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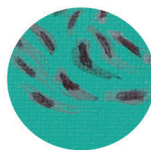
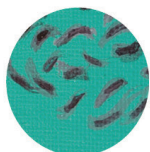
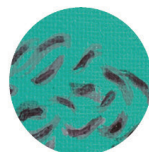


The handle <http://hdl.handle.net/1887/38545> holds various files of this Leiden University dissertation.

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Title: Mesenchymal stromal cell therapy for Crohn's disease : from perianal fistulizing disease to experimental colitis

Issue Date: 2016-03-15



CHAPTER 5

STANDARDIZATION OF LOCAL TREATMENT FOR PERIANAL FISTULIZING CROHN'S DISEASE

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ABSTRACT

Background: Durable remission rates of perianal fistulas in Crohn's disease (CD) are still low despite novel drugs and advanced surgical techniques. Local administration of mesenchymal stromal cells (MSCs) and anti-TNF in the fistula tract seem to improve patient's outcome.

Objective: To propose a standardized and validated protocol for the administration of local treatment of CD perianal fistulas.

Methods: A working group consisting of gastroenterologists and surgeons with expertise on perianal Crohn's from the Leiden University Medical Center (LUMC) in The Netherlands developed a perianal fistula map (PFM) for local MSC treatment of perianal fistulizing CD. The PFM was validated during our recently performed trial on allogeneic bone marrow-derived mesenchymal stromal cells for the treatment of refractory perianal Crohn fistulas (NCT01144962).

Results: Localization and classification of perianal fistulas with magnetic resonance imaging (MRI) and rectoscopy is of crucial importance prior to surgical intervention with local therapy administration. Examination under anesthesia (EUA) is necessary to incise and drain abscesses when present. Optimization of medical treatment when active luminal CD is present, is the first step before embarking on surgery and local therapy administration. In addition, strictures hindering the surgeon to adequately perform standard operating procedures (SOPs) have to be endoscopically dilated. Curettage of the fistula tract has an important role as long-standing CD perianal fistulas will not close without removal of epithelium. To diminish bacterial contamination of the fistula, the internal opening has to be closed. The origin of the fistula is the internal opening, therefore, efficacy of MSCs is presumably the highest when they are infused into the wall around the internal opening.

Conclusion: In this paper we proposed a standardized and validated protocol for the administration of local treatment of CD perianal fistulas to be able to reliably assess efficacy of local therapy.

INTRODUCTION

Despite all the available drugs and advanced surgical techniques durable remission rates of perianal fistulas in Crohn's disease (CD) still remain low.¹ The treatment outcome of perianal fistulas is dependent of multiple factors. Not only the activity of the underlying inflammatory disease, but also genetic and microbiological factors determine the clinical course of CD fistulas and the success rates of medical and surgical treatment. Anti-tumor necrosis factor (TNF) agents such as infliximab, especially combined with antibiotics, are effective in treating perianal fistulas.^{2,3} However, after infliximab treatment more than one third of the patients with an initially healed perianal fistula had a recurrence of their fistula after 5 years.⁴ Surgically, fistulotomy is an effective treatment for simple superficial fistulas with success rates of 80-100%.⁵ Unfortunately, most patients with perianal fistulizing Crohn's disease have complex fistulas often with multiple branches and involvement of the anal sphincters. A temporary non-cutting seton to promote drainage and diminish inflammation before embarking on surgery is crucial in the treatment of these patients. However, fistula healing rates after fibrin glue treatment (38%) or the insertion of an anal fistula plug (55%) are disappointing.^{6,7} A mucosal advancement flap is successful in 64% but incontinence occurred in almost 10% of the treated patients.⁸

Recently, the administration of local therapies for perianal fistulas is emerging. Various studies have demonstrated encouraging results of local injection of anti-TNF⁹⁻¹³ in the fistula tract and local cellular therapy with mesenchymal stromal cells (MSCs).¹⁴⁻¹⁷ MSCs are multipotent cells capable of modulating immune responses by for instance interfering in the differentiation of T cells¹⁸ and maturation of antigen presenting cells.¹⁹ In addition, MSCs are able to 'sense' inflammation as they appear to be capable of migrating to the damaged tissue to contribute in the repair processes.²⁰⁻²³ However, the number of MSCs that specifically migrate to the site of inflammation is low after systemic injection and therefore, local injection might enhance their therapeutic efficacy.²⁴⁻²⁶ Indeed, local injection of fibrin glue in combination with MSCs resulted in higher fistula healing rates compared to treatment with fibrin glue alone (71% vs 16%).¹⁴ Even a slightly higher complete closure rate of 82% was observed in a recently published phase II trial including 43 patients with perianal Crohn's who received MSCs locally proportioned to fistula length.¹⁷ Also without fibrin glue, local injection of MSCs originating from both adipose tissue and bone marrow induced reduction of draining CD perianal fistulas.^{15,16} Although this mode of therapy administration seems effective, preoperative workup and practice among surgeons regarding injection techniques is likely to differ substantially. In order to standardize local therapy for perianal Crohn's, we developed a new standardized approach and validated this during a recent study of MSC therapy for perianal fistulizing CD (NCT01144962).

METHODS

Process to consensus of the perianal fistula map (PFM)

An working group consisting of IBD-specialized gastroenterologists and -surgeons with expertise on perianal Crohn's from the Leiden University Medical Center (LUMC) in The Netherlands was formed to develop a perianal fistula map (PFM) for local MSC treatment of perianal fistulizing CD. The working group achieved decisions by consensus on the following four topics: (1) localization and classification of perianal fistulas, (2) surgical intervention prior to therapy administration, (3) local therapy administration, and (4) follow-up.

Validation of the PFM

Validation of the PFM was performed during our recently performed study on allogeneic bone marrow-derived mesenchymal stromal cells for the treatment of refractory perianal Crohn fistulas (NCT01144962). Eligible patients had refractory actively draining perianal fistulas with 1-2 internal openings and 1-3 fistula tracts. Patients with rectovaginal fistulas or complex perianal fistulas with more than 2 internal openings were not included in this trial.

RESULTS

Consensus of the PFM (figure 1)

(1) Localization and classification of perianal fistulas

Magnetic resonance imaging (MRI)

Classification of perianal fistulas by determining the location of the internal opening and the exact route of the fistula with respect to both sphincters is of crucial importance before embarking on surgery. Examination under anesthesia (EUA) is essential when an abscess is present to be able to incise and drain the abscess.

Rectoscopy

As proctitis complicates surgical procedures, rule out the presence of proctitis with a rectoscopy. If proctitis is present, it is important to optimize medical treatment before administrating local therapy for perianal Crohn's. Strictures hindering the surgeon to adequately perform the standard operating procedures (SOPs) are important to dilate endoscopically.

(2) Surgical intervention prior to therapy administration

It was agreed to be of utmost importance to prevent adverse effects by minimalizing the surgical trauma. Excessive and long-lasting stretch of the anal sphincter by introducing

rectal retractors must be omitted in order to reduce the risk of decreased continence. No fistulotomy should be performed for transsphincteric fistulas involving more than one third of the sphincter muscle. As long-standing CD perianal fistulas are often epithelialized, these fistulas will not close without curettage of the fistula tract. Closure of the internal opening is paramount to prevent continuous contamination of the fistula tract with feces.

(3) Local therapy administration

The goal of local treatment with MSCs is the reduction in the number of actively draining fistulas caused by CD. Therefore, it was decided that MSCs needed to be placed at the origin of the fistula where the inflammation resides: in the walls of the fistula around the closed internal opening. Moreover, leaving the suspension in the lumen leads to a waste of therapeutic agents as the majority of the suspension will just seep out of the lumen. The number of injection sites was kept to the minimum to ensure administration of enough MSCs per injection site.

(4) Follow-up

First 6 hours after local therapy administration

Observation of the patient with monitoring of the vital signs after local therapy administration is important to be able to quickly interfere when infusion reactions occur or when the patient develops fever during the first 6 hours after surgery. If no adverse events are observed, the patient can be discharged the same day.

6, 12 and 24 weeks after local therapy administration

Follow-up visits at the outpatients clinic are performed by an IBD-specialized gastroenterologist and/or -surgeon. The patient is checked for persistent or recurrent active fistulas, abscesses, wound infections and/or rebleedings. Efficacy defined as absence of discharge from the fistula(s) by gentle finger compression is determined at physical examination. MRI is not helpful in the evaluation of fistula healing within the first year after local therapy administration as radiological healing can lag behind clinical healing by a median of one year.²⁷ Most perianal abscesses are easily diagnosed during physical examination. However, when physical examination is not evident and an abscess cannot be completely rule out, ultrasound can be used as a quick and easy diagnostic tool.

Feasibility and outcomes of surgical intervention with local therapy administration

We validated the PFM in our recently performed study (NCT01144962). In our hands, 5 ml of 3×10^7 MSCs was enough to achieve a fistula healing rate of 85.7% compared to 33.3% in the placebo group at 12 and 24 weeks after local MSC administration. At these time points, 80%

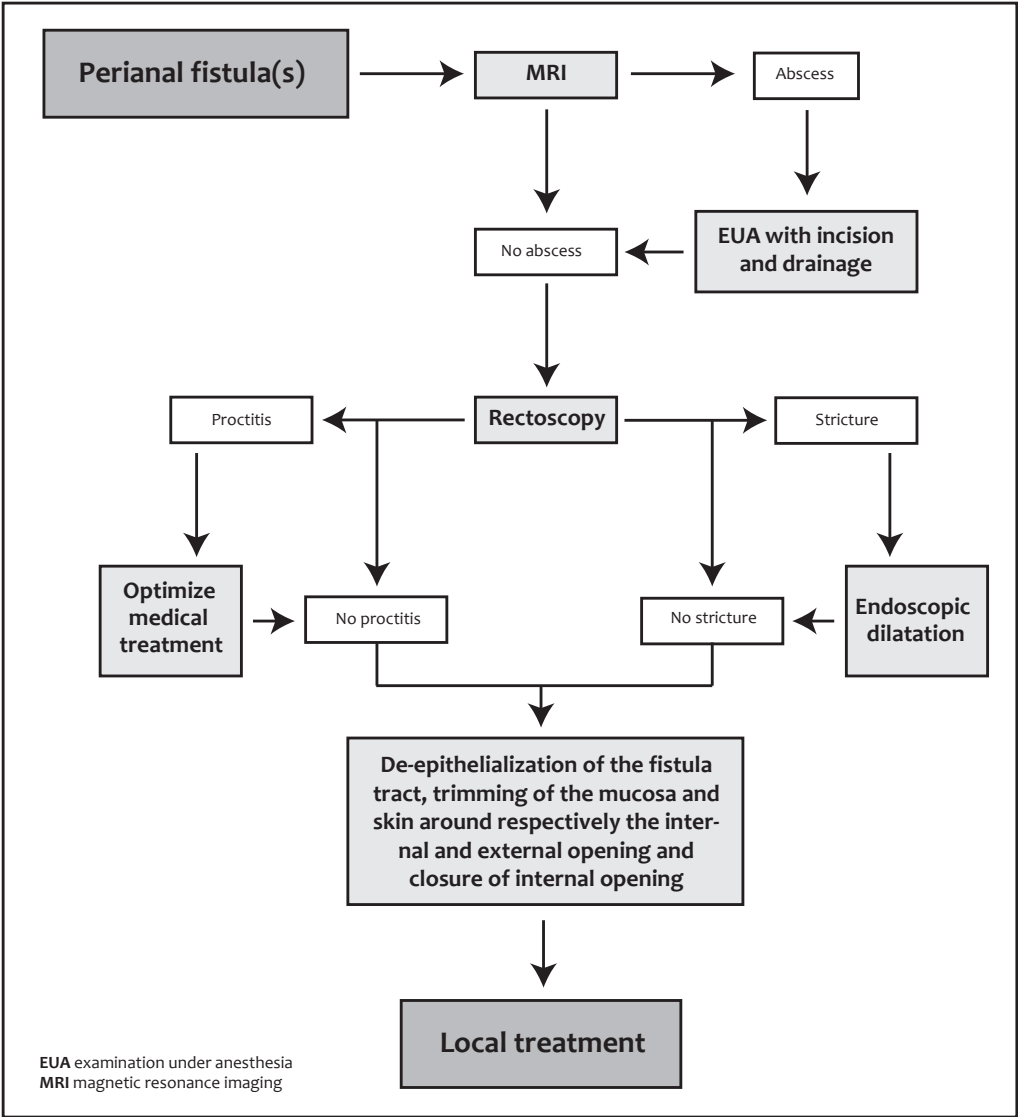


FIGURE 1 Consensus of the perianal fistula map (PFM).

of the patients had no draining perianal fistulas anymore after local treatment with 3×10^7 MSCs compared to 33.3% in the placebo group. Surgery with local injection of MSCs was feasible as we were able to perform all surgical SOPs in all included patients. In addition, the surgical procedures took only 20-40 minutes per patient depending on the number and complexity of the perianal fistulas. Surgical intervention was well tolerated by all patients: no wound infection or -bleedings were reported. Moreover, local MSC administration was without treatment related adverse events.

Standard operating procedures (SOPs) (figure 2)

(1) Localization and classification of perianal fistulas

Perform a MRI and a rectoscopy to describe the localization and classification of the perianal fistula(s) following the Parks and ‘simple/complex’ criteria.^{5,28,29} In figure 3 possible routes of perianal fistulas are schematically shown.

1. Locate the internal opening(s):
 - Use the ‘anal clock’ when patient is in lithotomy position to describe the location;
 - Use the anorectal junction to indicate the level of the internal opening: below, at or above
2. Determine the exact route of the fistula(s) with respect to both sphincters: intersphincteric, transsphincteric, suprasphincteric or extrasphincteric.
3. Locate the external opening(s): use the ‘anal clock’ when patient is in lithotomy position to describe the location.
4. Assess the presence of horseshoeing: intersphincteric, infra- or supralelevator.
5. Assess the presence of a rectovaginal fistula.
6. Assess the presence of perianal abscesses: superficial or supralelevator.
If present, perform an examination under anesthesia with incision and drainage of the abscess.
7. Perform a rectoscopy to assess luminal activity of CD.
 - If proctitis is present, optimize medical treatment before local therapy administration.
 - If a stricture that might hinder the surgeon to perform the SOPs is present, endoscopic dilatation of the stricture before local therapy administration is recommended.
 - Exclude patients from MSC treatment if dysplasia or a carcinoma (*in situ*) is present.

(2) Surgical intervention prior to therapy administration

Perform the surgery and local therapy administration under general anesthesia with the patient in lithotomy position.

1. Inspect perianal area for external openings. Use a Hill-Ferguson retractor for optimal exposure. Self-retaining devices such as the Parks' anal retractor are likely to increase the risk of postoperative incontinence.

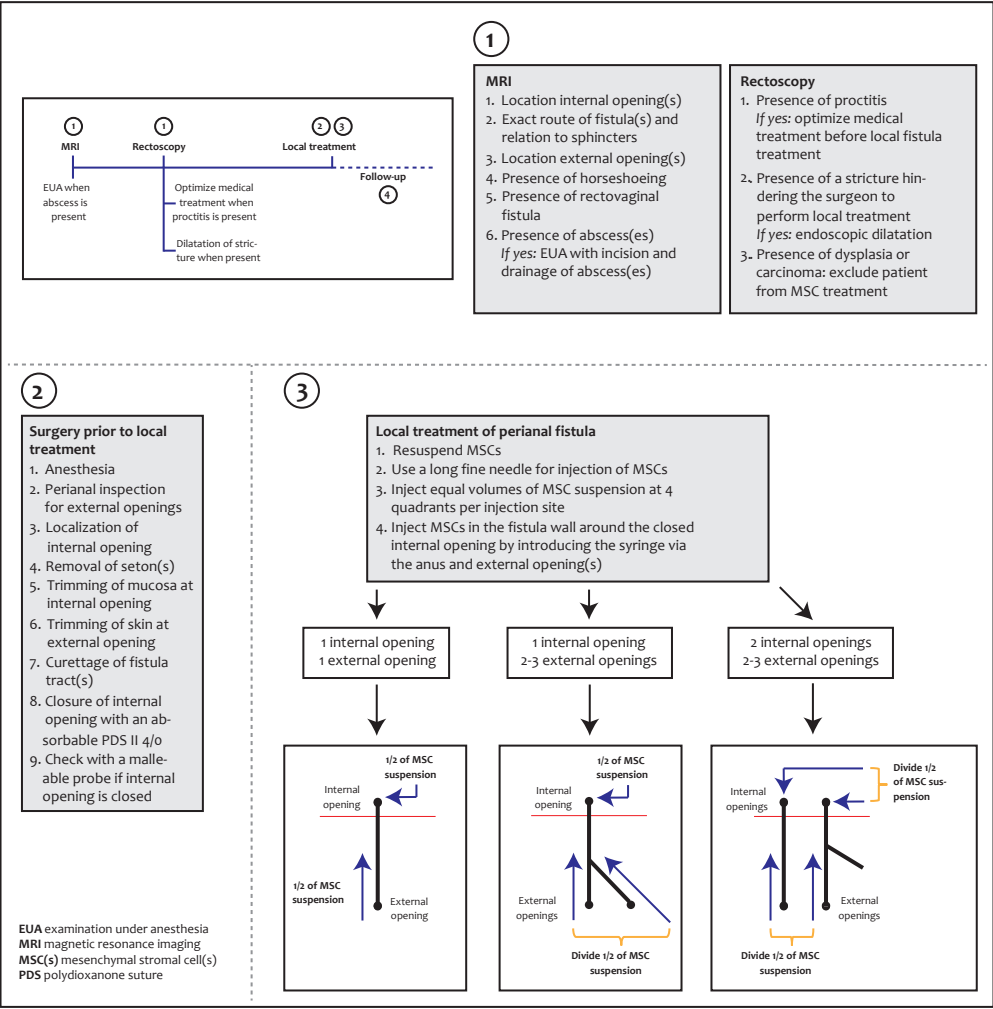


FIGURE 2 Standard operating procedures (SOPs). Timeline of SOPs. (1) Localization and classification of perianal fistulas. (2) Surgical intervention prior to therapy administration. (3) Local therapy administration.

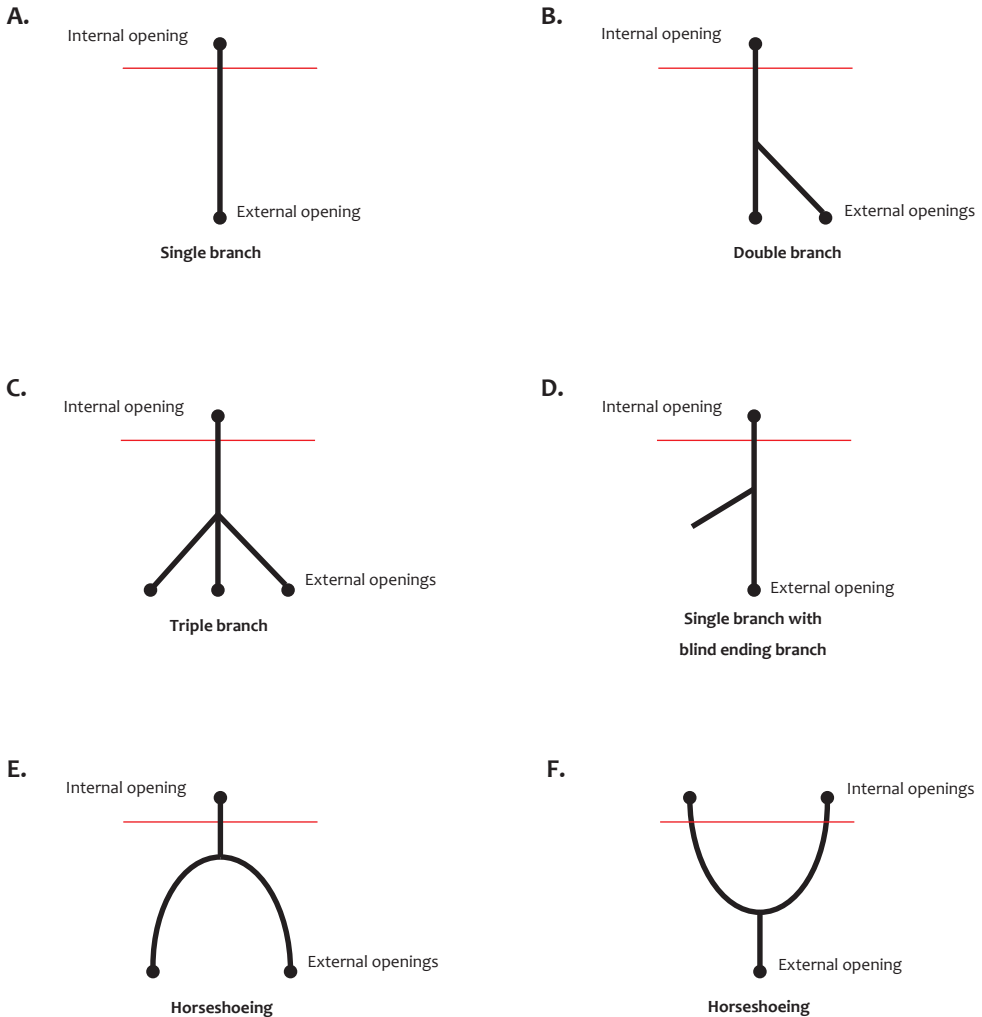


FIGURE 3 Schematic routes of perianal fistulas. (A) Single internal opening and single external opening. (B) Single internal opening and two external openings. (C) Single internal opening and three external openings. (D) Single internal opening with one blind ending tract and one external opening. (E) Single internal opening with horseshoeing and two external openings. (F) Two internal openings with horseshoeing and one external opening.

2. Explore the fistula tract(s) and identify the connection between the external and internal opening(s) by introducing a malleable probe via the external opening(s).
3. Remove seton(s) if *in situ*.
4. Trim the mucosa around the internal opening(s).
5. Remove the tissue surrounding the external opening(s).
6. Thoroughly scrape the entire fistula tract(s) using a curette.
7. Close the internal opening(s) with an absorbable polydioxanone (PDS) II 4/0 interrupted suture.
8. Check with a malleable probe by inserting it via the external opening(s) if the internal opening(s) is/are completely closed to prevent fecal contamination of the tract.
If not, repeat step 7.

(3) Local therapy administration

Inject equal volumes of MSCs at 4 quadrants per injection site. Inject the MSCs in the fistula wall around the closed opening.

1. Resuspend MSCs before injection.
2. Use a long fine needle for the administration of MSCs.
3. Half of the MSC suspension must be injected into the fistula wall around the closed internal opening(s) by introducing the syringe via the anus.
4. The second half must be divided equally into the fistula wall as close as possible to the closed internal opening(s) by introducing the syringe via the external opening(s) into the fistula tract(s) as far as possible.

(4) Follow-up

The patient needs to be monitored for adverse events related to the surgery and/or local therapy administration.

1. Observe the patient with monitoring of the vital signs during the first 6 hours after local therapy administration for infusion reactions and the development of fever. The patient can be discharged if no adverse events are observed after 6 hours.
2. Evaluate at the outpatients clinic the occurrence of wound infections and -bleedings, and changes in vital signs and laboratory measurements 6, 12 and 24 weeks after local therapy administration.
3. Evaluate efficacy of the local treatment by gentle finger compression at the external opening(s) to assess discharge from the fistula.
4. Use ultrasound when there is suspicion of a perianal abscess (fever and a fluctuating painful perianal swelling).

If present, EUA with incision and drainage of the abscess with subsequently placement of a non-cutting seton to promote drainage and reduce inflammation of the fistula is the first step before embarking on surgery. Antibiotics as ciprofloxacin and/or metronidazole are recommended.

DISCUSSION

In this paper we proposed a standardized protocol for the local treatment of perianal fistulizing CD which was validated in our recently performed clinical trial on allogeneic bone marrow-derived mesenchymal stromal cells for the treatment of refractory perianal Crohn fistulas (NCT01144962). Differences in surgical practice are likely to impact on treatment outcome. Therefore, standardization is crucial to assess the efficacy of current and future local treatment strategies.

The ultimate treatment goal is to achieve complete closure of the perianal fistulas. Unfortunately, treatment of perianal fistulizing CD remains challenging despite a range of both medical as surgical options, and is often accompanied by multiple relapses.¹ Therapeutic efficacy might be enhanced when drugs are locally administrated. Promising results after local administration of MSCs have been published with fistula healing rates of 69-82% in uncontrolled trials¹⁴⁻¹⁷ and 80% in our recently performed randomized double-blind placebo-controlled trial. In addition, local therapy with anti-TNF agents might also resulted in fistula closure, however, until now only open-label studies with small sample size and no randomized controlled trials have been reported.⁹⁻¹³ Fistula remission rates varied largely after local administration of anti-TNF drugs (36-88%) possibly as a result of additional fistulectomy in the trial with the highest efficacy rate.¹¹

Although fistula healing rates after local therapy with either MSCs or anti-TNF are encouraging, there are substantial differences in the techniques of administration, making it difficult to reliable assess the effect of local therapy. Therefore, we developed a standardized PFM. Although this protocol was validated with MSCs, we are convinced that these SOPs can also be implemented for local therapy with anti-TNF agents. Our rationale behind the location of injection is similar for all CD perianal fistulas and is not dependent on the type of local treatment. In all CD perianal fistulas, the origin of the fistula is the internal opening. Therefore we believe that local treatment should be injected around the closed internal opening. Closure of the opening prevents bacterial contamination of the fistula. Moreover, the treatment has presumable the most efficacy when it can reside at the site of injection. Therefore, administration of the drugs should be inside the walls of the fistula since suspension that is left in the lumen of the fistula will automatically be discharged from the tract. In addition, in the majority of the patients with long-standing perianal fistulas

epithelialization of the fistula tract is present.³⁰ Therefore, curettage of the entire fistula tract is required as the presence of epithelium inside the tract hinders the closure of the fistula. The possibility to locally treat different CD related fistulas such as rectovaginal and enterocutaneous fistulas, has not been examined. However, it is plausible that this standardized protocol can also be used in the treatment of different types of fistulas as the origin of the fistula remains the internal opening in the intestine.

Our proposed SOPs were validated in 21 patients and resulted in 100% feasibility since we were able to perform all SOPs in all included patients without treatment related side effects. However, when the syringe with MSC suspension was introduced via the external opening, we cannot ensure that all MSCs were indeed injected around the internal opening. It is likely that in patients with a longer or tortuous fistula tract, we were not able to reach the internal opening via this route. However, only half of the MSC suspension was injected by introducing the syringe via the external opening, the other half was always administered into the wall around the closed internal opening by introducing the syringe via the anus.

In this article, a proposal to standardize the method of local treatment administration for perianal fistulizing CD was made. We suggest to use our standardized and validated protocol for the administration of local treatment of CD perianal fistulas to be able to reliably assess efficacy of local therapy in future.

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