Standardization of mesenchymal stromal cell therapy for perianal fistulizing Crohn's disease

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Background Local administration of mesenchymal stromal cells (MSCs) into the fistula tract seems to improve patient outcome in perianal fistulas due to Crohn's disease (CD). In this paper we propose a standardized and validated protocol for the local administration of MSCs for CD perianal fistulas to be able to reliably assess efficacy.

Materials and methods A working group consisting of gastroenterologists and surgeons with expertise in the treatment of perianal CD developed a consensus perianal fistula treatment protocol for local MSC treatment of perianal fistulizing CD. The treatment protocol was validated during a trial of allogeneic bone marrow-derived MSCs for the treatment of refractory perianal Crohn's fistulas.

Results Localization and classification of perianal fistulas with MRI and rectoscopy is of crucial importance prior to surgical intervention with local therapy administration. Examination under anesthesia 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 preventing the surgeon from adequately performing the surgical procedure have to be endoscopically dilated. Curettage of the fistula tract has an important role as long-standing CD perianal fistulas close poorly without removal of their epithelial lining. 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 injected into the tissue around the internal opening.

Conclusion In this article, we propose a standardized method of local MSC administration for perianal fistulizing CD. The use of this standardized and validated protocol for the administration of local treatment of CD perianal fistulas will allow reliable comparison of the efficacy of local therapies in future. Eur J Gastroenterol Hepatol 30:1148–1154

Introduction

Despite all the recent advances in medical and surgical therapy for Crohn's disease (CD), durable remission rates for perianal fistulas in CD still remain low [1]. The treatment outcome of perianal fistulas is dependent on multiple factors. It is likely that 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. Antitumor necrosis factor (TNF) agents such as infliximab, especially combined with antibiotics, are effective in treating perianal fistulas [2,3]. However, more than one-third of the patients with an initially healed perianal fistula after infliximab treatment had a recurrence of their fistula within 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 CD have complex fistulas, often with multiple branches and involvement of the anal sphincters. A temporary noncutting seton attempts to promote drainage and fibrosis and aims to decrease inflammation, which is crucial before embarking on fistula closure. 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 occurs in almost 10% of the treated patients [8].

Recently, the administration of local therapies for perianal fistulas has emerged as an alternative approach. Various studies have reported encouraging results of a local injection of anti-TNF [9–13] into the fistula tract and local cellular therapy with mesenchymal stromal cells (MSCs) [14–17]. Last year, the results of a large multicenter phase III trial on allogeneic adipose-derived stem cells for the treatment of refractory complex perianal fistulas in patients with CD were published [18]. Fifty percent of the patients receiving MSCs compared with 34% in the placebo group achieved remission of their perianal fistulas. MSCs are multipotent cells capable of modulating immune responses by interfering in the differentiation of T cells [19] and maturation of antigen-presenting cells [20]. In addition, MSCs are able to 'sense' inflammation as they appear to be capable of migrating to the damaged tissue to contribute toward the repair process [21–24]. 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

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efficacy [25–27]. Indeed, local injection of fibrin glue in combination with MSCs resulted in higher fistula healing rates compared with treatment with fibrin glue alone (71 vs. 16%) [14]. An even 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 without additional fibrin glue [17]. Further studies using a local injection of MSCs originating from both adipose tissue and bone marrow without fibrin glue have also shown reductions in the number of draining CD perianal fistulas [15,16]. Although this mode of therapy administration seems to be effective, preoperative workup and practice among surgeons in terms of injection techniques is likely to differ considerably. 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 [28].

Patients and methods

Development of a standardized treatment protocol

A working group consisting of inflammatory bowel disease (IBD)-specialized gastroenterologists and surgeons with expertise on perianal CD from the Leiden University Medical Center in the Netherlands was formed to develop a standardized protocol for the local MSC treatment of perianal fistulizing CD. The working group reached decisions by consensus on the following four topics: (a) localization and classification of perianal fistulas, (b) surgical intervention prior to therapy administration, (c) local MSC administration, and (d) follow-up.

Validation of the standardized treatment protocol

Validation of the standardized treatment protocol was performed during our recently published study on allogeneic bone marrow-derived MSCs for the treatment of refractory perianal Crohn's fistulas [28]. 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 two internal openings were not included in this trial. In total, 21 (57.1% male) patients with a mean age of 38.0 years were included. The mean fistula duration was 5.5 years and most fistulas were complex (65.2%) and trans-sphincteric (65.2%) [28].

The study was approved by the Medical Ethical Committee of the Leiden University Medical Center (LUMC) and the Central Committee on Research involving Human Subjects (The Hague, Netherlands) and all patients gave written informed consent.

Results

Consensus of the standardized treatment protocol

Localization and classification of perianal fistulas

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 (Fig. 1).

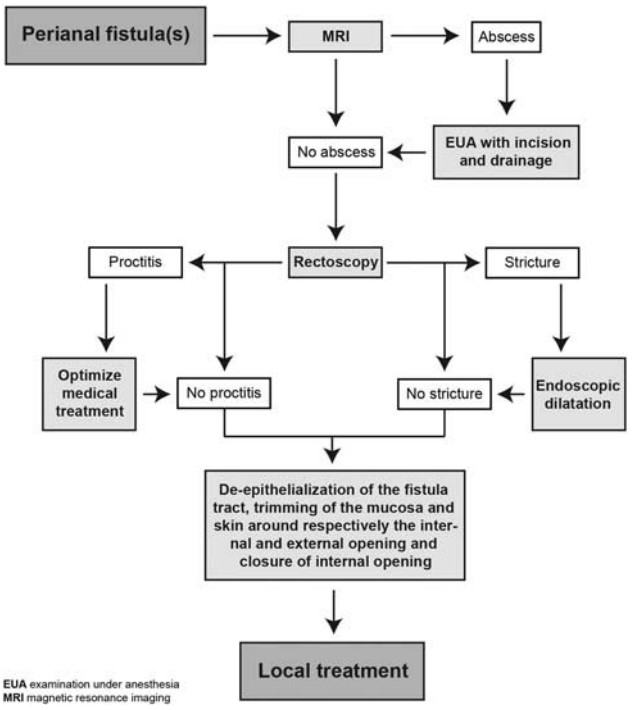

Fig. 1. Consensus of the standardized treatment protocol. EUA, examination under anesthesia.

Rectoscopy: active proctitis complicates surgical procedures. It is therefore advisable to rule out the presence of proctitis with a rectoscopy. If proctitis is present, it is important to optimize medical treatment before administering MSCs. Strictures that prevent the surgeon from adequately performing the surgical intervention and therapy administration as described below should be treated first by (repeated) endoscopic dilatation. If severe strictures persist, MSC treatment should not be performed.

Surgical intervention before therapy administration

Fistulas must be drained adequately for at least 2 weeks before embarking on the administration of MSC to prevent abscess formation after MSC treatment. If not drained adequately, do not administer MSC therapy, but optimize drainage with the use of setons.

It was agreed to be of utmost importance to prevent adverse effects by minimalizing surgical trauma to the anal sphincter. Excessive and long-lasting stretch of the anal sphincter during surgery must be avoided to reduce the risk of decreased continence. 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.

Local administration of mesenchymal stromal cells

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 need to be injected at the origin of the fistula where the inflammation resides, specifically in the walls of the fistula around the closed internal opening. Injection into the fistula lumen should be avoided as this is a waste of the therapeutic agent as the

majority will just seep out of the fistula opening. The number of injection sites was kept to a minimum to ensure administration of enough MSCs per injection site.

Follow-up

First 6 h after local administration of MSCs: patients should be observed closely for the first 6 h after local therapy administration by continuously monitoring the temperature, blood pressure, and pulse to be able to quickly intervene when infusion reactions occur. If no adverse events are observed after 6 h, the patient can be discharged the same day.

After local administration of MSCs: in the first 1–2 weeks after local MSC administration, patients can experience anal pain and discharge from the treated fistula(s). However, patients should be instructed to contact their IBD-specialized gastroenterologist and/or – surgeon when fever or a fluctuating painful perianal swelling develops to exclude a perianal abscess. Most perianal abscesses can be diagnosed easily during a physical examination. However, when a physical examination is not conclusive and an abscess cannot be ruled out completely, ultrasound can be used as a quick and easy diagnostic tool.

The efficacy of the local treatment, defined as an absence of discharge from the fistula(s) by gentle finger compression, is determined at physical examination. MRI is not useful 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 1 year [29].

Feasibility and outcomes of surgical intervention with local mesenchymal stromal cell administration

We validated the standardized treatment protocol in our recently published study [28].

Two trained surgeons performed the surgical procedures. Allogeneic bone marrow-derived MSCs were cryopreserved at passage 1 in the good manufacturing practice facility and then, 2 weeks before the injection, the cryopreserved MSCs were thawed and replated to ensure sufficient numbers of MSCs at the time of surgical intervention. The MSCs were spindle shaped, greater than 90% $CD73^+/CD90^+/CD105^+$, less than or equal to 1% $CD45^+$ and less than or equal to 0.01% $CD3^+$. The viability was measured and 3×10^7 viable cells were immediately transferred in a cold environment (4–8°C) to the operating room.

In our hands, 5 ml of 3×10^7 allogeneic bone marrow-derived MSCs (passage 2 in 20 patients; passage 3 in one patient) yielded a fistula healing rate of 85.7% compared with 33.3% in the placebo group at 12 and 24 weeks after local MSC administration. Surgery with a local injection of MSCs was feasible as we could perform the standard surgical procedure in all included patients. In addition, the surgical procedures took only 20–40 min/patient depending on the number and complexity of the perianal fistulas. Surgical intervention was well tolerated by all patients. There were no cases of wound infection or bleeding reported. Moreover, local MSC administration did not lead to treatment-related adverse events.

Standardized treatment protocol

Localization and classification of perianal fistulas

MRI and a rectoscopy were performed to describe the localization and classification of the perianal fistula(s) following the Parks and 'simple/complex' criteria (Fig. 2) [5,30,31]. In Fig. 3, possible routes of perianal fistulas are schematically shown:

(1) Locate the internal opening(s):
   (a) Use the 'anal clock' when the patient is in the lithotomy position to describe the location.
   (b) 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, trans-sphincteric, suprasphincteric, or extrasphincteric.
(3) Locate the external opening(s): use the 'anal clock' when the patient is in the lithotomy position to describe the location.
(4) Assess the presence of horseshoeing: intersphincteric, infralevator, or supralevator.
(5) Assess the presence of a rectovaginal fistula.
(6) Assess the presence of perianal abscesses: superficial or supralevator.
   If present, perform an EUA with incision and drainage of the abscess.
(7) Perform a rectoscopy to assess luminal activity of CD:
   (a) If proctitis is present, optimize medical treatment before local therapy administration.
   (b) 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.

Surgical intervention before local mesenchymal stromal cell administration

Perform the surgery and local MSC administration under general or epidural anesthesia with the patient in the lithotomy position.

(1) Inspect the perianal area for external openings. Use a Hill–Ferguson retractor for optimal exposure. To optimize visibility, especially of the anal canal, use a headlamp.
(2) Explore the fistula tract(s) and identify the connection between the external and internal opening(s) by introducing a malleable probe through 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 through 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.

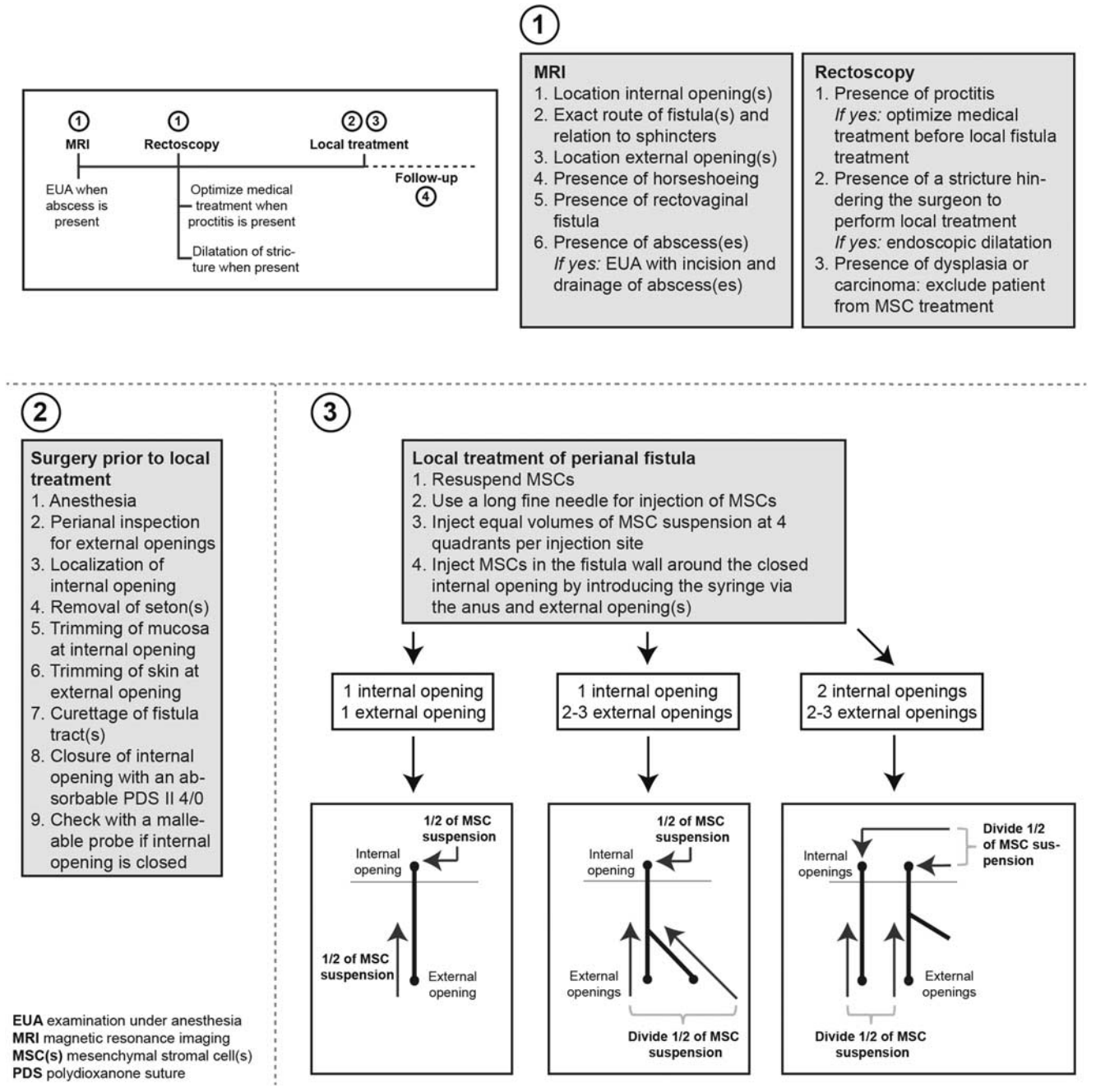

Fig. 2. Standardized treatment protocol. Timeline of procedures. (1) Localization and classification of perianal fistulas; (2) surgical intervention before MSC administration; (3) local MSC administration. EUA, examination under anesthesia; MSC, mesenchymal stromal cell; PDS, polydioxanone suture.

Local mesenchymal stromal cell administration

Inject equal volumes of MSCs at four quadrants around the closed opening. Inject the MSCs into the fistula wall.

(1) Resuspend MSCs before injection.
(2) Use a long fine needle (20 G) for the administration of MSCs.
(3) Half of the MSC suspension must be injected into the fistula wall at four quadrants around the closed internal opening(s) by introducing the syringe through the anus.
(4) The second half of the MSC suspension must be injected into the fistula wall at four quadrants as close as possible to the closed internal opening(s) by introducing the syringe through the external opening(s) into the fistula tract(s) as far as possible.

Follow-up

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

(1) Observe the patient with monitoring of the vital signs during the first 6 h after local MSC administration for infusion reactions. The patient can be discharged if no adverse events are observed after 6 h.

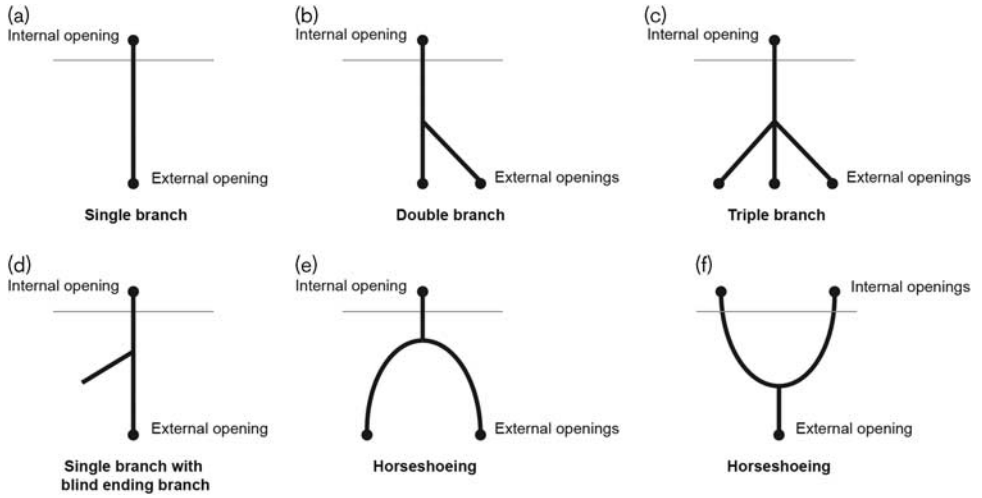

Fig. 3. Schematic routes of perianal fistulas. (a) Single internal opening and single external opening; (b) single internal opening and two external openings; (c) single internal opening and three external openings; (d) single internal opening with one blind ending tract and one external opening; (e) single internal opening with horseshoeing and two external openings; (f) two internal openings with horseshoeing and one external opening.

(2) Instruct patients to contact their IBD-specialized gastroenterologist and/or – surgeon if they develop a fever and/or a fluctuating painful perianal swelling.
(3) Use ultrasound when there is suspicion of a perianal abscess. If present, EUA with incision and drainage of the abscess with subsequent placement of a noncutting seton to promote drainage and reduce inflammation of the fistula is the first step before embarking on surgery. Antibiotics such as ciprofloxacin or metronidazole are recommended.

Discussion

In this paper, we propose a standardized protocol for the local administration of MSCs for perianal fistulizing CD that we have validated in a recently performed clinical trial on allogeneic bone marrow-derived MSCs for the treatment of refractory perianal Crohn's fistulas [28]. Differences in surgical practice are likely to have an 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 and surgical options, and is often accompanied by multiple relapses [1]. Therapeutic efficacy might be enhanced when drugs or MSCs are locally administered. Promising results after the local administration of MSCs have been published, with fistula healing rates of 69–82% in uncontrolled trials [14–17] and 50–80% in recently performed randomized double-blind placebo-controlled trials [18,28]. In addition, local therapy with anti-TNF agents might also result in fistula closure; however, to date, only open-label studies with small sample sizes and no randomized-controlled trials have been reported [9–13]. Fistula remission rates vary considerably after local administration of anti-TNF agents (36–88%) possibly as a result of the additional fistulectomy performed in the trial with the highest efficacy rate [11].

Although fistula healing rates after local therapy with MSCs are encouraging, there are substantial differences in the techniques of administration, making it difficult to reliably assess the effect of local therapy. Therefore, we sought to develop and test a standardized treatment protocol. Although we have only validated this protocol for use with MSCs, it is possible that this protocol could also be implemented for local therapy with anti-TNF agents. Our rationale behind the location of injection is the same for all CD perianal fistulas and is likely to hold true irrespective of the type of local treatment. The origin of the fistula is the internal opening in all CD perianal fistulas. Therefore, we believe that local treatment should be administered around the closed internal opening. Closure of the opening prevents bacterial contamination of the fistula. Moreover, the treatment is presumably most efficacious when it remains for as long as possible at the site of injection. Therefore, administration of the drugs should be into the tissue surrounding the fistula as a treatment agent 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 [32]. Therefore, curettage of the entire fistula tract is recommended as the presence of epithelium inside the tract prevents fistula closure. The possibility of local treatment of other types of CD-related fistulas such as rectovaginal and enterocutaneous fistulas has hardly been explored. Only one small trial of five patients with CD-related rectovaginal fistulas who were treated with allogeneic adipose-derived MSCs has been published. Complete healing of the fistulas was observed in three of the five patients [33]. It is plausible that our standardized protocol could also be used in the treatment of other types of fistulas as the origin of the fistula remains the internal opening in the intestine.

Our proposed protocol was validated in 21 patients. The protocol was feasible in 100% of patients as we were able to perform the standardized procedures in all included patients without treatment-related side effects. Although the number of viable MSCs in the syringes leaving the good manufacturing practice facility was 3×10^7, it is possible that the actual number of injected MSCs is lower as MSCs may adhere to the plastic syringe despite careful resuspension.

In addition, when the syringe with MSC suspension was introduced through the external opening, we could not 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 through this route. However, only half of the MSC suspension was injected by introducing the syringe through the external opening; the other half was always administered into the wall around the closed internal opening by introducing the syringe through the anus.

In this article, we propose a standardized method of local MSC administration for perianal fistulizing CD. The use of this standardized and validated protocol for the administration of local treatment of CD perianal fistulas will enable a reliable comparison of the efficacy of local therapies in future.

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Conflicts of interest

There are no conflicts to interest.

References

1 Molendijk I, Nuij VJ, van der Meulen-de Jong AE, van der Woude CJ. Disappointing durable remission rates in complex Crohn's disease fistula. *Inflamm Bowel Dis* 2014; 20:2022–2028.
2 West RL, van der Woude CJ, Hansen BE, Felt-Bersma RJ, van Tilburg AJ, Drapers JA, Kuipers EJ. Clinical and endosonographic effect of ciprofloxacin on the treatment of perianal fistulae in Crohn's disease with infliximab: a double-blind placebo-controlled study. *Aliment Pharmacol Ther* 2004; 20:1329–1336.
3 Dewint P, Hansen BE, Verhey E, Oldenburg B, Hommes DW, Pierik M, *et al*. Adalimumab combined with ciprofloxacin is superior to adalimumab monotherapy in perianal fistula closure in Crohn's disease: a randomised, double-blind, placebo controlled trial (ADAFI). *Gut* 2013; 63:292–299.
4 Bouguen G, Siproudhis L, Gizard E, Wallenhorst T, Billioud V, Bretagne JF, *et al*. Long-term outcome of perianal fistulizing Crohn's disease treated with infliximab. *Clin Gastroenterol Hepatol* 2013; 11:975–981.
5 Sandborn WJ, Fazio VW, Feagan BG, Hanauer SB. AGA technical review on perianal Crohn's disease. *Gastroenterology* 2003; 125:1508–1530.
6 Grimaud JC, Munoz-Bongrand N, Siproudhis L, Abramowitz L, Senejoux A, Vitton V, *et al*. Fibrin glue is effective healing perianal fistulas in patients with Crohn's disease. *Gastroenterology* 2010; 138:2275–2281.
7 O'Riordan JM, Datta I, Johnston C, Baxter NN. A systematic review of the anal fistula plug for patients with Crohn's and non-Crohn's related fistula-in-ano. *Dis Colon Rectum* 2012; 55:351–358.
8 Soltani A, Kaiser AM. Endorectal advancement flap for cryptoglandular or Crohn's fistula-in-ano. *Dis Colon Rectum* 2010; 53:486–495.
9 Poggioli G, Laureti S, Pierangeli F, Rizzello F, Ugolini F, Gionchetti P, Campieri M. Local injection of Infliximab for the treatment of perianal Crohn's disease. *Dis Colon Rectum* 2005; 48:768–774.
10 Asteria CR, Ficari F, Bagnoli S, Milla M, Tonelli F. Treatment of perianal fistulas in Crohn's disease by local injection of antibody to TNF-alpha accounts for a favourable clinical response in selected cases: a pilot study. *Scand J Gastroenterol* 2006; 41:1064–1072.
11 Alessandroni L, Kohn A, Cosintino R, Marrollo M, Papi C, Monterubbianesi R, Tersigni R. Local injection of infliximab in severe fistulating perianal Crohn's disease: an open uncontrolled study. *Tech Coloproctol* 2011; 15:407–412.
12 Tonelli F, Giudici F, Asteria CR. Effectiveness and safety of local adalimumab injection in patients with fistulizing perianal Crohn's disease: a pilot study. *Dis Colon Rectum* 2012; 55:870–875.
13 Maggi L, Capone M, Giudici F, Santariasci V, Querci V, Liotta F, *et al*. CD4 + CD161 + T lymphocytes infiltrate Crohn's disease-associated perianal fistulas and are reduced by anti-TNF-alpha local therapy. *Int Arch Allergy Immunol* 2013; 161:81–86.
14 Garcia-Olmo D, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, *et al*. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum* 2009; 52:79–86.
15 Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, *et al*. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut* 2011; 60:788–798.
16 De la Portilla F, Alba F, Garcia-Olmo D, Herrerias JM, Gonzalez FX, Galindo A. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. *Int J Colorectal Dis* 2013; 28:313–323.
17 Lee WY, Park KJ, Cho YB, Yoon SN, Song KH, Kim DS, *et al*. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells* 2013; 31:2575–2581.
18 Panés J, Garcia-Olmo D, van Assche G, Colombel JF, Reinisch W, Baumgart DC, *et al*. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet* 2016; 388:1281–1290.
19 Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005; 105:1815–1822.
20 Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Asian H, *et al*. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. *Blood* 2005; 105:2214–2219.
21 Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol* 2008; 180:2581–2587.
22 Chen Y, Xiang LX, Shao JZ, Pan RL, Wang YX, Dong XJ, Zhang GR. Recruitment of endogenous bone marrow mesenchymal stem cells towards injured liver. *J Cell Mol Med* 2010; 14:1494–1508.
23 Liang L, Dong C, Chen X, Fang Z, Xu J, Liu M, *et al*. Human umbilical cord mesenchymal stem cells ameliorate mice trinitrobenzene sulfonic acid (TNBS)-induced colitis. *Cell Transplant* 2011; 20:1395–1408.
24 Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, *et al*. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. *Circulation* 2005; 112: 1451–1461.
25 Anjos-Afonso F, Siapati EK, Bonnet D. In vivo contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. *J Cell Sci* 2004; 117:5655–5664.
26 Ruster B, Gottig S, Ludwig RJ, Bistrain R, Muller S, Seifried E, *et al*. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood* 2006; 108:3938–3944.

27 von BL, Batsis I, Moll G, Hogg M, Szakos A, Sundberg B, *et al*. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. *Stem Cells* 2012; 30:1575–1578.
28 Molendijk I, Bonsing BA, Roelofs H, Peeters KC, Wasser MN, Dijkstra G, *et al*. Allogeneic bone marrow-derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. *Gastroenterology* 2015; 149:918–927.
29 Tozer P, Ng SC, Siddiqui MR, Plamondon S, Burling D, Gupta A, *et al*. Long-term MRI-guided combined anti-TNF-alpha and thiopurine therapy for Crohn's perianal fistulas. *Inflamm Bowel Dis* 2012; 18:1825–1834.
30 Parks AG, Gordon PH, Hardcastle JD. A classification of fistula-in-ano. *Br J Surg* 1976; 63:1–12.
31 Bell SJ, Williams AB, Wiesel P, Wilkinson K, Cohen RC, Kamm MA, *et al*. The clinical course of fistulating Crohn's disease. *Aliment Pharmacol Ther* 2003; 17:1145–1151.
32 van Koperen PJ, ten Kate FJ, Bemelman WA, Slors JF. Histological identification of epithelium in perianal fistulae: a prospective study. *Colorectal Dis* 2010; 12:891–895.
33 García-Arranz M, Herreros MD, González-Gómez C, de la Quintana P, Guadalajara H, Georgiev-Hristov T, *et al*. Treatment of Crohn's-related rectovaginal fistula with allogeneic expanded-adipose derived stem cells: a phase I-IIa clinical trial. *Stem Cells Transl Med* 2016; 5:1441–1446.