REVIEW

Emerging treatments for complex perianal fistula in Crohn's disease

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Received: April 12, 2009 **Revised:** August 12, 2009

Accepted: August 19, 2009

Published online: September 14, 2009

INTRODUCTION

Crohn's disease is a chronic inflammatory disease of the intestine of unknown etiology. It is characterized by focal or segmental transmural inflammation which can occur in any part of the digestive tract with occasional granuloma formation. This transmural inflammation disrupts intestinal mucosal integrity, favoring the development of abscesses and fistulas. When fistulas form, they can track between intestinal segments or between an intestinal segment and other organs (bladder, vagina), adjacent tissue or the skin. Fistulas are classified as internal when they communicate with adjacent organs (e.g. entero-enteric and rectovaginal fistulas) and external when they communicate with the dermal surface (e.g. enterocutaneous, peristomal and perianal fistulas).

The cumulative incidence of perianal fistulas in Crohn's disease varies between 20% and 25% in population studies[1-3]. Perianal disease is associated with high morbidity and, typically, with local pain and discharge; it therefore has a very negative impact on the quality of life of the affected patients. In Crohn's patients, perianal disease is more common when the colon is affected and particularly when the rectum is involved[2].

The ideal therapeutic goal in perianal fistulizing Crohn's disease is complete and sustained closure of the fistulas without the development of abscesses, thereby avoiding the need for surgical interventions, and improving the patients' quality of life. In an appreciable number of patients, complete closure cannot be achieved despite intensive medical treatment (including infliximab) and surgery in accordance with normal practice. Currently the goal of therapy for these patients has shifted from complete fistula closure to reduction in drainage from the fistula tract in order to improve their

Abstract

Complex perianal fistulas have a negative impact on the quality of life of sufferers and should be treated. Correct diagnosis, characterization and classification of the fistulas are essential to optimize treatment. Nevertheless, in the case of patients whose fistulas are associated with Crohn's disease, complete closure is particularly difficult to achieve. Systemic medical treatments (antibiotics, thiopurines and other immunomodulatory agents, and, more recently, anti-tumor necrosis factor-α agents such as infliximab) have been tried with varying degrees of success. Combined medical (including infliximab) and less aggressive surgical therapy (drainage and seton placement) offer the best outcomes in complex Crohn's fistulas while more aggressive surgical procedures such as fistulotomy or fistulectomy may increase the risk of incontinence. This review will focus on emerging novel treatments for perianal disease in Crohn's patients. These include locally applied infliximab or tacrolimus, fistula plugs, instillation of fibrin glue and the use of adult expanded adipose-derived stem cell injection. More well-designed controlled studies are required to confirm the effectiveness of these emerging treatments.

quality of life. In these patients with complex perianal fistulizing disease that persists despite intensive medical and surgical treatment, a therapeutic gap exists for new treatments which aim for complete and sustained closure of the perianal fistulas.

In this review, after a brief discussion of the diagnosis, characterization, classification, and current systemic treatments of perianal fistulas in patients with Crohn's disease, we shall discuss some of the newer local therapies and their potential applications.

DIAGNOSIS AND CHARACTERIZATION

The starting point for management of perianal fistulas is a complete and accurate diagnosis of the lesions, which requires careful exploration of the anal and perianal region. An inadequate examination which fails to detect occult lesions (abscesses or fistula branches) may result in perianal disease becoming persistent or recurrent. An endoscopic examination is needed to determine the presence of macroscopic inflammation in the rectum and/or rectal stenosis, as such findings are important for the prognosis and treatment of the disease. There is consensus among the American Gastroenterological Association (AGA)[4,5] and European Crohn's & Colitis Organization (ECCO)[6] working groups concerning the need to complement the study of perianal disease with other diagnostic tools such as examination under anesthesia (EUA), magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS).

In the hands of expert surgeons, EUA is considered the gold standard against which other techniques are compared. EUA has an accuracy of 90% for diagnosis and classification of fistulas and abscesses[7]. With this technique, it is possible to perform concomitant surgery of the lesions: incision and drainage of abscesses with seton placement, and other procedures to treat fistulas. MRI has an accuracy of between 76% and 100% for diagnosis and classification of perianal fistulas[7,8]. With MRI, the surgeon who performs EUA can obtain important additional information in 15%-21% of patients[7,9]. In view of its harmless nature and the additional information it provides, MRI is the initial diagnostic technique of choice according to the ECCO consensus statement[6]. EUS offers a diagnostic accuracy of between 56% and 100%, and the findings change the surgical approach in 10%-15% of cases[7,10], helping to guide medical-surgical treatment of perianal fistulas in Crohn's disease, resulting in a high response rate[11]. Sometimes, the pain caused by the lesions or stenosis makes EUS difficult.

The combination of either of these imaging techniques (MRI or EUS) with EUA yields a diagnostic accuracy of 100% for perianal disease[7]. Imaging techniques are essential to provide surgeons with a virtual view which allows them to treat all lesions during the surgical procedure.

CLASSIFICATION OF PERIANAL FISTULAS

A number of classification systems have been proposed

in the past[12,13], but perhaps the most anatomically accurate is the Parks classification[14], which takes the external anal sphincter as the central reference point and describes 5 types of perianal fistula: superficial, inter-sphincteric, trans-sphincteric, supra-sphincteric and extra-sphincteric. The Parks classification is however limited in that it does not take into account the presence of abscesses and/or connections with other organs such as the vagina or bladder, even though such information is important for determining the medical and surgical management of the disease.

The AGA technical review proposed a more clinically useful classification system with just 2 categories: simple and complex fistulas[4,5]. Simple fistulas are low (superficial, low inter-sphincteric or low intra-sphincteric), have a single external opening and are not associated with perianal abscess, connection to the vagina or bladder, rectal stenosis or macroscopic proctitis. In contrast, complex fistulas are high (high inter-sphincteric, high intra-sphincteric, supra-sphincteric or extra-sphincteric) and/or can have several external openings and may be associated with perianal abscess, connection to the vagina or bladder, rectal stenosis or macroscopic proctitis. This classification has greater clinical relevance: simple fistulas respond better to treatment whereas complex ones have lower cure rates with medical treatment and, with this type of fistula, an aggressive surgical procedure will often lead to incontinence.

MEASURES OF HEALING

The Crohn's Disease Activity Index (CDAI) is widely used in Crohn's disease as an outcome measure but it is not designed or able to assess perianal fistulous disease activity, thus the Perianal Disease Activity Index (PDAI) is often used as an equivalent of this index for measurement of morbidity associated with perianal disease[15]. This index assesses 5 categories related to fistulas: discharge, pain, restriction of sexual activity, type of perianal disease and degree of induration. The advantage of the PDAI is that it assesses aspects of the quality of life that are most affected in patients with perianal disease and that it has been validated in recent clinical studies[16].

The most widely used instrument for assessing treatment outcomes in clinical trials is the Fistula Drainage Assessment. This measure classifies fistulas as open (i.e. purulent material is expelled with gentle pressure) or closed[17]. A fistula has to remain closed for 2 consecutive visits (at least 4 wk apart) to be considered in remission. The Fistula Drainage Assessment does not consider changes in anal pain, which is also an important marker of treatment response.

Consideration of complete re-epithelization of the external openings supported by MRI studies could represent a major improvement in the assessment of fistula closure. Long term maintenance of the healing is of great therapeutic relevance. Indeed, the drafted guideline of the European Medicines Agency (EMEA)

on "The clinical development of new medicinal products for the treatment of Crohn's Disease (Doc. Ref. CPMP/EWP/2284/99 Rev. 1)" states that "the therapeutic goals of management of fistulizing Crohn's disease are to close fistulas and maintain their closure, to reduce the incidence of infections in persisting fistulas, and to limit the need for surgical interventions. Clinical studies in fistulizing Crohn's disease should reflect this. The primary endpoint of the clinical trials should then be complete closure of fistulas and maintenance of a closed fistula without development of new fistulas."

An MRI-based activity score was developed to asses the anatomical evolution of perianal fistulas in Crohn's disease[18]. MRI imaging demonstrates that despite closure of draining external orifices after infliximab treatment, inflammatory changes in the fistula track persist for a long time. It has been suggested that this residual activity may cause recurrent fistulas and pelvic abscesses but a *post hoc* analysis of the ACCENT Ⅱ study showed that maintenance infliximab therapy does not result in increased abscess development in patients with fistulizing Crohn's disease[19].

TREATMENT

Crohn's disease cannot be cured by medical or surgical treatment. The aim of therapy is to alleviate symptoms and treat complications of the disease in order to improve the patients' quality of life. The strong negative impact of symptomatic perianal disease on quality of life justifies aggressive treatment to facilitate healing. The spontaneous cure rate for perianal fistulas is very low, ranging from 6% to 13% in the placebo arm of 3 controlled studies[17,20,21].

Medical treatments

Antibiotics: Bacteria may in theory play a role in the appearance and persistence of perianal fistulous disease. Thus antibiotics are sometimes used as first-line therapy for fistula healing. In other cases antibiotics, in view of their prophylactic effects against infections and abscesses, are used as adjuvant (or bridging) therapy. Most of the studies of perianal fistulizing disease treated with antibiotics are uncontrolled and the sample sizes are small. In these studies, both metronidazole[22-24], and ciprofloxacin[25], as well as a combination of the 2 drugs[26], showed an initial beneficial effect on the perianal fistula. Response typically occurs after 6 to 8 wk of treatment and is usually manifest in the form of decreased drainage. Fistula closure is uncommon and symptoms tend to recur after suspending treatment[24].

Recently, a small randomized, double-blinded, placebo-controlled study evaluated ciprofloxacin or metronidazole for the treatment of perianal fistulas in patients with Crohn's disease[27]. Twenty-five patients were randomized to ciprofloxacin 500 mg (10 patients), metronidazole 500 mg (7 patients) or placebo (8 patients) twice daily for 10 wk. Response ($\geqslant$ 50% reduction in the number of draining fistulas) at week 10 was seen in 4 patients (40%) treated with ciprofloxacin,

1 patient (14.3%) treated with metronidazole, and 1 patient (12.5%) treated with placebo (P = 0.43). One patient from both the ciprofloxacin and placebo arm and 5 (71.4%) treated with metronidazole dropped out of the study (P < 0.02). This study was probably too small to detect differences between treatment arms.

In two studies, antibiotics were used as an adjuvant or a bridge to other drugs. The use of metronidazole and/or ciprofloxacin induced a response ($\geqslant$ 50% reduction in the number of draining fistulas) at week 8 with fistula closure occurring in 25% of cases[28]. At week 20, those patients who received additional azathioprine therapy had a better medium-term response (48% *vs* 15%). It should be pointed out that most of the patients in that study had simple fistulas and that only 9 of the 52 cases were classed as complex fistulas. In a placebo-controlled study, all patients received infliximab (3 induction doses at weeks 6, 8 and 12) and were randomized to receive either 500 mg ciprofloxacin twice daily or a placebo for 12 wk[16]. The response rate (defined as $\geqslant$ 50% reduction from baseline in the number of draining fistulas) at week 18 showed a tendency in favor of ciprofloxacin in combination with infliximab compared to infliximab alone (OR: 2.37; 95% CI: 0.94-5.98)[16].

Thiopurines: Azathioprine and 6-mercaptopurine have shown efficacy in the treatment of Crohn's perianal fistulas. In a meta-analysis of 5 controlled studies, a response (defined as complete closure or decreased drainage) was found in 54% of the patients treated with azathioprine or 6-mercaptopurine compared to 21% in the placebo group (OR: 4.44; 95% CI: 1.50-13.2)[29]. This meta-analysis is limited in that fistula response was a secondary endpoint and not the primary one in all of the studies included. There have been no controlled trials in which the primary endpoint was assessment of the effect of thiopurines on the closure of fistulas in patients with Crohn's disease.

Anti-tumor necrosis factor (TNF)-α agents: The efficacy of the anti-TNF-α antibody infliximab in fistulizing perianal disease refractory to 3 mo of conventional treatment has been shown in a controlled clinical study[19]. The most favorable outcomes were obtained at doses of 5 mg/kg body weight and 3 induction infusions at 0, 2 and 6 wk. This regimen achieved complete fistula closure (no drainage in 2 visits 4 wk apart) in 55% of the patients compared to only 13% in the placebo group. The mean time to response was 2 wk and the mean duration of response was 12 wk after the last infusion. The ACCENT Ⅱ study later confirmed the response rate to induction (69% at week 14) in the open-label extension[30]. Responders were randomized to infliximab 5 mg/kg body weight or placebo every 8 wk. At week 54, 36% of the patients on infliximab were able to maintain complete closure compared to 19% of those on placebo (P = 0.009). Similar results have also been reported in clinical practice in a large uncontrolled series[31].

Infliximab maintenance treatment has been shown to decrease the use of hospital resources (fewer hospitalizations and less need for surgery) in patients with fistulizing Crohn's disease[32]. Nevertheless, it has been reported that in perianal disease, early relapse was common after stopping infliximab treatment, with only 34% of patient maintaining remission at 1 year[33].

Adalimumab, another anti-TNF-α antibody, may also prove effective in perianal Crohn's disease. In the CHARM study, 33% of the patients randomized to adalimumab achieved long term complete fistula closure versus 13% in the placebo group (secondary endpoint; placebo vs adalimumab group combined; P = 0.043)[34]. Complete fistula healing was sustained for up to 2 years by most of the patients in the open extension trial ADHERE. In a prospective open-label study in patients with active perianal fistulous disease who stopped responding or developed intolerance to infliximab, adalimumab induction therapy (160 mg at week 0 and 80 mg at week 2) induced complete fistula closure at week 4 in 23% of the cases[35].

Other immunomodulators: Randomized studies designed specifically to assess the efficacy of cyclosporine in the closure of fistulas in patients with fistulizing Crohn's disease have not been published. However, there are several uncontrolled case series which used continuous cyclosporine infusion in patients who had failed conventional therapy[36]. Many patients showed an initial response and were switched to oral cyclosporine; however the response was rapidly lost on drug withdrawal.

Uncontrolled case series suggested that tacrolimus may be useful in the treatment of perianal disease[37,38]. In a small controlled clinical trial, patients treated with tacrolimus (0.2 mg/kg per day) had a higher response rate (defined as closure of at least 50% of fistulas) at week 4 compared to placebo (43% vs 8%), but no differences were observed in terms of complete fistula closure (10% vs 8%)[21].

In a retrospective study methotrexate was used in patients with fistulizing Crohn's disease; after 6 mo 44% of the patients had partial or complete fistula closure[39]. An early case series suggested that the antimetabolite agent mycophenolate mofetil could be effective in Crohn's perianal disease[40]. In a more recent uncontrolled study from the same group mycophenolate mofetil induced a partial response in 7 out of 8 patients with perianal fistulas, but the response was subsequently lost in 5 of these 7 patients for several reasons including side effects[41].

In refractory Crohn's disease, small uncontrolled series showed that thalidomide may be effective in treating complex perianal fistulas[42,43]. Severe side effects, including neuropathy, were common and limited the long term use of the drug. Lenalidomide, an analogue of thalidomide, with lower toxicity and powerful anti-TNF properties was not effective in active luminal Crohn's disease[44], and has not yet been tested for perianal Crohn's disease.

Miscellaneous therapies: A pilot open-label study provided data suggesting granulocyte colony-stimulating factor (GM-CSF) is a safe and potentially effective agent for the treatment of active perianal Crohn's disease[45]. GM-CSF has been used in a placebo-controlled study in patients with luminal Crohn's disease, some of whom had draining fistulas at study entry. At 6 mo, 4 out of 8 patients in the GM-CSF group and 2 out of 5 in the placebo group had complete fistula closure[46].

Octreotide, a somatostatin analogue, may have a role in treating Crohn's enterocutaneous fistulas, but has not been used in perianal disease[47]. The effect of elemental diet on perianal Crohn's disease has been studied in a small retrospective series. Fistulas improved in some patients but early relapse occurred in almost all the cases[48]. In a review of 22 patients with active and refractory perianal Crohn's disease treated with hyperbaric oxygen, 73% achieved a response[49]. In a randomized, placebo-controlled trial oral, spherical adsorptive carbon was effective for the control of perianal fistulas in patients with Crohn's disease (remission rates were 29.6% vs 6.7% for placebo)[50]. There is not sufficient evidence for any of these agents to support their use in patients with Crohn's perianal fistulas outside of clinical trials.

Summary of medical treatment in current guidelines: Despite methodological limitations in the supporting studies, antibiotics and azathioprine or 6-mercaptopurine are considered first-line therapy in complex perianal disease in the ECCO consensus statement[6], and infliximab is reserved as a second-line treatment in case of failure. In the AGA technical review[4,5] infliximab is recommended for treatment of complex perianal disease along with azathioprine or 6-mercaptopurine and antibiotics for the induction phase. Maintenance is recommended with azathioprine or 6-mercaptopurine, in association with infliximab in some cases.

Surgical treatment

Surgical treatment of complex perianal fistulizing disease aims to control sepsis through abscess drainage and intervention in the fistula tracts, including placement of non-cutting setons[51]. Fistulectomy or fistulotomy are rarely indicated in complex fistulas in view of the high rate of proctectomy because of nonhealing or incontinence associated with the procedure[6,51,52]. In severe cases with high fistulas, endorectal flaps are useful[51,53]. In patients with severe refractory disease, diversion with ostomy (loop ileostomy or end sigmoid colostomy) or even proctectomy might be necessary.

In an uncontrolled study carbon dioxide laser ablation has been used as an alternative treatment in patients with perianal Crohn's disease[54].

Combined medical and surgical treatment

The ideal treatment goal for complex perianal fistulas associated with Crohn's disease is the closure of all the fistulas and the prevention of recurrence. The

best outcomes have been achieved in studies using a combination of medical and surgical therapy[55,56].

Surgery may offer some advantages when combined with medical treatment, for example, infliximab. There is concern, however, that use of infliximab may cause abscesses by inducing rapid closure of the fistulas. This problem might be reduced by performing MRI or EUS-guided EUA to detect all fistula tracks and to insert draining setons. Regueiro *et al*[55] observed that patients who underwent an EUA before infliximab administration were significantly less likely to have fistula recurrence compared to those treated with infliximab alone (44% *vs* 79%).

Hyder *et al*[57] investigated such a strategy in patients with perianal Crohn's disease. After EUA, 12 out of 22 patients required abscess drainage and 17 out of 22 had at least one drainage seton inserted. The short-term efficacy of that strategy was as high as 85% as measured by the PDAI; although the authors noted that long term healing rates were low. Talbot *et al*[58] reported a similar strategy in patients with complex fistulas-setons were inserted and removed after the second infliximab infusion. Complete healing of the perianal fistula was obtained in 47% of the patients and all showed at least a partial response.

The presence of active proctitis has a negative impact on the outcome of the surgical treatment of Crohn's fistulas. A pilot study suggested that infliximab treatment has a beneficial additive effect in the multistep treatment to first improve the proctitis before performing surgery in complex perianal Crohn's disease with active proctitis[59].

In one recent retrospective study, 21 patients with Crohn's perianal fistulas and symptomatic perianal disease were treated according to a treatment protocol of serial EUS examinations[60]. Surgical and medical therapy was tailored to the results of the EUS findings with seton placement and incision and drainage procedures performed when appropriate. Follow-up EUS examination guided when to remove setons or when to stop infliximab or antibiotics. Median follow-up was 68 wk (35-101 wk). No abscesses developed in any patient. Eighteen out of 21 patients (86%) had complete drainage cessation initially, and 16 out of 21 (76%) had long term cessation of drainage. Eleven (52%) had no persistent fistula activity on EUS. In 7 of these, the fistula remained closed after stopping infliximab or antibiotics while the remaining 4 continued infliximab for mucosal disease. This study showed that EUS-guided combination surgical and medical treatment with infliximab had high short and long term fistula response rates[60].

In a recent randomized prospective study, 10 patients with active Crohn's perianal fistulas were randomized to either EUS guidance or control[61]. All patients underwent an initial EUS. Patients in the EUS cohort were evaluated by a colorectal surgeon who had access to the EUS findings. The surgeon was blinded to the results of the initial EUS for those in the control group. EUA with seton placement or incision and drainage

was done at the surgeon's discretion, and all patients received optimal medical therapy including antibiotics, azathioprine or 6-mercaptopurine and infliximab. Patients in the control group received further therapy at the surgeon's discretion without EUS guidance. Those in the EUS cohort had additional EUS evaluations at weeks 22 and 38 with further therapy based on EUS results. One of 5 (20%) in the control group and 4 of 5 (80%) in the EUS group had complete cessation of drainage. In this small study, EUS guidance for combination medical and surgical therapy in perianal Crohn's disease appeared to improve outcomes.

Local treatments
Fistula healing is not possible in a significant percentage of patients with complex fistulizing Crohn's disease managed according to the currently accepted treatment algorithms[4-6]. In addition, systemic medical treatments may be subject to intolerance or loss of response and surgical treatments such as fistulotomy should be used with caution given the risk of incontinence. Thus there is a therapeutic gap in the management of perianal Crohn's disease, and a number of local therapies which aim to achieve complete closure are under development. Table 1 summarizes studies of these new local treatments. The following sections will discuss these new local treatments in more detail.

Topical tacrolimus: Topical tacrolimus has been used successfully in the treatment of skin diseases with an immune component such as atopic dermatitis[62]. Casson *et al*[63] therefore decided to investigate whether an in-house-prepared topical formulation could be beneficial in a series of pediatric patients with different manifestations of Crohn's disease including one case of perianal fistula; the patient responded to treatment although details of fistula healing were not presented.

The efficacy of topical tacrolimus in perianal Crohn's disease was recently investigated in a randomized placebo-controlled study[64]. In that study, 19 patients, 12 of whom had fistulizing perianal Crohn's disease, were randomized to topical tacrolimus (1 mg in 1 g ointment applied twice daily) or placebo for 12 wk. In the case of patients with fistulas, the primary outcome measure was improvement defined as $\geq$ 50% decrease in actively draining fistulas on 2 consecutive visits. Treatment showed a beneficial effect on anal and perianal ulcerating disease but lacked efficacy in the treatment of fistulizing Crohn's disease.

Fibrin glue: Instilling fibrin glue into fistulas is a simple and safe procedure which does not preclude the use of other techniques or repeat procedures in the case of failure[65]. Several studies have been published of series of patients treated with fibrin glue and success rates vary from 0% to 80% (Table 1). This variability can be attributed, among other things, to the different types of fistulas treated (simple or complex; cryptoglandular, Crohn's, or traumatic etiology), and the differences in the definition of healing.

Table 1 Summary of studies of local treatments

Intervention reference	Study design and patients	Main findings
Topical tacrolimus		
Casson et al[63], 2000	Case study of series of pediatric Crohn's patients including 1 patient with perianal fistula	Response reported in patient with perianal fistula
Hart et al[64], 2007	Randomized, placebo-controlled study in 19 patients (12 Crohn's)	Treatment found not to be beneficial in perianal fistulas
Fibrin glue		
Abel et al[79], 1993	Uncontrolled study of use of fibrin glue in 10 patients (2 Crohn's)	0/2 patients with Crohn's disease achieved healing
Cintron et al[80], 2000	79 patients (6 Crohn's) assigned to 3 types of fibrin glue treatment	2/6 Crohn's patients (33%) achieved healing (no drainage)
Lindsey et al[66], 2002	Randomized trial comparing fibrin glue with conventional surgery (fistulotomy or loose seton placement) in 42 patients (6 Crohn's and complex perianal fistula)	Healing (no drainage) in 2/6 Crohn's patients (33%) who received fibrin glue. No Crohn's patients received conventional surgery
Sentovich[81], 2003	Uncontrolled study: 48 patients (5 Crohn's) underwent seton placement followed by instillation of fibrin glue	Healing in 4/5 (80%) Crohn's patients
Zmora et al[82], 2003	Retrospective review of 37 patients with perineal fistula (7 Crohn's) treated with fibrin glue	Healing in 3/7 Crohn's patients (43%) (2 patients also treated with endorectal advancement flap)
Loungnarath et al[83], 2004	Retrospective review of 42 patients with perianal fistula (13 Crohn's) treated with fibrin glue	Lasting fistula healing in 4/13 (31%)
Singer et al[84], 2005	Randomized trial comparing fibrin glue + antibiotics, fibrin glue + surgery, and fibrin glue + antibiotic and surgery in 75 patients (3 Crohn's)	Treatment failed in all 3 Crohn's patients (fibrin glue + antibiotic in 1 patient and fibrin + antibiotic and surgery in 2 patients)
Intralesional infliximab		
Poggioli et al[68], 2005	Uncontrolled study of 15 Crohn's patients with complex perianal fistulas	Healing in 10/15 patients after 3-12 infusions
Asteria et al[69], 2006	Uncontrolled study of 11 Crohn's patients with complex perianal fistulas naïve to infliximab	8/11 patients responded (≥ 50% reduction in fistula drainage) to treatment
Adipose-derived stem cell (ASC) therapy with fibrin glue		
García-Olmo et al[72], 2005	Uncontrolled proof-of-concept study in patients with fistulizing Crohn's disease, including 1 perineal fistula	Perineal fistula healed after 8 wk
Garcia-Olmo et al[73], 2009	Randomized controlled phase II study comparing fibrin glue + ASCs with fibrin glue in 49 patients with complex perianal fistula (14 Crohn's)	Healing in 5/7 Crohn's patients (71%) in fibrin glue + ASCs group compared to 1/7 (14%) in the control group
Fistula plugs		
O'Connor et al[75], 2006	Uncontrolled study of fistula plug in 20 Crohn's patients with fistula tracts not amenable to fistulotomy	Success rate of 80%, lower in the case of complex fistulas
Schwandner et al[76], 2008	Uncontrolled study of 19 patients (7 Crohn's) with trans-sphincteric anorectal fistulas	Treatment success in 6/7 patients with Crohn's disease (86%)
Ky et al[77], 2008	Prospective analysis of 45 patients (20 with complex fistulas and 14 with Crohn's disease) receiving anal fistula plug	Healing in 4/14 Crohn's patients (29%) after a median follow-up of 6.5 mo

Only one controlled study with patients with Crohn's disease has compared fibrin glue with surgical treatment not involving fibrin glue. In that study, Lindsey et al[66] randomized patients with simple and complex fistulas to treatment with fibrin glue or conventional treatment (fistulotomy or loose seton placement with or without subsequent flap advancement). For the purposes of the study, complex fistulas were defined as high fistulas, fistulas associated with Crohn's disease, and low fistulas with compromised sphincters. Both Crohn's patients with complex fistulas reported healing, in one case after a second procedure. Healing among Crohn's patients in the other arm was not reported.

Intralesional infliximab: Although systemic infliximab administration is considered one of the more efficacious therapeutic options available for complex perianal fistulas associated with Crohn's disease, several authors have investigated the efficacy of local application of this drug. The main rationale for this approach is to try and avoid the potential systemic toxicity associated with infliximab. The first study to employ this approach was

published by Lichtiger et al[67] in 2001. Nine patients with perianal Crohn's disease refractory to antibiotics or 6-mercaptopurine were treated with a circumferential and intrafistulous injection of infliximab at 0, 4, and 7 wk. Remission or partial response was achieved in 83% of the patients.

Since then, a number of uncontrolled studies have been conducted to assess the feasibility of local infliximab therapy. Poggioli et al[68] included 15 patients with complex perianal fistulas associated with Crohn's disease. In 9 of these, intravenous infusion of infliximab was felt to be contraindicated because of fibrostenotic disease. The patients were injected with 15-21 mg of infliximab at the internal and external openings and along the fistula tract. The injections were well tolerated and 10 of the 15 patients achieved healing after 3 to 12 injections. A similar study was reported by Asteria et al[69], although patients were excluded if they had received prior treatment with infliximab. Up to 3 injections of 20 mg of infliximab were made along the fistula tract and at both openings. The efficacy endpoint was reduction in fistula drainage of 50% or more (response)

or complete cessation of fistula drainage for at least 4 wk (remission). Overall, 8 patients achieved a response (73%) and, of these, 3 achieved remission (27%). After a longer follow-up (mean 10.5 mo, range 7-18 mo), 6 patients were responders and 4 were in remission.

Adipose-derived stem cell therapy: Adult stem cell therapy has promising applications in a number of areas of medicine and has no ethical concerns. Given that liposuction is a relatively safe procedure, an appealing source of adult stem cells is lipoaspirate[70]. The stromal cells obtained are subsequently cultured and expanded to produce autologous adipose-derived adult stem cells (ASCs). Trials of ASCs in the treatment of fistulizing Crohn's disease have delivered the expanded ASCs by injecting them around the fistula opening and directly into the fistula tract.

The first procedure of this type published in the literature was a case report of a 33-year-old woman with Crohn's disease and a rectovaginal fistula refractory to treatment[71]. ASCs were injected into the rectal mucosa, close to the sutured internal opening. After resection of the posterior vaginal wall and construction of an advancement vaginal flap, the accessory perineal hole was sealed with 2 mL of fibrin glue. One week after the intervention, the wound had completely healed. In 3 mo of follow-up, no recurrence of the rectovaginal fistula was reported.

A subsequent phase I study assessed 9 ASC injection procedures in 4 patients with fistulizing Crohn's disease[72]. The series included 3 rectovaginal fistulas and 1 perineal fistula. As before, cells were injected into the rectal mucosa, close to the sutured opening and fistula tracks were then filled with fibrin glue. Two of the 3 rectovaginal fistulas and the perineal fistula had healed after 8 wk.

One randomized clinical trial using ASCs has also been conducted. In a recently completed phase II study, 49 patients with perianal fistula-14 of whom had Crohn's disease-were randomized to receive ASC therapy and fibrin glue or to receive fibrin glue alone (control group)[73]. The primary outcome measure was the proportion of patients with complete fistula closure. An investigator blinded to the treatment confirmed healing by examination of a digital photograph. Five of the 7 patients with Crohn's disease assigned to ASC therapy achieved healing compared to one of 7 patients in the control group. The difference was not statistically significant but the study was not powered to detect differences in small subgroups such as those with Crohn's disease. Healing in this trial was defined as the absence of drainage, as well as complete epithelization of external openings. No severe adverse events related to ASCs have been reported when utilized for fistulizing Crohn's disease.

Fistula plugs: Recently, the use of bioprosthetic plugs made from porcine intestinal submucosa has been tried in patients with perianal fistula. In a prospective study which excluded patients with Crohn's disease, Johnson

et al[74] randomized patients with high transsphincteric or deeper fistulas to either fistula plug or fibrin glue therapy. Of the 10 patients who underwent fibrin glue treatment, 6 (60%) had persistence of one or more fistulas at 3 m compared to 2 out of 15 (13%) of those who underwent the procedure with the fistula plug (*P* < 0.05). In a subsequent prospective but uncontrolled study, 20 Crohn's patients with a total of 36 fistula tracts not amenable to fistulotomy were treated with fistula plugs[75]. After irrigation with hydrogen peroxide, each primary opening was occluded with a fistula anal plug. The authors found an overall success rate of 80%, although they noted that patients with complex fistulas with multiple primary openings were less likely to achieve success. Success appeared to be independent of the presence of setons or the use of anti-TNF-α therapy.

Schwandner *et al*[76] have reported their experience in a series of 19 patients with transsphincteric anorectal fistulas. Seven of these patients had Crohn's disease. The surgical procedure comprised irrigation of the fistula tract and placement and internal fixation of the anal fistula plug without flap advancement or excision of the fistula tract. Success was defined as closure of both the internal and external openings with no further interventions and absence of abscess formation. Six of the 7 patients with Crohn's disease achieved success (85.7%).

In another retrospective study, anal fistula plugs were more successful in the treatment of simple anorectal fistulas but were associated with a high failure rate in complex perianal fistulas and particularly in patients with Crohn's disease (closure rate of 26.6% of fistulas in this group)[77].

In a recent retrospective review reported at the 2008 Digestive Diseases Week conference[78], the use of anal fistula plugs was associated with a lower success rate than previously reported: only 2 of the 22 (15%) Crohn's disease-associated fistulas healed. In 87% of the procedures the reason for failure was sepsis. These controversial results in an uncontrolled series await confirmation by randomized trials.

CONCLUSION

Symptomatic perianal fistulas in patients with Crohn's disease can have a large negative impact on quality of life. Treatment of complex perianal fistulas remains a difficult problem. Use of anti-TNF-α antibody therapy is widespread and supported by randomized clinical trials. Nevertheless, some patients fail anti-TNF-α treatment and, given the risk of incontinence associated with aggressive surgical procedures, there remains an unmet therapeutic need. Some of the emerging local therapies have obtained promising results in patients with fistulizing Crohn's disease in uncontrolled studies and case series but, for the most part, this promise has still to be confirmed in randomized trials. Adipose-derived stem cell therapy has compared favorably with fibrin glue alone in a randomized phase II trial, and high

healing rates were observed. Once the efficacy of these new local therapies has been confirmed, further effort will be required to optimize their use in the management of fistulizing Crohn's disease, which is necessarily complex and multidisciplinary.

ACKNOWLEDGMENTS

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