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Titolo della tesi:
Role of endoscopic scoring, mesenteric excision, and anastomosis technique in Crohn's Disease recurrence after ileocecal resection

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Chapter 1

Pathophysiology of Crohn's Disease and Principles of Treatment

Background

Crohn's Disease (CD) is a chronic inflammatory intestinal disease, first described as regional ileitis by Crohn, Ginzburg and Oppenheimer in a case series presented at American Medical Association annual meeting in 1932 [1]. CD inflammation interests the whole intestine, being the most frequently affected part the distal ileum. Patients with CD experience periods of flares and periods of remissions during their disease course. Pathogenesis results from the interactions of environmental factors, immune system, susceptibility genes and host's microbiome changes, leading to disruption of the intestinal mucosa. The role of inflammatory cells in maintaining an active disease is well known and most of the therapies aim to stop the cascade of inflammatory and proinflammatory cytokines. Treatment of CD is multidisciplinary: medical treatment is focused on mucosal healing and symptoms reduction; surgery maintains a key-role in treating complications such as stenosis, perforations, fistulas, and abscesses. [2, 3]. Although most CD patients will be treated surgically at least once in their lives, postoperative recurrence (POR) of the disease has proven virtually inevitable [4–8].

Endoscopic evidence of CD recurrence in the pre-anastomotic ileum is found in approximately 60% [9] of patients at six months and 80-90% at 12 months [10] from the index operation [11, 12]. Endoscopic POR is not a synonym for clinical recurrence although generally the former heralds the latter [13]. 15-30% of patients will complain of florid CD symptomatology at 12 months [14–17], 20-40% at 24 months, and so on [18]. Re-do surgery occurs in up to 50% of patients with long-term follow-up [19]. The precise pathogenesis of postoperative recurrence is not known although many studies have shed light on the pathways involved. Inflammation is the ultimate mechanism of tissue destruction that is reinitiated in POR. A deeper understanding of recurrence mechanisms may help clinicians and surgeons refine treatment and improve outcomes.

Pathogenesis and inflammation

Crohn's disease pathogenesis is based on tissue inflammation, caused by an unrestrainable immune response against luminal bacterial antigens (Fig. 1). Immune cells like CD4 T-Cells, CD8 T-Cells, B-Cells, CD14 monocytes and natural killers, are involved in this process as they infiltrate the gut of CD patients. Part of the immune-mediated susceptivity to CD resides in some innate mechanisms of defense form infectious diseases and the intestinal mucus secretion is part of those. It has been shown that variants of the Muc2 gene reducing mucus production are associated to CD in a mouse model. Moreover, molecules that are mediating bacterial adhesion have been correlated to the disease. This is the case of FUT2, which encodes for the fucosyltransferase enzyme, responsible for the secretion of soluble forms of the ABO antigens. People harboring a FUT2 variants decreasing the secretion of the antigens, have an altered interaction with bacteria and are more prone to developing CD [20]. The pathogenesis is also sustained by the interaction of these cells with integrins, adhesion molecules and multiple chemokines, responsible to produce elevated levels of inflammatory cytokines, representing the target of immune and non-immune cells and the promotion of mucosal inflammation. As such, among many adhesion molecules, some evidence on the involvement of the leucocyte MAcCAM-1, receptor for the α4β4 integrin, seems to play a crucial role. Together with leucocyte adhesion, the role of the extracellular matrix on their activation has been explored. Proteins like CD44 and CD26 were shown to play a role as well as metalloproteins (MMP), being MMP1 and MMP3 abundant in the granular tissue close to CD sites of inflammation, therefore responsible for leucocytes activation [21–24]. In the mucosa of CD patients, a dysregulation of various components of the immune system is invariably found. The most pronounced alteration is the hyperactivity of T cells with excessive production of cytokines, between which IL-12 and IFN-γ, promoting a TH1 lymphocytic phenotype, opposed to the TH2 one, correlating to ulcerative colitis. Moreover, TNF-α production has also been demonstrated to increase the number of CD4+ FoxP3+ TREG cells, especially in the mucosa of children affected by CD [25]. The inhibition of the effector cytokines, like TNF-α, attenuates the detrimental effects in subsets of CD patients. Furthermore, the expression of the interleukins, a subgroup of cytokines implicated in the enhanced or inhibition of other cytokines in many different regulatory pathways such as maturation, growth, and responsiveness of immune cells population, is to be considered anomalous in CD patients [26]. Further analysis of T cell subsets has revealed the presence of TH1 and TH17 cells in CD, whereas the cytokines considered more involved are TNF, IL12 and IL23.

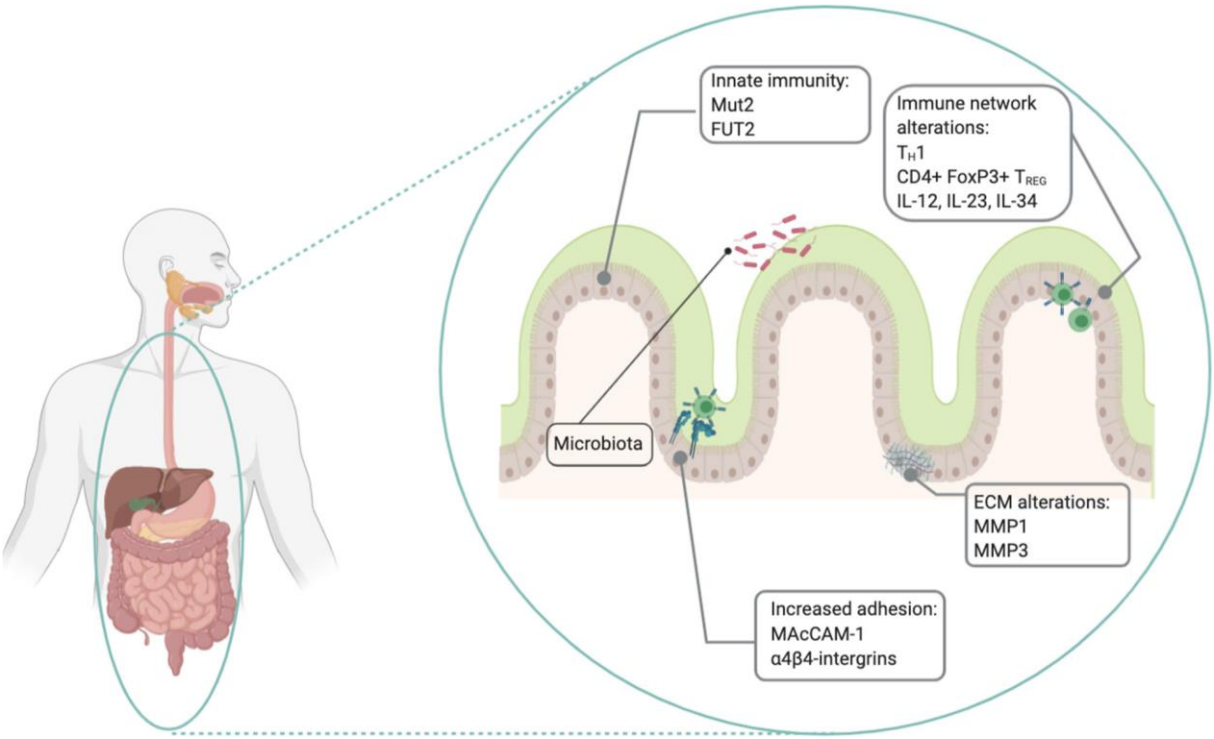

Fig. 1 Immuno-mediated pathogenesis of Crohn's disease. Crohn's disease is a multifactorial pathology in which a major role is played by alterations at the level of immunity and inflammation. Innate immunity is involved in terms of defects in the mucous barrier (Mut2 and FUT2 genes) while adaptive immunity relies on a TH1 lymphocitic response and TREG cells mediated by cytokines like TNF-α, IL-12, IL-34 and IL-23. The increased migration to the sites of inflammation is also determined by a reshaping of the extra cellular matrix through the action of metalloproteins (MMP-1 and MMP-3) and the overexpression of adhesion molecules such as MAcCAM-1 and integrin α4β4. Finally, also the host pathogen interaction between the intestinal epithelium and the microbiota has been linked to the evolution of the disease.

Apart from the cited cytokines, IL-34 has also been associated to IBD and CD in particular. IL34 expression is more pronounced in the areas of active inflammation, especially in CD, and seems to induce TNF-α and IL6 expression through an ERK-mediated mechanism. Moreover, IL-34 has been described as an inducer of CCL20 through the interaction with its receptor the M-CSFR1, abundantly expressed in the inflamed colonic epithelium but not in the healthy controls. On the contrary, IL-25 inversely correlates to the inflammatory state of the patients with IBD, being reduced in CD patients as opposing to healthy subject and being reduced in the affected areas of the colon if compared to the surrounding adjacent normal tissue from the same subject. Among all the possible interleukins associated to CD pathogenesis, IL- 12 and IL-23 represent the target of still inadequate therapies because of potential side effects, such as increased risk for infection, and the blockade of specific immunological targets, capable of induction of alternative signaling or homing pathways. The latter mechanism may also partially explain the frequent lack of response to therapy with biologics such as Infliximab (Remicade©), a chimeric monoclonal antibody used to treat autoimmune diseases, that works by binding to TNF-α causing the reduction of IL-34 expression, implicated in monocyte and macrophage differentiation, survival, and function [27–31]. Although T-Cells are the main effector lymphocytes in intestinal tissue inflammation, also humoral immune system plays a crucial role. Plasma cells differentiation indeed is promoted by CD4 T-Cells, through a mechanism that is firmly dependent on IL-2, overproduced in CD patient's gut. IL-21 converts naive B-Cells into B Cells expressing granzyme-B: it

possesses a cytotoxic activity on the intestinal mucosa and perpetuates the epithelial damage. These proofs indicate that an altered balance between effector and counter regulatory factors is probably involved in the sustainment of the tissue damaging immune response in CD [32]. Gut microbiota also plays a recognized role in designing the inflammatory response in IBD and especially in CD. There is growing evidence that some microbial gene products can influence gene expressions in the host [33–35]. The complex network arising from this assumption is referred to as the microbial-associated molecular pattern (MAMP) which is sensed by toll-like receptors on immune cells, contributing to their activation in the context of the chronic inflammation [36]. Microbiome moreover represent a source of potential pathogenic inputs that can be approached through the methods used in the omics era, such as metagenomics studies, also impacting on our knowledge on geographical variations on the clinical manifestation of the disease [37–40]. The inflammation is generally transmural, and, on pathology examination, granulomas may be identified on biopsies, with a discontinuous distribution along the longitudinal axis. This inflammatory process often leads to irreversible tissue damage in the form of intestinal stenosis or fistulas, inflammatory masses, or intra-abdominal abscesses. Patients can develop one or more of these disease behaviors and they often tend to evolve from inflammatory to penetrating or stricturing disease [41].

Medical management

Medical management (Fig. 2) should be tailored based on various factors such as disease severity, subtype, behavior, and location [42]. Moreover, it is important to consider other factors such as age at diagnosis, extension of the lesions and extra-intestinal manifestations [43]. As matter of fact, none of the drugs used in the treatment of CD has been demonstrated to be curative or completely safe. Mesalazine, which belong to the 5-ASA compounds category, has been evaluated in many studies and it has never shown to induce or maintain remission in CD. Its benefits are related to its safety outline [44]. Antibiotics are principally recommended to treat septic complications; like mesalazine, they do not show a real efficacy in the treatment of CD, except in the short-term treatment of perianal fistula in association with anti-TNF. Most frequently used agents are fluoroquinolone and metronidazole [45]. Systemic corticosteroids show a fast onset of action and are indicated to induce remission. Unfortunately, steroid dependency or steroid resistance can jeopardize their use that is often accompanied to a wide range of side effects like obesity, hypertension, glaucoma, cataracts, and adrenal insufficiency [46]. Another major part is played by immunosuppressant like thiopurines and methotrexate: thiopurines are used to maintain remission in moderate CD, usually in combination with steroids. Before starting thiopurines treatment, it is mandatory to assess TPMT (Thiopurine S-methyltransferase) activity, crucial for their metabolism [47]. Methotrexate may be considered as an option for steroid-dependent patients. Included in the side effects are hepatotoxicity and more rarely myelosuppression; they are prohibited during pregnancy because teratogenic and abortifacient [48]. Anti-TNF drugs are considered the most powerful tools to treat moderate and severe form of CD, alone or in association with immunomodulators to obtain and maintain remission. The most frequently used anti-TNF are Infliximab (Remicade©), a chimeric antibody that is administered intravenously; Adalimumab (Humira©), a fully humanized monoclonal antibody administered subcutaneously; Certolizumab (Cimzia©), a FaB antibody fragment of humanized anti-TNF molecule [49]. More recently super-selective target

monoclonal antibodies have been developed, directed against a specific pattern of inflammation. In this class there are Vedolizumab (Entyvio©), that targets the adhesion molecular inhibiting leukocyte migration [50], and interleukininhibitors like Ustekinumab (Stelara©), a fully humanized monoclonal antibody targeting the p-40 subunit of IL-12 and IL-23 [51].

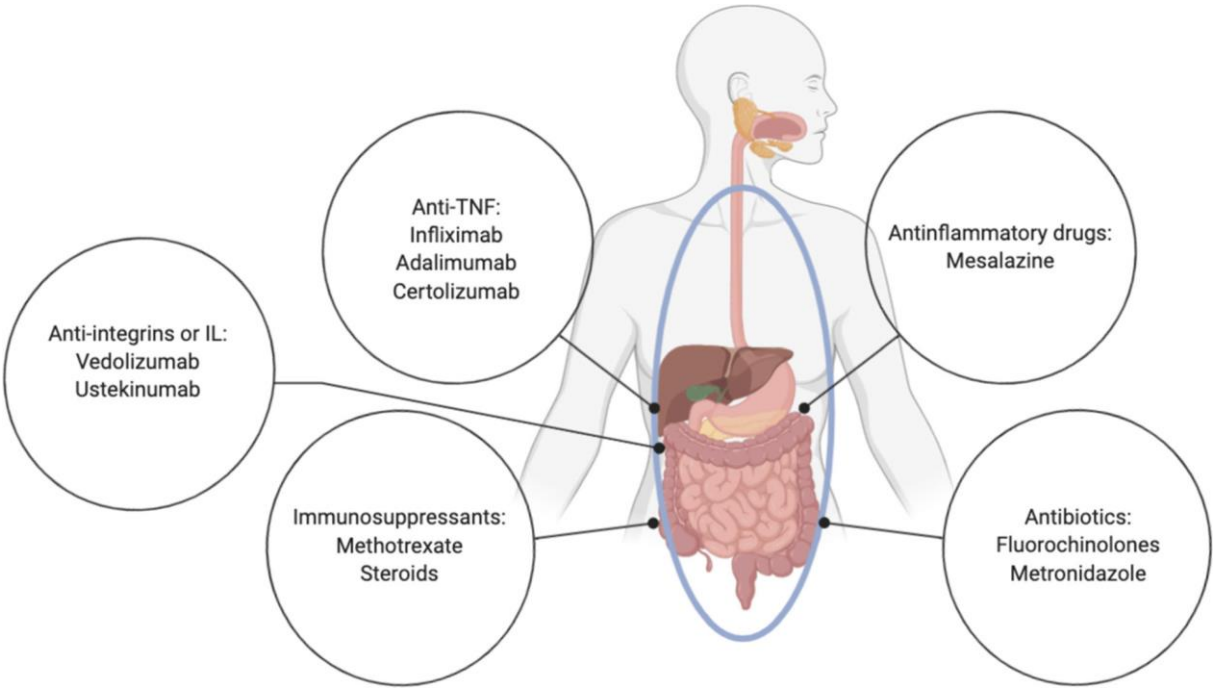

Fig. 2 Medical treatments of Crohn's disease. Many medical options for the treatment of Crohn's disease are available, but still not resolutive. Among them, anti-inflammatory drugs as mesalazine, antibiotics such as fluorochinolones and metronidazole and immunosuppressants (methotrexate). More targeted treatment options are directed towards TNF-α (Infliximab, Adalimumab, Certolizumab) or against integrins (Vedolimumab) and interleukins IL-12 and IL-23 (Ustekinumab).

Surgical management and Crohn's Disease recurrence

When CD was described for the first time, therapy was exclusively surgical. Since the beginning of CD surgery experience, there was no consensus on the optimal procedure. At the Mount Sinai Hospital in New York, Dr. Berg was the surgeon who operated fourteen patients presented by Crohn. The "Berg" operation, also known as "Mount Sinai," operation, implied exclusion bypass of the ileocecal region, transecting small bowel proximal to the diseased ileum, over sewing distal ileum, and anastomosing the proximal ileal end into the mid-transverse colon [52, 53]. In fact, this was a staged management, being the second planned step the resection of the diseased bowel. Performing more and more cases, Mount Sinai surgeons noted that during the second stage of surgery, the bypassed bowel seemed to have "healed" in several cases. Starting from this observation and as patients manifested clinical improvements, they decided to omit the planned second procedure. Obviously, a great debate about this procedure and all early and late risks linked to it arose blow out of the blind end, reactivation of CD in the excluded segment with abdominal pain and infections, deprivation of a large portion of the colon for water absorption. Eventually, bypass procedure was abandoned

due to findings of adenocarcinoma occurring in the excluded segment [54-56]. Surgical resection of the diseased bowel emerged as the procedure of choice for most patients with CD of the terminal ileum or with ileo-colitis, including complicated cases [57]. In tandem, advances in perioperative care, such as nutritional improvement, anesthesia and fluid and electrolyte management, guaranteed CD surgery improvements and safety. At this point physician focused on the amount of resection. In fact, it was commonly accepted practice for surgeons to resect all macroscopically involved intestine with large resection margins. Consciousness of CD as a panenteric affection and, above all, the evidence of the inevitable recurrence and possible development of short bowel syndrome due to repetitive surgery, suggested the adoption of a conservative policy, avoiding wide resections [58]. For this reason, to avoid short bowel syndrome - in particular in those scenarios characterized by extensive jejunal-ileitis with fibrotic stenosing segments scattered along the diseased intestine - Lee from Oxford, in 1986, reported another advancement in surgical management. Lee was inspired by the work of Indian surgeons on the management of tuberculous strictures: they observed that small bowel preservation could be achieved in patients with multiple tuberculous strictures by strictureplasty [59]. Lee applied strictureplasty to the short intestinal strictures of Crohn's disease and from that moment, with a great variability on techniques according to the different situations, strictureplasty assumed a fundamental role for CD surgeons [60,61]. Different strictureplasty techniques have been described. Heineke-Mikulicz strictureplasty consists of a longitudinal enterotomy closed in a transverse direction and it is best applied to stricture up to 7 cm in length [62]. Finney strictureplasty is used for longer stenosis, up to 10–20 cm: after an antimesenteric longitudinal incision, the opened bowel segment is bent into a U shape and posterior and anterior layers are close with continuous absorbable suture [63]. Michelassi strictureplasty is indicated for the treatment of multiple strictures, interesting up to 90 cm long bowel segment; in this case, a segment of diseased bowel is anastomosed to a nonaffected segment of intestine [64]. Interestingly this technique has shown to induce remission in the diseased part. The mechanism is still unknown, however there seems to be a process in CD whereby obstruction is responsible for the pathogenesis of many complications [65]. This technique allows the mitigation of fecal stasis, which may play a central role in postoperative mucosal healing, modifying the microbial-mucosal interaction. Possibly, the resolution of chronic obstruction may interrupt the cascade of events causing active disease [66–68]. Another cornerstone in CD surgery was represented by the advent of minimally invasive surgery. Feasibility and safety of laparoscopic ileo-cecal resection has been assessed and it was found not inferior in terms of outcomes when compared to open surgery [60–71]. Nowadays, laparoscopy is largely accepted as the first line approach for CD, in the presence of adequate expertise [72]. Laparoscopy demonstrated advantages in terms of cosmetics and postoperative recovery and assured some long-term advantages, including fewer incisional hernias, fewer adhesions, and a significant impact on female fertility [73, 74]; unfortunately, no clear differences on time to recurrence was found. From a surgical point of view CD recurrence should be considered as an inevitable consequence. The same factors that underline the pathogenesis of CD at its first stages are thought to be responsible for post-operative recurrence (POR) setting, being the result of interplay of microbial, environmental, immunological, and genetic variables [75]. Within this contest, microbial flora role seems to be linked to the fecal stream, as demonstrated by Rutgeerts et al. [76, 77] investigating the rapid recurrence of microscopic inflammation in the mucosa of excluded ileum when newly interested by fecal content. The term post-operative recurrence is used to define the appearance of new lesions after bowel resection. Active surveillance for an early diagnosis is considered mandatory. Rutgeerts endoscopic index is possibly the most

widely used scoring system to detect recurrent lesions [78, 79]. Previous studies demonstrated that the lesions are located more often in or near the area of anastomosis, usually reproducing the same initial pattern of the disease, though it has been suggested that postresection lesions should be considered new. The presence of microscopic lesion, detected during endoscopic examinations 1 year after surgery, reinforces their role as precursors of POR. Timing of endoscopic surveillance has been discussed considering available evidence; recommendation is to perform endoscopic examination after 6 months from surgery or within the first year [80, 81]. Other techniques have been investigated to assess POR [82]; in particular, there is a great interest in non-invasive techniques such as ultrasonography (US). Among several emerging US technique, Small Intestine Contrast Ultrasonography (SICUS) resulted more sensitive in detecting small bowel lesions in CD patients [83]. SICUS has been demonstrated to be comparable to ileocolonscopy, also after surgery, allowing the visualization of extra luminal lesions related to CD (bowel wall thickness, mesenteric and lymph nodes enlargement). Hence, in expert hands, SICUS could be considered a valid alternative for the follow-up and early diagnosis of POR after surgery in CD [84–87].

Chapter 2

Postoperative Recurrence after Ileocecal Resection

Pathogenesis of Post-Operative Recurrence of Crohn's Disease

Postoperative recurrence of CD is possibly an even more obscure event, yet again, it is beyond doubt that the host's immune system plays a major role. A summary of pathogenic mechanisms can be found in Table 1 and Figure 2.

Innate Immune System Role in Postoperative Recurrence

The innate immune system represents the body's first line of defense. It includes physical barriers (such as cells of the epidermis or mucosa, which can also actively contribute with their secretions) and immune cells such as granulocytes, monocytes, dendritic cells, and natural killer (NK) lymphocytes. Most of these elements have been shown to be involved in CD, but little evidence exists on the innate system's role in CD POR. Nonetheless, mechanisms involved in disease pathogenesis may also be implied in disease recurrence, while others appear to be specific for POR.

Table 1: Elements involved in CD recurrence.

Domain	Specific element	Putative mechanism of action	Level of evidence
Innate immune system	NOD2 gene rs2066844 polymorphism	Improper activation of the immune response (pattern recognition receptor)	Low-moderate
Innate immune system	SMAD3	Drive fibrosis after initial surgical scarring	Low
Innate immune system	CARD8	IL-1 production, immune dysregulation	Low
Innate immune system	Matrix metalloproteinases	Initiation of inflammation/fibrosis	Low-moderate
Innate immune system	Macrophages	Bacterial trafficking to mesenteric nodes	Low
Innate immune system	Dendritic cells and basophils	Set up a potent and durable acquired immune response	Low
Adaptive immune system	Effector T-cells	Bring about most recurrent inflammation	Moderate-high
Adaptive immune system	Memory T-cells	Persist after resection in mesenteric lymph nodes (and blood to a lesser degree) and conserve immunological memory	Moderate-high
Adaptive immune system	B-cells	Produce anti-GM-CSF immunoglobulins; others	Low
Immune system as a whole	Dysbiosis	Increased bacterial trafficking; others	Low
Immune system as a whole	Increased mucosal IL-6; decreased mucosal IL-10; RNASET2 polymorphism	Increase mucosal inflammation	Low
Immune system as a whole	Inflammation at resection margins	Persistence of activated cells	Low
Immune system as a whole	Myenteric and submucosal plexitis	Unknown	Low
Immune system as a whole	Diet	Stimulate the immune system through poorly understood mechanisms	Low-moderate
Other risk factors	Smoking	Unknown; may increase mucosal T-cell hyperclonality	High
Other risk factors	Penetrating disease at index surgery, perianal disease, prior intestinal surgery extensive small bowel resection (>50 cm)	Unknown	High

Derangements in the Pattern Recognition Receptor and Other Genes.

First, genetic analysis has identified a number of genes associated with greater odds of recurrence. The first gene to be correlated to CD has been NOD2/CARD15. This gene is an intracellular pattern recognition receptor (PRR), involved in the recognition of peptidoglycans and activation of innate immune responses. While its role in CD pathogenesis is certain, its implication in recurrence, although reasonable, is less definite and a meta-analysis [88] has failed to demonstrate a significant association [89]. A more recent study has identified instead, at multivariable analysis, an independent association between recurrence and a particular NOD2 polymorphism, rs2066844, which was not previously investigated [90]. Another gene, SMAD3, has never been linked to CD pathogenesis but was found to be an independent predictor of recurrence after ileocolic resection [91]. The authors speculate that SMD3 polymorphism may pathologically increase the scarring process after surgery and enhance fibrosis, resulting in accelerated recurrence and the need for reoperation. Finally, a recent study on 137 patients with bowel resection for CD analyzed more than 200 genetic variables [92]. Only CARD8, a gene that is highly expressed in monocytes and in the gut epithelium was independently predictive of surgical recurrence. CARD8 is a negative regulator of nuclear factor-κβ (NF-κβ), which is in turn a suppressor of apoptosis, and interacts with the inflammasome, which mediates IL-1 production and has a role in the maintenance of gut homeostasis. These functions place CARD8 as a regulator of innate and adaptive immunity. However, the exact pathways through which CARD8 enhances surgical recurrence have yet to be elucidated.

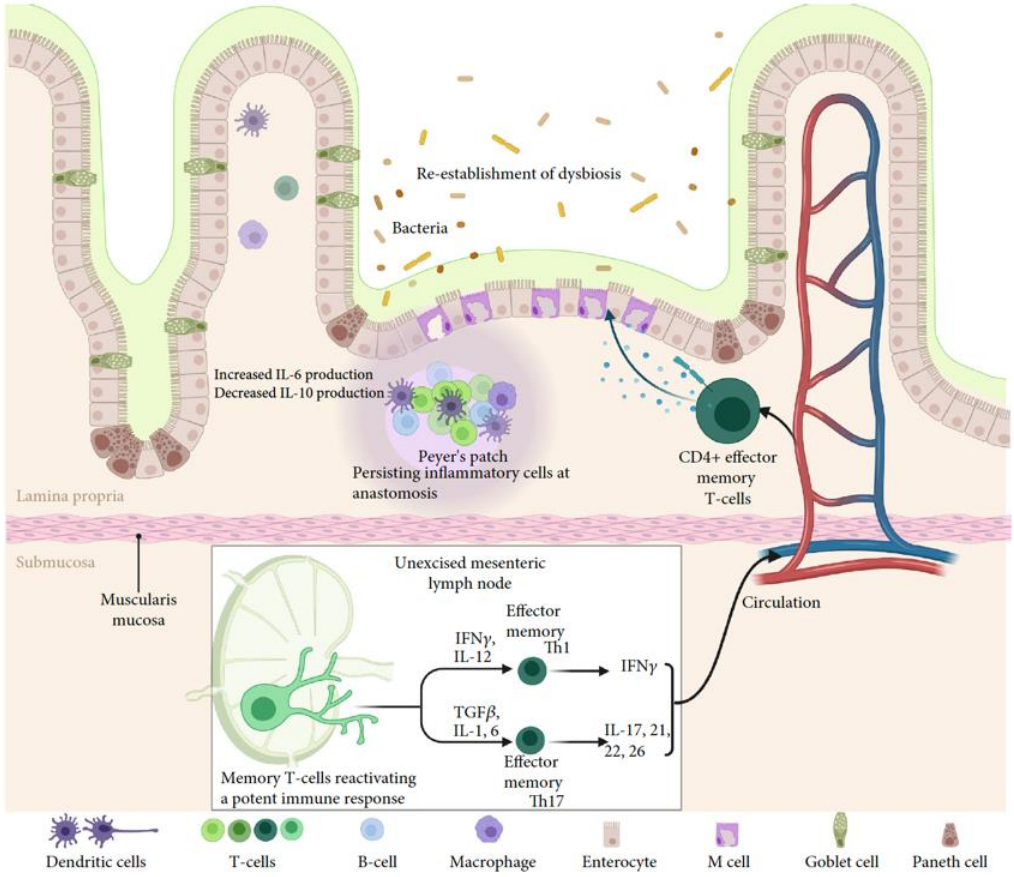

FIGURE 2: Pathogenesis of Crohn's disease recurrence. Created with https://BioRender.com

Matrix Metalloproteinases.

Matrix metalloproteinases (MMPs) are a group of endoproteinases with proteolytic activity directed toward various matrix proteins, secreted by inflammatory cells (chiefly macrophages). Tissue inhibitors of metalloproteinases (TIMPs) regulate their activity. In the inflamed mucosa of CD, the MMP/TIMP balance is tipped in favor of MMPs. One study found a TIMP-1 variant to be correlated to surgical recurrence but not to diagnostic recurrence, implying a relatively mild- or late-onset influence [93]. Investigators have also shown that higher expression of TIMP-1 and TIMP-2 in the noninflamed mucosa after resection was protective from diagnosis of recurrence and from repeated resections. The magnitude of this effect is quite impressive: the median time to recurrence in patients with low levels of TIMP-1 was 10 years, compared to 17 years in the high expression group.

Cells of the Innate Immune System, Their Response to Commensal Microbiota, and Clinical Implications.

Neutrophils represent the main kind of cell involved initially in acute inflammation and are the main actor in the typical cryptic abscesses of IBD. No study specifically correlates these cells to CD recurrence, but their presence in intestinal nervous plexuses has been implicated in some studies (see Immune Function as a Whole). Macrophages are very important innate immunity cells, which not only are major actors of acute inflammation but also are also involved in chronic inflammatory states and represent a major link with

acquired immunity. Their role in CD pathogenesis is multifaceted and includes the production of proinflammatory cytokines (in response to inappropriate TLR stimulation), generation of granulomas, mediation of fibrosis, and disruption of intestinal epithelial barrier function [94–96]. Less is known about specific actions in POR. Their infiltration in the mucosa of the neoterminal ileum of POR-free patients suggests their driving role of inflammatory events that bring about recurrence [97]. One mechanism through which they may be involved in POR has been highlighted in experimental studies that involve CX_3CR1^{hi} cells. These are mucosal resident mononuclear phagocytes, whose role is to capture luminal bacteria, to compartmentalize the immune response and avoid inflammation. These cells therefore represent the "second line" of defense. While previously reported to be a nonmigratory population, Diehl et al. [98] have shown in experimental models that they can be endowed with the ability to migrate and traffic bacteria (or their antigens) to mesenteric lymph nodes, key immune inductive sites. This may well start the inflammatory process. Not only have the authors described this phenomenon but, even more interesting, they also have unveiled a tight connection with luminal microbiota. In fact, it appears that dysbiosis is a potent activator of bacterial trafficking through this route. This mechanism has not been studied in recurrence. The effects of microbiota on recurrence have been studied. Sokol et al. have demonstrated that surgery produces profound changes in ileal microbiota, reducing dysbiosis typical of CD such as increased Gammaproteobacteria and declined Lachnospiraceae and Ruminococcaceae [99]. The magnitude of these changes was significantly reduced in patients who developed early postoperative endoscopic recurrence, so much so that biopsies at follow-up showed that diversification in microbiota decreased in parallel with an increase in the Rutgeerts score. The authors went so far as to develop a predictive score for recurrence that was based primarily on microbiota composition at surgery. Unfortunately, this score was not applicable to patients who had undergone antibiotic therapy in the last month before surgery (a considerable fraction of CD patients) and even more importantly did not predict the outcome in patients who were administered anti-TNF preventive therapy. Putting these facts together, one might conclude that dysbiosis reestablishment after surgery induces a new bout of bacterial trafficking, immune induction, and inflammation. This model is purely speculative, and it is likely that immune memory would have a greater role than a new activation. Nonetheless, if it does have a role, two strategies would have the potential to maintain the surgery-induced changes in microbiota and slow down recurrence: probiotic administration and fecal transplantation. A multicenter randomized controlled trial investigated the effect of probiotics on early endoscopic recurrence but failed to demonstrate a protective effect [100]. It must be said that in this study, a specific probiotic strain, Lactobacillus johnsonii, was used: whether other probiotics could better serve this purpose and revive this practice is unknown. Fecal transplantation brings the "probiotic strategy" to the extreme. This intervention has been extensively shown to be effective in the treatment of ulcerative colitis, but high-quality evidence lacks in CD. No randomized trial exists to date [101], and there is considerable heterogeneity in many aspects of therapy including donor selection, fecal conditions (fresh/frozen), delivery route, and antibiotic pretreatment [102–104]. Metanalyses of cohort studies have shown that it may have a good rate of clinical remission induction [105]. To date, no study exists on the evaluation of this therapy after surgery. Given the strong rationale for its use, this may indeed be the focus of upcoming trials. Another fundamental function of innate immunity is that of dendritic cells (DCs). They are potent antigen-presenting cells whose role in CD is well established: activating site-specific Th1 and Th17 responses [106–109]. Specific populations of dendritic cells direct inflammation and dictate CD location (by inducing homing markers) [110, 111]. These cells therefore set up a potent and

durable immune response that is thought to be the principal driver of CD recurrence. In this way, they drive recurrence indirectly. Whether they also play a more direct role by reviving this mechanism after resection is not known to date. Basophils have been recently found capable of serving a similar function to DCs [112].

Acquired Immune System Role in POR

The acquired immune system represents the body's more refined defense machinery. It mainly relies on the action of activated lymphocytes that are capable of initiating, amplifying, and perpetuating an adaptive, antigen-specific response. Lymphocytes include B-cells responsible for humoral immunity and T-cells, which conduct the cell-mediated arm.

T-Cells.

Cell-mediated immunity could be the real protagonist of the recurrence process. There is evidence that the mucosa of the affected bowel harbors significantly increased clonal expansions of T-cells compared to that of healthy controls [113]. Whether this clonality principally concerns CD4+ or CD8+ cells have not been consistent, as both have been implicated in different studies [114]. In any case, the presence of high clonality seems to be the harbinger of endoscopic recurrence, with greater percentages of clones associated with a greater risk of recurrence. Furthermore, these highly expanded clones are different from clones found in healthy tissue, supporting a pathogenic role of Tcells [115]. The T-cell receptor (TCR) repertoire seems to be different in different individuals, with no commonly targeted antigens, highlighting the singularity of each CD patient's immune response. Another observation was that the TCR repertoire in the mucosa could change with surgical resection. Postoperative persistence of a TCR repertoire similar to the ones before resection also correlates to the development of endoscopic recurrence. In one study, the investigators could identify a particular clone, highly enriched in active CD tissues, and found its presence to be correlated to the severity of endoscopic recurrence (based on Rutgeerts score). Another study identified a subset of T-cells, CD4+NKG2D+ T-cells, whose presence at the ileal mucosal margins of resection predicted endoscopic disease recurrence. In fact, this subset was found to be persistent in the mucosa of patients with recurrence. CD4+NKG2D+ T-cells display high expression of tumor necrosis factor alpha (TNF-α) and other proinflammatory and cytotoxic molecules. Possibly, they would be left behind in the macroscopically healthy bowel and then induce recurrence. It is unclear however how they (being already present before operation) would induce active CD only after surgery. An alternative and more consistent explanation would be that they persist in large numbers in the mesenteric lymph nodes draining the resected bowel (which instead are not removed in current CD surgery) and are then free to home back into the neoterminal ileum. In fact, lymph nodes not only are a site of immune induction but also represent the anatomical location where the majority of memory T-cells reside [116-117]. Therefore, mesenteric lymph nodes (MLNs) may be reasonably considered the dwelling of immune memory in CD. Bsat et al. have studied MLNs of CD patients in depth and found them to be enriched in Th17 memory Tcells and in particular effector memory Th17 cells (TEM). The same group has also elucidated an IL-12-dependent pathway through which these memory cells are activated in the MLN and switched to a Th1 phenotype (i.e., cytokine/- molecular profile) before being recruited back to the mucosa through homing mechanisms. This places the MLN at the center of a memory-driven immune reactivation in CD and therefore, potentially, of recurrence. Enhancement of MLN in imaging

studies is, in fact, predictive of active disease [118]. The so-called trafficking blocking strategy, based on the fact that lymphocytes have to home back to the, until then, healthy gut from the nodes where they reside, has been given much prominence in recent drug development with promising ongoing trials [119]. Immunological memory was also implicated by genetic studies on BACH2 (a highly conserved transcription factor that is a fundamental element in the maturation of B-lymphocytes and differentiation of T-cells, especially in regulatory T-cells) which independently correlate it to surgical recurrence [120]. The authors speculate the underlying mechanism to be the inability to memorize an appropriate (tolerant) response to luminal antigens.

B-Cells.

The part played by B-cells in CD inflammation is less clear [121]. So far, the most studied immunoglobulins have been peripheral Anti-Neutrophil Cytoplasmic Antibodies (pANCA) and Anti-Saccharomyces cerevisiae Antibodies (ASCA). Taken together, they are good serologic markers for CD. Other antigens eliciting a humoral response in CD are Omp-C (outer bacterial membrane protein, E. coli-derived), CBir1, A4-Fla2, and Fla-X (flagellin subunits, Clostridiumderived). In one study, anti-Fla-X positivity at baseline was associated with endoscopic recurrence at 18 months while anti-Omp-C with multiple surgeries [122]. The significance and the processes underlying these findings are yet unclear. Antibodies could be inducing recurrence also through the neutralization of protective cytokine signaling. Granulocyte- Macrophage Colony-Stimulating Factor (GM-CSF) enhances innate immune responses to luminal microbial antigens, thereby confining inflammation, avoiding antigen trafficking and activation of secondary immunity, and contributing to homeostasis. Anti-GM-CSF antibodies have been recorded in patients with CD, and their titers positively correlated with a shorter time to the second surgery [123]. It is possible that serology will be eventually used to predict the postoperative course and identify patients who would profit from a more aggressive, proactive treatment strategy.

Immune Function as a Whole Role in POR

Some factors that have been associated with recurrence cannot easily be categorized as “innate” or “adaptive” immunity but must be considered affecting the immune system as a whole.

Cytokines.

Cytokines are the signaling molecules of the immune system. They contribute to a variety of functions and are fundamental for a proper inflammatory response to take place. Some specific cytokines are known to be involved [124–130] and represent current therapeutic targets, others have been identified, and their targeting holds promise. In one study on 36 patients undergoing bowel resection, IL-6 was found to be elevated in most patients despite clinical remission, indicating persistent subclinical (systemic) inflammation through the IL-6-C-reactive protein (CRP) cascade [131, 132]. Patients with surgical recurrence of their disease had particularly high IL-6 levels, which resulted in a significant predictor of recurrence in this series. Yet this was extrapolated from only three patients, and two of them had recurred within 5 months. Even though very early surgical recurrence is a possibility, this more often represents a complication of the first intervention or preexisting disease that was overlooked or judged nonsignificant at the first operation. Nonetheless, this study

highlights the ongoing inflammation that underlies the postoperative CD course and eventually recurrence. More evidence on the subject comes from a Japanese group [133]. They took blood samples and ileal biopsies from 36 resected patients: 16 of them had clinical recurrence within 1 year. In this group, only ileal mucosal IL-6 levels correlated significantly with clinical recurrence, but not serum possible that patients with surgical recurrence are a group with more aggressive and advanced disease and therefore present high IL-6 levels systemically rather than only at the primary inflammatory site. Reduction of anti-inflammatory cytokines is another mechanism observed to correlate with endoscopic lesions after surgery. In particular, IL-10 levels in the ileal mucosa predict the development of postoperative recurrence. Different IL-10 haplotypes exist and are associated with greater or lesser production of the cytokine, yet these were not able to predict recurrence [134]. It has been therefore speculated that other local environmental factors, so far not predictable, are responsible for IL-10 production in the mucosa. RNASET2, a gene shown to be involved in enhancement of IFNγ production (the main cytokine involved in Th1 responses), has been observed to be associated with more severe recurrence (Rutgeerts score > 2) and, in line with this, to shorten time to repeat surgery [135]. A putative mechanism would therefore be an enhancement of adaptive cellular immunity and a quicker and more severe postoperative reestablishment of disease. Other observations come from direct clinical practice. The most used drug for the prevention of postoperative recurrence is infliximab, a monoclonal antibody directed against TNF-α. Infliximab is of proven benefit in this situation and is recommended by current guidelines. There is high-quality evidence from a randomized controlled trial that it reduces the incidence of endoscopic recurrence (by a half!) at one year from surgery [136]. At the same time, it did not reduce clinical recurrence in the same period. Yet, clinical recurrence occurs for most patients beyond this period, and therefore, the real clinical impact of infliximab has yet to be revealed with longer-term follow-up. Moreover, there is some evidence pointing to fewer operations for CD during the anti-TNF era [137]. This may indeed have an impact on surgical recurrence rates although this is so far unproven. Finally, the effect of anti-TNF therapy on endoscopic remission may be more pronounced in patients who were previously naïve or had undergone therapy with only one anti-TNF agent [138].

Inflammation in Resection Margins and Intestinal Nervous Plexuses.

Another analyzed factor is histologic evidence of inflammation at resection margins. Inflammation is defined in different studies in many ways and includes inflammation of the mucosa, of the submucosa, and even of the nerve plexuses. The first reports on microscopic evidence of inflammation at resection margins have done much to shape the current concepts of surgery for CD. It was shown that the presence of features of chronic inflammation or diagnostics of CD at resection margins did not influence recurrence rates [139, 140]. This was the basis to advocate as short a resection as possible, on the macroscopically healthy bowel [141]. This approach has been the standard practice for decades and is thought to have reduced the risk of short bowel syndrome. Lately though, this subject has been revived by new studies that have given us a slightly different perspective. Poredska et al. have reported resection margin inflammation (at both the proximal and distal margins) to be related to endoscopic POR [142]. In their study, “inflammation” included signs of both the acute and chronic forms. One recent report has focused instead on active inflammation at resection margins (defined as a Geboes score > 3) and found that its presence on the colonic margin was the only histologic predictive factor for clinical POR [143]. They failed to demonstrate any correlation for other histologic factors like myenteric plexitis and granulomas. Other investigators have studied both factors widely, so far

with nondefinitive results. Granulomas' presence in the histologic specimen seems to predict surgical POR and a shorter time to the second surgery in a large meta-analysis [144]. Myenteric plexitis was reported to be a risk factor for endoscopic POR in a landmark study by Ferrante et al. in 2006 [145]. More research focused on submucosal plexitis and found a similar association (again, not consistently) [146, 147]. Finally, one study incriminated increased enteric plexus glial cells [148]. The trouble to compare these studies is that most are retrospective and that they define "inflammation," "plexitis," etc. in different ways, as there is no consensus or grading system specific for these features in CD [149]. In fact, results are variable as to which feature is predictive as much as where to assess it and how. In general, predicting POR through a baseline histologic assessment on the index specimen is a promising approach but one that still requires confirmation and refinement before entering common clinical practice (or trials).

Diet.

Another very interesting factor is diet. CD patients are at high risk of malnutrition, in both the active and quiescent diseases, and dietary evaluation is fundamental in improving nutritional status, thus ameliorating disease outcomes [150]. Some investigators have specifically assessed diet influence on POR. An elemental diet (ED) has been advocated to reduce bowel inflammation. Most studies on the subject come from Japan, where it is used as standard therapy, in contrast to the USA or Europe [151]. It consists mainly of amino acids, which do not possess any intrinsic antigenicity and therefore are bereft of proinflammatory capacity. While large experience has been accumulating with its use in relapses, there are relatively few studies in the postoperative setting. The major limitation to ED is that patient compliance is generally low as it can be quite distasteful. When large amounts of calories must be taken as ED, the use of a self-inserted nasogastric tube is commonly advised. This practice has its own obvious limitations, and adherence is limited. Yamamoto et al. conducted a prospective controlled study on long-term postoperative ED in 40 patients. Patients were not randomized but arbitrarily assigned to non-ED vs. ED based on predicted compliance with the regimen. The two groups differed significantly at 1 year in terms of endoscopic remission [152]. At 5 years, clinically significant recurrence was impressively in favor of the ED group (10 vs. 45%) [153]. Surgical recurrence also showed a trend in favor of the ED group but was not significant. Another study showed similar results on endoscopic POR [154]. Despite fascinating results, they should still be considered preliminary as the number of patients was small and the study design was not devoid of bias. Today, the method has not entered common practice and has not gathered excessive interest in western countries.

Other Risk Factors for Postoperative Recurrence

Other risk factors for POR that are not strictly related to the immune system are as follows: penetrating disease at index surgery, perianal disease [155], prior intestinal surgery extensive small bowel resection (>50 cm), and smoking [156]. No clear explanation exists for any of these: probably, they simply herald a more aggressive phenotype. Smoking is undoubtedly the most studied with a higher level of evidence [157–160]. An interesting finding by Allez et al. is that smoking is associated linearly with hyperclonality of T-cells in the affected mucosa. This data therefore links smoking to the actual inflammatory processes that lead to recurrence: specific CD4+ T-cell clones. Ileocolic disease may recur more often than disease at other sites when assessed morphologically, yet it is less often symptomatic, and rates of clinical recurrence are lower.

One study has tried to put these elements together in a nomogram to predict early disease recurrence [161]. Interestingly, they have also included low preoperative serum albumin and “excessive perioperative inflammation” (expressed as high CRP levels). This attractive instrument however has not been widely validated so far.

Chapter 3

Prevention of Postoperative Recurrence

Prevention

We have so far analyzed several risk factors for the development of CD recurrence. The usefulness of this knowledge would be to detect patients in greater peril and to use treatment strategies accordingly. This concept is already in place for what concerns postoperative drug treatment, with anti- TNF and immunosuppressants being used in patients at high risk or with signs of endoscopic POR. Other pharmaceutical targets will surely be investigated in the coming years. Different medical pathways may also prove of benefit in the future such as fecal transplantation and hematopoietic stem cell transplantation. New surgical approaches are also promising. A summary of preventive strategies may be found in Table 2.

Hematopoietic Stem Cell Transplantation.

In the last decade, much work has been focused on one objective: resetting the entire immune system. This may be accomplished through a treatment well known to hematologists: hematopoietic stem cell transplantation (HSCT). HSCT for CD has been inspired by two parallel phenomena. The former was the

observation of prolonged (yet not indefinite) remission in patients with HSCT performed for other (hematologic) indications [162]. The latter was the success of this strategy in patients suffering from other autoimmune diseases like systemic sclerosis and systemic lupus erythematosus. Of course, given the high toll requested by allogenic HSCT, trials have used autologous HSCT. Many investigators have reported encouraging results [163]. The main drawback of this approach is the high rate of complications and mortality that persists even with the autologous form [164]. The highest level of evidence comes so far from the ASTIC trial. This trial failed to show the superiority of HSCT in achieving the primary outcome [165]. Yet, the primary outcome was an extremely ambitious one: sustained disease remission at 12 months, defined as a composite of Crohn'sDiseaseActivity Index ›CDAI < 150, no active treatment for at least 3 months, and no endoscopic evidence of disease (erosion/ulceration). A different analysis of data coming from the same trial, using conventional endpoints used in trials for CD, highlighted a significant benefit in the treatment arm [166]. When relapse occurs, patients tend to respond to conventional treatment to which they had become refractory prior to transplantation [167]. A new randomized trial has successively been set up, ASTIC lite, using a lower dose regimen in an effort to maximize the benefit/hazard ratio [168]. No trial is currently evaluating HSCT in the postoperative setting.

Surgical Approach and Diagnostic Procedures.

Surgical intervention and endoscopic scoring have also been at the center of much research in an endeavor to diminish POR, but the surgical approach did not appear to have any consequence on the short- or long-term disease course [169–175]. Nevertheless, the following are currently the more promising research fields in understanding POR in CD On which we focused to develop the three main research projects of this doctoral thesis.

Table 2: New perspectives for recurrence prevention.

Domain	Prevention strategy	Description	Rationale
Medical	Fecal transplantation	Transfer of fecal bacteria from healthy subjects	Avoid dysbiosis reestablishment
Medical	Hematopoietic stem cell transplantation	Bone marrow ablation followed by autotransplantation of multipotent stem cells	Reset immune system
Surgical	Michelassi strictureplasty	Side-to-side isoperistaltic strictureplasty	Induce mucosal healing by resolution of chronic obstruction
Surgical	Kono anastomosis	Fashioning of antimesenteric wide lumen anastomosis	Increase the diameter and stability/durability of anastomosis
Surgical	Mesenteric excision	Excision of the associated mesentery	Removal of pathological tissue
Surgical	Pathophysiological excision	Excision of draining mesenteric lymph nodes	Removal of most memory T-cells, drivers of inflammation and recurrence

Role of Endoscopic Scoring

According to guidelines (i.e. European Crohn's and Colitis Organisation (ECCO)), patients should be

assessed by endoscopy at six to twelve months postoperatively to diagnose endoscopic recurrence. In case of endoscopic recurrence therapeutic medical therapy is (re)initiated; and if prescribed prophylactically, optimized in order to prevent long-term complicated disease. (176) The guidelines recommend the use of the Rutgeerts classification to assess recurrence severity. Lesions in the category i2-i4 are considered endoscopic recurrence. (177-178) The classification was modified to discriminate pure anastomotic lesions, which are considered more likely due to postoperative changes (i2a), from the presence of more than five aphthous lesions in the neo-terminal ileum, with or without anastomotic lesions (i2b). (179-180) Despite this modification, the reproducibility of the classification is disputed. (181-183) Moreover, the mRS is (yet) not advised in ECCO guidelines.

Adequate endoscopic scoring is important, as it is used to tailor medical therapy and to monitor the effect of therapy. Especially in this asymptomatic patient group, where endoscopy is used as postoperative surveillance, a reliable scoring system is of utmost importance. After all, improper diagnosis of recurrent disease results in prescribing medical therapy unnecessary. This will impair the patient's quality of life and increase health care consumption. Moreover, the Rutgeerts classification is used as a primary outcome in clinical trials.

For these reasons, the objective of the first study (Chapter 4) of this doctoral thesis was to assess the variance in endoscopic recurrence in patients after ICR for CD, using the most common classification systems, the Rutgeerts- and modified Rutgeerts classification.

Role of Mesenteric Excision

There is a growing interest in modifying surgical techniques aiming to reduce recurrence rates. (184-193) Ongoing research investigates the role of the mesentery and the influence of type of anastomosis on postoperative recurrence rates.

The involvement of the mesentery in CD was first described in 1932 by Burrill Crohn himself, and the creeping fat is generally considered one of the hallmarks of macroscopic CD. Initially, radical surgical resection was performed, which was associated with major morbidity and mortality at the time. (194) In current daily practice, the mesentery is usually left in situ, as advised by the European Crohns and Colitis Organisation (ECCO) guidelines.

Coffey et al. have shown a significant decrease in postoperative surgical recurrence in their retrospective series of patients, by adopting a more radical, “mesenterybased” surgical approach. Despite the magnitude of reported results being impressive, the inherent limitations in study design and specific aspects of the work lend themselves to perplexity and criticism [195]. Furthermore, the rationale for its use is scarce: very little literature supports the hypothesis of a “mesentery-driven” disease [196–202]. On the contrary, some evidence exists that important proinflammatory mechanisms are unique to the mucosa and cannot be observed in the mesentery [203]. Finally, the proposed approach seems very difficult to standardized as the correct amount of the mesentery to be removed is unknown and the suggested “mesenteric disease score” to evaluate the severity of mesenteric affection probably suffers from great interobserver variability [204].

However, prospective data from a randomised controlled trial for patients with ileocecal CD are lacking.

Therefore, an international prospective randomised controlled trial to analyse the effect of extended mesenterectomy on postoperative recurrence rates was conducted (Chapter 5) as a second project of this

doctoral thesis. It was hypothesised that extended mesenteric resection is superior, with a decreased endoscopic recurrence rate when compared to mesenteric sparing resection in ICR for CD.

Role of Anastomotic Technique

The anastomosis type and fashioning technique have also been the objective of many investigations. Stapled anastomosis (despite boasting lower leak rates) is not superior to handsewn in terms of recurrence rates [205]. The same considerations are true for side-to side vs. end-to-end anastomosis [206, 207]. To reduce recurrence, some investigators have proposed new surgical techniques [208, 209]

In a retrospective cohort study, a newly introduced anastomosis, the Kono-S, appeared to be associated with less endoscopic recurrence than the stapled side-to-side anastomosis [210]. Although the study results were notably flawed, the study reignited interest in the various types of anastomoses and their impact on disease recurrence. However, a recently published study comparing Kono-S with stapled side-to-side anastomosis, demonstrated no difference in endoscopic recurrence, illustrating the controversies in the current literature [211].The currently recommended anastomosis after ileocolic resection is the stapled side-to-side anastomosis due to its ease of construction and safety [212, 213]. The third type of anastomosis, the end-to-end handsewn anastomosis appears to be less popular due to its technical complexity [214]. However, a recent study comparing end-to-end with stapled side-to-side anastomosis, showed reduced healthcare consumption in favour of the end-to-end anastomosis [215]. In terms of functionality, the end-to-end anastomosis theoretically outperforms the Kono-S and stapled side-to-side anastomosis. Both have a saccular design that is presumed to be associated with luminal content stasis and consequently functional problems and recurrent Crohn's disease [216, 217]. To date, prospective data from a randomized controlled trial comparing these three anastomoses are lacking, and it remains unclear which anastomotic technique is superior in terms of endoscopic recurrence. The aim of those two multicentre international randomized studies proposed as a protocol as a third part of this doctoral thesis (Chapter 6)is to determine which anastomoses handsewn (end-to-end versus Kono-S anastomosis) or side-to-side stapled anastomosis after ileocolic resection for Crohn's disease is superior with respect to endoscopic recurrence, gastrointestinal function, and (healthcare) costs.

Part II

Role of Endoscopic scoring system in diagnosis of recurrence

Chapter 4

Major variance in scoring of endoscopic recurrence after ileocolic resection for Crohn's disease –a systematic review and meta-analysis

Methods

The study protocol was prospectively registered at PROSPERO (registration number: CRD42022363208), and the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidance was followed throughout the process. (218) The final search was performed on January 4th, 2022.

Search strategy

MEDLINE (PubMed), Embase (Ovid) and the Cochrane Library were searched systematically with the assistance of a clinical librarian.

Medical subject headings (MeSH) and free-text terms used included 'Crohn's Disease', 'ileocolic resection', 'ileocecal resection', 'ileocaecal resection', 'modified Rutgeerts score', 'Rutgeerts score', 'anastomotic ulcer'. There were no restrictions considering the publication date or language, and no other methodological

filters were applied.

Author	Year	Design	Centre(s)	Country	N	Definition	Timing endoscopy
Auzolle et al.	2018	prospective	MC	France	225	both	6 months
Bachour et al.	2022	retrospective	SC	USA	240	mRS	1 year
Bellinger et al.	2014	retrospective	SC	France	46	RS	1 year
Bislenghi et al.	2021	retrospective	SC	Belgium	47	mRS	6 months
Bonanga et al.	2014	retrospective	MC	USA	50	RS	1 year
Bommelaer et al	2020	RCT	MC	France	62	mRS	6 months
Boschetti et al.	2016	prospective	SC	France	86	RS	6 months
Boschetti et al.	2015	prospective	MC	France	37	RS	6 months & 1year
Bourreille et al.	2006	prospective	SC	France	31	RS	6 months
Buisson et al.	2021	retrospective	MS	France	63	both	6 months
Buisson et al.	2022	retrospective	SC	USA	316	RS	6 months
Calabrese et al.	2009	prospective	SC	Italy	72	RS	6 months
Camargo et al.	2019	retrospective (abs)	NR	USA	261	RS	1 year
Caprilli et al.	1996	RCT	MC	Italy	98	RS	6 months
Caprilli et al.	2003	RCT	MC	Italy	84	RS	1 year
Cerrillo et al.	2019	prospective	SC	Spain	32	mRS	6 months
de Barcelos et al.	2016	retrospective	MC	brazil	127	RS	6 months
de Bruyn	2021	RCT	MC	Netherlands	142	mRS	6 months
De Cruz et al.	2022	retrospective of RCT	MC	Australia	85	mRS	6 months
De Cruz et al.	2015	retrospective of RCT	MC	Australia	101	RS	6 months
Djelouah et al.	2021	retrospective	SC	France	40	mRS	1 year
Domenech et al.	2008	prospective	SC	Spain	53	RS	1 year
Domenech et al.	2017	retrospective	MC	Spain	116	RS	1 year
Echarri et al.	2012	NR (abs)	SC	Spain	36	RS	6 months
Fortinsky et al.	2017	retrospective	SC	Canada	171	RS	1 year
Glick et al.	2019	retrospective	SC	USA	70	RS	1 year
Golovey et al.	2019	prospective (abs)	SC	Israel	33	RS	1 year
Han et al.	2018	retrospective	SC	China	83	RS	6 months
Hanauer et al.	2004	RCT	MC	USA	131	RS	1 year
Hirten et al.	2020	retrospective	SC	USA	182	RS	6 months
Huang et al.	2021	retrospective	SC	China	87	RS	1 year
Joustra et al.	2022	retrospective	MC	Netherlands	142	both	6 months
Keshteli et al.	2018	retrospective	SC	Canada	38	RS	6 months
Kono et al.	2011	retrospective vs prospective	MC	Japan	132	RS	1 year
Kotze et al.	2015	retrospective	MC	Brazil	168	RS	6 months
Laffin et al.	2018	prospective	MC	Canada	45	RS	6 months

Lasson et al.	2014	prospective	MC	Sweden	30	RS	1 year
Lavelle et al.	2017	prospective (abs)	SC	Ireland	64	RS	6 months
Lemmens et al.	2017	prospective	SC	Belgium	74	mRS	6 months
Lopes et al.	2016	prospective	SC	Portugal	99	both	6 months
Lopez Sanroman et al.	2017	RCT	MC	Spain	61	mRS	1 year
Luglio et al.	2020	RCT	SC	Italy	83	RS	6 months
Lukas et al.	2014	retrospective (abs)	SC	Czechia	106	RS	6 months
Machiels et al.	2020	prospective	SC	Belgium	120	mRS	6 months
Machiels et al.	2016	prospective (abs)	SC	Belgium	30	RS	6 months
Maggriori et al.	2019	prospective	MC	France	346	RS	1 year
Manosa et al.	2013	RCT	MC	Spain	50	RS	6 months & 1 year
McLeod et al.	2009	RCT	MC	Canada	139	RS	1 year
Mocciaro et al.	2019	retrospective (abs)	NR	Italy	64	RS	6 months
Monteiro et al.	2017	retrospective	SC	Portugal	42	RS	1 year
Nakamura et al.	2019	retrospective (abs)	MC	USA	123	RS	6 months
Nakao et al.	2017	retrospective	SC	Japan	40	RS	1 year
Noben et al.	2014	NR (abs)	NR	Belgium	100	RS	6 months
Olaison et al.	1992	prospective	SC	Sweden	30	RS	1 year
Ollech et al.	2019	retrospective	SC	USA	207	mRS	1 year
Onali et al.	2016	prospective	SC	Italy	40	RS	1 year
Papamichael et al.	2012	retrospective (abs)	SC	Greece	59	RS	6 months
Paredes et al.	2013	prospective	SC	Spain	60	RS	1 year
Poredeska et al.	2020	prospective	SC	Czech Republic	107	RS	6 months
Primas et al.	2021	retrospective	SC	Austria	79	mRS	1 year
Riviere et al.	2021	retrospective	MC	Belgium	365	both	6 months
Rutgeerts et al.	1990	prospective	SC	Belgium	89	RS	1 year
Rutgeerts et al.	2005	RCT	MC	Belgium	61	RS	1 year
Satsangi et al.	2017	RCT	MC	UK	240	RS	1 year
Savarino et al.	2013	retrospective (abs)	SC	Italy	51	RS	1 year
Shen et al.	2018	prospective	SC	China	40	RS	1 year
Siva et al.	2016	retrospective (abs)	NR	USA	131	RS	1 year
Sooben et al.	2018	retrospective (abs)	SC	Australia	97	RS	6 months
Suzuki et al.	2014	retrospective (abs)	MC	Brazil	96	RS	6 months
Tang et al.	2020	retrospective	SC	China	101	RS	6 months
Veyre et al.	2021	prospective	SC	France	55	RS	6 months
Viazis et al.	2016	NR (abs)	SC	Greece	36	RS	6 months
Walshe et al.	2021	prospective	MC	Canada	206	RS	6 month

Walters et al.	2011	NR (abs)	SC	Canada	93	RS	1 year
Yamamoto et al.	2016	prospective	SC	Japan	40	RS	6 months
Zaghiyan et al.	2014	prospective (abs)	SC	USA	165	RS	6 months

Table 1. Included studies with POR rates within one year following surgery. MC multi centre, SC single centre, abs congress abstract, NR not reported.

Study selection

All studies describing primary ileocecal or ileocolic resection in patients with Crohn's disease with ER rates were included. ER was defined as Rutgeerts score (RS) ≥2 or modified Rutgeerts score (mRS) ≥i2b at six to twelve months postoperative.

Studies not reporting primary ICR or ER defined as RS or mRS at six to twelve months after surgery were excluded. Animal studies, reviews, case reports and letters were excluded. Other exclusion criteria were patients aged less than 18 years and less than 30 patients included in the study. In case of overlapping study cohorts, the original study or - if POR was not reported - the study with the most included patients was included.

Two reviewers (EDW, VB) separately screened the titles and abstracts of the retrieved articles and independently assessed the full text of the remaining articles. Disagreements concerning the selection were resolved by joint discussion and, when necessary, the opinion of a third researcher (initials) was obtained.

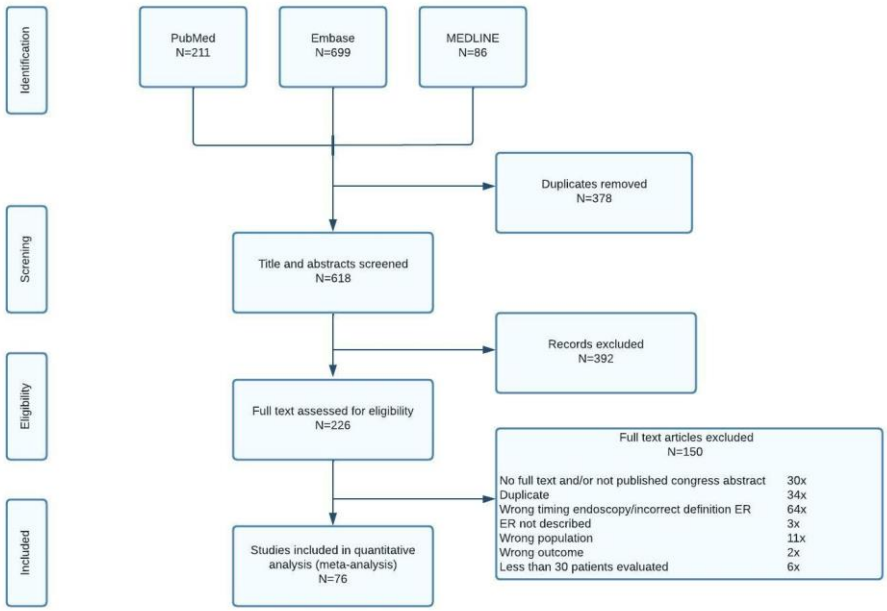

Figure 1. PRISMA flowchart

Outcomes

The primary outcome was range of ER rates defined as RS ≥i2 or mRS ≥i2b within one year after ICR.

Secondary outcomes were 1) the range of ER rates defined as RS ≥i2 within one year after surgery; 2) the range of ER rates defined as mRS ≥ i2b within one year after surgery; 3) the range of ER according to subcategories (i0, i1, i2a, i2b, i3 and i4); 4) the difference in ER rates comparing RS and mRS in studies reporting mRS; and 5) the range difference based on time interval after surgery, six months versus one year.

Quality assessment

The methodological quality of cohort studies was assessed by the Newcastle-Ottawa Quality Assessment Scale. In three different domains (selection, comparability and outcome) stars were assigned, with a total maximum of nine stars. In the outcome domain, a minimum follow-up period of twelve months and a maximum proportion of five per cent of subjects lost to follow-up was considered acceptable. Studies were rated as good, fair or poor depending on the number of stars, following the Agency for Healthcare Research and Quality (AHRQ) standard.(219) For randomized controlled trials (RCTs) the Cochrane Collaboration's tool for assessing risk of bias was used. This tool focuses on selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias, rated as low, high or unclear risk. Supplementary tables 3 and 4.

Data extraction and statistical analysis

Study and patient characteristics collected included first author, year of publication, number of patients, design, single- versus multicentre and ER rates within twelve months. The ER rates were extracted directly from the results or calculated by subtracting number of recurrences from the recurrence rates. Within the group of patients with ER defined as ≥i2b, the ER rates according to the Rutgeerts score (≥i2) for each studies individually was calculated manually. Studies reporting mRS were divided into subcategories in order to calculate the range of ER according to subcategories. The per protocol analysis was selected in case both the intention to treat and per protocol analysis available.

Data analysis was done using IBM SPSS Statistics Data Editor, version 28. Weighted mean differences of the percentage of POR with 95% confidence intervals (CIs) were computed. A histogram of the percentages was computed to show the distribution of the percentages reported by the included studies. A meta-analysis with forest plot was not preferable due to the large number of included studies. The independent t-test was used to determine the difference in means between the two time intervals, six months postoperatively and one year postoperatively.

RESULTS

Study selection

The initial literature search identified 966 studies in PubMed, in Embase and from the Cochrane Library, after removal of duplicates 618 studies remained. Subsequently, titles and abstracts were screened. Eventually, 222 potentially eligible publications were assessed based on full text, of which 76 studies met the inclusion criteria and were included in this review and meta-analysis (Fig. 1), including eleven RCTs (220-231) and 65 cohort studies (232-297).

In total, 76 studies including 7751 patients investigated ER defined by RS ≥2, mRS ≥i2b or both – within one year after primary ICR for CD, table 1. A pooled analysis showed a weighted mean of the ER rates of 43.99% (95 per cent CI 43.56 – 44.43). The range of reported ER (defined as either RS ≥i2 or mRS ≥i2b) was 5.0% – 93.0%. A histogram shows the distribution of the ER percentages weighted by number of patients as reported in the included studies, figure 2.

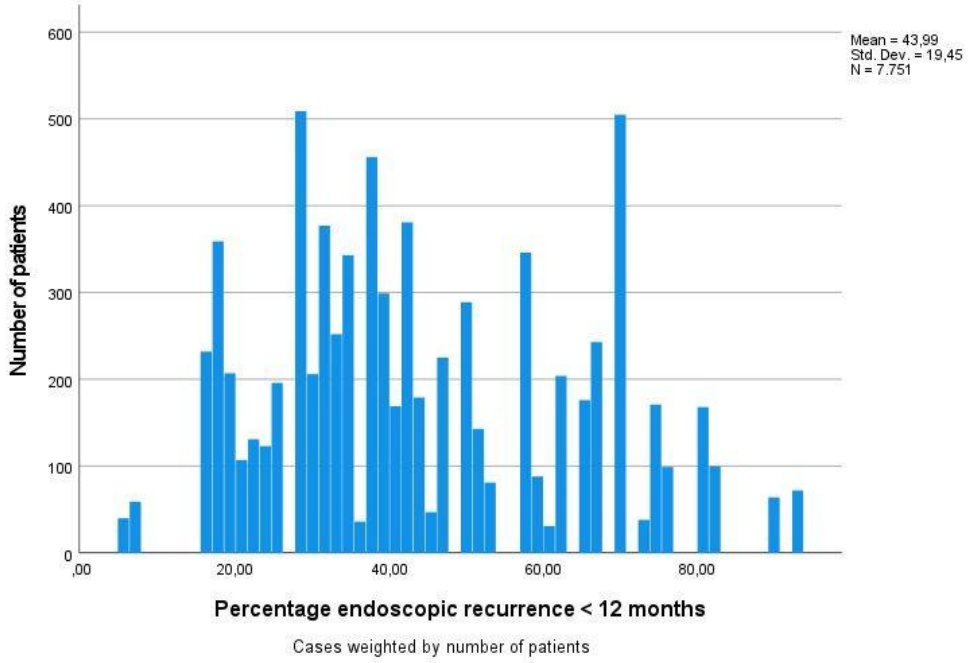

Figure 2. Histogram of distribution reported ER percentages weighted by number of patients

Range of ER rates defined as RS ≥i2

Of the 76 included studies, 64 studies including 6562 patients, investigated the Rutgeerts score (RS ≥i2) within twelve months following surgery. The weighted mean of the ER rates was 44.02% (95 per cent CI 43.53 – 44.51); range 5.0% – 93% (range 88%), figure 3.

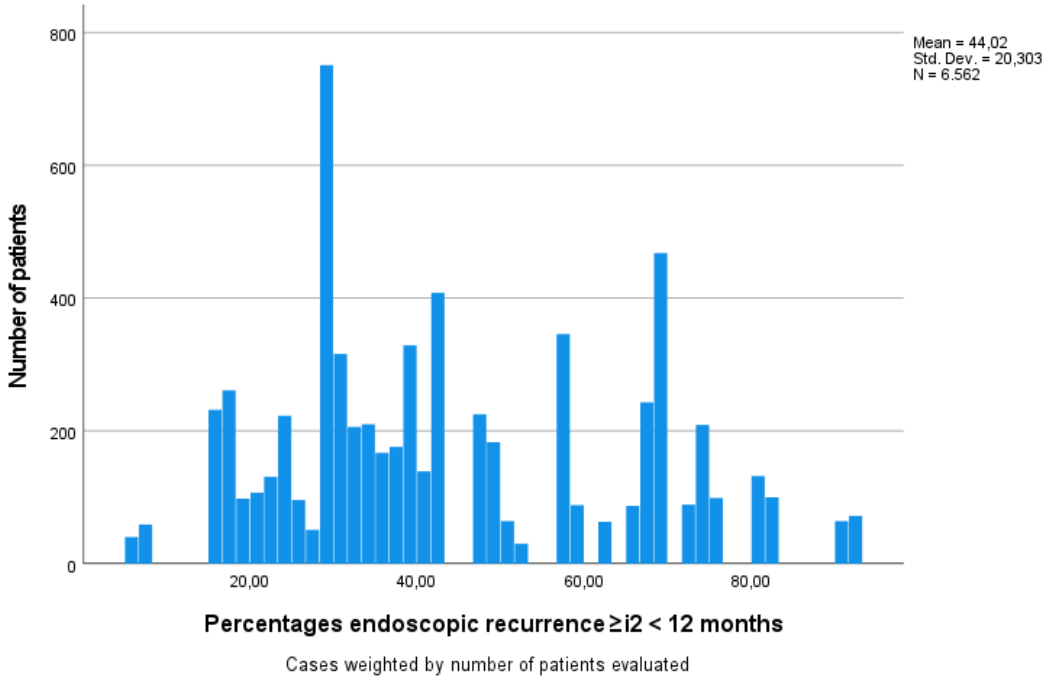

Figure 3. Histogram of distribution reported ER percentages weighted by number of patients

Range of ER rates defined as RS ≥i2b

Some 17 studies, including 2083 patients, investigated ER rates according to mRS (≥i2b), within twelve months following surgery. The weighted mean of ER rates for this group was 41.06% (95 per cent CI 40.60 – 41.52), range of 19.8% – 62.9% (43.1%), figure 4.

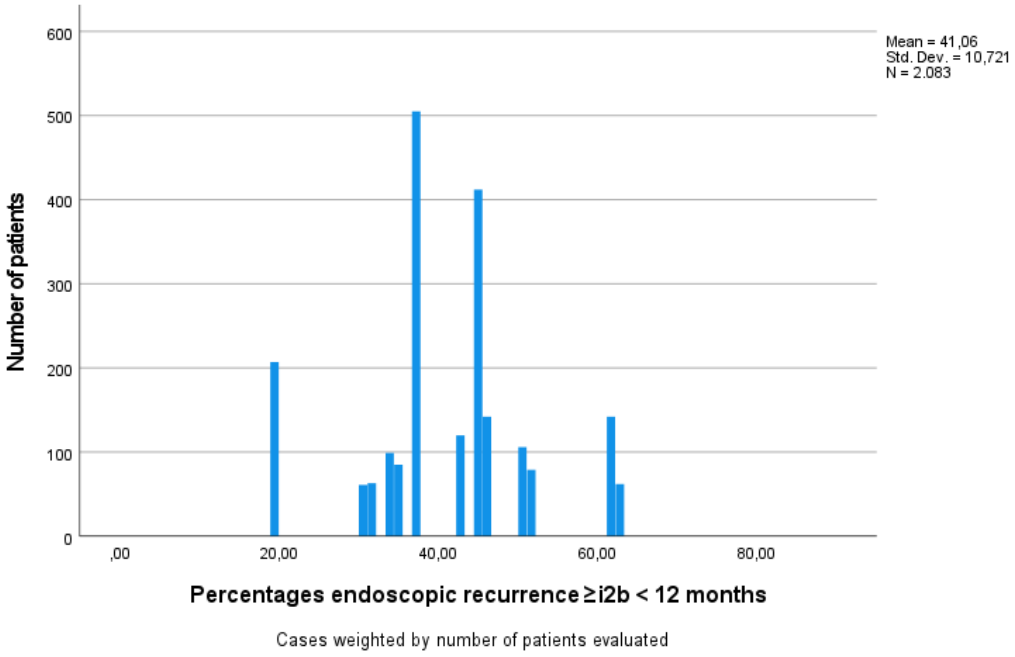

Figure 4. Histogram of distribution reported ER percentages weighted by number of patients in included studies according to the mRS classification.

Range of ER according to subcategories (i0, i1, i2a, i2b, i3 and i4)

Within 17 studies reporting mRS (table 2), the weighted mean and range of ER were evaluated per subcategory: nine studies reported the percentages for all categories, one study reported i2a and i2b, and one other study only reported the percentage for i2b. A minimum of 1209 patients was evaluated for each category and weighted means were calculated, table 3.

Author	Year	Patients (n)	Endoscopic recurrence < 1 year (%)
Djelouah et al.	2021	40	37,50
Lopez Sanroman et al.	2017	61	31,10
Buisson et al.	2021	63	44,40
Lemmens et al.	2017	74	50,00
Primas et al.	2021	79	51,90
Lopes et al.	2016	99	76,00
Ollech et al.	2019	207	19,80
Joustra et al.	2022	142	67,60
de Bruyn et al.	2021	142	62,00
Auzolle et al.	2018	225	47,00
Riviere et al.	2021	365	69,60
Cerrillo et al.	2019	32	50,00
Bachour et al.	2022	240	37,50
Bislenghi et al.	2021	47	44,70
Bommelaer et al.	2020	62	62,90
De Cruz et al.	2022	85	35,30
Machiels et al.	2020	120	43,00

Table 2. Studies reporting mRS.

mRS	Patients evaluated (n)	Weighted mean (95 percent CI)	Range
i0	1209	23.61% (22.94 – 24.29)	8.5% - 45.9% (37.4%)
i1	1209	11.88% (11.58 – 12.17)	4.2% - 22.0% (17.8%)
i2a	1449	22.20% (21.85 – 22.55)	11.4% – 42.0% (30.6%)
i2b	1481	20.86% (20.54 – 21.18)	10% – 34.3% (24.3%)
i3	1209	9.16% (8.94 – 9.37)	3.5% - 15.0% (11.5%)
i4	1209	10.04% (9.77 – 10.32)	2.9% - 22.8% (19.9%)

Table 3. Weighted mean and range of POR rates according to subcategories.

ER rates for mRS versus RS

Of eleven studies who reported the recurrence percentages for mRS and RS (reported or calculated), including 1497 patients, the difference in ER rates was calculated for ≥i2 versus ≥i2b. (20, 22, 28, 36, 41, 46, 47, 53, 61, 66, 92) In studies reporting ER defined as ≥i2, the weighted mean was 61.32% (95 per cent CI 60.61 –

62.03); range 39.1% – 84.7%. In those reporting POR defined as ≥i2b, the weighted mean of ER was 40.60% (95 per cent CI 40.01 – 41.18); range 19.8% – 62.0%.

Time interval after surgery: 6 months versus 1 year

Some 44 studies described ER six months postoperatively with a mean POR of 42.94%, versus 34 studies reporting ER up to one year postoperatively with a weighted mean of 45.03%, p <0.001 (95 per cent CI -1.22 – 1.22).

Data on the use of medication within the two different groups could not be determined, as this was either not reported or not reported unambiguously in all included studies. Therefore, no reliable analysis could be performed.

Discussion

This systematic review and meta-analysis, consisting of 76 articles, showed a wide range of ER rates according to both the RS as the mRS within one year after ICR for CD (ranging from 5% to 93%). Moreover, data from this study suggest that scoring ER according to the original RS could even lead to a 20% increase in diagnosis of disease recurrence compared to using the mRS. When used correctly, the mRS should theoretically be an accurate classification.

Earlier studies showed that the interobserver agreement and reproducibility of the original Rutgeerts was suboptimal. The agreement on the distinction between lesions classified as <i2 versus >i2 was low. (9, 12, 93, 94) The original Rutgeerts considers <5 lesions in the terminal ileum, an i1. One explanation for the large variance might be that patients with <5 lesions in the neo-terminal ileum in combination with lesions at the anastomosis, were not considered i1, but interpreted and therefore over-scored as i2. The same might be the case in scoring according to the mRS, where the variance in subcategories i2a and i2b is up to 30%. A combination of anastomotic ulcerations with < 5 neo-terminal ileum lesions might be over-scored as i2b instead of i2a.

The weighted means for subcategories i2a and i2b were doubled compared to subcategories i1, i3 and i4: 20 percent versus 10 percent respectively, with a wider range for i2a and i2b compared to the others. This may suggest that the definitions for subcategories i1, i3 and i4 are less prone to interobserver variability in comparison with the i2 subcategories i2a and i2b.

It can be debated whether the discrimination between i2a and i2b is clinically significant. A recent individual patient data meta-analysis concluded that the probability of both surgical and clinical recurrence is not different in patients with i2a compared to patients with i2b. This could be explained by the fact that the variance in scoring of i2a and i2b was large as a result of inadequate scoring. Not surprisingly, the postoperative course was not affected by type i2 (i2a or i2b).

The other subcategory that showed a wide variation was subcategory i4, both in RS and mRS. Large ulcers with diffuse mucosal inflammation in between, as well as a non passable stricture are classified as i4. In the case of an anastomotic stricture this may have been caused by a sealed anastomotic leak healed stricture formation or stricture, as seen in stapled anastomoses. If these strictures do not show inflammation in the terminal ileum, they should theoretically be scored as i0.

Finally, this study even showed some variation in category i0. This could be due to the differences in pre-

and postoperative treatment and monitoring strategies in the included studies in this review. No sub-analyses of the effect of the type of treatment and monitoring were done for this study. This was insufficiently monitored.

Theoretically, the modified Rutgeerts classification seems to be an adequate scoring system for ER, if used as intended. Interpretation of the endoscopic recurrence and classification according to the mRS are clearly not easy. Considering the clinical impact of recurrence on patients' QoL and health care consumption, there seems to be an unmet need to improve the diagnosis of endoscopic recurrence. Gastroenterologist should be trained in the correct assessment of anastomoses. Standard operating procedures for videotaping the anastomosis are important to allow central reading by an expert panel to reduce the unacceptable variance in scoring recurrence. (297,298) Dziki et al. described in 1991 that stapled anastomoses (serosa-serosa adaptation) heal differently compared to hand-sewn anastomoses (mucosa-mucosa adaptation). The inverted staple line heal with linear ulcers on the staple line due to ischaemia and secondary wound healing. (299) Recently, Does de Willebois et al. demonstrated that both patients with stapled anastomoses after resection for colorectal cancer as well as for CD show these ulcerations at six months after surgery. (300) These ulcerations might influence endoscopic scoring of recurrence and might lead to over-scoring. In fact, when hand-sewn and Kono-S anastomoses are compared with stapled anastomoses, this normal wound healing phenomenon of the stapled anastomosis may even interfere with the diagnosis disease recurrence when assessing endoscopic recurrence. (301) The type of anastomose being hand-sewn or stapled is therefore of importance. Only in 25 out of the 76 papers included in this review the type of anastomosis was reported.

In conclusion, this study demonstrates a high variance in the scoring of endoscopic recurrence after ICR for CD. This indicates a high likelihood of inadequate diagnosis of disease recurrence, with major implications for quality of life of the patient and health care consumption. For this reason, there is an important unmet need to improve endoscopic scoring of recurrent disease using training programs and central reading. Since the greatest variance and over-scoring was observed when using the original Rutgeerts classification, this classification should no longer be used. Moreover, since the type of anastomotic healing of stapled anastomoses might impact the discrimination of i2a from i2b and the diagnosis i4, it should be considered to neglect pure anastomotic lesions or pure anastomotic stricturing to avoid over-diagnosis of recurrent Crohn's disease, with all its consequences.

Part III

Role of Mesentery

Chapter 5

Mesenteric sparing or extended mesenterectomy in primary ileocolic resection for Crohn's disease in order to reduce postoperative endoscopic recurrence – results of an international randomised controlled trial

Study design

The SPICY trial is an international randomised controlled superiority trial, conducted in six hospitals and tertiary care centres in the Netherlands and Italy (four centres in the Netherlands and two in Italy). The study was conducted in accordance with the Good Clinical Practice guidelines and with the principles of the Declaration of Helsinki. (302) The reporting was in accordance with the CONSORT guidelines. The trial was approved by the Medical Ethic committee of the Amsterdam UMC Centre as well as by corresponding parties in all participating centres. The study protocol and predefined statistical analysis plan are available online, NCT04538638. (303)

Patients

Eligible patients were aged ≥16 years and had CD previously confirmed by endoscopy in the terminal ileum or ileocolic region (L1 or L3 disease), with a recent update (within the last 3 months) of imaging (ultrasound, MRI, or CT enterography) and were discussed by a multidisciplinary team. Exclusion criteria were a previous ileocolic resection; clinically significant medical conditions within six months before the operation (for example, myocardial infarction, active angina, congestive heart failure, or other conditions that would, in the opinion of the investigators, compromise the patient's safety); previous diagnosis of cancer with influence on the patient's prognosis; emergent operation; pregnancy or breastfeeding; inability to comply with postoperative assessments. All participants provided written informed consent.

Randomisation and masking

Randomisation was done by an internet randomisation module (CASTOR EDC). Patients were assigned 1:1 to either the intervention- or the control group. Patients and endoscopists were blinded to treatment allocation. Surgeons could not be blinded to the treatment.

Procedures

Patients allocated to the extended mesenterectomy group underwent resection of the mesentery up to the level of the ileocolic trunk. Standardization of the technique was ensured by distributing a video vignette and a standard operating procedure to all participating centres. (304, 305) The mesenteric resection was performed following the lower border of the ileocolic artery, to preserve these vessels, enabling a similar level of transection on the colon as in the sparing group (figure 5). Patients allocated to the mesenteric sparing resection, underwent surgery according to the currently advised procedure in the ECCO guidelines, dividing the mesentery close to the bowel. (3) A wide-stapled side-to-side anastomosis was proposed as the standard anastomosis, as recommended by current ECCO guidelines. Other anastomoses, such as Kono-S and end-to-end, were discouraged to avoid the introduction of additional bias. Perioperative care was according to the local enhanced recovery pathway.

Outcomes

The primary outcome was the endoscopic recurrence rate at six months postoperatively, defined as a modified Rutgeerts score of ≥i2b, according to blinded central reading by two experts (a gastroenterologist and a surgeon). Secondary outcomes were the degree of postoperative endoscopic recurrence according to blinded central reading – classified according to the modified Rutgeerts score (i1 – i4); operative time; per-operative blood loss; conversion rate; postoperative morbidity <30 days (length of hospital stay, anastomotic leakage and Clavien dindo score (20); histopathological data (length of resected colon, length of resected ileum, inflammation resection margins) and the use of postoperative Crohns' medication (continuation of Crohns' medication immediately after surgery, endoscopically guided start of Crohns' medication).

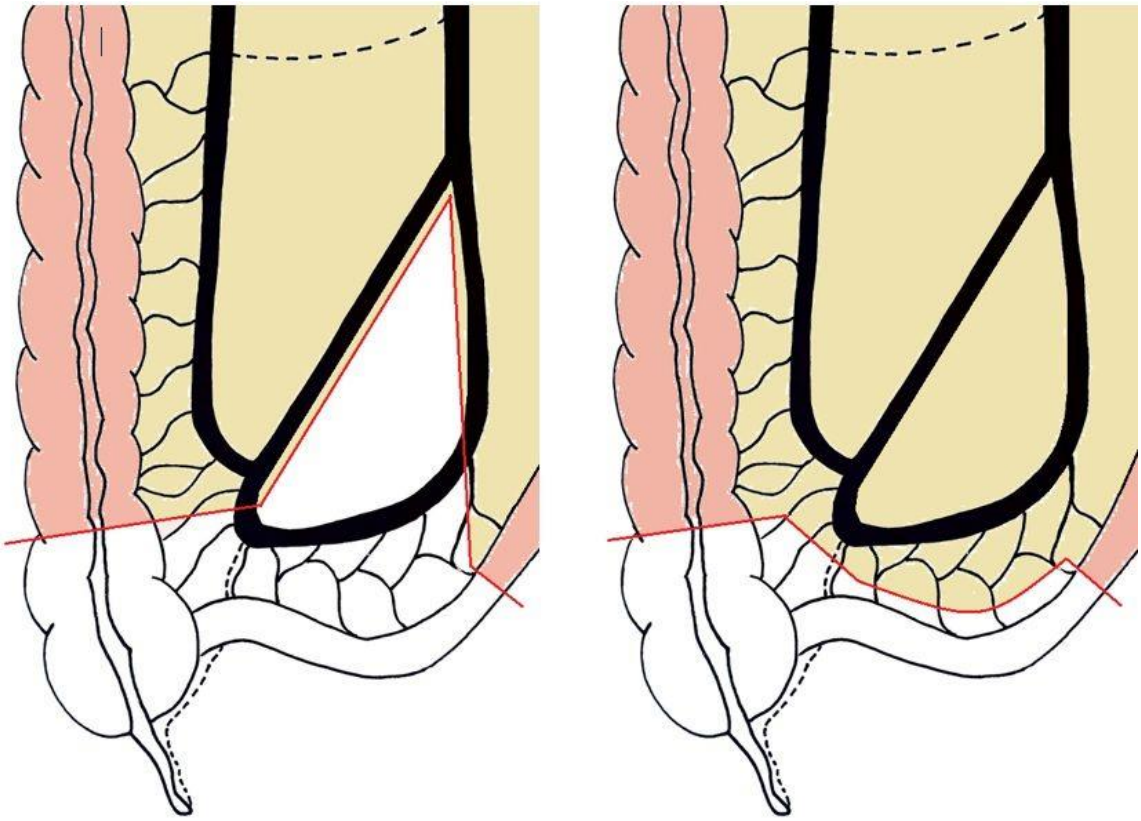

Figure 5. A) Extended mesenteric resection, up to the level of the ileocolic trunk. **B)** Mesenteric sparing resection

Statistical analyses

To attain a power of 80 percent and a two-sided α-level of 0.05, inclusion of 62 patients in each surgical arm was necessary. To accommodate an estimated loss to follow up of 10%, the target sample size was 138 patients. All analyses are based on the intention-to-treat principle. Categorical variables for baseline characteristics and outcomes are summarised as numbers and percentages in each category. These data are analysed using the chi-square or Fisher's exact test, as appropriate. Continuous variables that are normally distributed are summarised by mean and standard deviation, median and interquartile range in case of non-normal distribution. The distribution was checked using histograms and boxplots. Analyses of continuous variables are performed using the sStudent's t-test and the Mann-Whitney U-test. The degree of endoscopic recurrence is presented as bar chart with percentages. All statistical tests are two-sided, and results are presented with 95% confidence intervals. P-values of less than 0.05 are considered statistically significant. For statistical analyses, SPSS Statistics version 28 (IBM corp.) was used.

Role of funding source

The SPICY trial is an investigator study, with funding from TKI-LSH. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between February 19^{ths} 2020 and April 24th, 2023, a total of 217 patients were assessed for eligibility, of whom 78 were excluded due to refusal to participate or failure to meet the inclusion criteria. Ultimately, 139 patients were enrolled and randomly assigned to either extended mesenterectomy (n=71) or mesenteric sparing resection (n=68) (figure 6). Following randomisation six patients were excluded, due to withdrawal of consent (n=2), postoperative diagnosis other than CD (n=2) and no anastomosis performed (in case of a stoma) (n=2). This resulted in 133 included patients of whom 57 (42.9%) were male with a median age of 36 years (IQR 25-54) at time of surgery. Two patients were lost to follow up, and an additional two patients deviated from the protocol by undergoing investigations other than endoscopy at six months following surgery (i.e. MR or ultrasound). Demographic and clinical baseline characteristics were comparable in both groups (table 4).

Endoscopic recurrence in the intention to treat population (n=131), occurred in 28/66 (42.4%) in the extended mesenterectomy group and in 28/65 (43.1%) in the mesenteric sparing group (p=1.0), after a median time until endoscopy of 6 months (IQR 6-7) (table 5). Excluding the two patients who deviated from protocol by undergoing investigations other than endoscopy, the endoscopic recurrence rates were 41.5% and 42.2%, respectively. The degree of endoscopic recurrence was comparable in both groups (figure 7).

Operative data are summarised in *table 6*. Operative time, per-operative blood loss and conversion rate were comparable in both groups. Complications categorised as Clavien-Dindo IIIa or higher were observed in 12/131 (9.2%), with seven (10.6%) in the extended mesenterectomy group and five (7.7%) in the mesenteric sparing group (table 7). Among the six patients (4.6%) with an anastomotic leakage, three (2.3%) underwent ileostomy construction. All stomas were subsequently reversed within 1.5 years of follow up. The median hospital stay between operation and discharge, for both groups, was five days.

Histopathological data are summarised in *table 8*. The length of resected ileum specimen and resected colon specimen were comparable in both groups. In the mesenteric sparing group, significantly more patients had inflammation in the distal resection margin 18.5% versus 4.5%, p=0.023. There was no difference in the number of patients with inflammation in the proximal resection margin 25.8% versus 21.5%.

The use of postoperative Crohns medication was subdivided in medication started immediately after surgery (prophylactic) and endoscopically guided start of medication (therapeutic). *Table 9* summarises postoperative medication use. Numerically, more patients in the extended mesenterectomy group used Crohns medication immediately after surgery, 20/66 (30.3%), of whom 18 continued to use biologics. In the mesenteric sparing group: 12/65 patients (18.5%) continued medication, nine of whom continued biologics. Endoscopic guided medication was initiated in 36/131 (27.5%): 14 patients (21.2%) in the extended mesenterectomy group and 22 patients (33.9%) in the mesenteric sparing group. This resulted in an overall comparable number of patients being on medication after six months: 34/66 (51.5%) in the extended mesenterectomy group and 34/65 (52.3%) in the mesenteric sparing group, p=1.0.

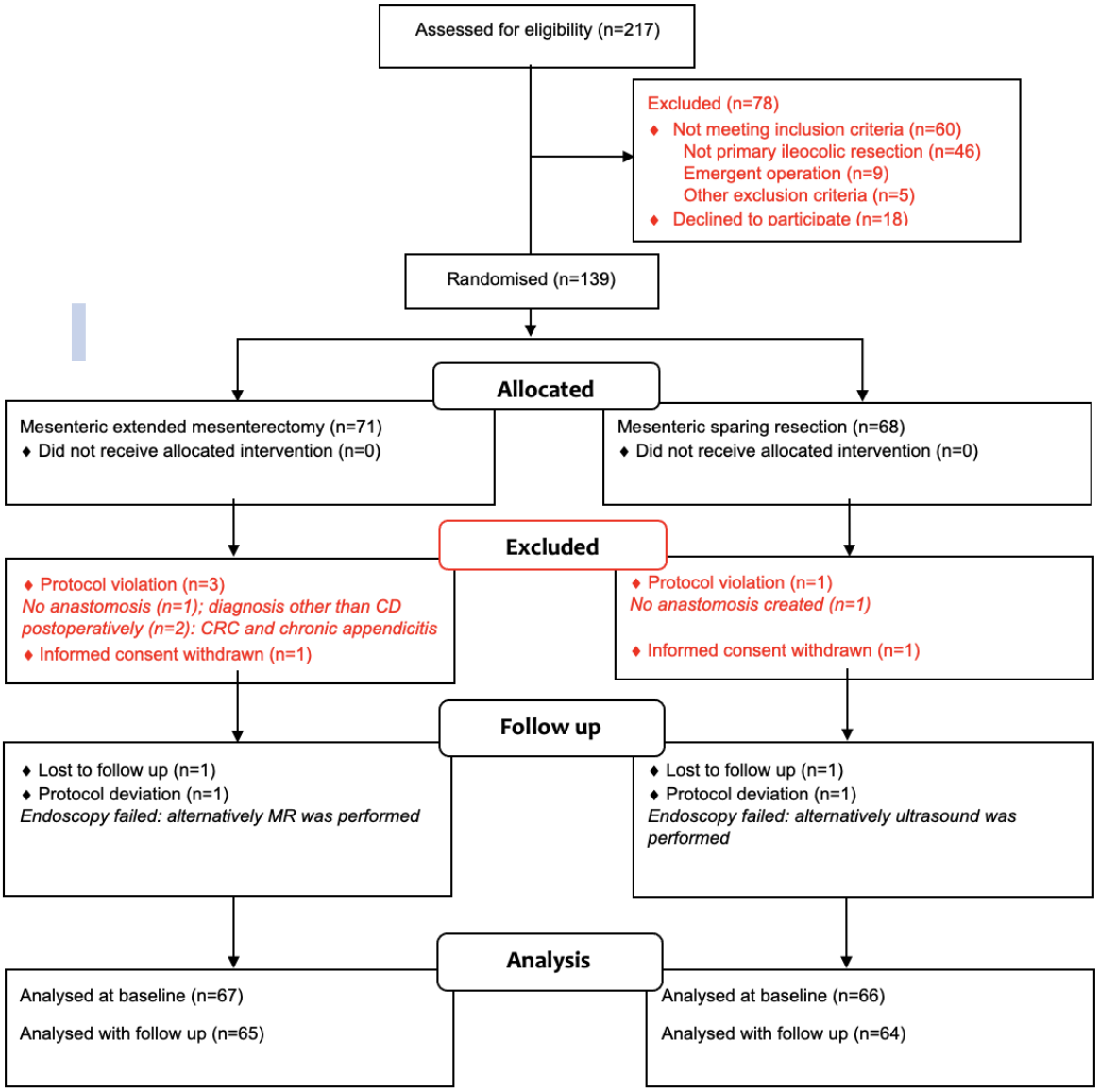

Figure 6. Consort flow diagram, intention to treat analysis for primary outcome parameter.

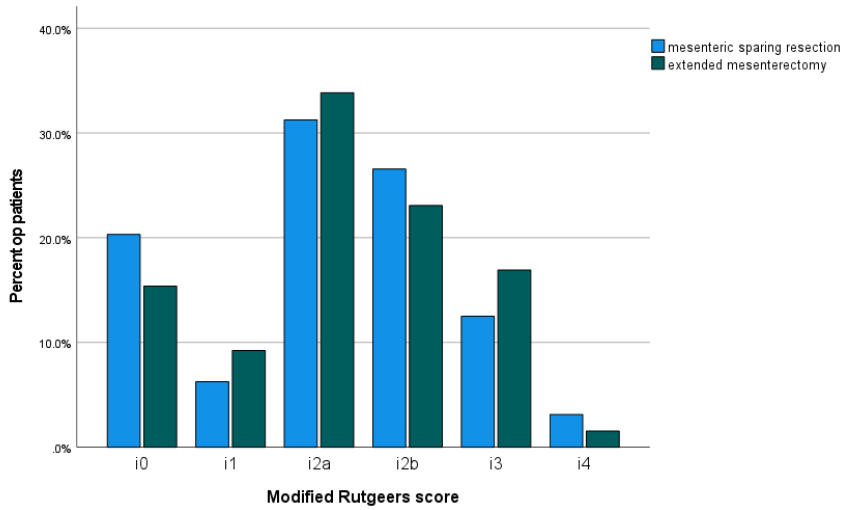

Figure 7. Degree of endoscopic recurrence according to central reading, defined as a modified Rutgeerts classification ≥i2b.

n(%); median (IQR)	Extended mesenterectomy (N=67)	Mesenteric sparing (N=66)	p-value
Age at surgery, years	36 (27-58)	36.5 (24-48)	0.222
Sex, male	29 (43.3)	28 (42.4)	1.0
Disease duration at surgery, months	53 (21-102)	45 (9-122)	0.596
Smoker			0.457
Active	15 (22.4)	16 (24.2)	
Ex-smoker	15 (22.4)	9 (13.6)	
Age at onset			0.302
A1, ≤16 years	4 (6.0)	8 (12.1)	0.242
A2, 17-40 years	38 (56.7)	40 (60.6)	0.726
A3, ≥41 years	25 (37.3)	18 (27.3)	0.276
Behaviour of disease			
B1, inflammation	22 (32.8)	15 (22.7)	0.246
B2, stenosis	28 (41.8)	29 (43.9)	0.862
B3, penetrating	17 (25.4)	22 (33.3)	0.345
Perianal disease	5 (7.5)	12 (18.2)	0.074
Location of disease			
L1, terminal ileum	44 (65.7)	40 (60.6)	0.592
L3, ileocolic	23 (34.3)	26 (39.4)	0.592
Stapled anastomosis	66 (98.5)	66 (100)	1.0
Medication <12weeks before operation			
None	24 (35.8)	28 (42.2)	0.480
Mesalazine	0 (0)	1 (1.5)	0.496
Thiopurines	8 (11.9)	10 (15.2)	0.621
Biologicals	35 (52.2)	25 (37.9)	0.118
Small molecules	0 (0)	2 (1.5)	0.244

Table 4. Demographic and clinical baseline characteristics.

n(%); median (IQR)	Extended mesenterectomy (N=66)	Mesenteric sparing (N=65)	p-value
Endoscopic recurrence rate	27 (43.5)	27 (42.2)	1.0
Time surgery until endoscopy, months	6 (6-7)	6.5 (6-8)	0.967

Table 5. Endoscopic recurrence ITT population, including patients with protocol deviation

n(%); median (IQR)	Extended mesenterectomy (N=66)	Mesenteric sparing (N=65)	p-value
Operative time, hours	2:47 (2.22 - 3:15)	2:50 (2:29 - 3:26)	0.396
Per-operative blood loss, cc	50 (10-200)	50 (20-150)	0.840
Conversion rate	0 (0)	2 (3.2)	0.240

Table 6. Operative data

n(%), median (IQR)	Extended mesenterectomy (N=66)	Mesenteric sparing (N=65)	p-value
Anastomotic leakage, total	5 (7.6)	1 (1.5)	0.208
Anastomotic leakage + deviating ostomy	2 (3)	1 (1.5)	1.0
Postoperative complication CD ≥ IIIa*	7 (10.6)	5 (7.7)	0.763
CD IIIa	0 (0)	2 (3.1)	0.244
CD IIIb	6 (9.1)	3 (4.6)	0.492
CD IV	1 (1.5)	0 (0)	1.0
CD V	0 (0)	0 (0)	1.0
Length of hospital stay, days	5 (4-7)	5 (3.3-7)	0.998

**IIIa: endoscopic clipping of the stapled anastomosis because of a staple line bleeding (n=1); percutaneous drainage of an abscess (n=1); IIIb: reoperation (n=9) V: admitted to the ICU (n=1) because of respiratory insufficiency.*

Table 7. Postoperative morbidity <30 days after surgery

n(%), median (IQR)	Extended mesenterectomy (N=66)	Mesenteric sparing (N=65)	p-value
Radical resection			0.126
Both sides no inflammation	45 (68.2)	39 (60.9)	
Distal inflammation	3 (4.5)	12 (18.5)	0.023
Proximal inflammation	17 (25.8)	14 (21.5)	0.797
Missing	2 (3.0)	3 (4.6)	
Length of resected ileum, cm	19 (15-34)	25 (16-36)	0.125
Length of resected colon, cm	7 (6-9)	7 (5-8)	0.302

Table 8. Histopathological data

n(%), median (IQR)	Extended mesenterectomy (N=66)	Mesenteric sparing (N=65)	p-value
3-6 months after surgery on medication	20 (30.3)	12 (18.5)	0.154
Mesalazine	1 (1.5)	0 (0)	1.0
Thiopurines	1 (1.5)	2 (3.1)	0.619
Biologics	18 (27.3)	9 (13.8)	0.093
Small molecules	0 (0)	1 (1.5)	0.496
Endoscopic guided start of Crohns medication	14 (21.2)	22 (33.9)	0.120
Mesalazine	0 (0)	0 (0)	1.0
Thiopurines	1 (1.5)	4 (6.2)	0.208
Biologics	13 (19.7)	18 (27.7)	0.310
Small molecules	0 (0)	0 (0)	1.0
Total on medication after 6 months	34 (51.5)	34 (52.3)	1.0
Mesalazine	1 (1.5)	0 (0)	1.0
Thiopurines	2 (3)	6 (9.2)	0.164
Biologics	31 (47)	27 (41.5)	0.599
Small molecules	0 (0)	1 (1.5)	0.496

Table 9. Postoperative use of Crohns medication

Discussion

This is the first RCT to present data on the effect of extended mesenteric resection compared to mesenteric sparing resection in ileocecal CD. Refuting the original hypothesis, the results of this study showed no superiority of extended mesenteric resection with regard to endoscopic recurrence at six months following surgery. Furthermore, there were no significant differences in procedure related outcomes, such as blood loss, conversion rate and length of resected colon. However, there was a non-significant higher incidence of anastomotic leakage in the intervention group. This may be attributed to potentially more extensive devascularisation, although the overall leakage rate is consistent with the existing literature. (306) Interestingly, in the mesenteric sparing group, a significantly higher number of patients had active inflammation in the distal colonic resection margin. Preoperatively, there were non-significantly more patients using biologics in the extended mesenterectomy group. However, no other factors appeared to be associated with the difference in distal inflammation, making this finding clinically less relevant. Furthermore, patients in the intervention group more frequently received medical therapy immediately after surgery. This was likely a consequence of practice variation, as some patients continued medication for concurrent disease (colonic or extra-luminal), while others started prophylactic therapy as advised by the ECCO guidelines. Nevertheless, the number of patients on medication after six months was similar in both groups. Overall, as expected in an RCT, only minor differences were observed between the two groups, likely influenced by variation in practice. Performance of an international RCT effectively reduced the most substantial differences and the remaining differences were unlikely to affect the study results.

There is increasing interest in the possible role of the mesentery in the pathophysiology and clinical course of CD. (307-309) However, data on the role of the mesentery in CD are conflicting. The results of the SPICY

study are not consistent with some recent reports suggesting that excision of the adjacent mesentery (comparable to an oncological resection) could reduce postoperative recurrence rates significantly. Coffey et al showed in a retrospective cohort study a significantly lower reoperation rate in favour of extended mesenteric resection, compared to close bowel resection (2.9% versus 40%). However, there were some important confounding factors in this retrospective cohort study besides the lack of randomisation. Firstly, there was a notable difference in follow-up time, favouring the historical control group. Consequently, this latter group had more time to develop surgical recurrence. In addition, there have been advancements in postoperative surveillance strategies and new (prophylactic) medication options were introduces over time, making it difficult to directly compare the outcomes of these two groups. Newer data on outcomes after standard mesenteric sparing ileocolic resection show a similar rate of surgical recurrences. In the LIRIC study, resections were performed around the same time as the intervention group in the Coffey study (with the same surveillance strategies and availability of medical treatment options). The surgical recurrence rate after five years was 0%, which reinforces the conclusions of current study. Another retrospective study of Zhu et al, with the same methodological shortcomings, also demonstrated that mesenteric sparing resection was independently associated with surgical recurrence. However, this study included a very heterogeneous population. All patients who underwent colorectal resection were included, with nearly half of the patients having isolated colonic (L2) disease, which is a different phenotype with a different prognosis when compared to L1 disease.

Other research shows a paradoxical correlation of the mesentery with CD and postoperative outcomes. Clinical data from the Remedy study, a cohort study, are in line with the outcomes of current study and demonstrated no effect of mesenteric resection on endoscopic recurrence rates. (310) Additionally, there was no observed association between mesenteric thickness and lymphadenopathy with the development of recurrence. (311) Ha et al demonstrated that the creeping fat serves as an adipose tissue barrier to prevent systemic dissemination of bacteria. This was in line with our own fundamental research demonstrating that the ileocecal mesentery did not contain high amounts of pro-inflammatory macrophages adjacent to the inflamed tissue, but a gradient towards a more pro-inflammatory phenotype was seen in the central mesenteric area. (312) As the mesentery is suggested to be a continuous organ, it is not possible to radically resect the affected central mesentery in ileocolic CD. In contrast, the relevance of radical mesenteric excision was demonstrated in patients undergoing proctectomy for CD. The performance of a total mesorectal excision when compared to close rectal dissection was associated with reduced perineal complications. However, it is important to emphasize that the purpose of performing an extensive mesenteric resection during proctectomy was to improve the wound healing process in cases of complex perianal disease. Radical resection cleared the ‘inflammatory rectal tunnel’ of disease, which was characterized by a decrease in pro-inflammatory macrophages. This contributed to an overall improvement in wound healing. The primary goal of the SPICY study was different; namely, to reduce recurrence at the anastomotic site. Current study showed that this could not be achieved by a more extended (but still partial) resection of the mesentery near the affected bowel. Finally, what further contradicts the need to remove mesentery to improve outcomes for ileocolic CD is the observation that both macroscopic and microscopic abnormalities return to a more ‘normal’ state after stricturoplasty. This implies that the mesentery responds to disease originating from the intestine and is not the driver of disease. The theory proposed by Ha et al. may clarify the rationale behind the recovery of both the bowel and mesentery. Removal of the stricture has the potential to reduce stasis, potentially reducing the

translocation of bacteria. [312-315]

The outcomes of the SPICY trial should not be misinterpreted as a negligible role of the mesentery in CD. The current data rather suggest that the natural disease course in ileocecal CD may not be altered by mesenteric removal. The long-term results of the SPICY trial, clinical and surgical recurrence, will contribute to further understanding of the role of the mesentery and the effect of partial mesenteric excision. A major strength of this study, besides the randomisation, was the blinded central reading. A recent meta-analysis highlighted a major variation in endoscopic recurrence rates. (See part II) This variation is attributed to interobserver variability and the potential confounding effects of wound healing of inverted stapled anastomoses, typically healing with ulcerations. (316- 318) To reduce the degree of interobserver variability, two experts centrally read all images and were blinded to the allocation arm. Another strength of the study was the standardization of the type of anastomosis and the comparable length of resected colon in both groups. The identification of vascular structures can be challenging in the presence of a hostile, thickened mesentery. Consequently, devascularisation may necessitate additional bowel resection. Standardization of the technique was ensured by distributing a video vignette to all participating centres. In this RCT, external validity was considered more important than internal validity. It was not possible to predict in advance which patients with ileocolic CD (i.e., inflammatory, stricturing or penetrating disease) might benefit from extended mesenterectomy. Therefore, a study that was generalizable to the entire population with ileocolic disease (L1 and L3 disease) was preferred.

In conclusion, the results of this study showed no superiority of extended mesenteric resection with regard to endoscopic recurrence or other perioperative outcomes. These data suggest opting for a sparing approach, as there is no difference in endoscopic recurrence and a lower risk of severe complications. Whether recurrence can be modulated by the type of anastomosis, is currently investigated in the End2End, Hand2End and the MEErKAT studies (NCT05578235, ISRCTN16900055). (see part IV. Third research of this doctoral thesis)

Part

IV

Role of Anastomosis

Chapter 6

Optimising surgical anastomosis in ileocolic resection for Crohn's disease with respect to recurrence and functionality: Two international parallel randomized controlled trials comparing handsewn (end-to-end or Kono-S) to stapled anastomosis (HAND2END and the End2End STUDIES)

Objectives

The primary objective of these two multicentre international randomized studies is to determine which anastomosis, handsewn (end-to-end versus Kono-S anastomosis) or side-to-side stapled anastomosis after ileocolic resection for Crohn's disease is superior with respect to endoscopic recurrence. Secondary objectives include evaluating gastrointestinal function, postoperative morbidity, quality of life and costs.

Methods/Design:

This study protocol is written in accordance with the SPIRIT guidelines [319, 320]. The SPIRIT checklist is provided in an supplementary file 1.

Study design

The HAND2END and End2End studies are two separate studies with similar designs conducted respectively in both Italy and the Netherlands. Three Italian centres and 12 centres in the Netherlands will participate in this prospective study. Both studies are multicentre randomized controlled superiority trials consisting of two phases. In phase one the two handsewn anastomoses (Kono-S and end-to-end) will be compared with the side-to-side stapled anastomosis. In this phase, participants will be randomized in a 2:1 ratio between (1) handsewn end-to-end (Fig.1) or Kono-S anastomosis (Fig.2) and (2) stapled side-to-side anastomosis (Fig.3). If the interim analysis shows superiority of the handsewn anastomosis, phase two of the trial will compare the end-to-end (Fig. 1) with the Kono-S anastomosis (Fig. 2). In the Netherlands, three academic and eight non-academic teaching hospitals have agreed to participate in this national initiative. This multicentre (international) collaborative will greatly facilitate the (inter)national implementation of the study results. The attached video clips will demonstrate one way of performing these anastomoses (Supplementary files 2-4).

Study population

All patients diagnosed with CD undergoing primary ileocolic resection or resection of recurrent disease of the neoterminal ileum will be considered for inclusion. To be eligible, a subject must meet all of the following criteria: adults of either sex, age ≥ 16 years or ≥ 18 years (in the Netherlands or Italy, respectively), diagnosed with either disease of the (neo)terminal ileum or ileocolic disease (L1 and L3 disease), previously confirmed by endoscopy with an indication for resection. Patients need to have a recent update of imaging (for example, ultrasound, MR/CT enterography) before surgery, the clinical case should have been discussed at the local multidisciplinary meeting and patients need to be able to comply with the study protocol and provide written informed consent. A potential subject who meets any of the following criteria will be excluded from participation in this study: inability to provide informed consent, patient under the age of 16 or 18 years (in the Netherlands or Italy, respectively), clinically significant medical condition within 6 months before the operation (for example, myocardial infarction, active angina, congestive heart failure, or other condition that would, in the opinion of the investigators, compromise patient's safety), history of cancer of less than 5 years that might influence the patient's prognosis, emergent operation, pregnancy or breastfeeding.

Ethical considerations

The trials will be conducted according to Good Clinical Practice guidelines and the principles of the Declaration of Helsinki (2013). The End2End study was approved by the Medical Ethical Committee of the Amsterdam UMC, Amsterdam (MEC 2022.0533). The protocol is registered by the Dutch Central Committee on Research Involving Human Subjects (NL81981.018.22). The HAND2END study was approved by the Medical Ethical Committee of the IRCCS San Raffaele, Milan (EC-13/06/2022, version 2.0).

Informed consent procedure

All eligible patients referred for outpatient consultation for surgical resection will be screened for inclusion criteria during outpatient clinic visits. Patient who meets all the inclusion criteria will be informed on the details of the study and the possibility of participating in the trial. The informed consent procedure includes explanation of the study, the provision of a patient information sheet and ample time for addressing questions before signing the study consent form prior to the surgical procedure. Written informed consent is obtained by a participating surgeon, resident or trained research nurse before any study procedures. All included patients will be registered in Castor EDC and will be assigned a six-digit study number. A log of the assigned subject number will be maintained by each study site.

Randomization

Randomization of consented study participants to either handsewn (end-to-end or Kono-S) or stapled side-to-side anastomosis will be done using an online-based system for allocation concealment (Castor EDC). Allocation concealment will be ensured, as the service will not release the randomization code until the patient has signed for informed consent and has been recruited into the trial. Randomization will be stratified for primary or repeated ileocolic resection. The randomization block sizes will not be disclosed, to ensure concealment. There is no blinding of treatment allocation for the treating surgeon. The treatment will be blinded for all other physicians (for example, gastroenterologists, central readers) and patients. A statistician blinded to treatment allocation will analyse the data.

Study outline

Preoperative

Adult patients with ileocolic Crohn's disease (CD) or recurrent disease of the terminal ileum will be screened during outpatient clinic visits. Qualified subjects expressing interest will be offeredparticipation in the trial following multidisciplinary team discussion and will sign a written consent form if they agree to be enrolled. Medical treatment will be advised to be discontinued before surgery: glucocorticoids will be weaned to < 20 mg per day, and biologic agents and immunomodulators will be stopped at time of surgery. Prior to surgery, all patients must have had a colonoscopy with biopsy to confirm disease of the (neo)terminal ileum, and a recent update of imaging (for example ultrasound, MR or CT enterography). Baseline characteristics will be collected. Patients will all be treated in an enhanced recovery protocol.

Surgery

At the time of surgery, all the procedures will be initiated as a laparoscopic procedure with conversion to an open operation only if clinically indicated. Close bowel ileocolic resection is advised to preserve vascularization.

Group I: End-to-end handsewn or Kono-S anastomose. The end-to-end anastomosis is fashioned either by enlarging the small bowel diameter with an antimesenteric incision to fit the large bowel lumen or by tailored resection of a part of the staple line of the cross stapled colon. The Kono-S anastomosis is made according to

the description by Kono. The length of the actual anastomosis should be approximately 7 cm (figure 8-9).
Group II: The side-to-side anastomosis is done according to local practice using a linear stapler either anisoperistaltic or isoperistaltic (Fig. 10)
The rest of the operation will be identical. Care should be taken to ensure that, as a routine, the colon is anastomosed just beyond the ileocolic angle. Operative, postoperative and pathology data will be collected.

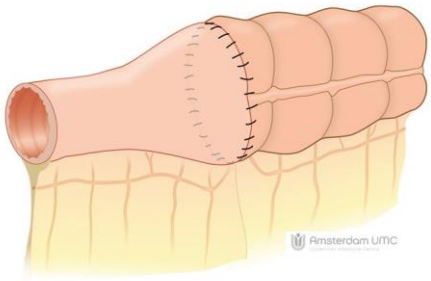

Fig.8 Handsewn end to end anastomosis

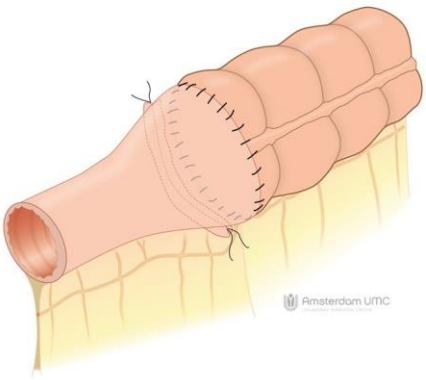

Fig.9 Handsewn Kono-S anastomosis

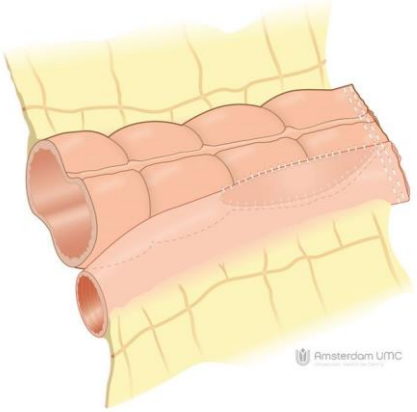

Fig.3 Stapled side to side anastomosis

Quality control of surgical procedure

To ensure that the surgical technique is performed correctly in all centres, quality control will be conducted. A video vignette of the surgical procedure will be shared with all participating centres as an example (video). Furthermore, the participating centres will be asked to provide photos of the anastomoses and the resected specimen. Finally, each centre will provide videos of the procedure when requested.

Postoperative

1) Postoperative morbidity

Data on postoperative morbidity will be collected. All adverse events (AEs) and severe adverse events (SAEs) will be documented throughout the hospital stay and outpatient visits.

2) Assessment of primary endpoint (endoscopic recurrence)

All patients will undergo endoscopy with biopsies taken from the distal ileum andproximal colon on either side of the anastomosis to assess endoscopic and histologic recurrence at 6 months after surgery. Endoscopic results will be classified according to the SES-CD subdivided according to location and the (modified) Rutgeerts scoring system. [321] All colonoscopies must be video recorded according to a standard operational procedure (video). The quality of endoscopic scoring and over scoring will be assessed comparing the scoring of the local gastroenterologist with that of the central reading panel.

After endoscopic assessment at 6 months, medication can be initiated according to the severity of endoscopic recurrence according to the guidelines and the treating physician's preference.

3) Assessment of clinical recurrence, quality of life and health care consumption.

The Crohn's Disease Activity Index (CDAI) is determined at baseline and at 6 and 12 months postoperatively based on seven-days scoring by the patient before to these visits. Quality of life questionnaires are administered at each visit (preoperative, postoperative at 3 months, at 6 months and 12 months: the EuroQol, (EQ-5D-5L), 36-Item Short Form Health Survey (SF-36), and Inflammatory Bowel Disease Questionnaire (IBDQ). All types of healthcare consumption within the first year will be recorded, including readmission, emergency room visits, examinations, medical therapy etc.

4) Medical management

Patients will be treated according to the ECCO guidelines with respect to prophylactic medication after surgery.

5) Assessment of surgical recurrence

Patients will be followed up for up to 5 years after surgery to determine the reoperation rate for recurrent disease at the anastomotic site.

Primary and secondary outcomes

The primary outcome is endoscopic recurrence at six months following ileocolic resection, defined as Rutgeerts score ≥ i2b assessed by local and central reading separately and blinded for the type of anastomosis. The quality of endoscopic scoring and over scoring will be assessed comparing the local scoring by the treating gastroenterologist with the central reading scoring for the different types of anastomoses.

Secondary outcomes include postoperative morbidity, the 6 months histologic and clinical recurrence rate and the need of restarting immunosuppressive medication within the first year postoperatively for endoscopic or clinical recurrence. For long term results, patient will be followed for at least 5 years to determine reoperation rate for recurrentdisease at the anastomotic site. Additionally, quality of life will be measured with IBDQ, EQ-5D and SF- 36 together with health care consumption and costs.

Sample size calculation

The primary endpoint is the post-operative endoscopic recurrence of Crohn's disease at 6 months.

The study is powered to detect a clinically relevant difference of 25% endoscopic recurrence at 6 months between the two randomized surgeries, 50% versus 25%. Assuming a test with continuity correction with 80% power, and a two-sided alpha-level of 0.05, in 2:1 ratio a total of 63 patients in each surgical arm will be required, for a total enrolment of 189 patients including an anticipated dropout of 10% in the Italian HAND2END study. In the Dutch End2End study, a total of 55 patients per arm will be required, for a total enrolment of 165 patients allowing, for a 10% dropout.

After inclusion of the 189 patients in Italy requiring 2.5 years with a 0.5-year follow-up or 165 patients in the Netherlands requiring 2 years with a 0.5-year follow-up, an interim analysis is done to determine whether the handsewn anastomoses are superior to stapled side-to-side anastomosis. If the interim analysis shows superiority of the handsewn anastomosis, the trial will continue with the end-to-end and the Kono-S anastomosis only. To find a reduction in endoscopic recurrence from 28 to 13% within the two handsewn groups (end-to-end and Kono-S), another 88 patients need to be included for a total of 277 in the HAND2END. In Italy a total 189 or 277 patients over 3 or 4 years will be needed. In the Dutch study, another 71 patients per group need to be included, leading to a total of 307 patients. In the End2End a total of 165 or 307 patients are required over 2.5 or 4 years. If no superiority within the hand sewn is expected from the interim analysis the study will be stopped after inclusion of the 189 or 165 patients in Italy or the Netherlands, respectively, plus those that are already included in the period needed to reach the 0.5-year follow-up.

The Dutch sample size has been adjusted according to the ZonMW first-round review committee's preference; hence the Dutch numbers are slightly different from those in the Italian study.

Statistical analysis

Descriptive statistics will be used to report baseline patient and surgical variables. Baseline patient and procedure characteristics and perioperative outcome parameters are categorical, continuous and dichotomous variables, and will be presented accordingly. The chi-squared or Fisher's exact test will be used to analyse the differences between the proportion of patients with recurrence.

Primary endpoint

Differences in endoscopic recurrence at six months between the handsewn and stapled anastomosis groups will be analysed using logistic regression, which will be corrected for stratification factors and other potential confounders. Univariate associations with the risk of recurrence will be assessed using a Cox proportional hazards model, results will be reported as a hazard ratio (HR) and 95% confidence interval (CI).

Multiple variable models will be considered in the same way depending on the number of recurrences identified. The two-sided alpha-level will be set at 0.05 for statistical significance. Differences in QoL will be analysed using mixed-model analysis of variance for repeated measures. At the time of analysis, the most recent version of the statistical program IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.will be used.

The statistical analysis plan will be finalized before the data is locked for analysis. Decisions will be made regarding planned subgroup analyses and how to address protocol violations and any potential baseline imbalances.

Safety reporting

This study is considered a low-risk trial, because it involves the comparison of three well-established and commonly performed techniques in IBD surgery. No data and safety monitoring board (DSMB) will be installed. All adverse and serious adverse events (SAEs) will be monitored until they have abated or until a stable situation has been reached. The reporting procedure applies to all (S)AEs occurring up to 30 days after the surgical procedure and to any SAE that occurs after the 30-day period, if it is considered to have a reasonable possibility to be related to the protocol treatment or study participation. The study coordinator will report all SAEs to the accredited institutional review board that approved the study protocol.

Data handling and monitoring:

Each enrolled patient will be assigned a six-digit study number and all communication will occur using this number. The full name and date of birth of the patient will only be documented on the informed consent form, and these details will be securely kept in the participating hospital. In each of the participating hospitals, one surgeon acts as local investigator who is primarily responsible for execution of the study in compliance with the study protocol and ensuring the accuracy and completeness of the case report form (CRF). Data will be digitally collected and stored using the electronic data management system Castor EDC version 1.6 (www.castoredc.com). Continuous data monitoring will ensure complete and real-time prospective recording of data. Independent monitoring of the study progress and study quality will be performed by the Sponsors Clinical Monitoring Center (CMC).

Public disclosure and publication policy

The HAND2END and End2End trials are registered at www.ClinicalTrials.gov, with registration numbers rNCT05246917 and NCT05578235, respectively. The results of both studies will be submitted separately to peer-reviewed journals, regardless of study outcomes. Additionally, the results will be presented at international conferences and disseminated to relevant surgical and IBD associations. Co-authorship will be based on the International Committee of Medical Journal Editors (ICMJE) guidelines.

Discussion

Surgery for Crohn's disease has been performed since the discovery of Crohn's disease by Burrill Crohn in 1932 [322]. It is remarkable that, even after 90 years of surgical advancements, we still do not know the preferred anastomosis technique after ileocolic resection concerning safety, diagnosis of Crohn's disease recurrence, functional outcome, and costs. The proposed trials aim to answer this unmet need.

The primary endpoint of the present study is postoperative endoscopic recurrence after 6 months. This is a crucial endpoint, because the diagnosis of endoscopic recurrence signifies disease recurrence. Disease recurrence typically necessitates the resumption of medical therapy which is in general long-term immunosuppressive or biological therapy. This entails a sequence of biological treatments for as long as the clinical symptoms do not require another surgical intervention. On the contrary, clinical recurrence has been the primary outcome in many studies in the postoperative setting, but its definition remains debated. Most importantly, there does not seem to be a strong correlation between clinical recurrence and endoscopic recurrence [323]. Furthermore, functional disorders resulting from ileocolic resection might interfere with symptoms indicating clinical recurrence.

According to ECCO guidelines, it is recommended to initiate t medical therapy after surgery if the patient has one risk factor for recurrence (smoker, prior intestinal surgery, penetrating disease, perianal disease, granulomas or myenteric plexitis) [324]. All other patients should undergo endoscopy within 6-12 months to assess endoscopic recurrence. Notably, assessment of endoscopic recurrence has shown a high degree of variability (10-70%) with significant implications for both patients and society (diagnosis of recurrence and starting expensive medication) [325, 326].
The Rutgeerts score has been developed to classify endoscopic recurrence with a score of ≥ 2 indicating its presence. The modified Rutgeerts classification was introduced to differentiate between patients with ulcerations solely at the anastomotic line (2a) and those with ulcerations extending into the neoterminal ileum (2b). Nevertheless, endoscopic scoring remains highly variable, probably due to observer bias as evident from the significant variability between local and central readings [227].
As recommended in ECCO guidelines, the currently most popular anastomosis technique is the stapled side-to-side anastomosis. Technically, in side-to-side stapled anastomosis, both inverted and everted staple lines are present. The longitudinal staple line of both the isoperistaltic and anisoperistaltic side-to-side anastomoses is an inverted staple line, while the cross-staple line is an everted staple line. The different adaptation of the cut ends of the bowel determines the type of wound healing. Direct mucosa-to-mucosa adaptation in an everted anastomosis will result in primary wound healing, whereas the serosa-to-serosa adaptation of the longitudinal staple line will heal with secondary intent [328]. This secondary intent healing involves a process where the mucosa needs to re-epithelialize over the staple line. It was already demonstrated over thirty years ago that inverted stapled side-to-side anastomosis heal with ulcerations due to secondary wound healing where reepithelialisation is necessary and due to ischemia caused by the staples [329]. Hypothetically, these Crohn's disease-unrelated ulcerations on the staple line could lead to systematic overscoring of endoscopic recurrence, resulting in unjustified restarting of often expensive and potential harmful drugs, reduced QOL and increased costs. This might also explain the significant variation in diagnosing endoscopic recurrence, particularly in stapled anastomoses.
Hand sewn end-to-end and the Kono-S anastomoses heal with primary intent due to mucosa-mucosa adaptation on both sides and have a lower risk of overscoring endoscopic recurrence based on different types of wound healing.

To avoid anastomotic ulcerations from being misinterpreted d as disease recurrence, the primary endpoint is

assessed by both the local gastroenterologist and a central reading panel. A standard operating procedure (SOP) for recording the endoscopic evaluation of the anastomosis has been developed.

In addition to the potential for overscoring due to method of anastomosis construction (handsewn versus stapled), the anastomosis configuration itself might contribute to disease to recurrence. One hypothesis regarding the cause of disease recurrence is gastrointestinal luminal stasis. In this context, saccular side-to-side and Kono-S anastomoses are at risk for stasis, which may cause gastrointestinal functional disturbance and recurrent disease . With this regard, the disease recurrence often occurs more proximal to the inlet of the actual anastomosis rather than in the small bowel part of the anastomosis (fig 11a-11b).

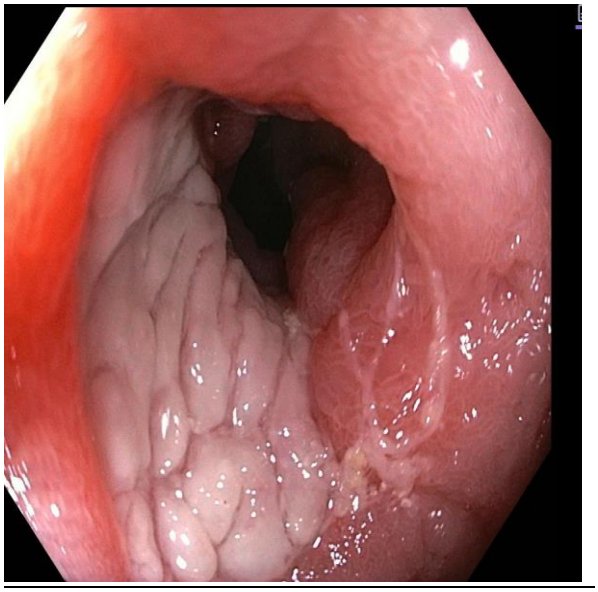

Fig 11a. Endoscopic recurrence at the inlet of the side to side anastomosis

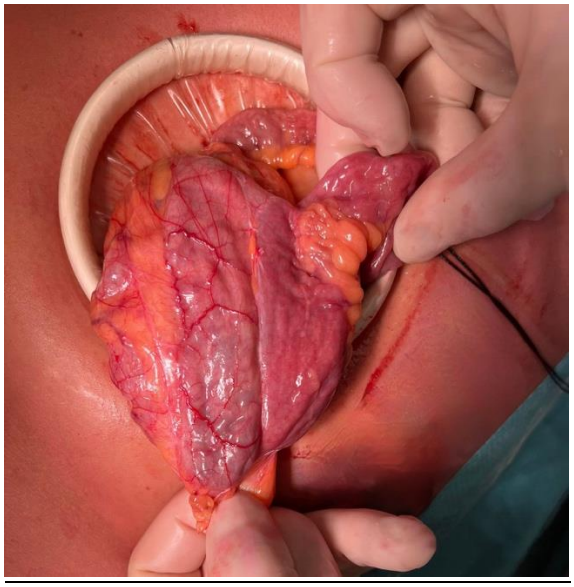

Fig 4b. Macroscopic recurrence at the inlet of the anastomosis.

The observations of Gajendran et al. support this hypothesis. In their study, they compared the side-to side stapled with the handsewn end-to-end anastomosis and noted that the former configuration resulted in worse QoL and higher healthcare consumption in the two years after surgery due to increased abdominal complaints and consequently diagnostic measures.[330]

For these reasons, hypothetically the handsewn end-to-end anastomosis has the best characteristics with a lower risk of overscoring and less stasis, followed by the handsewn Kono-S (less overscoring, but potentially stasis). The expected worst anastomosis, the currently advised stapled side-to-side, potentially suffers from both overscoring and stasis.

Data from the only randomized trial focused on difference in surgical recurrence between handsewn and stapled anastomosis in ileocolic resection demonstrated the superiority of the stapled anastomosis. The recurrence rate was 9.1% in the stapled group compared to 28.8% in the handsewn group, although the number of patients in each group was only 11 vs 21 [331]. The CAST trial by McLeod found no differences in endoscopic and clinical recurrence in patients undergoing ileocolic resection for Crohn's disease after a mean FU of 11.9 months [332]. A randomized trial conducted by Luglio et al. comparing the Kono-S anastomosis with the stapled side-to-side showed higher endoscopic recurrence rates of the stapled

anastomosis, while the clinical recurrence after one year did not differ [333]. It is unclear, whether the differences in endoscopic recurrences were caused by systematic overscoring of ulcerations on the anastomotic line in the side-to-side stapled group.

The two similar studies conducted in both Italy and in the Netherlands aim to conclude which anastomosis is associated with the least recurrence of Crohn's disease, the best function, and the least healthcare consumption. The purpose of performing two similar multicentre trials in two westernized countries, separately sponsored, is to increase the likelihood of obtaining a rapid answer, and increase the external validity and generalizability. If patient accrual is disappointing, the trails can be merged to deliver a timely result.

Part V

Conclusion

Chapter 7

Summary and future perspective

This comprehensive investigation, spanning one systematic review, a randomized controlled trial (SPICY), and a prospective protocol (End2End) analyze 3 main field of research in surgical recurrence of Crohn's Disease after Ileocecal Resection.

In the systematic review encompassing 76 articles, the significant variability in endoscopic recurrence (ER) scoring after ileocolic resection for Crohn's disease (CD) came to the forefront. Notably, ER rates within one year ranged widely, from 5% to 93%. The Rutgeerts score (RS) and the modified Rutgeerts score (mRS) demonstrated considerable variance, with the mRS showing potential advantages in accuracy. The study raised concerns about interobserver agreement, particularly for subcategories i2a and i2b, indicating a need for standardized training. It underscored the importance of discerning between hand-sewn and stapled anastomoses, calling attention to the healing variations that might impact recurrence diagnosis.

The SPICY trial, the first randomized controlled trial exploring the impact of extended mesenteric resection,

challenged existing hypotheses. Contrary to expectations, extended mesenteric resection did not exhibit superiority concerning endoscopic recurrence at six months post-surgery. The trial shed light on potential complications, with a non-significant increase in anastomotic leakage in the intervention group. It also highlighted variations in inflammation patterns and postoperative medication use, emphasizing the complexity of mesenteric involvement in CD.

The End2End protocol addresses the longstanding uncertainty surrounding the preferred anastomosis technique after ileocolic resection. The focus on postoperative endoscopic recurrence after six months as the primary endpoint aligns with the pivotal role of endoscopic diagnosis in guiding subsequent medical therapy. The potential impact of anastomosis construction on recurrence rates, with stapled side-to-side anastomoses posing challenges, is a crucial aspect under examination. The hypothesis that different anastomosis configurations might contribute to disease recurrence, particularly due to luminal stasis, adds a layer of possible pathophysiological explanation to the ongoing trial.

Collectively, these studies illuminate the complexities of CD, urging a reevaluation of conventional practices. The plea for improved ER scoring standardization, the SPICY trial's unexpected findings, and the ongoing quest for the optimal anastomosis technique converge to form a narrative that advances our understanding of CD.

In conclusion, this amalgamation of evidence from ER propels us into a future where scientific comprehension evolves. As this thesis concludes, it serves not just as a culmination of scientific inquiry but as a humble step towards unraveling the complexities of Crohn's disease. The ongoing End2End trial, coupled with reflections on SPICY's long-term outcomes, opens avenues for future investigations, marking the beginning of a new chapter in CD research.

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