# Perianal fistulizing Crohn's disease: pathogenesis, diagnosis and therapy

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Abstract | Perianal fistulizing Crohn's disease has a major negative effect on patient quality of life and is a predictor of poor long-term outcomes. Factors involved in the pathogenesis of perianal fistulizing Crohn's disease include an increased production of transforming growth factor β, TNF and IL-13 in the inflammatory infiltrate that induce epithelial-to-mesenchymal transition and upregulation of matrix metalloproteinases, leading to tissue remodelling and fistula formation. Care of patients with perianal Crohn's disease requires a multidisciplinary approach. A complete assessment of fistula characteristics is the basis for optimal management and must include the clinical evaluation of fistula openings, endoscopic assessment of the presence of proctitis, and MRI to determine the anatomy of fistula tracts and presence of abscesses. Local injection of mesenchymal stem cells can induce remission in patients not responding to medical therapies, or to avoid the exposure to systemic immunosuppression in patients naive to biologics in the absence of active luminal disease. Surgery is still required in a high proportion of patients and should not be delayed when criteria for drug failure is met. In this Review, we provide an up-to-date overview on the pathogenesis and diagnosis of fistulizing Crohn's disease, as well as therapeutic strategies.

Patients with Crohn's disease might present with a variety of disease-related perianal lesions that include anal skin tags, anal canal lesions including fissures, ulcers and strictures, perianal fistulas and abscesses, and cancer. The first description of perianal fistulas in patients with 'regional enteritis' was made in 1938, 6 years after the original report of Crohn's disease[1]. The course of perianal fistulizing Crohn's disease is characterized by prolonged periods of active pus drainage through the external fistula openings and frequent disease relapses with a major negative effect on the patient's quality of life[2]. The presence of perianal fistulizing disease is a predictor of poor long-term outcome in patients with Crohn's disease[3] and optimal care of patients with perianal Crohn's disease requires a multidisciplinary approach for assessment and treatment of this aggressive manifestation. Advances in the use of imaging modalities and the development of new therapeutic approaches, including cell therapies, mandates a redefinition of the management of perianal Crohn's disease with new diagnostic and therapeutic strategies. The focus of this Review is on perianal fistulizing Crohn's disease; for a detailed description of other lesions and their management, we refer the reader elsewhere[4,5].

## Epidemiology and natural history

Perianal lesions affect one-quarter of the global Crohn's disease population, with 18% of cases presenting as penetrating lesions: fistulas or abscesses[6]. The cumulative incidence of perianal Crohn's disease increases with disease duration. In a population-based study, the cumulative probabilities of developing any type of perianal Crohn's disease were 29.5% and 42.7% at 10 and 20 years after diagnosis, respectively, and for perianal fistulas in particular, the cumulative probabilities were 16.9% and 28.3% at 10 and 20 years after diagnosis, respectively[7]. These data are in keeping with a previous study, also population-based, which reported a frequency of 12% at 1 year, 15% at 5 years, 21% at 10 years and 26% at 20 years[6]. The prevalence of perianal fistulas in Crohn's disease varies according to disease location, with fistulas least common in isolated ileal disease (12%) or ileocolonic disease (15%), and most common in colonic disease (41%), particularly in cases with rectal involvement (92%)[8]. Perianal lesions can be the first manifestation preceding the diagnosis of Crohn's disease by >6 months in 17.2% of patients; in 26.9%, perianal disease presents from 6 months before to 6 months after the diagnosis of Crohn's disease, whereas perianal disease is first observed >6 months after Crohn's disease diagnosis in the remaining 55.9%[7]. Presence of anorectal stricture is associated with an increased prevalence of fistulizing disease: perianal fistulae have been observed in 61% of patients with Crohn's disease with rectal strictures compared with 34.3% of patients with Crohn's disease

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**Key points**

- Factors involved in fistulizing perianal disease pathogenesis include a genetically determined altered immune response with increased production of cytokines, leading to upregulation of matrix metalloproteinases and epithelial-to-mesenchymal transition
- Essential evaluation of fistulas includes a clinical assessment of external openings, endoscopic assessment of proctitis and MRI to determine the anatomy of fistula tracts and presence of abscesses
- A top-down approach with medical therapy might provide maximal benefit for treatment of this aggressive manifestation of Crohn's disease
- Local injection of mesenchymal stem cells can induce remission in patients not responding to medical therapies, or avoid the exposure to systemic immunosuppression
- Surgery is still required in a high proportion of patients and should not be delayed when criteria of drug failure is met

matched for age, gender and duration of disease but without anorectal strictures ($P = 0.001$)[9]. Furthermore, the presence of perianal abscesses was more frequent in patients with Crohn's disease and anorectal strictures than in those without (61% versus 34.3%, $P = 0.001$)[9].

Data on the natural history of perianal Crohn's disease coming from historical series of reference centers[10,11] and population-based studies[6,8] show that this manifestation of Crohn's disease also follows a relapsing and remitting course, with frequent recurrences occurring within a period of 18 months after achieving remission in 44% of cases[10,11]. A population-based study from Stockholm County, Sweden, that included 184 patients with perianal fistulas at the time of Crohn's disease diagnosis observed that 51% were healed after a mean duration of 9.4 years, 9% had persisting active drainage or abscesses and 40% had undergone proctocolectomy[8]. In a cohort of 33 patients with Crohn's disease and perianal fistulas from Olmsted County, USA, 52% of patients required minor surgery, 24% required major surgery (mainly proctectomy) and 24% were managed with medical interventions[6].

Among the factors influencing fistula outcome, the presence of proctitis has a major effect. Active proctitis is an independent predictor of reduced fistula healing and increased recurrence rates[11]. Furthermore, rectal involvement is associated with higher proctectomy rates compared with rectal sparing (29.0–77.6% versus 4.0–13.6%)[12,13]. Fistula anatomy also has prognostic implications, as complex fistulas are less likely to heal than simple fistulas (64.6% versus 88.2%)[14], and durable remission at the end of a 10-year period is less likely to occur in complex fistulas compared with simple fistulas (37.0% versus 66.7%)[15].

Fistula-associated neoplasia is a rare event and the risk is dependent on the duration of perianal fistulizing disease[16]. A Dutch multicentre study including >6,000 patients with Crohn's disease observed fistula-associated cancer in only four patients[17]. The malignancies developed, on average, 22 years after the diagnosis of Crohn's disease was established and 9 years after the diagnosis of perianal fistula. The tumour is frequently a mucinous carcinoma[18].

## Pathophysiology of fistulas

Our understanding of the pathophysiology of Crohn's disease-associated fistulas is not complete. Two mechanisms seem to have a major role: epithelial-to-mesenchymal transition (EMT) and matrix remodelling enzymes[19] (FIG. 1). In EMT, differentiated epithelial cells transform to mesenchymal-type cells and acquire the ability to migrate and penetrate adjacent tissues[20]. This process is essential in embryogenesis, organ development and would healing, and has also been documented to occur in tumour growth and metastasis[21]. EMT results in the formation of transitional cells that express epithelial cell markers such as cytokeratin 8 and cytokeratin 20, together with mesenchymal markers such as vimentin and smooth muscle actin. These transitional cells downregulate expression of adhesion molecules such as E-cadherin, and upregulate transcription factors such as SNAI1 and SLUG (also known as SNAI2)[20]. Known inducers of EMT include transforming growth factor (TGF)β and TNF[22]. The notion that the EMT process is involved in fistula formation is supported by findings of transitional cells in fistula tracts of patients with Crohn's disease with all the characteristics described, as well as high levels of TGFβ in the zone between transitional cells and epithelial cells, and marked upregulation of TNF and its receptor in transitional cells[20,23]. Other molecules that are upregulated in transitional cells of fistula tracts in Crohn's disease include IL-13, the IL-13 receptor, ETS1 (also known as protein C-ets-1) and DKK1 (dickkopf-related protein 1)[20,22,24]. EMT might also be involved in the pathogenesis of fistula-associated neoplasia[25].

Matrix metalloproteinases (MMPs) can degrade virtually all components of the extracellular matrix. Increased MMP activity has been found in experimental and human IBD[26], and MMP inhibitors afford protection against the development of intestinal inflammation in animal models[27]. A markedly upregulated expression of MMPs has been documented in Crohn's disease fistula tracts, in particular MMP3 protein and mRNA levels in mononuclear cells and fibroblasts[28].

Clinical observations showing that antibiotic therapy and faecal diversion might be of benefit in the management of perianal fistulizing Crohn's disease suggest that the gut microbiota might also have a pathogenic contribution. However, few studies have assessed microbiological changes in Crohn's disease fistulae. In one study, the contents of fistula tracts were obtained from perianal fistulas of patients with Crohn's disease and from idiopathic fistula[29]. In patients with Crohn's disease, Gram-positive organisms, in particular streptococci, staphylococci and *Corynebacterium* spp., predominated over Gram-negative enteric organisms, and differed from idiopathic fistulas, in which the microbiota were largely of gastrointestinal origin[29]. Another study in 2015 confirmed differences in the microbiota content of Crohn's disease and idiopathic fistulas, finding the total number of operational taxonomic units and number of species identified substantially higher in fistula tracts of patients with Crohn's disease than idiopathic fistulas[30]. The most abundant species in the Crohn's disease group were *Bradyrhizobium pachyrhizi* followed by *Pseudomonas azotoformans* and

*Prevotella oris*[30]. Additional investigations are needed to clarify the role of the microbiota in perianal fistulizing Crohn's disease.

Heterogeneity in the phenotype of Crohn's disease, including development of perianal disease, has fostered the study of genetic predispositions. The gene *PUS10* (coding for pseudouridylate synthase 10) has a substantial protective effect against the development of perianal disease[31], and a C allele at the *CDKAL1* rs6908425 variant and the absence of *NOD2* variants have also been independently associated with perianal fistulas[32]. Interestingly, the genetic factors associated with the development of internal fistulas turned out to be different in the two studies that separately analyzed internal and perianal fistulas[31,32]. Pathway analyses of genetic associations implicated the Janus kinase (JAK)–STAT pathway more prominently in patients with perianal fistulizing Crohn's disease than in patients with Crohn's disease without perianal fistula[33].

## Classification of perianal fistulas

Two major anatomical classifications of perianal fistulas have been used: the Parks classification[34] and the St. James's University Hospital classification[35,36] (FIG. 2). The Parks classification provides a detailed description of the course of the fistula tract in relation to the external sphincter and the levator plate (FIG. 3). However, this classification does not provide any information regarding the complexity of the fistula (secondary tracts or presence of abscesses) or the presence of proctitis. By contrast, the St. James's Hospital classification offers detailed information on the primary fistula tract, its relation to the sphincter, and on secondary tracts and related abscesses (FIG. 2). However, this classification is not simple in daily clinical practice.

To simplify the classifications of perianal fistulas, the American Gastroenterological Association proposed a classification that aimed to generate a practical approach for perianal lesion assessment[4], in which perianal fistulas are divided into two categories: simple or complex (FIG. 2). This division is made on the basis of fistula tract anatomy, number of external openings, presence of abscesses and/or proctitis. This classification has prognostic relevance for fistula healing as patients with more complex fistula are less likely to achieve clinical remission than patients with simple fistulas[37]. However, the 'complex fistula' category is based on multiple variables and does not permit proper individualization of treatment; depending on whether the fistula is classified as complex on the basis of tract anatomy, presence of abscess or presence of proctitis, the therapeutic approach should be different.

Anatomical descriptions of fistulas should include the type of fistula, location of internal and external openings, and the presence of secondary branches and abscesses. Internal and external openings should be described using the 'anal clock' system (surgeon's view of the perianal region when the patient is in the lithotomy position; anterior perineum is 12 o'clock, posterior is 6 o'clock)[38]. Fistula tract location should be classified based on their presence in the intersphincteric space, supraelevator space or in the roof of the ischioanal fossa.

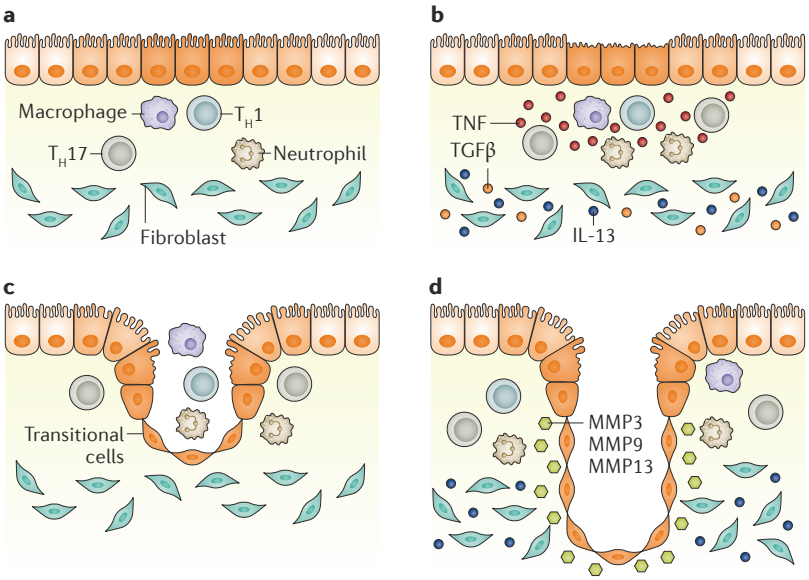


Figure 1 | **Proposed pathogenesis of perianal fistulas. a** | Inflammatory infiltrate at sites of fistulas in Crohn's disease contains $CD4^+$ and $CD161^+$ T cells with type 17 T helper ($T_H17$), type 1 T helper ($T_H1$) and/or $T_H17/T_H1$ phenotypes, plus an underlying band of $CD68^+$ macrophages, neutrophils, a dense infiltrate of $CD20^+$ B cells, and fibroblasts[19]. **b** | TNF produced by inflammatory cells, transforming growth factor β (TGFβ) and IL-13 produced by fibroblasts, induce epithelial-to-mesenchymal transition (EMT) and tissue remodelling[20]. **c** | In EMT, epithelial cells downregulate epithelia-specific proteins such as E-cadherin and claudin-4, lose epithelial-specific cell contacts and apicobasal polarity, and gain qualities of mesenchymal cells including increased motility and cell spreading, and upregulation of mesenchymal proteins such as vimentin[21]. **d** | IL-13, also produced by transitional cells, and its receptors act in an autocrine manner, favouring spread of EMT cells into deeper layers, a process facilitated by upregulation in the inflamed intestine of matrix metalloproteinases (MMPs) that degrade all components of the extracellular matrix. MMP3, MMP9 and MMP13 are highly expressed around fistulas in Crohn's disease[2,26].

## Measurement of fistula disease activity

The measurement of perianal fistula activity should enable evaluation of disease severity and response to therapy. In this regard, the lack of a robust and validated outcome measure has constrained research in this area. In clinical practice, the accepted approach to assessment of perianal fistulas takes into account information from physical examination, endoscopy and pelvic MRI, including anatomical description and parameters of inflammation.

### *Clinical scores*

Various clinical indices have been derived to measure the activity of perianal disease, the advantages and limitations of these indices are summarized in TABLE 1. The Perianal Disease Activity Index (PDAI) is based on the assessment of quality of life and perianal disease severity (fistula discharge, type of perianal disease and degree of induration, rating each item on a five-point scale)[39]. The reliability and the responsiveness of this index were validated against physicians' and patients' global assessment[39]. Despite its wide use in clinical practice and in trials, one of the major limitations of this index is the lack of established optimal cutoff for determining a substantial clinical response.

| Parks classification | | St. James's Hospital classification | | AGA classification |
|---|---|---|---|---|
| **Superficial** | Superficial fistula without crossing any sphincter or muscular structure | | | **Simple fistula**<br>• Low (superficial or low intersphincteric or low trans-sphincteric origin of the fisula tract)<br>• Single external opening<br>• No pain or fluctuation to suggest perianal abscess<br>• No evidence of a rectovaginal fistula<br>• No evidence of anorectal stricture |
| **Intersphincteric** | Fistula tract between the internal anal sphincter and external anal sphincter, in the intersphincteric space | **Grade 1** | Simple linear intersphincteric fistula | |
| | | **Grade 2** | Intersphincteric fistula with intersphincteric abscess or secondary fistulous tract | **Complex fistula**<br>• High (high intersphincteric or high trans-sphincteric or extrasphincteric or suprasphincteric origin of the fisula tract)<br>• Multiple external openings<br>• Presence of pain or fluctuation to suggest a perianal abscess<br>• Rectovaginal fistula<br>• Anorectal stricture |
| **Trans-sphincteric** | Fistula tract crosses the external anal sphincter | **Grade 3** | Trans-sphincteric fistula | |
| | | **Grade 4** | Trans-sphincteric fistula with abscess or secondary tract within the ischioanal or ischiorectal fossa | |
| **Suprasphincteric** | Fistula tract penetrates the intersphincteric space and continues over the top of the puborectalis and penetrates the levator muscle before reaching the skin | **Grade 5** | Supralevator or translevator disease | |
| **Extrasphincteric** | Fistula tract outside the external anal sphincter and penetrating the levator muscle | | | |

Figure 2 | **Classifications of perianal fistulas in patients with Crohn's disease.** Features of the Parks[34], St. James's University Hospital[35,36] and the American Gastroenterological Association[4] (AGA) classifications for perianal fistulas are shown. Overlaps between the different classifications are indicated by dotted lines.

The Anal Disease Activity Index was derived after the analysis of different symptoms related to anal disease using a linear analogue scale, and determined that spontaneous pain, pain limiting locomotion and pain at defecation were the most discriminative parameters to detect clinical improvement[40].

Fistula Drainage Assessment is a simple measure of fistula activity and response to medical treatment, classifying fistulas as being either open (draining) or closed (no drainage despite gentle finger compression). One of the major drawbacks of this clinical evaluation in early stages after medical treatment is that a fistula might be clinically classified as closed, but an active fistula tract might persist in more proximal sections[37,41]. In that regard, for the detection of complete tissue healing beyond the simple clinical response, a combined evaluation consisting of clinical assessment plus MRI examination is preferred, although resolution of fistula tracts in MRI might require a period of 12 months[42].

### *MRI scores*

Pelvic MRI represents the gold-standard imaging modality for the assessment of fistula characteristics and detection of abscesses. Although different clinical scoring systems have been developed for perianal Crohn's disease, far fewer exist for MRI. The use of T2-weighted sequences is essential to identify the fluid content in fistula tracts or abscesses, whereas gadolinium-enhanced images might be useful for differentiating pus from granulation tissue inside the fistula tract and inflammatory masses, and has been suggested to be an important method to determine changes after therapy[38,42]. MRI findings in a case of simple fistula with a low single tract and no abscesses are shown in FIG. 4. A case of complex fistula is shown in FIG. 5, with multiple fistula tracts, fistula extensions and presence of abscesses.

The most frequently used MRI index is the van Assche score[43]. This index was originally developed in response to the need for a standardized tool for quantitatively measuring the severity of perianal fistulizing Crohn's disease and response to medical therapy, thereby avoiding having patients serve as their own controls to determine whether perianal disease has improved or worsened relative to baseline[12]. This score combines the anatomical fistula characteristics with MRI findings linked to inflammation (TABLE 2). Changes in the van Assche score have a good correlation with clinical response to immunosuppressant treatment[43–45], and the index has been partially validated in small studies demonstrating that this score is responsive to medical therapy[41,42]. Nevertheless, the responsiveness of each individual item of the score was not assessed; determining this information represents a relevant unmet need, as it might be key to developing a more robust index for assessing fistula healing. The assumption that the decrease in fistula T2 signal is associated with fistula healing has not been validated[46] and, in complex fistulas, a decrease of the global Van Assche score might be observed owing to disappearance of fluid collections or a reduction of T2 signal intensity of the fistula tract, even if the fistula tract remains active. Moreover, some evidence indicates that gadolinium enhancement can help in determining perianal disease activity[44], and the disappearance of gadolinium enhancement in the fistula tract might better

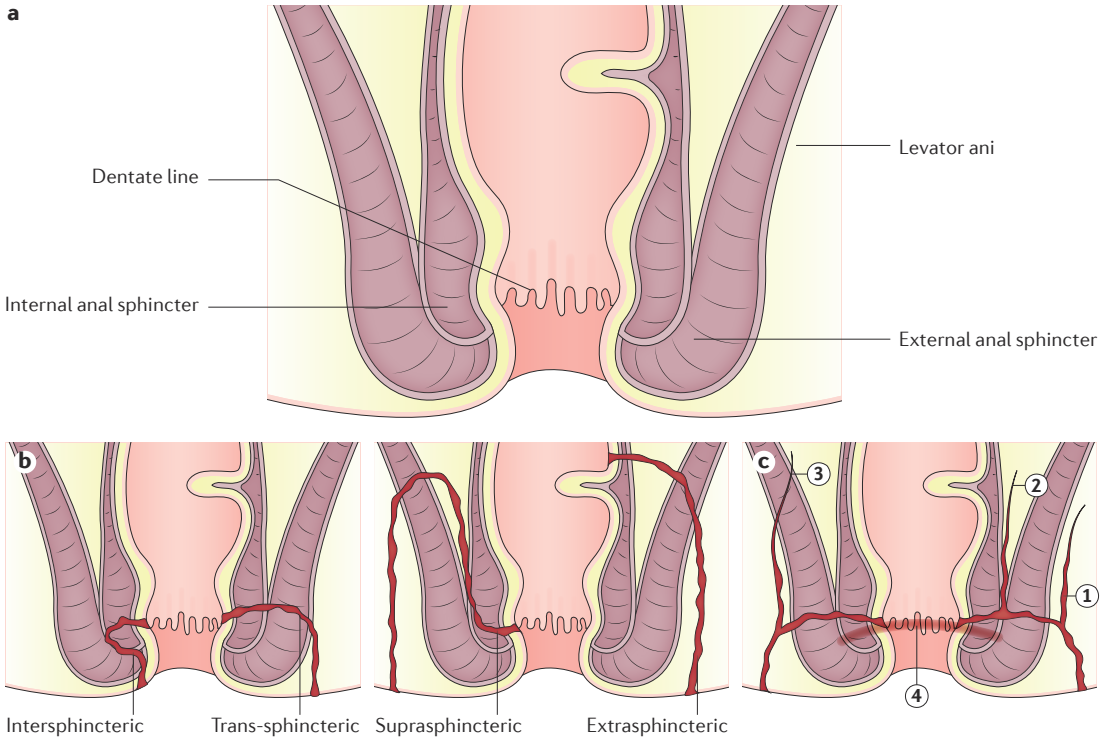


Figure 3 | **Classifications of perianal fistulas. a** | The anatomic structures essential for fistula classification. **b** | Illustrations of the Parks classification of perianal fistula in a coronal plane. **c** | Illustrations of fistula extensions: extension into roof of ischioanal fossa (infraelevatoric) (1); supraelevatoric extension from apex of trans-sphincteric fistula (2); supraelevatoric extension from trans-sphincteric fistula (3); and horseshoe extension (4).

predict the achievement of deep fistula remission than the T2 signal[46]. Gadolinium-enhanced sequences should be included in the development of a fully validated MRI-based evaluative instrument that can eventually be applied in clinical trials and in clinical practice.

### *Practical recommendations*

At the time of diagnosis, perianal Crohn's disease should be evaluated both clinically by visual inspection and rectal examination, and by imaging. Examination under anaesthesia, when performed by an experienced surgeon, is 90% accurate in detecting and correctly classifying perianal fistulas and abscesses[47]. Examination under anaesthesia should be the first diagnostic approach when the presence of abscesses is suspected as it enables concomitant therapy such as incision and drainage of perianal abscesses and placement of loose setons, which are loops of surgical thread passed along the fistula tract (from external to internal opening) and exteriorized through the anal canal that facilitate drainage of pus and contribute to fistula healing. Subsequent cross-sectional imaging techniques will confirm appropriate drainage of the cavities and fistula anatomy[48]. Pelvic MRI is the gold standard for detecting fistula anatomy, complexity and activity. As an alternative to MRI, endoanal ultrasound has been suggested to be useful but requires expertise and has limited accuracy for detecting lesions distant to the anal canal[38,49]. The identification of clinically relevant abscesses on MRI will require surgical drainage[50].

Monitoring of perianal disease in clinical practice can be based on clinical evaluation. Imaging follow-up is not part of the standard of care in most institutions; in patients achieving sustained closure of all fistula tracts, the added value of imaging assessment is not clear. Furthermore, optimal timing for imaging reassessment is also not well established. Thus, reassessment of perianal disease by imaging is limited to those patients with unfavourable clinical outcomes. In clinical trials, an objective demonstration of lesion healing is recommended and a combined clinical plus imaging definition of primary end points should be preferred to simple clinical evaluation[49–51].

## Management of perianal fistulas

### *Medical treatment*

***Aminosalicylates and corticosteroids.*** Systematic reviews and meta-analyses of clinical trial data show no clinically significant benefit of aminosalicylates for the treatment of luminal Crohn's disease, and these drugs have not been specifically tested in perianal Crohn's disease[52]. Corticosteroids have been used in one study in combination with ciclosporin for perianal fistulas in Crohn's disease[53]. Although all patients responded to the combination therapy, 56% had a recurrence when ciclosporin was stopped and low-dose corticosteroids continued. Another observational study reported a worsening of fistula discharge and increased surgery requirements in patients treated with high doses of corticosteroids (up to 60 mg prednisone per day or equivalent)[54].

***Antibiotics.*** Antibiotics improve symptoms of perianal fistulas and might contribute to healing. Metronidazole and ciprofloxacin are the two most common agents used for this condition. However, despite their widespread use, evidence of their efficacy is scarce. An early series, which included a small number of patients and was uncontrolled, reported fistula improvement after 6–8 weeks of antibiotic therapy (metronidazole 750–1,500 mg per day, ciprofloxacin 500–100 mg per day) but with high rates of relapse upon discontinuation of the antibiotic[55,56]. The only prospective randomized, double-blind, placebo-controlled trial assessing the efficacy of antibiotics in perianal Crohn's disease fistulas was underpowered. A total of 25 patients were randomized to metronidazole (7 patients) ciprofloxacin (10 patients) or placebo (5 patients), and no statistically significant differences were observed[57]. The potential for topical metronidazole (10%) ointment to exert therapeutic benefit in perianal Crohn's disease was assessed in a randomized, placebo-controlled trial including 74 patients, but no difference in PDAI improvement was observed between the two treatment arms (metronidazole, 22.4%; placebo, 22.2%)[58].

The efficacy of ciprofloxacin in combination with an anti-TNF drug has been tested in two randomized controlled trials[59,60]. The first study assessed the efficacy of combining ciprofloxacin and infliximab, observing a fistula response in 73% of patients receiving the combination therapy at week 18, compared with 39% of patients treated with infliximab monotherapy ($P = 0.12$)[59]. The second study assessed the efficacy of combining ciprofloxacin and adalimumab by comparison with adalimumab monotherapy, and showed a significant benefit of the combination therapy in terms of fistula response at week 12 (70.6% versus 47.2%, $P = 0.047$)[60].

Overall, these results support the efficacy of antibiotics in reducing fistula drainage, but not for inducing fistula healing, and indicate that antibiotics should be used as adjunctive treatment for fistulas.

***Immunosuppressants.*** Evidence on the efficacy of immunosuppressants for treatment of perianal fistulizing disease is scarce and all studies include a low number of patients.

No prospective randomized controlled trial data exists for thiopurines with fistula outcome as the primary end point. However, a meta-analysis has been performed that assessed the efficacy of azathioprine or mercaptopurine in a subgroup of patients with Crohn's disease included in randomized controlled trials that had perianal fistulizing disease at baseline[61]. This subgroup analysis observed fistula healing in 54% of patients treated with azathioprine or mercaptopurine compared with 21% of patients treated with placebo (odds ratio (OR) 4.44, 95% CI 1.5–13.2). A subsequent open-label study with 49 patients compared the efficacy of antibiotics (ciprofloxacin and/or metronidazole) with the combination of antibiotics plus azathioprine and observed a response, defined as at least a 50% reduction from baseline in the number of draining fistulas, in 48% of patients treated with the combination therapy in comparison with 15% of those treated with antibiotics alone ($P = 0.03$)[62].

Only one randomized, placebo-controlled trial assessed the efficacy of tacrolimus for treatment of perianal fistulas in Crohn's disease[63]. In this short-duration (10 week) study comparing a standard dose of tacrolimus (0.2 mg/kg body weight per day) versus placebo, fistula response (closure ≥50% of external openings) occurred in 43% of patients receiving tacrolimus compared with 8% of those receiving placebo ($P = 0.004$). Complete fistula closure was only achieved in 10% of patients who received tacrolimus and 8% of placebo-treated patients — the short duration of the study might have contributed to the low remission rates. Nephrotoxicity was observed in the tacrolimus arm and was managed by dose reduction. A subsequent trial using topical tacrolimus ointment showed no benefit in the treatment of perianal Crohn's disease[64].

Table 1 | **Summary of pros and cons of different clinical and imaging indices for measuring fistula activity**

| Index | Pros | Cons |
|---|---|---|
| Perianal Disease Activity Index (PDAI) | • Simple to apply in clinical practice<br>• Validated against physicians' and patients' global assessment | • Limited to clinical assessment, no objective measure of healing<br>• Further data is needed to determine the minimum clinically significant difference, and a cut-off value that indicates remission (ideally defined as a score of 0) |
| Anal disease activity index | • Includes discriminative parameters to assess disease activity | • Incomplete evaluation of manifestations of perianal disease<br>• Not validated |
| Fistula drainage assessment | • Simple measurement<br>• Accepted by regulatory agencies as endpoint | • Limited to clinical assessment, no objective measure of healing<br>• Fistula compression is investigator dependent |
| MRI score (Van Assche) | • Partially validated (against PDAI)<br>• Combines assessment of activity and complexity of fistulas<br>• Simple to calculate | • Not fully validated (conflicting data between studies)<br>• No cut-off to define clinically significant improvement or remission<br>• Lack of validation of changes on T2-weighted images for capturing inflammation improvement<br>• Responsiveness of each individual item of the score not determined |

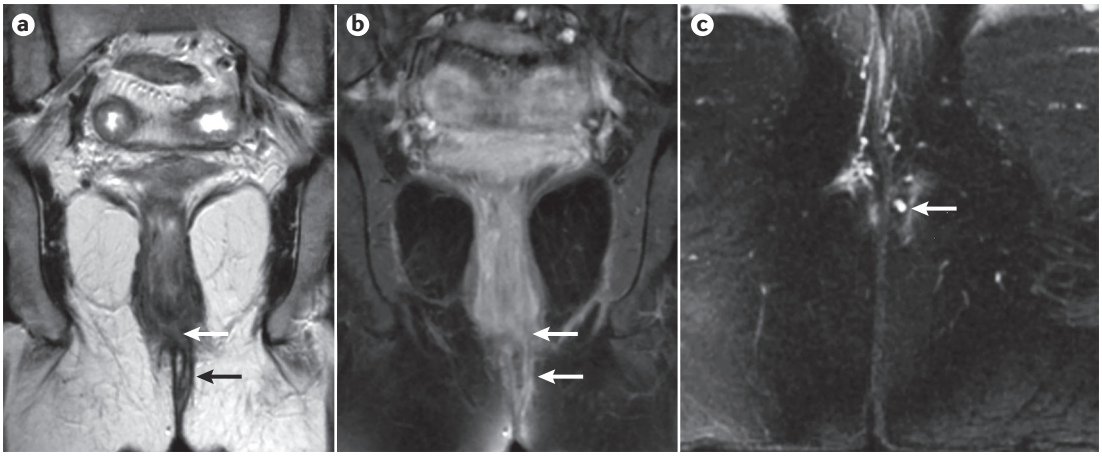


Figure 4 | **Simple perianal fistula. a** | Coronal T2-weighted MRI showing a single left low intersphincteric fistula (arrows) with a single external opening and without extensions or associated collection. **b** | Coronal gadolinium-enhanced T1 sequence with fat saturation showing the enhancement of the fistula track (arrows). **c** | Depicts the same fistula from (part **a**) and (part **b**) on axial T2-weighted imaging with fat saturation, showing marked signal intensity (arrow). The rectal wall (not seen) was normal on MRI. The total van Assche MRI activity score was 10.

Observational studies have reported on the efficacy of ciclosporin, observing rapid improvements in 50–80% of cases[65,66], but with high relapse rates after drug discontinuation. The effect of methotrexate on fistula drainage has only been assessed in two uncontrolled case series; both suggested that methotrexate might be effective in fistulizing Crohn's disease[67,68]. Currently, ciclosporin and methotrexate should only be considered for those failing other more proven therapies.

***TNF inhibitors.*** Among the three anti-TNF antibodies approved for treatment of Crohn's disease, only infliximab has been specifically evaluated for efficacy in treating fistulizing perianal Crohn's disease. The first was an induction study assessing the efficacy of two doses of infliximab of 5 mg/kg or 10 mg/kg body weight, or placebo, administered at weeks 0, 2 and 6 (REF. 69). The primary end point was fistula response, defined as cessation of drainage of ≥50% of fistula tracts present at baseline in two consecutive visits performed within 18 weeks. The primary end point was achieved by 26% of patients receiving placebo, 68% of patients receiving 5 mg/kg infliximab ($P = 0.002$), and 56% of patients receiving 10 mg/kg ($P = 0.02$). Additionally, complete remission, defined as cessation of drainage of all fistula tracts, was observed in 13% of patients receiving placebo, 55% of patients receiving 5 mg/kg infliximab ($P = 0.001$), and 38% of patients receiving 10 mg/kg ($P = 0.04$). However, the median length of time during which fistulas remained closed was short; 3 months. This observation led to the development of a maintenance study (ACCENT II) in which patients responding to induction therapy with 5 mg/kg body weight of infliximab on weeks 0, 2 and 6, were randomized to receive maintenance infliximab therapy of 5 mg/kg body weight every 8 weeks or matching placebo every 8 weeks[70]. Time to loss of response was longer in patients receiving infliximab maintenance than in patients on placebo maintenance (>40 weeks versus 14 weeks, $P < 0.001$) and, at week 54, the proportion of patients with complete cessation of drainage in all fistula tracts was superior in the infliximab group (36%) compared with those who received placebo maintenance after infliximab induction (19%, $P = 0.009$).

To date, no studies specifically designed to test the efficacy of adalimumab or certolizumab for fistulizing Crohn's disease have been performed. Inference of efficacy of these anti-TNF antibodies has been obtained from post-hoc analyses of the subpopulation of patients who had draining fistulas at baseline. Two induction studies with adalimumab[71,72] did not show superiority of adalimumab relative to placebo, although the number of patients with fistulas at baseline was low. In a maintenance study (CHARM) that included a larger population than previous studies, 117 patients that had draining fistulas at both screening and baseline were randomly assigned to either adalimumab ($n = 70$) or placebo ($n = 47$)[73]. Closure of fistulas at week 54 was obtained in a significantly higher proportion (33%) of patients treated with adalimumab compared with those receiving placebo (12%, $P = 0.016$)[73]. The subanalysis of fistula closure in two studies assessing the efficacy of certolizumab (PRECISE 1 and PRECISE 2) produced mixed results[74,75]. Complete fistula closure at week 26 was observed in 36% of patients on certolizumab compared with 17% on placebo ($P = 0.038$) but, by contrast, no statistically significant difference was found in the rate of fistula response, defined as >50% closure at two consecutive visits[76].

Data on the efficacy of concomitant use of immunosuppressants and anti-TNF therapy for treatment of perianal fistulas remains controversial. In the ACCENT II trial, response rates at 1 year were similar in patients on combination therapy compared with those receiving infliximab monotherapy[70], but another study does suggest a positive association between combination therapy and fistula closure[77].

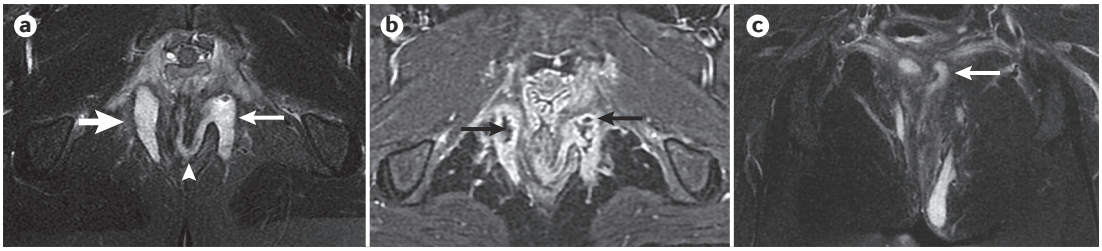


Figure 5 | **Complex perianal fistula. a** | An axial T2-weighted image with fat saturation, showing a left trans-sphincteric fistula with a marked hyperintensity component on T2 sequence. The internal opening is located on the posterior aspect of the anal canal (arrowhead). There is an associated abscess in the left ischioanal fossa (long arrow). A second abscess in the right ischioanal fosa can be noted (short arrow) associated with another fistula tract (not seen). **b** | Image of the same fistula corresponds to an axial contrast-enhanced T1-weighted image showing lack of enhancement on the central part of the abscesses confirming the presence of pus (arrows). **c** | Coronal T2 fat-saturated image that depicts a left ischioanal roof extension. The rectal wall (not seen) was normal. The total van Assche MRI activity score was 18.

***Other medical therapies.*** The anti-α4β7 integrin antibody vedolizumab showed efficacy for induction and maintenance of remission in Crohn's disease[78]. However, available data on the potential efficacy of the drug for treating perianal fistulizing disease is limited. In a small subgroup of patients that responded to vedolizumab induction, maintenance of 300 mg intravenous vedolizumab every 8 weeks achieved fistula closure at week 52 in 41.2% of patients, whereas those who received placebo during maintenance had a fistula closure rate of 11% ($P = 0.03$)[78].

Thalidomide has been studied for Crohn's disease fistulas in four small open-label trials and one retrospective study[51]. The largest study used 200 mg of thalidomide per day and, among the 13 patients included, 46% had complete cessation of drainage by week 12 (REF. 79). However, similar to other trials using this drug, all patients needed a dose reduction due to adverse events and two patients were withdrawn due to adverse events.

Table 2 | **The MRI-based van Assche index for perianal disease activity**

| Descriptor | Categories | Scoring |
|---|---|---|
| Number of fistula tracts | None | 0 |
| | Single, unbranched | 1 |
| | Single, branched | 2 |
| | Multiple | 3 |
| Location | Extrasphincteric or intersphincteric | 1 |
| | Trans-sphincteric | 2 |
| | Suprasphincteric | 3 |
| Extension | Infralevatoric | 1 |
| | Supralevatoric | 2 |
| Hyperintensity on T2-weighted images | Absent | 0 |
| | Mild | 4 |
| | Pronounced | 8 |
| Collections (cavities >3 mm in diameter) | Absent | 0 |
| | Present | 4 |
| Rectal wall involvement | Normal | 0 |
| | Thickened | 2 |

Hyperbaric oxygen, involving intermittent inhalation of 100% oxygen at pressures >1 atmosphere, has been tested in small and open-label studies. Reported cessation of drainage rates are 33–71%[80–82]. To date, no controlled study testing this type of therapy has been carried out.

The potential therapeutic benefit of oral spherical adsorptive carbon has been assessed in two placebo-controlled, randomized trials[83,84]. An initial study suggested a therapeutic benefit; compared with patients receiving placebo, patients treated with adsorptive carbon showed higher response rates (37% versus 10%, $P = 0.025$) and remission rates (29.6% versus 6.7%) at both weeks 4 and 8 (REF. 83). However, the benefit was not confirmed in a subsequent and larger ($n = 249$) study using the same product, in which no difference was found between adsorptive carbon and placebo at either week 4 or 8 (REF. 84).

### *Cell therapies*

Mesenchymal stem cells (MSCs) have the capacity to differentiate into various mesodermal cell lineages, including chondrocytes, tenocytes and myoblasts[85]. MSCs were originally isolated from bone marrow, but similar populations have been found in other tissues, including adipose tissue, placenta, amniotic fluid and fetal tissues such as fetal lung and blood[85]. MSCs possess immunomodulatory properties, suppressing T cell activation and proliferation, dendritic cell differentiation, maturation and function, B cell function, and natural killer cell proliferation[86]. The properties of MSCs could be particularly relevant in the context of perianal fistulizing Crohn's disease given the presumed involvement of lymphocyte and dendritic cell activation, as well as natural killer cell proliferation, in the pathogenesis of fistula formation[87,88].

The efficacy of local injection of MSCs in perianal fistulas of patients with Crohn's disease was first tested in open-label studies using autologous cells from an adipose[89–91] or bone marrow[92] origin; high fistula closure rates (75–77%) were observed in all studies. Considering the ability of MSCs to induce tolerance, subsequent studies tested allogeneic MSCs. An open-label study with 24 patients used 20–60 million adipose-derived MSCs and obtained closure on an average of 28% of fistula tracts after 24 weeks[93]. A placebo-controlled,

dose-ascending study including 21 patients with perianal fistulizing Crohn's disease used 20–90 million bone-marrow-derived MSCs and obtained closure of 33–80% of fistula tracts, compared to ~20% in the placebo group[94]. The first adequately powered randomized, placebo-controlled trial compared the efficacy of a single injection of 120 million allogeneic, adipose-derived MSCs or placebo in 107 and 105 patients, respectively[95]. In this study, patients underwent two surgical procedures: in the preparation surgery, fistula curettage, abscess drainage and seton placement, when necessary, was performed; in the second surgical procedure, setons were removed, the internal fistula opening was surgically closed and 60 million cells were injected around the closed internal opening, with an additional 60 million cells distributed into the tissue adjacent to all fistula tracts (FIG. 6). The primary end point was assessed at week 24 and had a stringent definition of combined closure of all fistula tracts and absence of collections >2 cm documented by MRI. Treatment success was achieved in a significantly ($P = 0.024$) higher proportion of patients treated with MSCs (50%) compared with controls (34%) and this benefit was sustained at week 52, meeting the combined endpoint in 56.3% of patients treated with MSCs compared with 38.6% of those receiving placebo. Importantly, a majority (>78%) of patients had previously failed anti-TNF therapy.

A modification of the technique to deliver MSCs in the fistula tract has been tested, which involved coating the autologous MSCs on fistula plugs. In a phase I study, 12 patients with a single draining fistula for at least 3 months without proctitis, and who had failed anti-TNF therapy, underwent intraoperative placement of the plug loaded with stem cells. Nine of 12 patients had complete fistula closure by 3 months, and 10 of 12 patients (83.3%) had complete fistula closure at 6 months[96].

Thus, MSCs represent an efficacious local intervention that might not only be optimal for patients with active perianal fistulizing disease, but also for patients with quiescent luminal disease naive to immunosuppressants or anti-TNF therapy, and those not responding to these forms of medical therapy who then avoid exposure to systemic immunosuppression.

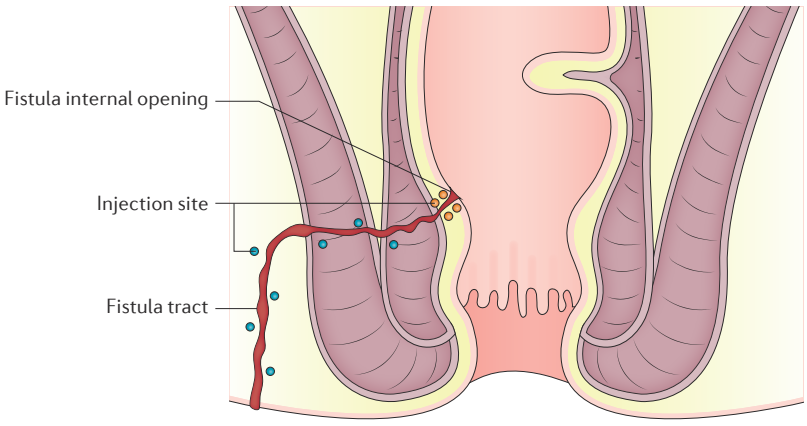


Figure 6 | **Injection of mesenchymal stem cells in perianal fistulas.** First the fistula internal opening is closed using polygalactin absorbable stitches. Subsequently, a suspension of 120 million mesenchymal stem cells are injected, distributing the cells into the tissue adjacent to all fistula tracts (60 million, blue spots) and internal openings (60 million, yellow spots).

### *Surgical treatment*

***Abscess drainage and setons.*** Active perianal fistulizing disease is commonly associated with the presence of perianal abscesses[97]. Surgical drainage of these abscesses should be complimentary to medical therapy. Placement of noncutting setons is the cornerstone of combination medical and surgical treatment. Setons maintain the patency of the fistula tracts, preventing premature closing of the external orifice and hence limiting recurrent abscess formation[98,99]. Loose setons preserve the integrity of the external anal sphincter and are preferred[100]. The optimal timing for seton removal is not well established. In the ACCENT II study, setons were removed at week 2 and abscess recurrence was 15%[70]; by contrast, in a small prospective study that maintained setons for the duration of infliximab therapy, abscess recurrence was 0%[101]. Removal at week 2 might, therefore, be too early and setons should be kept at least until completion of induction therapy. Complete closure of the fistula tract can be achieved after seton removal.

***Fistulotomy.*** Fistulotomy is the surgical longitudinal opening of a fistulous tract. Fistulotomy is the option of choice for patients with superficial and occasionally low intersphincteric fistulas, especially if they have failed a course of antibiotic treatment and have no active proctitis. Under these conditions, fistulotomy is a safe method with high healing rates and low recurrence[102]. Preservation of continence must always be a consideration and the procedure should be avoided in women with low trans-sphincteric fistulas located anteriorly, as these are associated with a high risk of incontinence[103].

***Fibrin glue.*** Fibrin glue consists of fibrinogen and thrombin. When the two agents are mixed and injected into a fistula tract, a fibrin clot is formed in the lumen of the fistula, sealing the tract and providing a matrix for fibroblast growth and collagen deposition[104].

Initial observational studies included a small number of patients ($n = 3$–13) and observed healing in highly variable proportions of patients (0–40%)[105–109]. Fibrin glue was subsequently evaluated in a prospective, randomized open-label trial including 77 patients with fistulizing Crohn's disease, and showed limited efficacy[110]. At week 8 after randomization, clinical remission — defined as cessation of drainage — was observed in 38% of the fibrin glue group compared with 16% in the observation group ($P = 0.04$). However, the benefit was not statistically significant for patients with complex fistulas, with only 25% achieving closure, and some patients in the fibrin glue group had an early recurrence after week 8 and no MRI assessment after treatment was performed[110]. As the benefit of fibrin glue is perceived as marginal, attempts have been made to combine fibrin glue with other types of therapies, but the combination with antibiotics, flap repair or suture closure of the internal opening have not increased its efficacy[111].

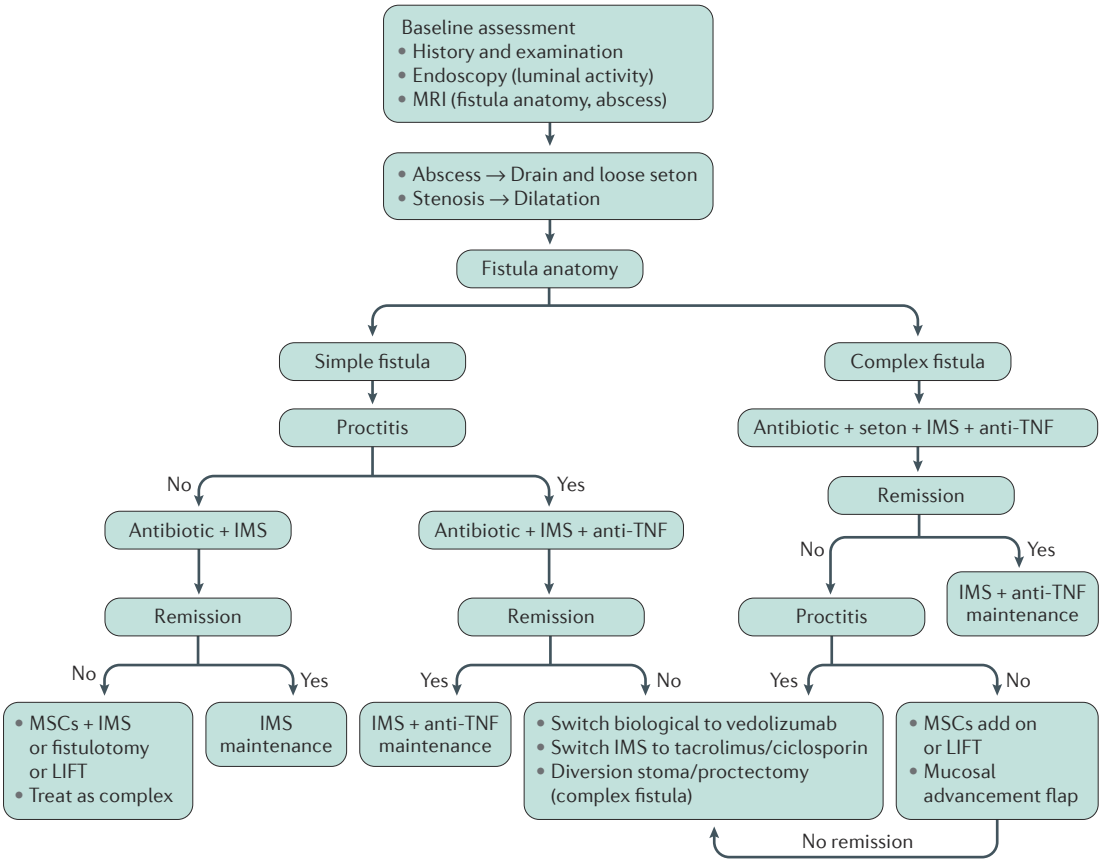


Figure 7 | **Treatment algorithm for perianal fistulizing disease.** Anatomic characteristics of the fistulas, presence of abscesses and presence of proctitis are the main determinants of the therapeutic strategy. For patients with complex fistulas and/or proctitis, a top-down approach is preferred. Timely assessment of response to medical therapy is essential to initiate alternative therapies or surgery. IMS, immunosuppressant; LIFT, ligation of the intersphincteric fistula tract; MSCs, mesenchymal stem cells.

***Bioprosthetic plugs.*** Plugs made of collagen or porcine intestinal submucosa can be used to fill the fistula tract, sealing the internal opening. A systematic review of 20 observational or retrospective studies that included 4–60 patients showed wide variations in the proportion of patients achieving fistula closure, ranging from 20–86%, with a pooled proportion of closures in patients with Crohn's disease of 55%[112]. To date, the only randomized trial completed using biosynthetic plugs was an open-label study that included 106 patients[113]. Fistula closure at week 12 was achieved in 31.5% patients in the anal fistula plug group and in 23.1% in the control group (relative risk stratified on American Gastroenterology Association classification: 1.31; 95% CI 0.59–4.02, $P = 0.19$)[113]. Overall, the evidence does not support a recommendation for using this therapy.

***Mucosal advancement flap.*** Mucosal advancement flap involves the mobilization of a rectal mucosal flap of tissue (mucosa, submucosa, circular muscle) to cover the internal opening of the fistula tract, leaving the sphincter complex untouched. The excluded fistula tract is expected to heal over time but this procedure can only be performed in patients without active proctitis. In a systematic review including 35 studies, the average success rate after a mean follow-up of 29 months was 64% (range 33–93%), with reoperation needed in 50% of patients[114]. Concomitant therapy with an immunosuppressant has also been advocated[115].

***Ligation of the intersphincteric fistula tract.*** This surgical approach consists of ligation of the fistula tract at the level of the intersphincteric space, close to the internal fistula opening, and removal of intersphincteric tract. Then, intense curettage in the rest of the fistulous tract is performed, removing all granulation tissue, followed by suturing of the defect at the external sphincter muscle[116]. In a prospective study of 15 consecutive patients with Crohn's disease and trans-sphincteric fistulas, none of the patients developed faecal incontinence and healing at the surgical site was seen in 67% at 12-month follow-up[117].

***Diverting stoma.*** The rationale for faecal diversion is the observed relationship between the recurrence of inflammatory lesions and restoration of faecal transit in patients undergoing resection surgery for Crohn's disease[118]. Faecal diversion might be considered in patients with severe perianal sepsis with insufficient response to drainage and seton placement, or as a temporary measure to improve the condition of the patient until

completing induction of medical therapy or proctectomy[119,120]. If other therapeutic measures are not introduced, recurrence of perianal fistulas upon stoma closure occurs in the majority of patients[120].

***Proctectomy.*** Proctectomy with permanent stoma is a last-resort treatment for severe refractory perianal fistulizing disease. Proctectomy is indicated when perianal disease is severe and chronically active, or if the disease results in sphincter damage causing disability due to incontinence. Proctectomy is required in 10–20% of patients with perianal fistulizing disease[12,120], and the introduction of anti-TNF therapy does not seem to have a major effect on this requirement[121]. Proctectomy can be complicated by poor wound healing and formation of a perineal sinus in up to 25% of patients[119].

## Conclusions

A complete initial assessment and classification of perianal fistulizing disease is the first and essential step for proper management. This approach should include the number and location of external fistula openings with active drainage, a rectal examination or examination under anaesthesia to assess the presence of anal or rectal strictures and existence of collections, an endoscopy to assess disease activity in the rectum, and an MRI (or anorectal ultrasound when there is local expertise) to have a full and detailed map of fistula tracts and the presence of abscesses. Clinical assessment together with MRI is also the basis for monitoring fistula response[49,122].

The goal of therapy is to achieve complete fistula healing and prevent recurrence of the lesions. A summary of controlled studies on treatment for perianal Crohn's disease published up to December 2016 is shown in Supplementary information S1 (table), and FIG. 7 illustrates a practical algorithm for treatment of perianal fistulizing disease. In patients with simple fistulas and no proctitis, therapy is based on a course of antibiotics and long-term immunosuppression therapy. If fistula drainage persists after 3 months of immunosuppression therapy, an anti-TNF drug should be added. If there is no response to this therapy after 14 weeks, then a combined medical and surgical approach is recommended. In patients with simple fistulas and without proctitis who do not respond to this therapy, local injection of MSCs or fibrin glue can be contemplated. If there is no response to local therapies, fistulotomy or ligation of the intersphincteric fistula tract are the surgical procedures of choice[4,51]. In cases with active proctitis, treatment should be optimized to control luminal disease by use of combination therapy with immunosuppressants and anti-TNF or vedolizumab[50].

Complex fistulas require a combined approach from the start. If abscesses are present, these should be drained, setons placed and a course of antibiotics administered. Anal strictures should be dilated and followed by combination therapy with an immunosuppressant (thiopurine) and an anti-TNF agent, or vedolizumab in cases of previous failure or intolerance to anti-TNF drugs. Patients without proctitis not responding to these therapies are candidates for local injection of MSCs. In case of no response to the local therapy, ligation of the intersphincteric fistula tract or an endorectal advancement flap can be considered. Faecal diversion and proctectomy might be considered in patients with severe perianal sepsis with insufficient response to the previous measures and in cases of sphincter damage causing disabling incontinence[4,50,51].

A considerable number of research questions on perianal Crohn's disease remain open. The study of the pathogenesis of this manifestation of Crohn's disease is challenging given the absence of relevant animal models and the difficulty in obtaining human tissue samples at the various stages of fistula development. The observation that some patients with very severe luminal disease never develop perianal fistulas, and the discordant response between luminal and perianal lesions that some patients exhibit, suggest that specific factors are involved in this complication that need to be discovered to develop more effective treatments. The efficacy of new therapies for Crohn's disease under development, including integrin immunoblockade, anti-IL-12 or IL-23 antibodies, JAK inhibitors and sphingosine 1-phosphate receptor modulators, have to be tested specifically in the treatment of perianal fistulizing Crohn's disease. Efficacy should not only be extrapolated from observations in luminal disease. Finally, better tools for outcome measures in these studies are also required, with development of accurate, responsive and reliable indices of activity based on MRI features.


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