusions, using antithrombotic drugs (ATDs), and using nonsteroidal anti-inflammatory drugs (NSAIDs).

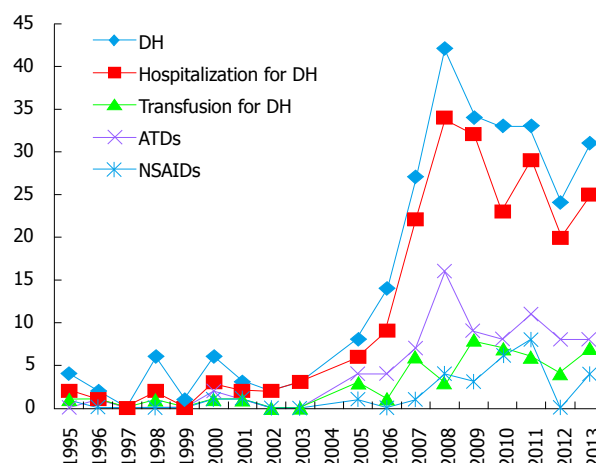


Figure 2 Changes in the number of patients with colonic diverticular hemorrhage: Number of patients requiring hospitalization, requiring transfusion, and using antithrombotic drugs or nonsteroidal anti-inflammatory drugs (1995 to 2013). The number of patients with diverticular hemorrhage (DH) started to increase rapidly in 2003, and peaked in 2008. This was associated with an increase in the number of patients hospitalized, receiving blood transfusions, using antithrombotic drugs (ATDs), and using nonsteroidal anti-inflammatory drugs (NSAIDs).

Comparison of patient characteristics between DH and non-DH patients

As shown in Table 3, compared with non-DH, DH showed higher frequencies of patients ≥ 70 years old ($P < 0.01$) and male patients ($P < 0.01$). Seventy-nine (28.9%) of the 273 DH patients and 229 (15.0%) of the 1530 non-DH patients received ATDs. ATDs were thus more commonly used in DH patients than in non-DH patients ($P < 0.01$). Thirty (11.0%) of the 273 DH patients and 85 (5.6%) of the 1530 non-DH patients took oral NSAIDs. Oral NSAIDs were thus also more commonly used in DH patients than in non-DH patients ($P < 0.01$).

In addition, obesity ($P < 0.01$), smoking ($P < 0.01$), alcohol drinking ($P < 0.01$), hypertension ($P < 0.01$), hyperlipidemia ($P = 0.02$), diabetes ($P < 0.01$), and requirement for blood transfusion ($P < 0.01$) were significantly more frequent in DH patients than in non-DH patients.

DISCUSSION

The incidence of LGIB increased from 20.5/100000 inhabitants/year in the early 1990s^[6] to 87/100000 inhabitants/year in 2010^[3]. In particular, the incidence of LGIB is high in elderly patients (690/100000 inhabitants/year)^[3], and is expected to continue increasing with the aging of the population in Japan. The aging rate (≥ 65 years old) of Japan was 12% in 1990, 20% in 2005. However, the aging rate of 2013 is higher than 25%, and aging advances^[9]. Our study also showed an annual increase in the number of patients undergoing colonoscopy for overt LGIB. One background factor was the approval of low-dose aspirin 100 mg in Japan as an antiplatelet drug in 2000, with initial marketing in 2001. In addition, ATDs such as clopidogrel and warfarin became more

widely used for risk reduction and secondary prevention of cerebral and cardiovascular events in Japan, based on 2002-2009 guidelines from the American College of Cardiology and American Heart Association^[10,11]. ATDs and NSAIDs have been reported as risk factors for LGIB^[2,3]. These background factors, together with the aging of the population, are thought to be key reasons for the rapid increase in the incidence of LGIB.

In particular, the incidence of colonic DH increased markedly from 5.9% in EG to 23.0% in LG, with a pronounced change in the specific diseases causing LGIB. In LG, colonic DH was the most common disorder causing LGIB. Moreover, colonic DH has also recently been reported as the most common cause of LGIB in Japan^[2,12]. Compared to a few decades ago, changes in the aging-associated diseases that cause LGIB have been occurring. In particular, the incidence of colonic DH has increased.

Colonic diverticulosis itself is increasing. During the period from 1960 to the 1980s, Kubo *et al.*^[13] reported that the prevalence of colonic diverticula was only 8.9% among patients who underwent barium enema examination. Since the 1990s, the prevalence of diverticulosis seen on barium enema examination or colonoscopy has increased to 15%-25%^[5,14]. In addition, more than 80% of patients who had diverticulosis were elderly. Diverticulosis is thus thought to increase with aging^[5]. As the absolute number of diverticula in colonic diverticulosis increases with the aging of the population, the risk of DH is increased. Moreover, as mentioned previously, the availability of low-dose aspirin has probably also contributed to the rapid rise in DH starting in 2001.

Of course, bleeding does not occur in all patients with diverticulosis. In fact, bleeding only occurs in 2%-5% of

Table 2 Comparison between early group and late group among patients undergoing colonoscopy for overt lower gastrointestinal bleeding from 1995-2013 *n* (%)

Age, sex and cause/site of bleeding	All patients (1995-2013) <i>n</i> = 1803	EG (1995-2006) <i>n</i> = 828	LG (2007-2013) <i>n</i> = 975	<i>P</i> -value	Adjusted <i>P</i> -value
Old age (≥ 70 yr)	582 (32.3)	196 (23.7)	386 (39.6)	< 0.01	< 0.01
Male	913 (50.6)	419 (56.0)	495 (50.8)	0.94	0.94
External/internal hemorrhoids	432 (24.0)	212 (25.6)	220 (22.6)	0.13	0.46
Ischemic colitis	316 (17.5)	143 (17.3)	173 (17.7)	0.79	0.1
Colonic DH	273 (15.1)	49 (5.9)	224 (23.0)	< 0.01	< 0.01
Advanced colonic cancer	153 (8.5)	80 (9.7)	73 (7.5)	0.1	0.06
Early colon cancer/colon adenomas/polyps	111 (6.2)	53 (6.4)	58 (5.9)	0.69	0.73
Infectious enteritis	68 (3.8)	37 (4.5)	31 (3.2)	0.15	0.4
Angiodysplasia	27 (1.5)	15 (1.8)	12 (1.2)	0.31	0.26
Drug-related enteritis	23 (1.3)	13 (1.6)	10 (1.0)	0.3	0.32
Others	248 (13.8)	137 (16.5)	111 (11.4)	-	-
No abnormal findings	152 (8.4)	89 (10.7)	63 (6.5)	-	-
Total	1803 (100)	828 (100)	975 (100)	-	-

Adjusted *P*-value age- and sex-adjusted *P*-value; No abnormal findings no site identified as origin of bleeding; Each set of values represents number (%). χ^2 test. EG: Early group: 1995-2006; LG: Late group: 2007-2013; DH: Diverticular hemorrhage.

Table 3 Comparison of old age, sex, and use of antithrombotic drugs or nonsteroidal anti-inflammatory drugs between patients with and without diverticular hemorrhage *n* (%)

	DH (<i>n</i> = 273)	Non-DH (<i>n</i> = 1530)	Adjusted <i>P</i> value
Old age (≥ 70 yr)	136 (49.8)	446 (29.2)	< 0.01
Male sex	172 (63.0)	741 (48.4)	< 0.01
Obesity (BMI ≥ 25 kg/m ²)	82 (30.0)	255 (16.7)	< 0.01
ATDs	79 (28.9)	229 (15.0)	< 0.01
NSAIDs	30 (11.0)	85 (5.6)	< 0.01
Smoking (current/past)	124 (45.4)	427 (27.9)	< 0.01
Alcohol drinking (current)	139 (50.9)	486 (31.8)	< 0.01
Cerebrovascular disease	36 (13.2)	134 (8.8)	0.67
Ischemic heart disease	34 (12.5)	109 (7.1)	0.33
Hypertension	178 (65.2)	480 (31.4)	< 0.01
Hyperlipidemia	74 (27.1)	281 (18.4)	< 0.05
Hyperuricemia	20 (7.3)	71 (4.6)	0.78
Diabetes	44 (16.1)	163 (10.7)	< 0.01
Chronic liver disease	13 (4.8)	93 (6.1)	0.1
Chronic kidney disease	8 (2.9)	35 (2.3)	0.94
Charlson Risk Index ≥ 2	79 (28.9)	311 (20.3)	0.67
Blood transfusion	56 (20.5)	74 (4.8)	< 0.01

P value age- and sex-adjusted *P*-value; χ^2 test; Each set of values represents *n* (%). DH: Diverticular hemorrhage; ATDs: Antithrombotic drugs; NSAIDs: Nonsteroidal anti-inflammatory drugs; BMI: body mass index.

patients during the natural history of diverticulosis, and most cases are relatively mild, resolving spontaneously in 75%-93% of cases^[15,16].

In a case-control study in Japan, risk factors for DH included the use of ATDs and NSAIDs, hypertension, diabetes, arteriosclerotic diseases such as ischemic disease mellitus and chronic kidney disease, age ≥ 70 years, obesity^[13,15,17,18]. Our study also found higher rates of use for both ATDs and NSAIDs, higher age and male sex, obesity, smoking, alcohol drinking, and arteriosclerotic diseases in patients with colonic DH compared to those with bleeding from other causes. ATDs and NSAIDs thus represent risk factors for bleeding in diverticulosis. Moreover, higher rates of DH in patients who are older, obesity, smoke, or drink alcohol may be

related to the association between older age, obesity, smoking, and alcohol drinking with arteriosclerotic disease.

Colonic diverticula develop at sites where the vasa recta penetrate the large intestinal wall when intestinal pressure increases. Blood vessels in the diverticula are separated from the bowel lumen only by the mucosa, and are easily injured. With repeated mechanical stimuli, intimal thickening of the vasa recta occurs, often with thinning of the media. These changes can cause segmental weakening of the vasa recta, which may lead to arterial hemorrhage in the bowel lumen^[19]. Thus, because DH represents a form of arterial bleeding, transfusion may be required more often than with bleeding due to other disorders.

Arteriosclerosis also plays a role in the pathogenesis

leading to rupture of blood vessels in diverticula^[20]. However, not all patients with diverticulosis experience bleeding; indeed, most patients remain asymptomatic. Therefore, in addition to arteriosclerosis, other factors increase the fragility of blood vessels in diverticula.

Our study was performed with adjustment for factors including sex and age, so analysis was performed independently of arteriosclerosis. The results identified NSAIDs and ATDs as a significant risk factor, suggesting that NSAIDs and ATDs have synergistic effects on injury to blood vessels in diverticula. Most NSAIDs inhibit prostaglandin synthesis, which disrupts the microcirculation and inhibits platelet aggregation. As a result, NSAIDs can lead to bleeding in patients with diverticulosis^[17]. DH in Japan is more common in men, whereas in Western countries, DH occurs equally in men and women^[21]. These findings suggest ethnic differences, but the exact factors involved are not yet well understood. DH in Japan is more common in men, because it may be one of the reasons the men listed as the risk of arteriosclerosis in the Japan Atherosclerosis Society Guidelines^[22].

Our study examined patients who underwent colonoscopy for LGIB over a 19-year period, including detailed information about underlying diseases and medications, and found an increase in overt LGIB during this time. The investigation included a relatively large cohort of 273 patients with colonic DH for comparison and analysis. We compared the baseline data of DH patients and those of non-DH patients among all subjects in this cohort. Therefore, there may have been little recall bias or selection bias in our study. We divided it in 2004 when we divided it for the same period or in 2000 when low-dose aspirin was approved as division of EG and LG. However, number of cases included a difference too much in EG and LG and was inappropriate for analysis. Thus, the 1803 patients were divided into two groups by time period, with each consisting of about half of the patients.

However, some limitations to the study must be considered. One was the retrospective nature of the study design, and the fact that only a single institution was involved. In addition, this was not a strictly controlled study, as no comparison with non-bleeding diverticulosis was conducted. We had not evaluated by carotid artery ultrasonography for arteriosclerosis in this study.

In conclusion, a rapid increase in the incidence of colonic DH has been seen with the aging population. One reason is the widespread adoption of ATDs since the early 2000s, based on guidelines to prevent ischemic heart disease and ischemic cerebrovascular disease. Colonic DH is the most frequent cause of LGIB.

DH is more likely in patients who are older, are men, obesity, use NSAIDs or ATDs, and have hypertension and diabetes associated with arteriosclerotic disease. These patients are also likely to have more severe anemia and require blood transfusions. These factors should be kept in mind when treating patients with LGIB.

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the translation to English.

COMMENTS

Background

With the aging of the population in Japan, dramatic changes in the incidence of lower gastrointestinal bleeding (LGIB) have been seen, particularly as an increase in colonic diverticular hemorrhage.

Research frontiers

Colonic diverticular hemorrhage (DH) is more likely in patients who are older, are men, obesity, use nonsteroidal anti-inflammatory drugs (NSAIDs) or antithrombotic drugs (ATDs), and have hypertension and diabetes associated with arteriosclerotic disease.

Innovations and breakthroughs

Older patients and those with colonic DH were more frequent in late group than in early group ($P < 0.01$). Patients using ATDs as well as NSAIDs, male sex, obesity, smoking, alcohol drinking, and arteriosclerotic diseases were more frequent in patients with DH than in those without.

Applications

Incidence of colonic DH seems to increase with aging of the population, and factors involved include use of ATDs and NSAIDs, male sex, obesity, smoking, alcohol drinking, and arteriosclerotic disease. These factors should be kept in mind when treating patients with LGIB.

Terminology

NSAIDs and ATDs have synergistic effects on injury to blood vessels in diverticula. Most NSAIDs inhibit prostaglandin synthesis, which disrupts the microcirculation and inhibits platelet aggregation. As a result, NSAIDs can lead to bleeding in patients with diverticulosis, including severe DH.

Peer-review

This is an interesting manuscript which analyses the spectrum of LGIB in a single center retrospective analysis at Fukuoka University Hospital in chikushino city in Japan, and provides further data on the incidence and risk factors for colonic DH. Amongst a total of 1803 Japanese patients with LGIB with a mean age of 59 years, 273 patients with colonic DH were separated into an early (1995-2006) and late (2007-2013) group.

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Prospective Study

Faecal incontinence and health related quality of life in inflammatory bowel disease patients: Findings from a tertiary care center in South Asia

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Abstract

AIM: To analyze the frequency and severity of faecal incontinence (FI) and its effect on the quality of life (QOL) in inflammatory bowel disease (IBD) patients.

METHODS: All patients who attended surgical and medical gastroenterology outpatient clinics in a tertiary care center with an established diagnosis of either ulcerative colitis (UC) or Crohn's disease (CD) over a period of 10 mo were included in this study. Before enrollment into the study, the patients were explained about the study and informed consent was obtained. The patients with unidentified colitis were excluded. The data on demographics, disease characteristics, FI (Vaizey score), and quality of life (IBD-Q) were collected. Data were analyzed using SPSS version 21.

RESULTS: There were 184 patients (women = 101, 54.9%; UC = 153, 83.2%) with a female preponderance for UC (male/female ratio = 1:1.5) and a male preponderance for CD (male/female = 2:1). Forty-eight (26%) patients reported symptoms of FI. Among the patients with FI, 70.8% were women ($n = 34$) and 29.2% were men ($n = 14$) with an average age of 52.7 years (range, 20-78 years). Average age of onset of FI was 48.6 (range, 22-74) years. Ten percent ($n = 5$) reported regular FI.

Incontinence to flatus was seen in 33.3% ($n = 16$), to liquid faeces in 56.2% ($n = 27$), to solid faeces in 6.2% ($n = 3$) and to all three in 4.1% ($n = 2$). Twenty-one percent ($n = 10$) complained of disruption of their physical and social activity. There was no association between FI and type of IBD. Significant associations were found between FI and age ($P = 0.005$) and gender ($P < 0.001$). QOL in our cohort of patients was significantly affected by FI.

CONCLUSION: In our study, nearly a quarter of patients reported FI. There was a significant correlation between FI and QOL. Therefore, enquiring about FI in IBD patients can lead to identification of this debilitating condition. This will enable early referral for continence care in this group of patients.

Key words: Inflammatory bowel disease; Quality of life; Faecal incontinence; Crohn's disease

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Core tip: This was a prospective study involving 184 patients with inflammatory bowel disease (IBD). It was designed to analyze the frequency and severity of faecal incontinence (FI) and its effect on the quality of life (QOL) in IBD patients in a tertiary care center. In our study, nearly 25% of patients reported the symptoms of FI. There was a significant correlation between FI and QOL. Therefore, enquiring about FI in IBD patients can lead to identification of this debilitating condition.

Subasinghe D, Navarathna NMM, Samarasekera DN. Faecal incontinence and health related quality of life in inflammatory bowel disease patients: Findings from a tertiary care center in South Asia. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 447-452 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v7/i3/447.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v7.i3.447>

INTRODUCTION

Faecal incontinence (FI) is defined as the involuntary passage of solid or liquid stools, which is a hygienic and social problem^[1]. It is a devastating personal and social problem which causes emotional distress leading to social isolation and loss of self-confidence^[2]. The prevalence rates of FI in the community vary between 2.2%-15% in adults^[3-7]. It is widely accepted that many patients with anal incontinence do not seek medical advice, thus making the true prevalence uncertain. Therefore under-reporting is common due to social embarrassment^[8,9].

FI can lead to social isolation. It also can adversely affect ability to maintain relationships, occupation and self-esteem aspects of the quality of life (QOL)^[10,11]. Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory conditions related to the gastrointestinal tract. There is a paucity of knowledge of FI in patients with inflammatory bowel disease (IBD), except in patients

with fistulas and those who underwent restorative proctocolectomy with an ileal pouch^[12]. FI is also known to be associated with vaginal delivery in women^[13,14]. In addition, in both genders, FI can be associated with a range of pelvic floor disorders and perianal surgeries (e.g., haemorrhoidectomy and sphincterotomy)^[15,16].

The only estimation of FI in IBD is from patients attending special clinics and the data from the Crohn's and Colitis Foundation of the United Kingdom, and the incidence ranged from 22%-33.5%^[17-19]. No previous study has reported on FI among patients with IBD in Sri Lanka or in South Asia.

Therefore, the main aim of this study was to determine the frequency and severity of FI, and its effect on QOL in IBD patients who presented to a tertiary care center in Sri Lanka, which is a South Asian country.

MATERIALS AND METHODS

Patients and methods

This study was conducted at the National Hospital of Sri Lanka, which is a tertiary care hospital. The patients were interviewed prospectively over a period of 10 mo. Before the interview, the patients were educated about the study and informed consent was obtained. All the patients who attended outpatient clinics with an established histological diagnosis of either UC or CD were included in the study. Diagnosis of IBD was made based on clinical, endoscopic, radiological and histological findings. Age younger than 18 years, lack of cooperation, diagnosed psychiatric illness, being too ill to participate, patients with neurological disorders and those with a previous traumatic anal sphincter injury were excluded from the study. The study was approved by the ethics review committee of the hospital.

All IBD patients were interviewed using an interviewer administered questionnaire, which consisted of two parts. The first part consisted of personal details of the patients including socio-demographic data, disease characteristics, management details and history. The second part of the questionnaire included FI severity (Vaizey score)^[20] and quality of life (IBD-Q) score.

FI

FI was assessed based on Vaizey score with a four point scale: Never, rarely, sometimes, and regularly. Vaizey score was selected because it has shown high clinical validity and utility^[20]. The Vaizey Incontinence questionnaire consists of seven questions. A score of 0 suggests no problems with bowel continence and a score of 24 suggests very severe problems with incontinence.

QOL

IBD-Q32 evaluates QOL in four main aspects (bowel symptoms, emotional health, systemic symptoms and social symptoms). Cumulative score reflects the overall QOL. For each aspect under specific category, score varies from one to seven. Score of one indicates very poor QOL and that of seven indicates excellent QOL. Total IBDQ

Table 1 Demographic characteristics of the study population *n* (%)

	Total IBD	UC	CD
Age at the diagnosis (yr)			
≤ 10	2 (1.1)	1 (0.7)	1 (3.2)
11-19	17 (9.2)	13 (8.5)	4 (12.9)
20-29	42 (22.8)	25 (16.3)	17 (54.8)
30-39	53 (28.8)	49 (32.0)	4 (12.9)
40-49	39 (21.2)	36 (23.5)	3 (9.7)
50-59	21 (11.4)	19 (12.4)	2 (6.5)
60-69	8 (4.3)	8 (5.2)	-
70-79	2 (1.1)	2 (1.3)	-
Gender			
Male	83 (45.1)	62 (40.5)	21 (67.7)
Female	101 (54.9)	91 (59.5)	10 (32.3)
Education			
Primary (Grade 1-5)	40 (21.7)	35 (22.9)	5 (16.1)
Secondary (Grade 6-13)	118 (64.1)	101 (66.0)	17 (54.8)
Higher (University or above)	26 (14.1)	17 (11.0)	9 (29.0)
Employment			
None	72 (39.1)	64 (41.8)	8 (25.8)
Student	11 (6.0)	11 (7.2)	-
Labourer	63 (34.2)	50 (32.7)	13 (41.9)
Professional	38 (20.7)	28 (18.3)	10 (32.3)

IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease.

score can range from 32 (very poor QOL) to 224 (perfect HRQOL).

Both IBDQ and Vaizey score were selected because of their simplicity and precision, which make those ideal for clinical practice to identify patients who require specialist help and in the clinical research setting to provide a sensitive measure of FI^[20,21].

Statistical analysis

The associations between categorical data were examined using χ^2 test. The association between categorical variables and IBDQ-32 scores was determined using Student's *t*-test. Factors statistically significant in the univariate analysis were included in a multivariate regression model to examine their associations with FI score and QOL. The differences were considered significant when $P \leq 0.05$. Data were analyzed using SPSS (Version 21, Chicago, IL, United States).

RESULTS

Demographic and disease characteristics

There were 184 patients (M:F = 83:101) with a mean age of 44.5 (range, 20-78) years. The majority of patients (83.2%, $n = 153$) had UC. The mean duration of disease was 8.17 (range, 1-28) years, while 33.7% ($n = 62$) of patients had IBD for more than 10 years. The participation rate of our population was high (184/188 = 97.87%). The majority of UC patients were female, with a male to female ratio of 1:1.5. A male preponderance was noted in CD (male to female ratio = 2:1). None of our patients had a positive family history. Mean age at diagnosis for UC was 36.3 (range,

Table 2 Descriptive statistics for the four domains and overall score of the IBDQ-32 and categories

	Minimum (Reference)	Maximum (Reference)	Mean
IBDQbowel	10	56	22.33
IBDQSystemic	6	34	12.5
IBDQEmotional	18	84	34.87
IBDQSocial	5	35	12.3
IBDQTotal	51 (32)	215 (224)	94.28

7-71) years. The patients with CD were diagnosed at a significantly younger age than UC patients (27.35 ± 10.22 years vs 38.14 ± 13.05 years, $P < 0.0001$). Peak age of onset was in the fourth decade for UC and in the third decade for CD (Table 1). Out of females ($n = 101$, UC = 91, CD = 10), the majority were unmarried ($n = 55$, UC = 47, CD = 8). Out of married females ($n = 46$), 25 had undergone lower segment caesarian sections and 10 had undergone vaginal deliveries, while 11 had no childbirth yet. There were no females with ongoing pregnancy in our sample.

QOL

The mean of IBDQ-32 scores of enrolled patients was 94.28 (51 to 215). Mean IBDQ scores of bowel symptoms, systemic, emotional, social categories of IBDQ are shown in Table 2. The social symptom and systemic symptom categories had the lowest HRQOL scores (12.3 and 12.5, respectively).

There was no significant difference between CD and UC, with regard to the mean IBDQ-32 (80.26 for CD and 79.52 for UC, $P = 0.778$) or mean Vaizey score (UC 13.79 vs CD 14.45, $P = 0.629$). Also, there was no significant difference in mean scores of bowel symptom (21.11 vs 22.39, $P = 0.220$), systemic (12.46 vs 12, $P = 0.560$) social (11.91 vs 11.10, $P = 0.297$) and emotional symptoms (34.04 vs 34.77, $P = 0.607$) between the two categories of UC and CD.

Determinants of QOL

Although females had a slightly higher mean IBDQ score (79.9 vs 79.34), it was not statistically significant ($P = 0.769$). In subgroup analysis, there was no significant difference in the four aspects of IBDQ categories ($P > 0.05$). Females had significantly higher incontinence scores than males (mean Vaizey score 79.9 vs 79.34, $P < 0.05$).

Twenty-six (14.1%) patients of the total study population underwent surgical treatment. In the UC group, 8.5% ($n = 13$) underwent surgical treatment, the commonest surgical procedure was restorative proctocolectomy ($n = 12$) and one patient underwent sigmoid colectomy. IBD patients who underwent surgery had significantly higher IBDQ bowel (23.48 vs 21, $P < 0.05$) and IBDQ total scores (81.83 vs 79.34, $P < 0.05$) compared to the patients who were on long-term medical management. However, the difference

Table 3 Details of surgical procedures for inflammatory bowel disease

Surgical procedure	Indication	n (%)
UC		
Restorative proctocolectomy and ileoanal pouch	Steroid resistance-7 Atypia on histology-4 Sigmoid colon cancer-1	12 (7.8)
Sigmoid colectomy	Stricture of sigmoid colon	1 (0.7)
CD		
Drainage and fistulectomy	Perianal abscess and fistula	1 (3.2)
Fistulectomy and repair	Recurrent enterocutaneous fistula	1 (3.2)
Incision and drainage	R/Ischiorectal fossa abscess	1 (3.2)
Repair of the fistula	Enterocutaneous fistula	2 (6.4)
R/hemicolectomy and ileo transverse anastomosis	Strictures of the colon	4 (12.9)
Total colectomy and ileostomy	Strictures of colon	2 (6.4)
Repair of the fistula	Recto vaginal fistula	1 (3.2)
Strictureplasty, R/hemicolectomy and ileo transverse	Two long segment narrowings –distal ileum	1 (3.2)
Anastomosis	multiple narrowings > 10 in jejunum and proximal ileum and strictures of ascending colon	-

UC: Ulcerative colitis; CD: Crohn's disease.

Table 4 Correlation between quality of life components and incontinence scores

Association	Pearson correlation coefficient (Rho value)
IBDQbowel vs Vaizey score	0.74
IBDQSystemic vs Vaizey score	0.13
IBDQEmotional vs Vaizey score	0.09
IBDQSocial vs Vaizey score	0.3
IBDQTotal vs Vaizey score	0.61

of incontinence scores was not significantly different between the two groups.

Mean IBDQ-emotional and IBDQ-social scores had significant association with the extent of colonic involvement by the disease. The mean total IBDQ scores did not show significant differences in relation to education level ($P = 0.676$), age ($P = 0.343$), duration ($P = 0.884$), extent of IBD ($P = 0.92$) or current symptoms of the disease ($P = 0.3$).

The relationships between psychosocial, clinical, and demographic variables and the overall score of IBDQ-32 are shown in Table 3.

FI vs QOL

The extent of colitis was significantly associated with the Vaizey scores ($P = 0.002$), where patients with distal colitis had higher scores. Association of total IBDQ and Vaizey score was statistically significant ($P < 0.001$). Pearson correlation was performed to determine the correlation between Vaizey score and components of QOL scores and total IBD-Q score. QOL scores for emotional and systemic components showed a weak association ($Rho < 0.3$), QOL score of social component showed a moderate association ($Rho 0.3-0.7$) and that of bowel symptoms showed a strong association ($Rho > 0.7$) (Table 4).

DISCUSSION

It is noted that the incidence of IBD is increasing in the Asian population^[22,23]. They are among the group of chronic disorders associated with periods of remission and unpredictable relapses. QOL measurement is especially pertinent in IBD, because it is a chronic disabling disease^[24] which commonly occurs in early adulthood and hence affects all aspects of life, mainly physical, social and psychological. The peculiarities of chronic disease over acutely resolving conditions are that they often have a long-term negative effect on the emotional and social life, which are most of the time not visually apparent^[25]. Feeling dirty and smelly following loss of bowel control, with resultant offensive body odours, unfulfilled potential in the work place and issues related to sexual relationships were the highly ranked concern in a survey of patients with IBD^[26].

In addition, fear of loss of bowel control and its unpredictability can lead to a profound effect on the individual's behaviour. In the majority of patients with IBD, this factor can lead to an avoidance of routine social events or impairment of daily activities^[27,28]. Recent work by Daniel *et al*^[27] and Hall *et al*^[29] showed that these patients only attend places with toilet facilities or avoid public places all together.

Our results showed that IBD patients who underwent surgery for UC and CD had significantly higher IBDQ bowel (23.48 vs 21, $P < 0.05$) and IBDQ total scores (81.83 vs 79.34, $P < 0.05$) than those who was on long-term medical management. This may be due to the long-term symptom relief and avoidance of chronic medicine intake leading to more convenient life style. According to our results, higher Vaizey scores were associated with lower IBDQ scores ($P < 0.001$). This shows that the fear of anal incontinence and its unpredictability had a profound effect on the individual's day-to-day activities. In our study, we found important variables significantly

related with lower QOL, suggesting that HRQOL analysis has an important role in understanding the true impact of the disease on patients. QOL score of social component showed a moderate association (ρ 0.3-0.7) and QOL of bowel symptom component showed a strong association ($\rho > 0.7$) with FI. This shows the significant impact of incontinence on social activities.

In conclusion, FI has adverse effects on social, emotional and other aspects of QOL in patients with IBD. Given the availability of specialist FI interventions and support, we recommend that sensitive questioning regarding FI should be part of routine disease surveillance in the outpatient setting to cater for this unmet need.

COMMENTS

Background

Severity and impact of faecal incontinence (FI) on quality of life (QOL) of inflammatory bowel disease (IBD) are not widely investigated. In general FI has adverse effects on daily activities, hence on QOL. The current study was designed to evaluate the severity and frequency of FI and its effect on QOL in IBD patients presented to a tertiary care center in a South Asia country.

Research frontiers

This study has showed that FI has more adverse effects on social, emotional and other aspects of QOL in IBD. Given the availability of specialist FI interventions and support, the authors recommend that sensitive questioning regarding FI should be part of routine disease surveillance in the outpatient setting.

Innovations and breakthrough

Current literature suggests various strategies to improve the management and outcome of chronic diseases such as IBD. This study provides evidence on improvement QOL by considering the FI as an important aspect of the management.

Applications

This study has showed that FI correlates with HRQOL in IBD patients. Therefore, these aspects should be addressed to improve the management of these patients having this chronic disease.

Terminology

FI is defined as the involuntary passage of passage of solid or liquid stools, which is a social and hygienic problem. Ulcerative colitis/Crohn's disease are chronic IBD affecting gastrointestinal tract.

Peer-review

A well-timed piece with pertinent clinical insight, and the information provided is relevant and could be interesting enough to warrant readers' attention.

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Efficacy and safety of botulinum toxin in treatment of anismus: A systematic review

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Data sharing statement: Technical appendix, statistical code, and dataset are available from the corresponding author at sameh200@hotmail.com. Informed consents of patients were obtained by the original studies included in the review.

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Abstract

AIM: To evaluate the efficacy and safety of botulinum toxin type A (BTX-A) in the management of patients with anismus.

METHODS: An organized search of published literature was conducted using electronic databases including: PubMed/MEDLINE, and Cochrane Central Register of Controlled Trials, also an internet-based search using "Google Scholar" service was conducted. Both comparative and observational studies were included. We excluded irrelevant articles, editorials, case reports, reviews, and meta-analyses. The studies that followed the patients less than 6 mo were excluded. Variables collected were demographic data of the patients, technique of BTX-A injection and number of sessions, short-term and long-term clinical improvement, post-injection changes in electromyography (EMG), defecography, manometry, and balloon expulsion test, and complications recorded after BTX-A injection.

RESULTS: Seven studies comprising 189 patients were included in the review. The median age of the patients was 41.2 years and female-to-male ratio was 1.3:1. The median dose of BTX-A injected per procedure was 100 IU (range, 20-100 IU). Lateral injection was done in five trials and combined lateral and posterior injections in two trials. Three studies used endorectal ultrasonography-guided technique, one study used EMG-guided technique,

whereas the remaining three studies used manual palpation with the index finger. The median percentage of patients who reported initial improvement of symptoms was 77.4% (range 37.5%-86.7%), this percentage declined to a median of 46% (range 25%-100%) at 4 mo after injection of BTX-A. Rates of improvement evaluated by balloon expulsion test, EMG, and defecography ranged between (37.5%-80%), (54%-86.7%), and (25%-86.6%), respectively. Fourteen (7.4%) patients developed complications after injection of BTX-A. Complication rates across the studies ranged from 0% to 22.6%.

CONCLUSION: Initial satisfactory improvement of symptoms after BTX-A injection remarkably deteriorated after 3 mo of the procedure. However, repeated injection may provide better sustained results with no additional morbidities. Further analysis of more patients is necessary to conclude the safety of BTX-A for the treatment of anismus.

Key words: Botulinum toxin; Botulinum toxin type A; Botox; Anismus; Puborectalis syndrome; Efficacy

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Core tip: Injection of botulinum toxin type A (BTX-A) is a simple, technically feasible outpatient procedure. The initial satisfactory improvement of symptoms after BTX-A injection remarkably deteriorates after three months of the procedure from a median rate of 77.4% to 46%. However, repeated injections may provide better sustained results with no additional morbidities. The endorectal ultrasonography- and electromyography-guided techniques do not add significant value regarding both initial and long-term improvement. Combined lateral and posterior injections do not offer better results than lateral injection alone, on the contrary they can lead to higher complication rates. Although most of the studies reported very low complication rates after BTX-A injection; further studies on a larger number of patients are necessary to conclude the safety of this treatment.

Emile SH, Elfeki HA, Elbanna HG, Youssef M, Thabet W, Abd El-Hamed TM, Said B, Lotfy A. Efficacy and safety of botulinum toxin in treatment of anismus: A systematic review. *World J Gastrointest Pharmacol Ther* 2016; 7(3): 453-462 Available from: URL: <http://www.wjgnet.com/2150-5349/full/v7/i3/453.htm> DOI: <http://dx.doi.org/10.4292/wjgpt.v7.i3.453>

INTRODUCTION

Anismus is a functional disorder of the defecation process that entails failure of relaxation or even paradoxical contraction of the puborectalis muscle and external anal sphincter (EAS) during defecation^[1]. The term "Anismus" was first described by Preson and Lennard-Jones^[2] in 1985. Anismus, also known as pelvic floor dyssynergia

and puborectalis syndrome^[3], commonly affects young and middle-aged females. The exact incidence of anismus is still unknown; however, it ranges between 20% and 70% of the general population^[4].

The pathophysiology of anismus is not clearly defined yet. Certain predisposing factors as physical and emotional stress, previous anorectal surgery or hysterectomy, and psychological disorders are associated with anismus^[5]. Sexual assault or abuse in childhood may also contribute to the development of anismus^[6].

Patients with anismus typically complain of symptoms of outlet obstruction and defecation difficulties. Frequent attempts of evacuation, prolonged straining, anal pain, and sense of incomplete evacuation are the common presenting features of this condition^[7]. On digital rectal examination (DRE), the puborectalis muscle and EAS fail to relax during straining, and sometimes a paradoxical contraction may occur. Although DRE can preliminarily diagnose anismus, additional physiologic tests such as anorectal manometry^[8], balloon expulsion test^[2], electromyography (EMG) of the puborectalis muscle and EAS^[9], and defecography^[10] are required to establish the diagnosis.

Anismus is initially managed in a conservative manner, starting with dietary modification focusing on high fiber diet, then using enemas and laxatives in increasing doses. However, conservative measures usually fail to solve the problem. Biofeedback (BFB) retraining was introduced by Bleijenberg and Kuijpers^[11] for the treatment of anismus. Results of BFB were conflicting with efficacy rates ranging from 31% to 89%^[3]. Surgical treatment in the form of partial myotomy of the puborectalis muscle has been described in a few reports with long-term success reaching up to 67% of patients^[12].

Botulinum toxin, the product of *Clostridium botulinum* anaerobic bacterium, divides into seven subtype (A-G) that share similar structure, yet have different antigenic properties. Botulinum toxin type A (BTX-A) functions through extracellular binding to glycoprotein structures on the presynaptic cholinergic nerve endings which prevents the secretion of acetylcholine causing neuromuscular blockage and muscle paralysis. In addition, BTX-A blocks the efferent autonomic fibers to the smooth muscles and to the exocrine glands. While BTX-A does not induce direct central nervous system effects, some indirect effects as reflex inhibition and intra-cortical inhibition have been observed^[13].

Injection of BTX-A neurotoxin directly into the puborectalis muscle is a non-operative method for the treatment of anismus^[14]. Similar to BFB, the results of BTX-A injection were also conflicting. While the short-term results were highly satisfactory, the long-term outcome was disappointing with success rates of around 50% necessitating repeated injections in order to maintain the initial clinical improvement^[15].

The primary objective of the current review was to analyze all the eligible articles that have evaluated the efficacy of BTX-A with regard to short and long-term

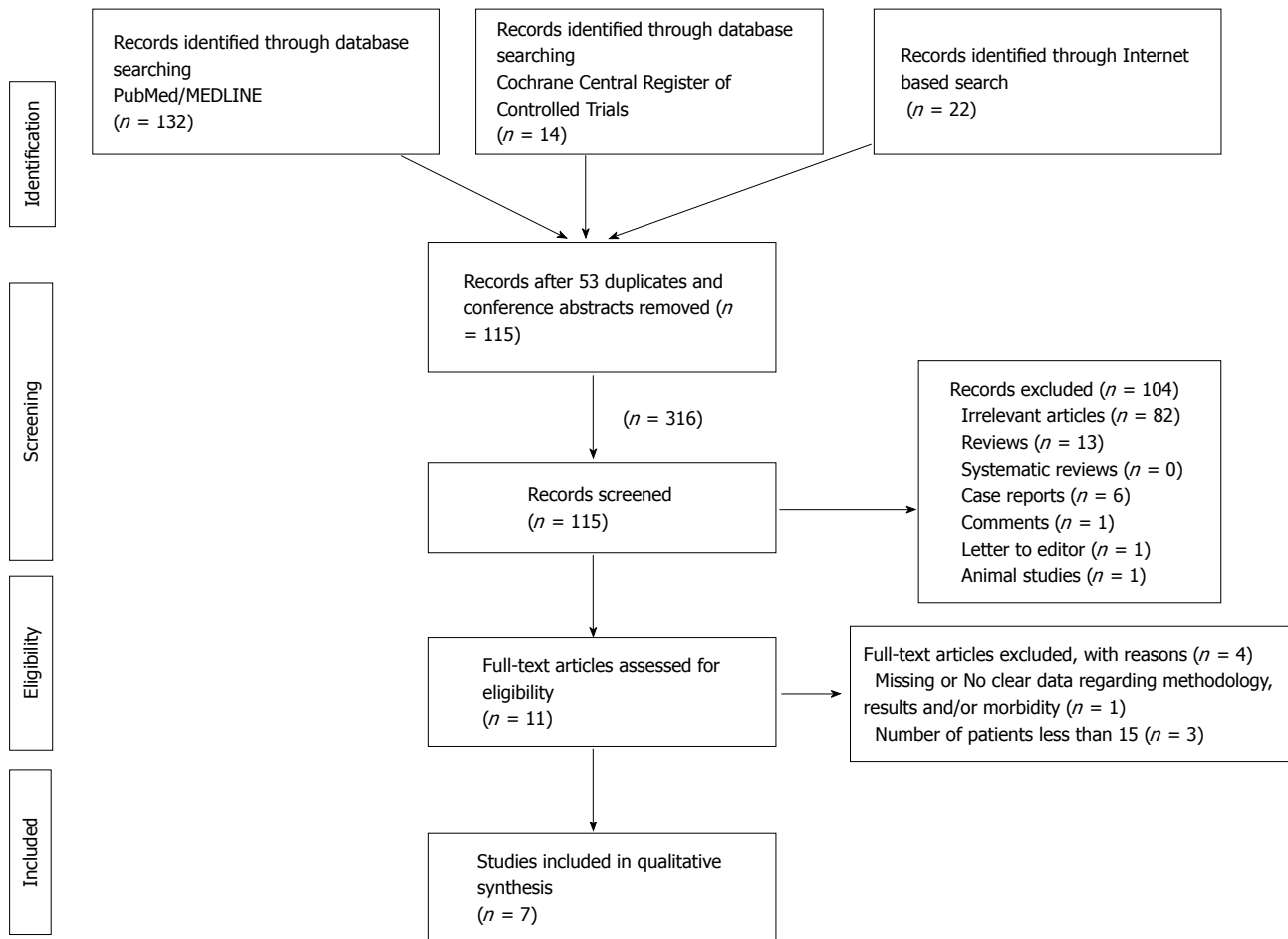


Figure 1 PRISMA flow-diagram.

outcomes. The secondary objective was to assess the side effects and complications encountered after the procedure to establish an evidence-based approach of treatment of anismus with BTX-A injection.

MATERIALS AND METHODS

Registration

This systematic review was registered online in the PROSPERO project under the registration number of CRD42016033892.

Search strategy

A systematic review of the literature for the role of BTX-A in the treatment of anismus was conducted following the screening guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Figure 1)^[16]. Electronic databases including: PubMed/MEDLINE, and Cochrane Central Register of Controlled Trials were searched for published studies until October 2015. A parallel internet-based search using "Google Scholar" service was also conducted. The PubMed function "related articles" was used to search further articles.

The keywords used in the preliminary search process included: Anismus, puborectalis, botulinum toxin,

BTX-A, puborectalis syndrome, efficacy, and safety. The following keywords syntax was utilized in the search process: (Botulinum toxin OR BTX-A) AND (Anismus OR Paradoxical contraction of puborectalis OR puborectalis syndrome).

Relevant articles mentioned in the references section of the initial publications were obtained and the related articles were also screened to add any relevant publications to the results. The full text versions of the selected articles were screened by two independent reviewers (Emile SH and Elfeki HA) to check eligibility.

Inclusion criteria

This systematic review included studies that involved patients with anismus who were treated with BTX-A injection. We included both comparative and non-comparative trials that evaluated BTX-A therapy for treatment of anismus with a sample size of at least 15 patients. No language restrictions were applied.

Exclusion criteria

We have excluded irrelevant articles, editorials, case reports, reviews, and meta-analyses. The studies that followed the patients less than six months were excluded. Duplicate reports were identified and excluded from this review. Articles that did not report the aim,

Table 1 Assessment of the methodological qualities of the comparative studies included in the review

Items	Farid <i>et al</i> ^[12] , 2009	Farid <i>et al</i> ^[20] , 2009
Inclusion criteria	1	1
Exclusion criteria	1	1
Comparable demographics	1	1
Number of participating centers is stated?	1	1
The number of surgeons is stated	0	0
Reporting where the authors were on the learning curve	0	0
Clearly stated diagnostic criteria for clinical outcomes	1	1
Adequate description of surgical technique	1	1
Standard surgical technique	1	1
Standard perioperative care	0	0
Age and range are given for patients in BTX-A group	1	1
Statement about any missing data	0	0
Age and range are given for patients in the comparative group	1	1
Patients in each group treated along similar timelines	1	1
The patients asking to enter the study, did they actually take part to it?	0	0
Statement about drop-out rates	0	0
Clearly defined outcomes	1	1
Availability of blind assessors	0	0
Assessment tools were standardized	1	1
Was the analysis by intention to treat?	0	0
Score	12	12

Yes = 1, no (not reported) = 0; Total score = 21, < 8 = poor quality; 8-14 = fair quality and > 14 = good quality. BTX-A: Botulinum toxin type A.

methodology, demographic data of patients, final results, and conclusion clearly were excluded after second thorough revision.

Types of included studies

After reviewing the full text of 11 articles, seven of them^[12,17-22] met the eligibility criteria of the review. Two studies were randomized comparative trials^[12,20], comparing BTX-A injection with BFB or partial division of the puborectalis muscle. The remaining five trials were observational cohort studies assessing the efficacy and complications of BTX-A injection.

Unfortunately, due to the high degree of heterogeneity among the studies included a formal meta-analysis could not be conducted in this review as two studies were comparative and five were case series studies. In addition, the studies reviewed had different methods for assessment of improvement of anismus and variable follow-up durations.

Assessment of the methodological quality and risk of bias within the studies included

Two reviewers (Emile SH and Elfeki HA) have independently assessed the methodological quality and risk of bias in each study. The revised grading system of the Scottish Intercollegiate Guidelines Network (SIGN)^[23] was used to assess comparative studies. The checklist for the quality of case series of the National Institute for Health and Clinical Excellence (NICE)^[24] was used for assessment of case series studies. Six of the assessed studies were of fair quality and one study^[18] was of good quality (Tables 1 and 2, Figure 2).

Variables of interest

We have extracted the following data from each study:

The demographic data of the patients, technique of BTX-A injection and number of sessions, short-term and long-term clinical improvement, post-injection changes in EMG, defecography, manometry, and balloon expulsion test, and complications recorded after BTX-A injection.

The clinical diagnostic criteria of anismus in the studies included were based on the established Rome criteria^[25]. The short-term and long-term clinical improvements were defined as the subjective feeling of improvement of symptoms within one month, and four months after injection, respectively.

Statistical analysis

The statistical methods of this study were reviewed by Professor Basem Eldeek, PhD, Mansoura University, Faculty of medicine. Data were extracted from the original articles into fields of Microsoft Excel spreadsheet. SPSS (Statistical Package for Social Science) version 22 under Microsoft Windows was used in the analysis of the collected data. Variables were expressed as median, normal range, and percentage of patients reported in each variable. *P* value less than 0.05 was considered significant.

RESULTS

Characteristics of the studies and the patients

Seven studies met the inclusion criteria of this review and were included. Two studies were retrospective and five were prospective. The median duration of follow-up was 14.6 (range, 6-19.2) mo.

The studies comprised 189 patients who were 108 (57%) female and 81 (43%) male with a female-to-male ratio of 1.3:1. The median age of the patients was 41.2 (range, 23.7-56) years. All patients complained

Table 2 Assessment of the methodological qualities of case-series studies included in the review

Items	Shafik <i>et al</i> ^[17]	Ron <i>et al</i> ^[18]	Maria <i>et al</i> ^[19]	Hompes <i>et al</i> ^[21]	Zhang <i>et al</i> ^[22]
Multi-center study	0	0	0	0	0
Clearly defined objective	1	1	1	1	1
Reported inclusion exclusion criteria	0	1	1	0	0
Clearly defined outcomes	0	1	1	1	1
Prospective data collection	1	1	1	1	1
Patients were recruited consecutively	0	1	0	0	0
Clearly described results of the study?	1	1	1	1	1
Stratified outcomes	1	1	1	1	1
Total Score	4	7	6	5	5

Yes = 1, no (not reported) = 0; Total score, 8; ≤ 3 = poor quality; 4-6 = fair quality; ≥ 7 = good quality.

Table 3 Characteristics of the studies included

Ref.	Country	Type	n	Male	Mean age (yr)	Duration of complaint (mo)	Follow up (mo)	Dose of BTX-A (IU)	Site of injection
Shafik <i>et al</i> ^[17]	Egypt	Prospective	15	2	41.2	105.6	14.6	25	Lateral (3, 9 o'clock)
Ron <i>et al</i> ^[18]	Israel	Prospective	24	9	23.7	Not reported	61.0	10-20	Lateral and posterior
Maria <i>et al</i> ^[19]	Italy	Prospective	24	10	56.0	28.0	39.0	60	Lateral (3, 9 o'clock)
Farid <i>et al</i> ^[12]	Egypt	Prospective RCT	15	15	34.7	71.1	14.7	100	Lateral (5, 7 o'clock)
Farid <i>et al</i> ^[20]	Egypt	Prospective RCT	24	7	34.7	Not reported	12.0	100	Lateral (5, 7 o'clock)
Hompes <i>et al</i> ^[21]	United Kingdom	Retrospective	56	20	47.5	Not reported	19.2	100	Lateral (3, 9 o'clock)
Zhang <i>et al</i> ^[22]	China	Retrospective	31	18	50.1	67.2	8.4	100	Lateral and posterior (3, 6, 9 o'clock)

RCT: Randomized controlled trial; BTX-A: Botulinum toxin type A.

of symptoms of outlet obstruction constipation for a median duration of 69.1 (range, 28-105.6) mo. The characteristics of each study are shown in Table 3. Only in two studies^[17,22] patients completed a course of BFB retraining before they were considered unresponsive and were shifted to BTX-A injection.

Technique of injection

The studies used BTX-A under variable commercial names (Dysport®, Botox® and Allergan®). Injection of BTX-A was performed as a day-case procedure, except in one study^[22] where patients were hospitalized after BTX-A injection. The injection was conducted under local anesthesia in one study^[21], caudal anesthesia in one study^[23], sedation in one study^[18], and without anesthesia in four studies^[12,17,19,20].

The median dose of BTX-A injected per procedure was 100 IU (range, 20-100 IU). The site of injection varied; five trials employed lateral injection either at 5 and 7 o'clock^[12,20], or at 3 and 9 o'clock^[17,19,21]. The remaining two trials used a combination of lateral and posterior injections^[18,22].

Three studies^[18,19,22] used endorectal ultrasonography-guided technique for injection, one study^[17] used an EMG-guided technique, whereas the remaining three studies used manual palpation with the index finger.

A single session of BTX-A injection was conducted in four studies, two sessions were conducted in two studies^[17,18], and more than two injection sessions were

required in one study^[19]. Auxiliary pelvic floor rehabilitation (BFB) program was employed after BTX-A injection in one study^[22].

Efficacy of BTX-A injection

Clinical improvement: The clinical improvement of symptoms was classified into initial and long-term improvement. The median percentage of patients who reported initial improvement of symptoms was 77.4% (range, 37.5%-86.7%). This percentage declined to a median of 46% (range 25%-100%) at 4 mo after injection of BTX-A (Figure 3). One study^[19] that employed repeated injections of BTX-A at two and four months reported long-term improvement in all patients.

Symptom assessment scores were not routinely used as only two studies^[12,22] submitted patients to Wexner constipation scale^[22] before and after BTX-A injection. The mean Wexner scores dropped from 11.2 and 14.3 before injection to 8.2 and 6.4 after injection, respectively. None of the studies used any of the scores designated for obstructed defecation syndrome.

Improvement according to anorectal manometry:

Two studies^[18,20] reported post-injection manometric relaxation in 28.5% and 70.8% of patients, respectively. No significant changes in anal pressures after BTX-A injection were observed according to two studies^[12,21]. Conversely, two studies^[19,22] reported significant decrease in the mean resting and squeeze anal pressures 3 mo

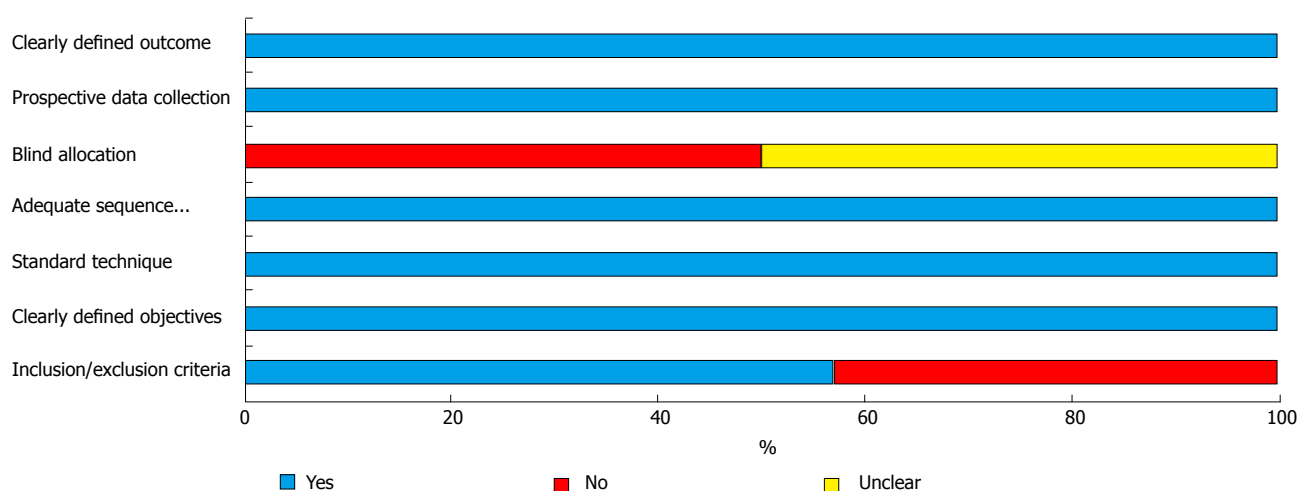


Figure 2 Assessment of the methodological quality of the studies included.

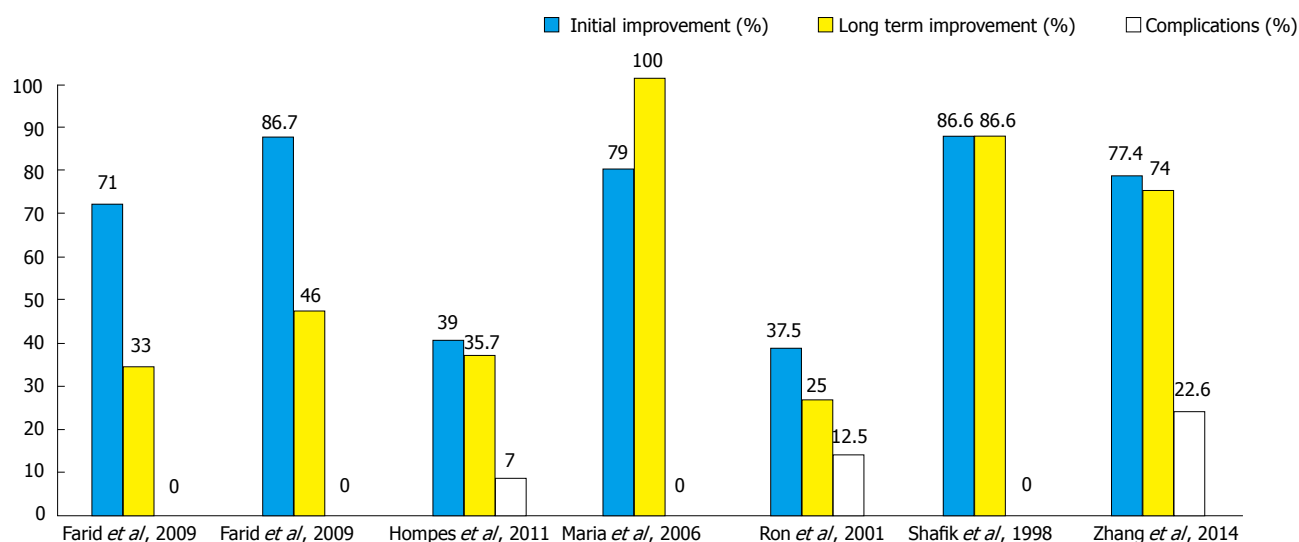


Figure 3 Improvement of symptoms and complications after injection of botulinum toxin type A.

after injection.

Improvement according to balloon expulsion test:

Positive balloon expulsion was reported in four studies^[12,18,20,22], with a median rate of 74.6% ranging from 37.5%-80%. Ninety-four patients were subjected to balloon expulsion test before and after injection, all of them failed the test before injection and 62 (66%) of them had a positive test within three month after BTX-A injection.

Improvement according to EMG: Based on EMG, three studies^[12,17,20] reported post-injection improvement of anismus ranging between 54% and 86.7%. Fifty-four patients were subjected to EMG before and after injection, 39 (72%) of them showed improvement in their post-injection EMG.

Improvement according to defecography: Four

studies^[12,17,19,20] reported improvement of 25%-86.6% of patients in the post-injection defecogram. Seventy-eight patients underwent defecography before and after injection, 50 (64%) of them showed resolution of signs of anismus in the post-injection defecogram. One study^[19] evaluated the anorectal angle (ARA) before and after injection, and reported a significant increase of the ARA during straining from $97^\circ \pm 11^\circ$ to $127^\circ \pm 10^\circ$ at two months after injection.

Complications

Fourteen (7.4%) patients developed complications after the injection of BTX-A. The median rate of complications across the studies was zero ranging from 0%-22.6% (Figure 3). Eleven (5.8%) patients developed minor transient fecal incontinence (FI) as reported by two studies^[21,22]. Two patients developed acute posterior anal fissure^[18], and one patient developed complete rectal prolapse^[18].

DISCUSSION

Anismus is a complex functional disorder with unclear pathophysiology and elusive diagnosis rendering it a difficult condition to treat. Conservative measures for treatment of constipation usually fail to provide any significant improvement to the patients with anismus. BFB retraining, surgical division of puborectalis muscle, and BTX-A injection are the main options for treatment of anismus that were described in literature. Nevertheless, the optimal treatment of anismus is still debatable.

BFB retraining depends on the concept of operant conditioning. During BFB patients learn how to control an unconscious physiologic function with the aid of an instrument that provides visual, auditory or verbal feedback of an action that can be reinforced until a satisfactory response is accomplished^[26,27].

Since the first report^[11] that concluded the utility of BFB in pelvic floor disorders, several other studies tried to evaluate the efficacy of BFB in the treatment of anismus. Gilliland *et al.*^[28] reported a success rate of 63% in patients who completed their training programs. Similarly, Rhee *et al.*^[29] reported that about 69% of anismus patients showed complete response on completion of their BFB training program. However, most of these studies were small non-controlled series with short follow-up durations.

A meta-analysis of randomized controlled trials^[30] evaluating BFB in the treatment of pelvic floor disorders concluded that symptomatic relief of anismus after BFB was six fold that obtained with other methods. Moreover, clinical improvement of symptoms after EMG-BFB was seven times higher than that after non-EMG BFB.

Surgical myotomy of the puborectalis muscle was described since 1960s with initial satisfactory results^[1,31]. However, subsequent trials reported disappointing outcomes and unacceptably high rates of FI following division of the puborectalis muscle^[32,33]. A recent pilot study^[34] devised a modified semi-closed technique in dividing puborectalis muscle stating that symptomatic improvement occurred in 75% of patients without encountering any significant postoperative complications. Nonetheless, the authors recommended conducting more studies before considering this technique a validated procedure.

Hallan *et al.*^[35] described direct injection of BTX-A into the puborectalis muscle. BTX-A is a potent neurotoxin that causes muscle paralysis by inhibition of release of acetylcholine at the presynaptic region^[13,14]. Injection of BTX-A emerged as a promising option in the treatment of anismus with the advantages of being less costly and technically easier than BFB retraining^[19]. BTX-A injection, unlike BFB, does not depend on patient's cooperation and compliance which are merely subjective.

As the effect of BTX-A is temporary for around three months after administration, BTX-A injection therapy was considered successful in terms of short-term symptomatic improvement of anismus. Longer term improvement

necessitates repeated injections in order to maintain the achieved clinical improvement^[18].

The objective of the current review was to assess the efficacy and safety of BTX-A injection in the management of anismus. Only seven studies were eligible to be included which reflects the paucity of trials in this regard. Patients were mostly middle-aged females coping with the literature^[4]. Most of the studies used BTX-A injection as a primary treatment except two studies^[18,23] that resorted to BTX-A after failure of BFB therapy.

Despite the availability of designated scores for obstructed defecation^[36-38], none of the studies reviewed employed any of these scores to assess patients with anismus. Instead, two studies used Wexner constipation score which is not specific for obstructed defecation syndrome. The clinical utility of the obstructed defecation scores in anismus remains debatable and needs further studies to be ascertained.

Some studies used endorectal ultrasonography- or EMG-guided techniques for BTX-A injection, yet none obtained superior results compared to the studies that used manual guidance, concluding no clear benefits for the guided techniques. Although Zhang *et al.*^[22] found ultrasonography-guided injection simplified the localization of the injection site which led to a long-term improvement rate of 74%; the adjuvant BFB course they have applied to the patients after BTX-A injection could have contributed to this good outcome, rather than the guided technique of injection.

Only two studies^[18,22] used combined lateral and posterior injections technique which was associated with higher complication rates with almost the same efficacy obtained by lateral injection alone. We can explain this phenomenon that posterior injection potentially affects part of EAS at the anorectal ring, subsequently this will lead to weakening of the sphincter complex and development of FI. While the site of injection played an important role in the development of complications, the dose of BTX-A did not have any special significance since the studies that used the least dose^[17,18] reported conflicting results with an efficacy close to that of higher doses.

The median rate of initial improvement of symptoms after injection was 77.4% reaching up to 86%. Unfortunately, these initial good results did not last longer as they dropped to a median of 46% after three months necessitating repeated injections of BTX-A in three studies. The studies that reported satisfactory long-term results had to repeat the injection twice or more. The reason why repeated injections attained better long-term results can be attributed to the cumulative effect of BTX-A on the puborectalis muscle. Interestingly, we found that the repeated injections do not necessarily induce higher complication rates, therefore repeated BTX-A injection can potentially provide sustained improvement in cases where BFB fails and surgical myotomy is contraindicated or refused by the patient.

Improvement of anismus as assessed by the physiologic tests was variable and rather confounding.

Anorectal manometry reported a decrease in anal pressures in two studies^[19,21]. Conversely, the remaining studies showed no significant change in the anal pressures, although clinical improvement was evident. The rate of improvement of anismus evaluated by balloon expulsion test, EMG, and defecography ranged between (37.5%-80%), (54%-86.7%), and (25%-86.6%), respectively. Interestingly, the highest rates of improvement according to clinical examination, EMG, and defecography were the same (86%) implying the harmony of these tests with the clinical examination.

Complications after BTX-A injection were detected in 7.4% of patients. The most common complication was FI which was only transient, for two weeks, and of a minor grade. FI was reported in two studies^[18,22], both applied combined lateral and posterior injections. Other morbidities as posterior anal fissure and complete rectal prolapse were observed only by one study^[18] that also used posterior injection in addition to lateral injection, hence demonstrating the negative impact of posterior injection that induces further weakness to the sphincter muscles.

In summary, BTX-A injection has distinct advantages as technical feasibility, lack of need for general or spinal anesthesia, being an outpatient procedure, and excellent initial results. On the other hand, BTX-A injection proved to be a temporary short-term solution with disappointing outcome on the long term. However, longer term results can be improved further by repeated injections, although satisfactory results are still not guaranteed.

Limitations

The heterogeneity of the studies included was a major limitation during the analysis and interpretation of their results, thus, a meta-analysis could not be conducted. Another limitation was the lack of data of some investigations that were not reported by some studies. In addition, most of the studies were observational with low grade of evidence; only two studies were randomized controlled trials which may influence the final outcome of the review.

Conclusions

The injection of BTX-A is a simple, technically feasible outpatient procedure. The initial satisfactory improvement of symptoms after BTX-A injection remarkably deteriorated after three months of the procedure. However, repeated injections may provide better sustained results with no additional morbidities.

The endorectal ultrasonography- and EMG-guided techniques did not add significant value regarding both initial and long-term improvement. Combined lateral and posterior injections technique did not achieve better results than lateral injection alone, on the contrary the studies that employed the combined injections technique reported higher complication rates. Overall, further analysis of more patients is necessary to conclude the safety of BTX-A in the treatment of anismus.

Recommendations

The present review suggests that injection of BTX-A in the puborectalis muscle is an effective short-term method for treatment of anismus, hence in case of deterioration of the initial satisfactory amelioration of clinical symptoms we recommend further sessions of BTX-A injection in order to maintain the clinical improvement.

From the results we obtained, we don't recommend combined lateral and posterior injections since this technique can result in higher complication rates, yet with no substantial benefits.

COMMENTS

Background

Anismus is considered one of the most important causes of obstructed defecation syndrome. The precise diagnosis and management of anismus have been a challenging problem for surgeons. While biofeedback (BFB) confers excellent results in many patients; some patients fail to respond to BFB, hence alternative methods for treatment are indicated. The injection of botulinum toxin type A (BTX-A) in the puborectalis muscle provided satisfactory short-term results, yet these good results tend to deteriorate with time. The aim of this review was to determine the overall efficacy and safety of BTX-A in treatment of anismus

Research frontiers

BTX-A has various indications in surgery as cervical dystonia, severe axillary hyperhidrosis, strabismus, and upper limb spasticity. Earlier attempts of using BTX-A for treatment of anismus date back to the nineties. BTX-A prevents the release of acetylcholine by binding to glycoprotein structures on the cholinergic nerve terminals, inducing neuromuscular blockage.

Innovations and breakthroughs

A number of trials have used BTX-A for treating anismus and pelvic floor dyssynergia using different approaches and dosage of BTX-A. Some authors used endorectal ultrasonography and EMG as a guide for the injection process. While some authors used lateral injection method; others tried combined lateral and posterior injections. The studies evaluating the efficacy and safety of BTX-A were reviewed by the authors and the data were extracted using a standardized collection tool.

Applications

This review suggests that BTX-A can be an effective method for treatment of anismus; however, the remarkable deterioration of symptom improvement may necessitate injection of further doses of BTA-X within an interval of 3-6 mo after the first injection.

Terminology

BTX-A stands for botulinum toxin type A, EMG stands for electromyography, and BFB stands for biofeedback.

Peer-review

This is a short review about the botulinum toxin treatment for patients with anismus. This review is well written.

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Response of irritable bowel syndrome with constipation patients administered a combined quebracho/conker tree/*M. balsamea Willd* extract

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Institutional review board statement: The study is exempt from IRB review and oversight pursuant to the terms of the United States Department of Health and Human Service's Policy for Protection of Human Research Subjects at 45 CFR and 46.101(b) since the data already exists in patient medical charts and this data was accumulated retrospectively. There was no experimentation on patients. The botanical extract was recommended to patients who chose to take the formulation.

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Abstract

The aim of this case series was to retrospectively examine the symptom response of irritable bowel syndrome with constipation (IBS-C) patients administered an herbal extract in a real-world setting. Twenty-four IBS-C patients in a community office practice were provided a combination over-the-counter dietary supplement composed of quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts (Atrantil™) and chose to take the formulation for a minimum of 2 wk in an attempt to manage their symptoms. Patient responses to the supplement were assessed by visual analogue scale (VAS) for abdominal pain, constipation and bloating at baseline and at 2 wk as part of standard-of-care. Patient scores from VAS assessments recorded in medical chart data were retrospectively compiled and assessed for the effects of the combined extract on symptoms. Sign tests were used to compare changes from baseline to 2 wk of taking the extract. Significance was defined as $P < 0.05$. Twenty-one of 24 patients (88%) responded to the dietary supplement as measured by individual improvements in VAS scores for abdominal pain, bloating and constipation symptoms comparing scores prior to administration of the extract against those reported after 2 wk. There were also significant improvements in individual as well as mean VAS scores after 2 wk of administration of the combined

extract compared to baseline for abdominal pain [8.0 (6.5, 9.0) *vs* 2.0 (1.0, 3.0), $P < 0.001$], bloating [8.0 (7.0, 9.0) *vs* 1.0 (1.0, 2.0), $P < 0.001$] and constipation [6.0 (3.0, 8.0) *vs* 2.0 (1.0, 3.0), $P < 0.001$], respectively. In addition, 21 of 24 patients expressed improved quality of life while taking the formulation. There were no reported side effects to administration of the dietary supplement in this practice population suggesting excellent tolerance of the formulation. This pilot retrospective analysis of symptom scores from patients before and after consuming a quebracho/conker tree/*M. balsamea Willd* extract may support the formulation's use in IBS-C.

Key words: Irritable bowel syndrome; Constipation; Abdominal pain; Bloating; Dietary supplement; Herbal; Botanical; Extract; Peppermint

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Core tip: Irritable bowel syndrome with constipation (IBS-C) is a diagnosis by exclusion which is defined by abdominal pain accompanied by reduced stool frequency and painful, hard bowel movements. Gas and bloating may also be present in many patients with this condition suggesting a role in fermentation of food producing gas by bacteria in the gut. Safe tannin byproducts from wineries used in cows to reduce gas that can impair milk and meat production are combined with saponins, shown to be antibacterial and promote intestinal motility, and peppermint oil for abdominal pain in this combination extract (Atrantil™) to manage key IBS-C symptoms.

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INTRODUCTION

One third of diagnosed irritable bowel syndrome (IBS) in the United States is constipation predominant and includes symptoms of abdominal pain, bloating, and constipation^[1]. Women experience IBS symptoms about twice as frequently as men^[2]. Irritable bowel syndrome with constipation (IBS-C) has a huge impact on quality of life and productivity especially in women^[3] with one investigator suggesting that IBS patients have worse health-related quality of life compared to patients with diabetes and end-stage renal disease^[4]. The symptom-driven quality of life altering condition can be due to the production of gas (hydrogen, methane) which causes bloating and contributes to alterations in motility in IBS-C patients. Gas production has been linked to the presence of methanogenic archaeobacteria^[5,6]. Methane production has been found to be associated with delayed transit

time^[7,8]. Individuals diagnosed with small intestinal bacterial overgrowth (SIBO) also produce more hydrogen and methane which can lead to abdominal pain and constipation^[9]. Fiber supplements^[10] and probiotics^[11] as well as drugs like rifaximin, neomycin^[12], laxatives^[13,14], lubiprostone^[15], and linaclotide^[16] all have variable effects in patients with IBS-C. There is still a need for safe agents to support GI health in patients with IBS-C.

Atrantil™, a dietary supplement composed of Quebracho, Conker Tree and *M. balsamea Willd* extracts, has been shown against placebo to statistically improve constipation and bloating in IBS-C subjects^[17]. Quebracho extract contains tannins which are large delocalized flavonoid structures that have been used safely in wine for decades^[18]. Tannins potentially have dual function^[19]: They act as molecular "sponges" for excess hydrogen and methane^[20] as well as disrupt and destroy bacterial lipid bilayers. Conker tree extract contains escins, also known as saponins. Saponins act as an antimicrobial agents, promote intestinal motility^[21] and directly reduce methane production/emission^[22,23]. *M. balsamea Willd* extract contains peppermint oil which has been shown to reduce abdominal pain and discomfort^[11].

Patients from a single, community physician practice, who had failed to respond to conventional therapy, chose to take a recommended over-the-counter dietary supplement composed of quebracho, conker tree and *M. balsamea Willd* extracts in attempt to manage symptoms of abdominal pain, bloating and constipation associated with IBS-C. Their medical chart responses were retrospectively analyzed for improvement in symptomatology.

CASE REPORT

Patient charts were retrospectively examined from a single physician's practice in this case report of 24 IBS-C patients who took the dietary supplement, Atrantil™ [quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts], after experiencing incomplete management of symptoms with other therapies. The quebracho extract has a 80%-82% polyphenol content with 72%-74% soluble tannins, primarily consisting by of profisetinidin subunits as part of trimeric, tetrameric and pentameric condensed tannins (about 75%) determined by MALDI-TOF and ¹H- and ¹³C-NMR fingerprint analysis. The Conker Tree extract is standardized to 20% saponin content by UV-Visible spectrophotometry and high performance thin layer chromatography (HPTLC) densitometry. Finally, pure peppermint oil content from *M. balsamea Willd* was determined by specific gravity, angular rotation and refractive index (USP29).

No IRB review or oversight was required in this analysis according to the terms of the United States Department of Health and Human Service's Policy for Protection of Human Research Subjects at 45 CFR and 46.101(b) since the data already existed in patient medical charts and this data was accumulated retrospectively. There was

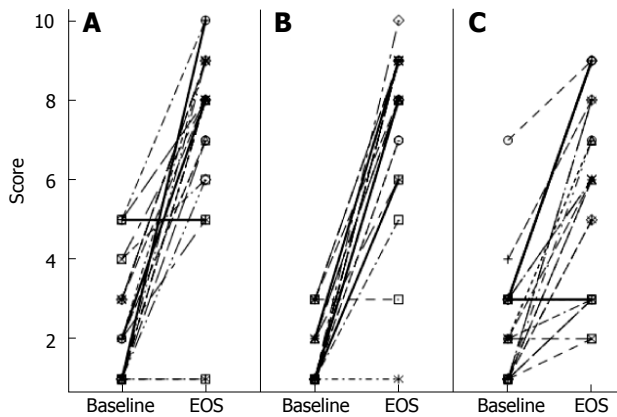


Figure 1 Visual Analogue Scores (0 = worst symptoms, 10 = no symptoms) were taken prior to administration of the dietary supplement (baseline) and at 2 wk end of analysis (end of analysis) for abdominal pain (A), bloating (B), and constipation (C). Each symbol represents a different, individual patient in the analysis.

no experimentation on patients. The dietary supplement was recommended to patients who chose to take the formulation after failing to respond to other treatments. The patients in this analysis were diagnosis with IBS-C for at least 6 mo prior to enrollment into the study (according to Rome III criteria) and had a history of uncontrolled symptoms of abdominal pain, bloating and constipation. Patients were previously on the FODMAP diet, probiotics and/or traditional drug treatments. The combined extract was administered for two weeks. Patient response to the combined extract was assessed by visual analogue scale (VAS) at baseline (before administration) and after 2 wk [End of Analysis (EOA)] for abdominal pain, bloating, and constipation as part of standard-of-care. The median and the 25th and 75th percentiles, the interquartile range (IQR), were used to summarize the scores. Sign tests, which make no assumptions about the shape of the distribution, were used to compare changes over time. Significance was defined as $P < 0.05$. Changes in therapy for rescue due to increased symptoms and side effects were also noted. All patients consented to have their data published.

The patients in this retrospective chart analysis ($n = 24$) ranged in age from 18 to 58. The population consisted of 2 men and 22 women with a racial composition of 21 Caucasian, 2 Hispanic or Latino, as well as 1 African American. There were also various co-morbidities of gastroesophageal reflux disease, rosacea, hypertension and fatigue, which did not contribute to their gastrointestinal condition. Patients were not taking any other therapies for IBS-C or SIBO when they were first administered the combined extract. By EOA, 21 out of 24 patients had responded with improved VAS scores for abdominal pain (Figure 1A), bloating (Figure 1B), and constipation (Figure 1C).

Overall, 88% of this gastroenterology practice population which had incomplete relief with traditional therapies responded to the combined herbal extract in the dietary supplement. A comparison of mean VAS scores for abdominal pain, bloating and constipation from

Table 1 Symptom response of irritable bowel syndrome with constipation patient population to combined herbal extract

Symptom	Baseline median (IQR)	EOA median (IQR)	EOA-baseline median (IQR)	<i>P</i> value
Abdominal pain	2.0 (1.0, 3.0)	8.0 (6.5, 9.0)	5.5 (3.5, 7.0)	< 0.001
Bloating	1.0 (1.0, 2.0)	8.0 (7.0, 9.0)	6.5 (5.5, 8.0)	< 0.001
Constipation	2.0 (1.0, 3.0)	6.0 (3.0, 8.0)	4.0 (2.0, 5.5)	< 0.001

EOA: End of Analysis (2 wk); IQR: Interquartile range (25%, 75%).

baseline and EOA showed a significant improvement in all three symptoms over time for the entire population while on the combined extract (Table 1).

A response rate of 88% in IBS-C patients with a significant reduction in abdominal pain, bloating, and constipation suggests very good efficacy in this difficult to treat population. No rescue medication was needed during the 2 wk course of the observation and there were no reported adverse events suggesting excellent tolerance of the herbal extract.

DISCUSSION

Over 90% of IBS patients suffer from bloating which is directly linked to abdominal pain and distention^[24]. These symptoms may be caused by SIBO or dysbiosis. No matter the cause, current therapeutics may not meet the needs of all patients. In a 10 wk study of rifaximin (550 mg TID) vs placebo in IBS patients, for example, the overall response rate was 40.8% vs 31.2% for placebo ($P = 0.01$)^[25]. Using a similar retrospective medical chart analysis to the one utilized in this study, Yang *et al.*^[26] found a 69% response rate to rifaximin and 44% to neomycin in 98 lactulose breath test positive IBS patients. Another study found that patients who had an abnormal lactulose breath test with follow up testing ($n = 47$) when treated with neomycin had a 75% response rate^[9]. Even with the success of antibiotic treatment, relapse remains a significant problem in SIBO patients^[27].

Other agents are also used for constipated patients. In an open-label extension study of lubiprostone ($n = 522$), a locally acting chloride channel activator, demonstrated a response rate of about 40%, but about 32% of participants in the extension part of the study required a rescue medication^[28]. Adverse effects for lubiprostone include dose-related nausea and dyspnea with chest tightness. For idiopathic constipation, linaclotide demonstrated about 50% response rate for pain and increase in stool frequency compared to placebo responses of about 35% and about 25%, respectively^[29,30]. About 20% of patients on linaclotide experienced diarrhea compared to about 3% in the placebo groups.

Nutritional approaches to IBS-C and SIBO include dietary fiber, the FODMAP (Fermentable oligosaccharides, disaccharides, monosaccharides and polyols) diet

and probiotics. Fiber can be effective in managing constipation, but bloating, distension, flatulence and cramping may limit the use of insoluble fiber. Water intake with fiber is very important. In patients with IBS, soluble fiber, such as psyllium may be effective, but insoluble fiber can exacerbate symptoms^[10,31]. The FODMAP diet has been found to decrease abdominal pain and bloating, but adherence to the diet can be difficult^[32]. Probiotics containing *Bifidobacterium lactis* DN-173 010, *Lactobacillus casei* Shirota, and *Escherichia coli* Nissle 1917 demonstrate favorable data on defecation frequency and stool consistency^[33]. Other approaches are still needed for patients with IBS-C and SIBO.

In a 2 wk randomized, double-blind placebo-controlled study of patients previously diagnosed with IBS-C ($n = 16$), there were significant improvements in the average constipation ($P = 0.0034$), bloating ($P < 0.001$) and constipation plus bloating scores ($P < 0.001$) in the Atrantil™ group compared to no improvement for the placebo arm^[17]. There were also no reports of AEs over the 2 wk period. In this retrospective chart analysis of 24 patients administered Atrantil™, there was a 3.2-fold average improvement in abdominal pain, a 5.1-fold improvement in bloating, and a 2.7-fold improvement in constipation. Twenty-one of 24 patients responded to therapy for an overall response rate of 88%. There were also no reported side effects to therapy. These consistent data suggest that the combined herbal extracts of quebracho, conker tree and *M. balsamea Willd* present in Atrantil™ decreases symptoms associated with IBS-C.

The quebracho extract consists primarily of tannins, the same used in for over 50 years to change the taste and texture of wine^[18]. Tannins are highly delocalized structures which are able to act as antiradical sinks or antioxidants^[34]. Tannins also directly limit methanogenesis by inhibiting the growth of methane producing bacteria by reducing the availability of hydrogen^[35]. Conker Tree extract contains the antimicrobial saponins^[21] which can also reduce the production as well as emission of methane presumably by limiting hydrogen availability^[22,23]. Saponins have also been found to improve intestinal motility in mice^[36] and improve passage of gas, GI sounds and bowel movements in postoperative colorectal surgery patients^[37]. The *M. balsamea Willd* extract contains peppermint oil which has been found to help reduce abdominal pain^[11]. Peppermint oil has also been shown to act as an antispasmodic attenuating contractile responses to acetylcholine, histamine, 5-hydroxytryptamine, and substance P^[38,39]. The combination of these extracts in Atrantil™ may have limited the availability of hydrogen by preventing growth of microorganisms which produce methane that contributes to abdominal pain, bloating and constipation in this IBS-C patient population. In addition, the combination extracts may also improve motility and intestinal transit time.

Though this pilot medical chart analysis was performed in a relatively small number of patients ($n = 24$) with IBS-C, the response rate was very high (88%). The small number of patients, the fact that they were

drawn from a single site and the uncontrolled nature of the analysis with only therapy adherent individuals being evaluated are limitations for this study. Still, the statistical improvement in symptoms of abdominal pain, bloating and constipation found in this retrospective study are consistent with a previous placebo-controlled clinical trial^[17]. Therefore, the results of this small open-label study of Atrantil™ may be a useful intervention for patients with IBS-C and SIBO. Further, larger double-blind, placebo-controlled studies are needed to confirm these results.

COMMENTS

Case characteristics

The primary symptoms experienced by this clinical practice cohort of patients were abdominal pain, bloating and constipation.

Clinical diagnosis

Significant improvements in abdominal pain, bloating and constipation were found after a 2 wk administration of the mixed quebracho/conker Tree/*M. balsamea Willd* extracts in Atrantil™ in irritable bowel syndrome with constipation (IBS-C) patients.

Differential diagnosis

Organic causes of constipation were excluded first for all patients in this practice cohort which were then diagnosed with IBS-C according to Rome III criteria for functional constipation including at least two of the following: (1) two or fewer defecations in the toilet per week; (2) At least one episode of fecal incontinence per week; (3) History of retentive posturing or excessive volitional stool retention; (4) History of painful or hard bowel movements; (5) Presence of a large fecal mass in the rectum; and (6) History of large diameter stools which may obstruct the toilet.

Laboratory diagnosis

Since there is no tissue or blood marker for IBS-C, no laboratory testing was performed in this case series.

Treatment

Twenty-four IBS-C patients in a single clinical practice were provided a combination over-the-counter dietary supplement composed of quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts (Atrantil™) and chose to take the formulation for a minimum of 2 wk in an attempt to manage abdominal pain, bloating and constipation.

Related reports

This case series is a follow up to a well-controlled pilot clinical study in IBS-C patients (Brown *et al.*, 2015) testing the same dietary supplement in IBS-C patients composed of quebracho (150 mg), conker tree (470 mg) and *M. balsamea Willd* (0.2 mL) extracts (Atrantil™).

Term explanation

All terms in this case series are standard and used in the field of gastroenterology.

Experiences and lessons

This case series shows the utility of a dietary supplement in drug refractory IBS-C patients formulated to act as a molecular sink for gas ions in the intestine, a bacteriostatic agent to inhibit the impact of bacteria in the small bowel and a component to aid in abdominal discomfort.

Peer-review

The limitations of this case series were that it was in a relatively small cohort of patients biased for compliance in consuming the therapeutic agent in an uncontrolled setting. This case series in combination with the previously

published pilot clinical trial suggests promise for Atrantil™ in IBS-C patients with the caveat that a larger, well-controlled study is needed.

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