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ORIGINAL ARTICLE

Micro-fragmented adipose tissue injection for the treatment of complex anal fistula: a pilot study accessing safety and feasibility

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Abstract

Background The aim of the present study was to evaluate the safety and efficacy of autologous, micro-fragmented and minimally manipulated adipose tissue injection associated closure of the internal opening in promoting healing of complex anal fistula.

Methods A pilot study was conducted on patients referred to our center with anal fistula, from April 2015–December 2016. Inclusion criteria were age over 16 years old and a diagnosis of complex anal fistula according to the American Gastroenterological Association classification The patients were divided into 2 groups; the "first time group" (Group I) in which micro-fragmented adipose tissue injection with closure of the internal opening was the first sphincter-saving procedure, and the "recurrent group" (Group II) consisting of patients who had failed prior sphincter-saving procedures. The procedure was carried out 4–6 weeks after seton placement. Follow-up visits were scheduled at 7 days, and 1, 3, 6 and 12 months after surgery. Fistula healing was defined as the closure of the internal and external openings without any discharge.

Results Out of 47 patients with complex transsphincteric anal fistula, 19 met the inclusion criteria and were selected to undergo the procedure. Twelve of these patients (Group I) had micro-fragmented adipose tissue injection as first-line treatment, and 7 (Group II) had failed previous sphincter-saving procedures. The mean operative time was 55 ± 6 min (range 50–70 min). The mean postoperative pain score measured with the visual analog pain scale was 2 ± 1.4 (range 0–4). No intraoperative difficulties related to the use of the kit were recorded. There were no cases of postoperative fever or abdominal sepsis related to the procedure and no post-treatment perianal bleeding or impaired anal continence. Only 3 cases of minor abdominal wall hematoma that did not require any treatment and 1 case of perianal abscess were observed. Patients were evaluated for a mean follow-up time of 9 ± 3.1 months (range 3–12 months). The overall healing rate was 73.7, 83.3% for Group I and 57.1% for Group II.

Conclusions The injection of autologous, micro-fragmented and minimally manipulated adipose tissue associated with closure of the internal opening is a safe, feasible and reproducible procedure and may enhance complex anal fistula healing.

Keywords Adipose tissue · Anal Canal · Muscles · Lipogems · Fistula · Lipectomy

Introduction

Perianal fistula is one of the most common anorectal diseases with a yearly incidence of 8–10 cases per 100,000 people [1]. The incidence rate can reach 13–39% in patients with Crohn's disease because perianal fistula is one of the more frequent clinical signs of the disease [2, 3]. Perianal fistulas may be divided in two categories according to the American Gastroenterological Association (AGA) classification: simple and complex [4]. Simple fistula includes intersphincteric and low transsphincteric with less than 30% of the external and internal anal sphincter involved. Complex fistula, instead, includes high transsphincteric with more than 30%

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of the external and internal anal sphincter involved, suprasphincteric, extrasphincteric, horseshoe, multiple tracks, anteriorly lying tracks in females, recurrent, inflammatory bowel disease (IBD), radiation, preexisting incontinence and chronic diarrhea [4]. Cutting seton or fistulotomy is the gold standard for the treatment of the simple type [5]. The treatment of the complex type is still very challenging, and a gold standard procedure is not available. Nowadays, the proposed techniques treat only the internal orifice (IO) or both the IO and the fistula tract. Advancement flap, ligation of the intersphincteric fistula tract (LIFT) and over the scope clips (OTSC®) belong to the first category [6, 7]. Fibrin glue sealant (FGS), anal collagen plug (ACP), Permacol™ collagen paste, fistula tract laser closure (FiLaC™), video-assisted anal fistula treatment (VAAFT) and platelet rich plasma (PRP) belong to the second category. Recently, new therapeutic approaches, such as the use of mesenchymal stem cells (MSCs), have been proposed and the regenerative capabilities of fat with mesenchymal properties [adipose-derived mesenchymal stem cells (ASCs)] have been explored [8–10]. MSCs have been reported to be able to activate and influence the microenvironment due to the secretion of mitogenic and immunomodulatory factors [11]. The use of ASCs either expanded or obtained by enzymatic digestion as stromal vascular fraction (SVF) has recently attracted great interest, and several studies have demonstrated their regenerative and immunomodulatory properties [12] both in vitro and in vivo. Nevertheless, enzymatic treatment or cell expansion has complex regulatory constraints. Hence, a minimally manipulated autologous adipose tissue as a therapeutic option would be preferable. The aim of the present study is to evaluate the safety and efficacy of autologous, micro-fragmented and minimally manipulated adipose tissue injection associated with closure of the internal opening in promoting complex anal fistula healing.

Materials and methods

Study population

All the patients referred to our center over 16 years old and with a diagnosis of complex anal fistula according to the American Gastroenterological Association classification [4] from April 2015–December 2016 were consecutively enrolled for a prospective study. The diagnosis of complex fistula needed to be confirmed by pelvic magnetic resonance or three-dimensional 360° transanal ultrasound. The patients underwent a first-step procedure with the placement of a loose seton 4–6 weeks before the sphincter-saving procedure. The patients were divided in two groups: the "first time group" (Group I) in which this was the first sphincter-saving procedure and the "recurrent group" (Group II) who had failed previous sphincter-saving procedures. Patient demographics and previous fistula treatment were collected. Written informed consent was obtained from all patients included in the study.

Exclusion criteria

The exclusion criteria were the following: Multiple fistula tracts, the presence of abscess, IBD, human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, rectovaginal fistula, therapy with anticoagulants, steroids or immunomodulants, previous pelvic radiotherapy, personal history of neoplasia within 5 years from the diagnosis, pregnancy, uncontrolled diabetes, coagulopathy or connective tissue diseases.

Surgical technique

The sphincter-saving procedure was carried out in one surgical step.

- Harvesting of the adipose tissue.

  The lower or the lateral abdomen was chosen as the donor site for adipose tissue harvesting. Before harvesting the fat, the subcutaneous fat of the anterior abdominal wall was injected with 360 ml (180 ml in each side right and left) of modified Klein solution (500 ml of saline solution, 20 ml lidocaine 20 mg/ml, 1 ml adrenaline 1 mg/ml) using a disposable cannula (17G) (Fig. 1). The adipose tissue was harvested using a 13G blunt cannula connected to a Vaclock® 20-ml syringe (Fig. 2a, b). The aspirated tissue was then collected in 10-ml syringes and positioned for decant (Fig. 2c, d).
- Processing of the adipose tissue with the Lipogems® device.

  The harvested fat was then processed in the Lipogems® processing kit, a disposable device that gradually reduces the size of the adipose tissue clusters while eliminating oily substances and blood residues with pro-inflammatory properties. The entire process was performed in complete immersion in saline solution minimizing any traumatic action on cell products. The resulting micro-fragmented adipose tissue was collected in a 10-ml syringe and positioned for decant the excess of saline solution. At the end, the product was transferred to several 1-ml syringes with a 22G and 30-mm length needle to be injected into the patient (Fig. 3).
- Curettage of fistula tract and closure of internal opening.

  The first step was the debridement of the internal opening, and then, the closure of the muscular layer was carried out with 2/0 polydioxanone (PDS) stitches after undermining the mucosal edges in order to raise an amount of tissue 1 cm wider than the size of the enlarged

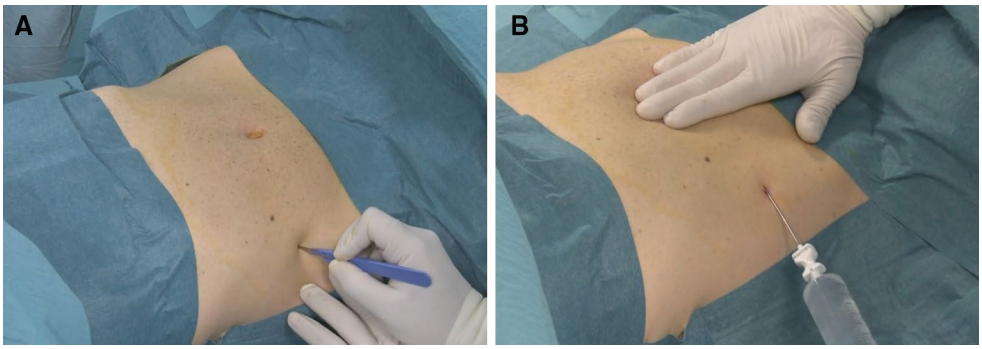

Fig. 1 **a** Skin incision along the anterior axillary line 2 cm above the iliac crest. **b** Infiltration of the subcutaneous tissue with modified Klein solution

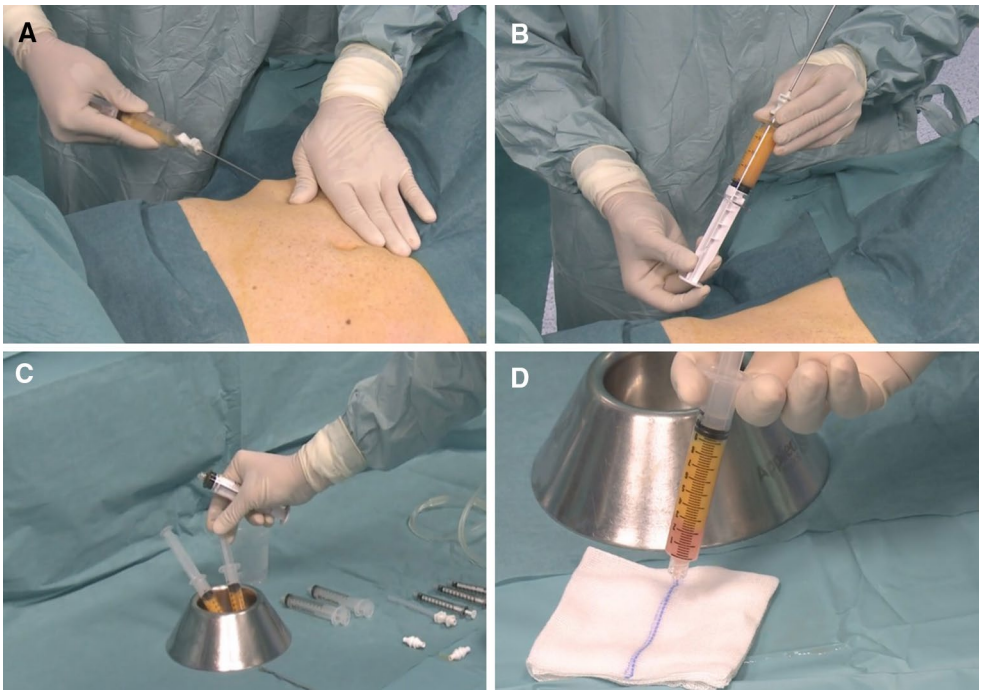

Fig. 2 **a**, **b** Liposuction is performed through a cannula (13G) connected to a Vaclock® syringe from both sides of the subcutaneous adipose tissue of the anterior abdominal wall. **c** The aspirated tissue is transferred to 10-ml syringes, and the piston is flipped upwards. This position allows the stratification of the fluids and tissue. **d** The fluid part of the aspirate is removed from the syringe before the contents are used

orifice. In addition, the mucosal flap was closed with 2/0 Vicryl stitches above the muscular layer (Fig. 4).

- Injection of micro-fragmented adipose tissue.

  The product was injected into the mucosal and muscular layers all around the internal opening (6 ml around the IO in the muscular layer and 4 ml around the mucosal flap) and around the fistula tract from the internal to the external opening. The amount of product injected may vary depending on the total amount harvested, but it should be not less than 6 ml, at least 2 ml per cm of fistula tract (Fig. 5).

Perioperative evaluations

Operative time, the volume of injected micro-fragmented fat and intraoperative difficulties related to the use of the processing kit were evaluated at the end of the surgical procedure. Postoperative complications including pain [0–10 visual analog scale (VAS)], fever, bleeding, abdominal or perineal hematoma and abdominal or perineal were prospectively evaluated at each follow-up visit. Impaired continence (Wexner incontinence score) was assessed before surgery and after surgery at each follow-up visit and then compared using Student's

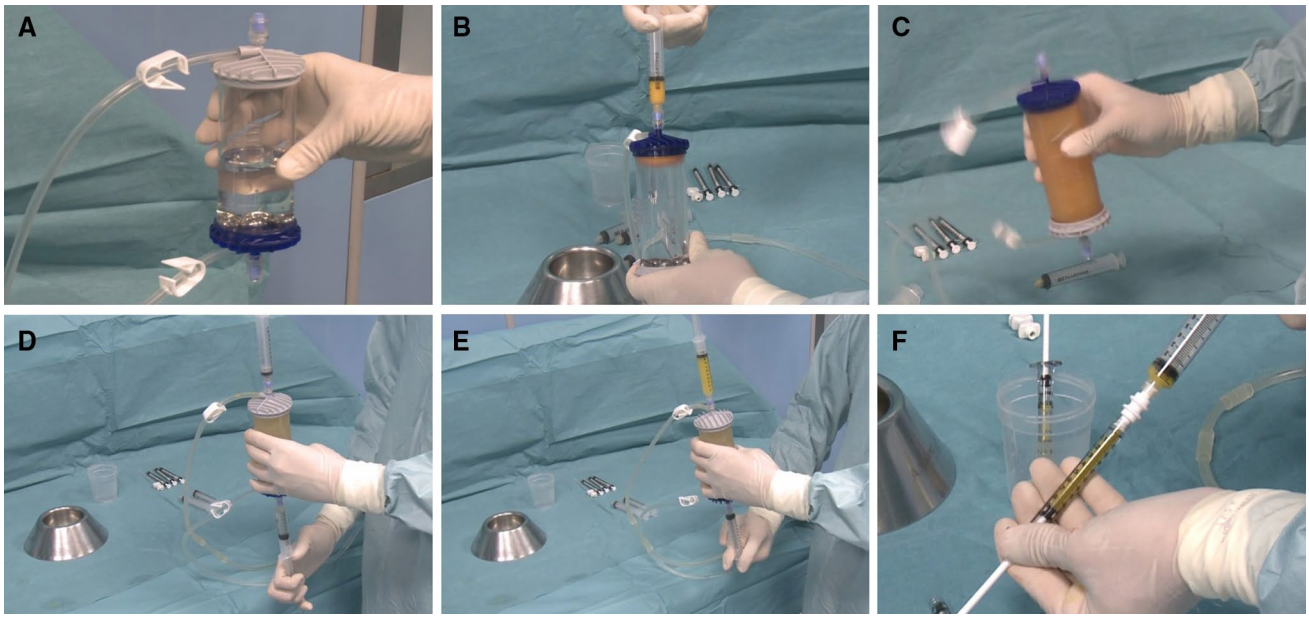

Fig. 3 **a** The blue filter hose is connected to the bag of saline solution. The cylinder is rotated with the grey filter upwards. Both hoses are opened. The cylinder is filled with the saline solution while held vertically. Once the cylinder is completely full, both hoses are closed. **b** The cylinder is replaced with the blue filter upwards. The blue filter hose is closed and the grey one opened. The syringe with the adipose tissue aspirate is connected to the blue filter pushing it inside the processing cylinder. **c** Both hoses are opened to allow a continuous flow of saline solution and the elimination of oily and bloody residual. The cylinder is shaken until the saline solution inside it becomes clear and then both hoses are closed. The action of the steel spheres emulsifies and micro-fractures the adipose tissue. **d** Two Luer-lock syringes are connected to the filters to remove the Lipogems® product. The processing cylinder is flipped so that the grey filter is at the top. The blue filter hose is opened and a full syringe of saline solution is drawn, and then, the hose is closed. **e** The cylinder is held vertically with the grey filter upwards. The saline solution is pushed from the syringe connected to the blue filter inside the cylinder. The final product will flow through the grey filter into the syringe connected to it. **f** The product is transferred to several 1-ml syringes with a 22G needle to be injected into the patient

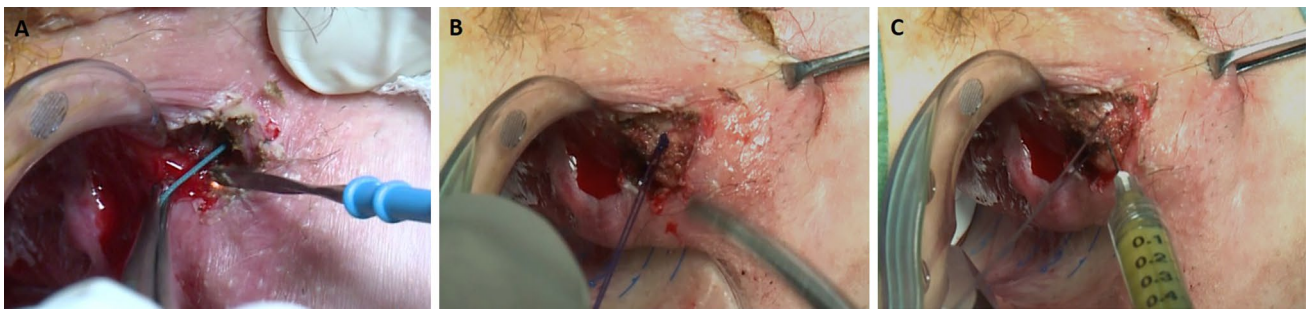

Fig. 4 **a** Debridement of fistula tract. **b** Closure of internal opening using 2/0 polydioxanone stitches for the muscular layer. **c** Injection of the final Lipogems® product through 1-ml syringes around the internal opening

t test. Follow-up visits were scheduled at 7 days, and 1, 3, 6 and 12 months after surgery. Fistula healing was defined as the closure of the internal and external openings without any discharge.

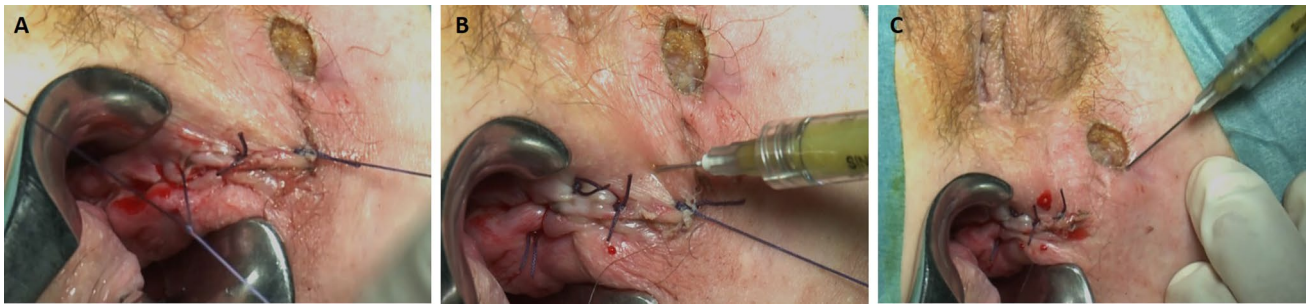

Fig. 5 **a** Mucosal flap closed with 2/0 Vicryl stitches. **b** Injection of the final Lipogems® product through 1-ml syringes around the mucosal flap. **c** Injection of the final Lipogems® product through 1-ml syringes around the fistula tract

Results

Demographics

From April 2015 to December 2016, 47 patients had sphincter-saving procedures for complex transsphincteric anal fistula, but only 19 (7 males and 12 females) with a mean age of 48 years (range 28–77 years) were enrolled in the present study. Twenty-eight were excluded because of Crohn's disease ($n = 11$ pt), multiple tracts ($n = 7$), anticoagulant therapy ($n = 7$ pt), coagulopathy ($n = 2$), ($n = 7$) and HCV infection ($n = 1$). Seven of these patients had undergone previous sphincter-saving procedures (Group II—2 fistula plug, 1 LIFT, 1 plug and LIFT, 1 Permacol paste, 2 advancement flap). Twelve patients were in Group I because they had this surgery as first line of treatment.

Intraoperative and postoperative complications

The mean operative time was 55 ± 6 min (range 50–70 min). The mean volume of injected micro-fragmented fat was 16 ± 2.9 ml (range 12–22 ml). No intraoperative difficulties related to the use of the kit were recorded.

The mean degree of postoperative pain measured with the VAS pain scale was 2 ± 1.4 (range 0–4) at 1 week and at 1 month after surgery. The mean value of VAS score was 0 at the subsequent follow-up visits. Only two cases of perianal discomfort lasted 2 months after surgery. There were no cases of postoperative fever, or abdominal sepsis related to the procedure, perianal bleeding or impaired anal continence after surgery. (The mean preoperative and postoperative Wexner score was 1.) Only 3 cases of minor abdominal wall hematoma that did not require any type of treatment and 1 case of perianal abscess were observed. The perianal abscess developed 3 months after surgery, and it was due to fistula recurrence.

Outcomes

Patients were evaluated for a mean follow-up time of 9 ± 3.1 months (range 3–12 months). The overall healing rate was 73.7, 83.3% (10 patients) in Group I and 57.1% (4 patients) in Group II. Overall, only 1 case of abscess and 4 cases of persistent discharge without closure of the internal and external openings were observed. In 10 patients, the fistula healing occurred between 3 and 6 months after surgery and was observed during the 6-month follow-up visit. In 2 cases, the fistula was already healed at the 3-month follow-up visit.

Discussion

The treatment of complex anal fistula is still challenging, and different treatment techniques have been proposed. The success rate of each technique is actually variable, as reported in recent systematic reviews [6, 13–20], because of the lack of uniformity and comparability among different studies and techniques. The increasing interest in finding new approaches to improve surgical outcomes, recovery time, and patient's quality of life has led to the development of innovative approaches, including the use of MSCs. It is well known that, besides being multipotent, these cells are able to promote tissue repair through the release of bioactive [21] and immunomodulatory molecules [22]. Adipose tissue is an optimal source of MSCs because of easier access and greater abundance than other sources such as the bone marrow. In addition, bone marrow differentiation capacity decreases with the donor's age [23–25]. Previous papers have reported the use of MSCs for the treatment of complex anal Crohn's-related [26] or cryptoglandular fistula [27, 28]. Garcia-Olmo et al. [28] in a phase II clinical trial on 35 participants reported a 71% healing rate at 1 year through the association of expanded ASCs and fibrin glue. Herreros and colleagues, instead, in a phase III randomized clinical trial on 200 participants reported a 57.1% healing

rate using expanded ASCs alone and a 52.4% healing rate using expanded ASCs associated with fibrin glue. In both cases, the internal opening was always closed [27]. Panès and colleagues in a randomized, double-blind, parallel-group, placebo-controlled study reported a 50% combined remission in the intention-to-treat group treated with MSCs versus 34% success rate in those treated with placebo. The treatment-related adverse events rate was 17% in the group treated with MSCs versus 29% in those treated with placebo [26]. MSCs have also been used to treat rectovaginal fistulas. Garcia-Arranz and colleagues in a phase I–II clinical trial on 10 patients with rectovaginal fistula related to Crohn's disease reported a healing rate of 60%. In some patients, the injection of MSCs was associated with a flap [29]. In all the above-mentioned studies, ASCs were isolated by enzymatic treatment and, in most cases, underwent further ex vivo expansion. In addition to the large number of processing steps, the significant costs and the restrictions related to the good manufacturing practice (GMP) regulations, the reported success rate was variable. Hence, the availability of a minimally manipulated autologous adipose tissue rich in these regenerative ASCs would have remarkable clinical relevance. For these reasons, we took advantage of a commercially available innovative technique (Lipogems®) that intraoperatively provides micro-fragmented adipose tissue in a very short time, without expansion or enzymatic treatment. With the aid of this technology, the adipose tissue is gently micro-fragmented and washed until free of pro-inflammatory oil and blood residues [22]. The results of our study demonstrate that the selected procedure is feasible, reproducible and safe. Indeed, no major complications nor adverse events were recorded. Only 3 cases of minor abdominal wall hematoma that did not require any type of treatment were observed. All the phases of the procedure are important, but a step that is fundamental for a good outcome is the double-layer closure of the internal orifice, which prevents the continuous passage of stool residue thorough the fistula. This results in an excluded fistula tract from which the inflammatory tissue was removed and healing may be enhanced with injection of micro-fragmented adipose tissue. For this reason, differently from other procedures, the external opening is left open to allow any discharge and to guarantee a closure from the inner to the external part. The micro-fragmented adipose tissue was injected in all the layers around the internal opening and around the fistula tract. We do not inject the tissue inside the tract because it does not have any bulking activity since ASCs act only as immunomodulatory agents to stimulate tissue regeneration sustaining the natural healing process [22].

Our preliminary results are very promising, with a healing rate of 83.3% in Group I. In Group II, the healing rate only reached 57.1%. This may be explained by the fact that in this "recurrent group," due to the abundance of fibrotic tissue in the chronic and recurrent fistulas, the regenerative stimulus failed to promote the healing. Conversely, tissue never treated before, as in Group I, has the potential to achieve complete and stable healing if adequately stimulated.

A possible bias of this study is the difficulty in discriminating between the contribution of the closure of the internal opening procedure and of the micro-fragmented adipose tissue injection. The closure of the internal opening differs from an advancement flap since the mucosa is raised laterally and on both sides of the defect. However, if we consider an average AF success rate of 66.7% (range 20–100%), based on published data [6], an important contribution to the healing rate, particularly in Group I, is provided by the micro-fragmented adipose tissue injection. The healing rate of Group II is even lower than the advancement flap alone possibly because of the chronic inflammatory process that generates an inert scar. However, it is not possible to make an adequate comparison between our technique and the advancement flap alone in these 2 different patient populations.

Conclusions

The injection of autologous, micro-fragmented and minimally manipulated adipose tissue associated with closure of the fistula opening is a safe, feasible and reproducible procedure and may enhance complex anal fistula healing. According to our preliminary results, we suggest the use of this combined technique in patients with complex anal fistula at their first sphincter-saving procedure.

Authors' contribution GN, AS and IG contribute to the conception and of the study and to the drawing up of the manuscript; AS, IG and BF contribute to the data acquisition, analysis and interpretation; AS, CM and GN contribute to the critical revision of the manuscript and to the approval of the version to be published.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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