Refractory Complex Crohn's Perianal Fistulas: A Role for Autologous Microfragmented Adipose Tissue Injection

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Background. Complex perianal fistulas represent one of the most challenging manifestations of Crohn's disease. Combined surgical and medical therapy with biologic drugs today represent the first-line treatment option, but its efficacy does not exceed 60%. Recently, new therapeutic approaches, such as the use of mesenchymal stromal cells, have shown promising results. The adipose tissue is an abundant and easy to access source. The effectiveness, safety, and feasibility of local injections of microfragmented adipose tissue in patients with refractory complex fistulizing perianal Crohn's disease (PCD) were evaluated.

Methods. Fifteen patients with persistent complex fistulizing PCD after biosurgical approach and subsequent surgical "rescue" repair were treated in S. Orsola-Malpighi Hospital with a single-local administration of microfragmented adipose tissue prepared using a minimal manipulation technique (Lipogems) in a closed system. Clinical outcomes were determined at 24-week follow-up assessing success rate, defined as combined clinical and radiological remission.

Results. Upon clinical examination at 24 weeks, 10 patients had combined remission (clinical and radiographic), 4 patients showed improvements, and 1 patient failed. The results were confirmed in all patients by pelvic MRI. No relevant postoperative complications nor adverse events were reported.

Conclusion. These results suggest that the local injection of autologous microfragmented adipose tissue is a safe and promising "rescue therapy" for patients with multiresistant complex fistulizing PCD. This approach might be proposed as routine because it is affordable, is minimally invasive, has no risk of sphincteric damage, and can be carried out in a day-surgery setting.

Key Words: perianal Crohn's disease, complex anal fistulas, microfragmented adipose tissue, mesenchymal stromal cells, regenerative medicine.

INTRODUCTION

Perianal Crohn's disease (PCD) affects up to 42% of patients with a CD diagnosis after a 20-year history of disease.[1, 2] During their lifetime, 90% of CD patients with proctitis will possibly be affected by a perianal abscess and/or fistula in anus.[2] These perianal septic complications represent one of the most challenging manifestations of CD, eventually resulting in the removal of the rectum with a permanent stoma in 31%–50% of patients.[3, 4] The treatment options of PCD, especially fistulous disease, were traditionally aimed at relieving symptoms (mainly pain and drainage), improving the patients' quality of life, and deferring or avoiding permanent stoma.[1]

Over the past 20 years, the introduction of tumor necrosis factor antagonist (anti-TNF-alpha) radically changed this point of view, switching the algorithm from simple control of sepsis to likely chances of healing. Biological therapy, when combined with surgical drainage of the perianal sepsis, now represents the gold standard treatment of fistulizing PCD.[1] Clinical effectiveness of systemic or local administration of anti-TNF-alpha agents has been demonstrated in published case series and controlled trials.[5] However, the overall success rate does not exceed 60%. Efficacy of bioprosthetic plugs made from porcine intestinal submucosa has been tested in some studies in patients with fistulizing PCD with controversial results. Recently, in a multicenter, randomized controlled trial, Senéjoux et al showed a 33% healing rate after anal fistula plug

insertion in patients with fistulizing PCD with no significant differences compared with seton removal alone.[6] Better results are reported using the endoanal mucosal advancement flap as repair surgery. However, this technique, described in 1998 by the Cleveland Clinic Group,[7] can be attempted only in highly selected cases with sparing or post-treatment healing of rectal mucosa and in the absence of vaginal fistulas.

The treatment of patients in which complete closure cannot be achieved despite the combination of biological therapy and surgery is still not well defined. These patients may benefit from innovative therapeutic approaches such as mesenchymal stromal cells (MSCs).[8–12] Mesenchymal stromal cells have been reported to have a perivascular origin (pericytes) and to influence the microenvironment by serving as "a site-regulated drug store."[13] Through trophic, immunomodulatory, and anti-microbial actions, these cells "sense" and "signal" changes in the microenvironment where they are located.[14] Bone marrow and adipose tissue are the most readily available sources of MSCs, and the adipose tissue is preferable because of its abundance, the easy access, and the simple isolation procedure.[15] The use of adipose-derived MSCs (ASCs), either culture-expanded or obtained by mechanical or enzymatic treatment as stromal vascular fraction,[16, 17] created a large interest because both in vitro and in vivo studies confirmed their anti-inflammatory and regenerative properties. However, enzymatic treatment and/or cell expansion are subjected to complex regulatory issues.[18, 19] Hence, the availability of minimally manipulated autologous adipose tissue as a therapeutic option would be of significant clinical relevance. Techniques for harvesting and processing the adipose tissue have rapidly evolved,[20] and published data show both the safety and efficacy of using fat and its derivatives.[21]

We employed a commercially available system that intra-operatively provides microfragmented adipose tissue in a short time, without expansion and/or enzymatic treatment, washing away pro-inflammatory oil and blood residues while preserving the stromal vascular niche.[22] The resulting product has been shown to be effective in the treatment of different pathologies, including osteoarthritis, anal incontinence, and orthognathic surgical corrections.[23–29] Therefore, we aimed to assess in a small pilot study its potential when combined with the surgical drainage of complex fistulizing PCD. The primary end point of the study was combined remission, defined as the clinical assessment of closure of all treated external openings that were draining at baseline and absence of collections >3 mm of the treated perianal fistulas assessed by pelvic MRI 24 weeks after the procedure.

MATERIALS AND METHODS

Study Design and Population

This is a nonprofit prospective pilot study (ClinicalTrials.gov number NCT03555773) where 15 patients with complex fistulizing PCD refractory to biological therapy were treated with autologous microfragmented adipose tissue. Patients were selected according to the following inclusion criteria: older than 18 years, diagnosis of CD confirmed by instrumental and histological methods, and presence of complex fistulizing PCD refractory to standard treatment (combination of surgical drainage of sepsis and local/systemic administration of anti-TNF-alpha for at least 1 year with subsequent surgical "rescue" repair by means of endo-anal mucosal flap or biological plug placement). Complex fistulas were defined according to the American Gastroenterological Association[30] and included high trans-sphincteric with involvement of more than 30% of the internal and external anal sphincter, suprasphincteric, extrasphincteric, horseshoe, and multiple tracks. Patients with more than 1 internal and 3 external openings, with ano- and recto-vaginal fistulas, active infections by Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Hepatitis B Virus (HBV), tuberculosis, septic uncontrolled conditions, abdominal acute localization of CD that could have required general surgery during the study, and oncological or lymphoproliferative active diseases were excluded. Patients from whom an adequate amount of lipoaspirate (at least 60 cc) could not be safely harvested were also excluded.

Concomitant treatments such as the administration of low bioavailability steroids or mesalamine in the 4 weeks before enrollment and azathioprine or 6-mercaptopurinein 6 months before enrollment were allowed in a stable dosage for the duration of the study and registered.

After the enrollment, medical history and clinical data were collected, and pre-operatory exams and laboratory tests (erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP]) were performed. All patients underwent pretreatment pelvic MRI. Perianal Disease Activity Index (PDAI) and health-related quality of life were assessed using a disease-specific (Inflammatory Bowel Disease Questionnaire [IBDQ]) and a generic (Short-Form 36 Health Survey [SF-36]) questionnaire.

Harvesting of the Adipose Tissue

The lower/lateral abdomen or—eventually—the inner/outer thigh were chosen as the donor site for adipose tissue harvesting under general or spinal anesthesia. Before the harvesting, the donor site was injected with 100 cc of Klein Solution (500 cc saline, 1 cc epinephrine 1/1000 IU, and 40 cc lidocaine 2%) using a disposable 17G blunt cannula connected to a 60-cc luer-lock syringe. The fat was then harvested (50 to 100 cc) using a 13G blunt cannula connected to a 20-mL VacLok syringe.

Processing of the Adipose Tissue With the Lipogems Device

The harvested fat was immediately processed in the Lipogems processing kit (Lipogems International Spa, Milan, Italy) as previously described.[22] Lipogems is a disposable device that mechanically reduces the size of the adipose tissue clusters while eliminating oily substances and blood residues with pro-inflammatory properties (Fig. 1). The entire process was carried out in 1 surgical step in complete immersion in
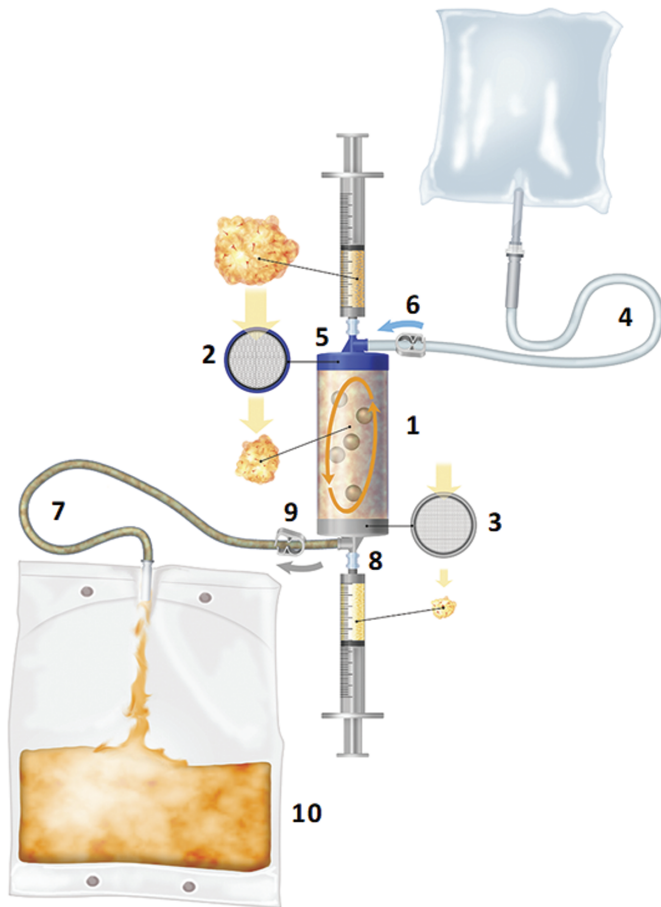
FIGURE 1. The Lipogems system operative scheme. The device consists of (1) a processing unit which contains five stainless steel balls; (2) an input sieve and (3) an output sieve; (4) a saline input line, connected to the blue side of the processing unit; (5) an access port with luer-lock connection; (6) a self-occluding valve at blue side to load the lipoaspirate; (7) a drainage line connected to the grey side of the processing unit; (8) an access port with luer-lock connection; (9) a self-occluding valve to unload processed material; (10) a collection bag for waste fluid. The processing unit, filled with normal saline solution, is maintained in flow condition by gravity. After saline priming, adipose tissue inserted in the device undergoes a first reduction and micronization of the lipid clusters by means of the input sieve. The mechanical action, exerted by shaking the processing unit, allows emulsion of the lipid mass and consequently the reduction of the clusters. The continuous flow of normal saline solution eliminates residues of oil emulsion and any remaining blood components.

physiological solution, minimizing any traumatic action on the cells and microarchitecture. The processed microfragmented fat was collected in a 60-cc syringe and positioned to decant the excess of saline solution. Finally, the product was transferred to several 10-cc syringes to be re-injected in the patient (Fig. 2).

Surgical Procedure and Microfragmented Adipose Tissue Injection

Examination under anesthesia was performed in order to identify all the fistula tracts and abscesses; eventual purulent material was drained and the fistula tracts curetted. Necrotic and inflamed tissues were excised using a "cone-like" fistulectomy at each fistula tract.[31] After local surgical drainage of the perianal disease, 20 cc of microfragmented adipose tissue was injected using a 21G needle circumferentially into the submucosa surrounding the internal fistula orifice and in the perianal tissue along the residual fistula tract (Fig. 2).

Follow-up Visits and Outcome Measures

All patients were assessed at 2 (T2), 4 (T4), 8 (T8), 12 (T12), and 24 (T24) weeks after the procedure. During all follow-up visits, laboratory tests (ESR, CRP) and clinical examination (absence of drainage upon gentle finger compression) were performed. The PDAI was completed at all follow-up visits by the same investigator. The IBDQ and SF-36 questionnaires were also administered. A second pelvic MRI was performed at T24 to radiologically assess the results. The radiological definition of fistula healing was based on the disappearance of T2 hyperintense signal and absence of abscess >3 mm.[32]

Treatment-emergent adverse events (TEAEs) were recorded up to T24.

Primary End Point

The primary end point of the study was combined remission at T24, defined as the clinical assessment of closure of all treated external openings that were draining at baseline and absence of collections >3 mm of the treated perianal fistulas assessed by pelvic MRI.

Secondary End Point

The secondary end point was clinical remission at T24 (ie, closure of all treated external openings that were draining at baseline despite gentle finger compression). At this time point, changes in CRP, ESR, PDAI, IBDQ, and SF-36 were also evaluated.

Other secondary end points included the clinical remission and relapse rate at 24 months follow-up, where relapse was defined as a clinically assessed re-opening of any of the treated external openings with active drainage.

Magnetic Resonance Imaging

A 1.5T MRI (Signa HDxt, GE medical system, Milwaukee, WI, USA) and an 8-channel phased array body, without endoanal coil, were used. The protocol included 5-mm thick slice acquisition for Axial T1 and T2 weighted Fast-spin-echo (T1-W FSE and T2-W FSE) sequences to assess morphological features and provide a good overall evaluation of the sphincter anatomy (necessary for the assessment of extensions in fistula-in-ano) and 3-mm thick slice acquisition for Axial, Sagittal and Coronal STIR (Short-Tau Inversion Recovery) sequences to provide a good contrast between fistula and adjacent tissues and a better evaluation of the edema in the soft

FIGURE 2. Left) Lipogems device; middle) processed microfragmented adipose tissue; right) surgical procedure: microfragmented adipose tissue injection circumferentially into the submucosa surrounding the internal fistula orifice and in the perianal tissue along the residual fistula tract.

tissue and of the extension of the inflammatory process in the perianal and perirectal areas. For a better anatomical evaluation, after analyzing the fistula orientation in previous acquisitions, Coronal T2-W FSE or Sagittal T2-W FSE was used if the fistula orientation was, respectively, latero-lateral or anterior-posterior.

Contrast-enhanced phases were not used according to literature. To guarantee standard operating procedures, MRI examinations were performed by the same experienced radiologist (AC) who was blinded regarding the clinical outcome.

Statistics

Given the explorative nature of the study, no formal sample size calculation was performed. In the design of the protocol, 15 patients were considered sufficient, from a clinical point of view, to obtain reliable results. Data is expressed as mean and standard deviation. Statistical analysis was performed using SPSS Statistics 25.0 software (IBM, USA). For statistical comparisons, the χ^2 test for all categorical data and Student t test for unpaired groups were used. A value of $P < 0.05$ was considered statistically significant.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of S. Orsola-Malpighi Hospital (protocol Lipogems-crohn2, number 4/2016/U/Sper, January 19, 2016). All the procedures mentioned in this study were carried out in accordance with the ethical standards and with the Helsinki Declaration of 1975, as revised in 2000. Patients were informed of risks and benefits and signed their informed consent.

RESULTS

A detailed description of the study population at the enrollment is reported in Table 1. The 15 patients (7 males [46.7%] and 8 females [53.3%]) ranged in age from 22 to 58 years old,

with a mean age of 40.1 years old (SD 11.7). Six out of 15 patients (40%) had been previously subjected to abdominal surgery (mostly ileocecal resection and subtotal colectomy), and 3 patients (20%) had a diverting ileostomy. All patients had a persistent anal fistula (n = 21) despite different multiple surgical treatments. Fistulectomy with seton was performed, at least once, in all the patients. Surgical strategies employed for the "rescue" treatment of the perianal disease after biosurgical approach were mucosal advancement flap in 12 of the 15 patients (80%) and plug placement in 3 of the 15 patients (20%). Anal substenosis was present in 3 patients (20%), and all were under self-dilation treatment with Hegar's dilators.

The pharmacological history of the population was also investigated at the beginning of the study and during the follow-up visits. All patients had been previously treated for at least 1 year with biological agents: infliximab in 7 (46.7%), adalimumab in 10 (66.7%), and vedolizumab in 3 (20%) cases.

Seven of the 15 patients (46.7%) were administered with pharmacological agents for the duration of the study: 1 with corticosteroids, 4 with mesalazine, and 2 with azathioprine.

At final follow-up (T24), 14 patients (93.3%) presented clinical remission with absence of drainage upon gentle finger compression on the external orifice, and 10 (66.7%) presented combined remission (average healing time: 10 weeks, Table 2 and Fig. 3). Only 1 patient (6.6%) failed with persistence of drainage from the fistula. At 24 months follow-up, the results were maintained with no relapses.

At final follow-up (T24), CRP, ESR, and PDAI were reduced compared with baseline (T0) values (CRP, 1.1 ± 1.0 mg/dL at T24 vs 1.7 ± 1.1 mg/dL at T0; ESR, 26.7 ± 12.8 mm at T24 vs 19.3 ± 14.1 mm at T0; PDAI, 4.3 ± 2.8 at T24 vs 7.5 ± 2.1 at T0). The IBDQ score increased (160.9 ± 57.8 at T24 vs 143.1 ± 38.5 at T0), while SF-36 physical (PCS) and mental scores (MCS) were not significantly changed (PCS, 40.1 ± 10.8 at T24 vs 41 ± 9.2 at T0; MCS: 39.1 ± 12 at T24 vs 33.7 ± 11.3 at T0, Fig. 4A, B, C). Although there was a clinically relevant trend of improvement

TABLE 1. Descriptive Characteristics of the Study Population at Enrollment

| ID	Gender	Age	Stenosis	Proctitis	Ileitis	Ostomy	CD diagnosis (years)	Surgery	Fistulectomy (nr)	Flap (nr)	Plug (nr)	Biological Agents (months of therapy)	Ongoing Therapy	Fistula Type	PDAI
1	M	22	n	n	y	n	7	n	y (4)	y (2)	y (1)	IFX (43)	ASA, AZA	posterior horseshoe transphincteric	7
2	F	47	y	n	n	n	23	n	y (4)	y (2)	n	IFX (21), Vedolix	n	posterior horseshoe transphincteric	9
3	M	31	n	n	n	n	10	n	y (2)	y (2)	y (1)	Adalim (39)	ASA	posterior horseshoe transphincteric	8
4	M	47	n	n	y	n	13	n	y (1)	y (2)	n	Vedoliz	Steroids	linear transphincteric	7
5	M	29	n	n	n	y	13	subtotal colectomy diverting ileostomy	y (2)	n	n	IFX (30), Adalim (4)	n	posterior horseshoe transphincteric	5
6	F	34	n	n	y	n	11	n	y (3)	y (1)	n	IFX (3)	ASA	linear transphincteric	3
7	F	45	n	n	y	n	23	ileocecal resection	y (3)	y (3)	n	IFX (36), Adalim (12)	n	posterior horseshoe transphincteric	8
8	F	55	y	y	n	n	38	intestinal resection ileocecal resection subtotal colectomy	y (1)	y (1)	n	IFX (10), Adalim (36)	ASA	posterior horseshoe transphincteric	8
9	M	33	n	y	n	y	13	intestinal resection subtotal colectomy diverting ileostomy	y (5)	y (1)	n	Adalim (68)	n	linear transphincteric	5
10	F	27	n	n	n	n	5	n	y (1)	y (1)	n	Adalim (12)	n	linear transphincteric	7
11	F	56	n	n	y	n	34	abdominal surgery	y (1)	y (1)	n	Adalim (12)	ASA, AZA	linear transphincteric	9
12	M	32	n	n	y	n	11	n	y (3)	y (1)	y (1)	IFX (24), Adalim (16)	AZA	linear transphincteric	7
13	F	35	n	n	n	y	15	ileocecal resection subtotal colectomy diverting ileostomy	y (2)	y (2)	n	Vedoliz	n	linear transphincteric	9
14	F	58	y	n	n	n	12	n	y (2)	n	n	Adalim (84)	n	linear transphincteric	12
15	M	51	n	y	n	n	26	n	y (1)	n	n	Adalim (80)	n	linear intersphincteric	8

M, male; F, female; n, no; y, yes; nr, number; IFX, infliximab; Adalim, adalimumab; Vedoliz, vedolizumab; ASA, mesalazine; AZA, azathioprine.

TABLE 2. Radiological Features of the Population at Enrollment (T0) and at Final Follow-up (T24)

ID	T0 MRI Fistula	T0 MRI Fistula Type	T0 External Draining Openings	T0 T2 Hyperintensity	T0 MRI Abscesses >0.3 mm	T24 External Draining openings	T24 T2 Hyperintensity	T24 MRI Abscesses >0.3 mm	Combined Remission
1	1 branching	transphincteric	2	moderate	absent	0	absent	absent	yes
2	1 branching	transphincteric	2	high	absent	1	moderate	absent	no
3	1 branching	transphincteric	2	absent	absent	1	moderate	absent	no
4	1 linear	transphincteric	1	high	absent	0	absent	absent	yes
5	1 branching	transphincteric	2	high	present	0	absent	absent	yes
6	1 linear	transphincteric	1	moderate	absent	0	absent	absent	yes
7	1 branching	transphincteric	2	moderate	present	0	absent	absent	yes
8	1 branching	transphincteric	2	moderate	present	0	absent	absent	yes
9	1 linear	transphincteric	1	moderate	absent	1	moderate	absent	no
10	1 linear	transphincteric	1	moderate	present	0	absent	absent	yes
11	1 linear	transphincteric	1	moderate	present	0	absent	absent	yes
12	1 linear	transphincteric	1	high	absent	1	moderate	present	no
13	1 linear	transphincteric	1	moderate	absent	0	absent	absent	yes
14	1 linear	transphincteric	1	high	present	1	high	present	no
15	1 linear	intersphincteric	1	high	absent	0	absent	absent	yes

observed in these parameters in the combined remission group (Table 3), the changes were not statistically significant due to the small sample size ($P > 0.05$). For this reason, the potential correlations between effectiveness and specific patient characteristics (age, gender, previous therapy with biologics, steroids, mesalazine and/or azathioprine, and presence of ileostomy diversion, proctitis, ileitis, oostomy) were evaluated but were not significant, except for anal stenosis, which seemed an unfavorable factor ($P = 0.018$). Indeed, 3 patients (60%) that did not reach combined remission presented with anal stenosis at enrollment.
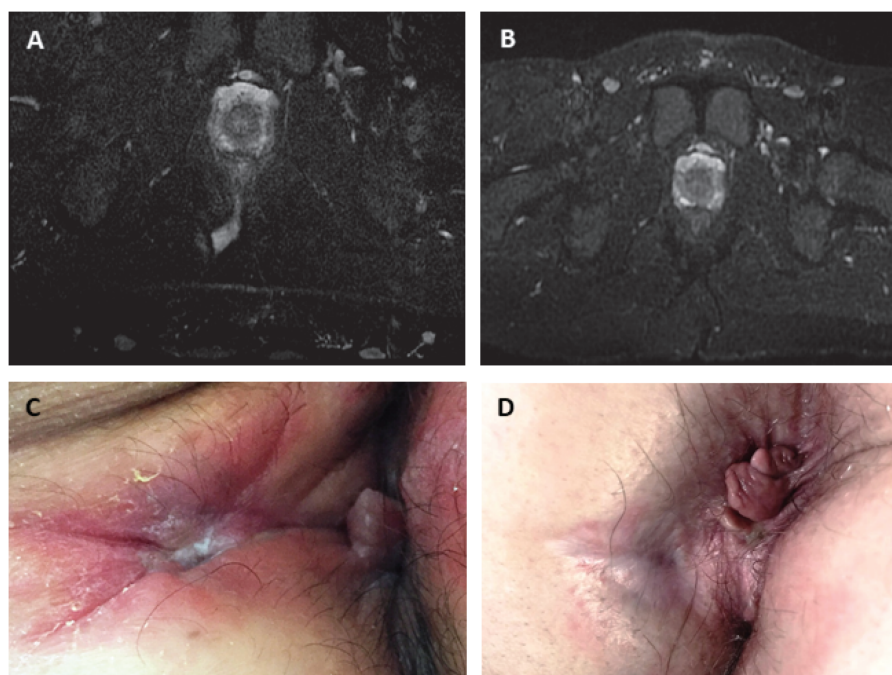
FIGURE 3. Clinical case example of a combined remission patient. D.A., male, 21 years old. MRI (A, B) and digital photograph (C, D) of the external fistula orifice. A, C, Pre-op; B, D, Six months post-op.
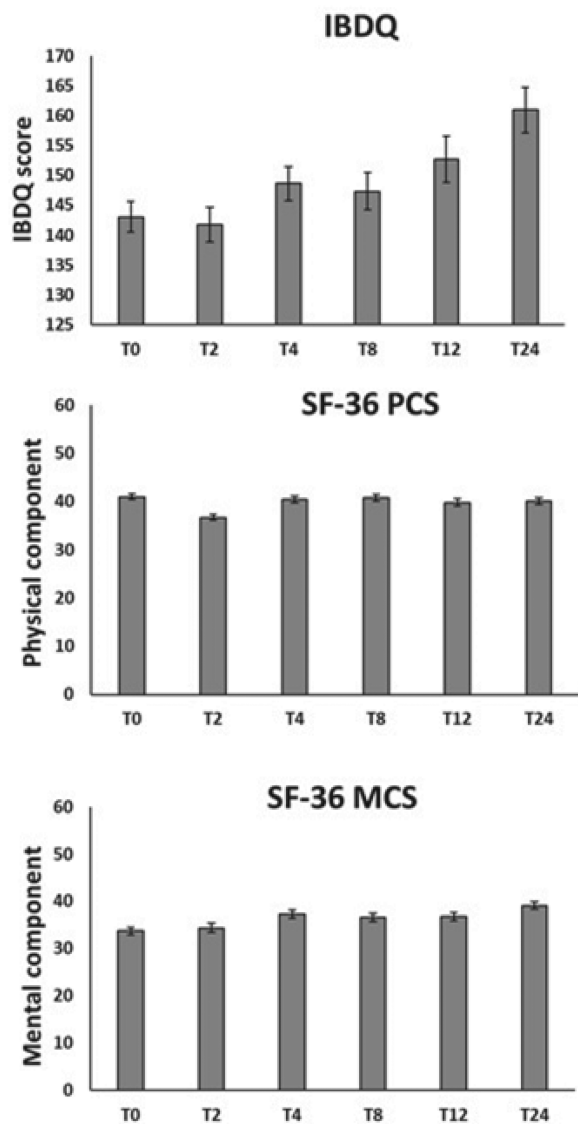
IBDQ

SF-36 PCS

SF-36 MCS

FIGURE 4. Trend of improvement of the health-related quality of life from baseline to T24 (24 weeks after the procedure). A, Inflammatory Bowel Disease Questionnaire (IBDQ) score; B, Short-Form 36 physical component score (PCS); C, Short-Form 36 mental component score (MCS). Results are expressed as mean and standard error. Black bars are combined remission patients; gray bars are noncombined remission patients.

Neither intra- nor postoperative major complications occurred. All patients, except 1 with hemorrhage from the perianal surgical wound conservatively resolved with suture placement, were discharged the day after the procedure. Only minor complications relating to the lipoaspiration procedure were recorded. In particular, subcutaneous hematoma occurred in about 20% of the patients, but none of these required additional treatments, and all spontaneously resolved in a few weeks.

TABLE 3. Summary of the Results

	Healed (n = 10)	Not Healed (n = 5)
CRP (mg/dL)		
Baseline	1.8 ± 1.0	1.6 ± 1.2
Week 24	1.3 ± 1.1	0.6 ± 0.5
Change from baseline	−0.5 ± 1.0	−1.0 ± 1.2
ESR (mm)		
Baseline	28.3 ± 11.5	23.4 ± 16.1
Week 24	16.9 ± 9.2	24.0 ± 21.5
Change from baseline	−11.4 ± 10.7	0.6 ± 24.8
PDAI		
Baseline	7.1 ± 1.9	8.2 ± 2.6
Week 24	2.9 ± 1.9	7.2 ± 2.2
Change from baseline	−4.2 ± 1.8	−1.0 ± 1.0
IBDQ		
Baseline	150.0 ± 33.9	129.2 ± 47.2
Week 24	175.3 ± 59.5	132.2 ± 46.8
Change from baseline	25.3 ± 45.3	3.0 ± 21.6
SF-36 PCS		
Baseline	42.8 ± 8.7	37.4 ± 10.2
Week 24	41.2 ± 11.4	37.8 ± 10.3
Change from baseline	−1.6 ± 7.8	0.4 ± 4.8
SF-36 MCS		
Baseline	36.4 ± 8.2	28.4 ± 15.7
Week 24	42.3 ± 10.3	32.8 ± 13.8
Change from baseline	5.9 ± 7.8	4.4 ± 10.6

Data are expressed as mean ± standard deviation. Healed, combined remission patients; not healed, patients who did not reach combined remission.

DISCUSSION

In this pilot study we report, for the first time, the successful use of autologous microfragmented adipose tissue, obtained with a nonenzymatic method in a closed system for the treatment of complex refractory fistulizing PCD.

Microfragmented adipose tissue injection has been recently tested in a series of 19 patients with complex idiopathic anal fistulas as first-line therapy or as a "rescue" therapy after the failure of surgical repair. The authors report a 73.7% healing rate, defined as "closure of the internal and external opening without any discharge." It has to be highlighted, however, that the injection of microfragmented adipose tissue was associated with the surgical closure of the internal opening by means of an endoanal mucosal flap.[26]

The 66.7% rate of complete healing we obtained with a single administration of microfragmented adipose tissue is quite encouraging and comparable to data previously described in the literature using different approaches. Our results are noteworthy if we consider the extremely challenging pool of selected patients, who were already treated without success using combined biosurgical approach and subsequent multiple repair surgeries.

To date, the first-line treatment for complex fistulizing PCD relies on combined surgical and medical therapy with biological drugs administered both systemically or locally, as confirmed in a number of controlled trials with healing rates ranging between 46% and 75%.[5, 31, 33–37] Nevertheless, the treatment of patients in which complete closure cannot be achieved despite the combination of biological treatment and surgery is not yet well defined. These patients may benefit from innovative therapeutic approaches exploiting mesenchymal stromal cells (MSCs)[8–12] because these cells have the capacity to serve as a "site-regulated drug store," inducing a reduction in inflammation and the activation of a reparative mechanism.[13, 14] Intra-fistula administration of autologous MSCs derived both from bone marrow[9, 38] and adipose tissue[8, 10–12, 39, 40] has been used, without side effects, in the treatment of refractory PCD in phase 2 and 3 clinical trials. Garcia-Olmo et al combined fibrin glue with adult adipose-derived MSCs (ASCs) achieving short-term success in more than 70% of cases, decreasing in the long-term to 58%.[11, 41] In a subsequent phase 3 randomized controlled trial conducted in several centers throughout Spain, the group confirmed favorable results for the ASC-enhanced fibrin glue only from the main author's pioneer center.[42] Using direct injection of bone marrow MSCs, Ciccocioppo et al achieved not only local success but also attenuation of the systemic inflammation.[9, 38] In a series of 10 patients with CD treated with ASC infiltration, Cho et al obtained a successful complete fistula closure rate of 44.4%.[8] Recently, the ADMIRE CD Group held a phase 3 randomized double-blind, placebo-controlled trial for the treatment of complex PCD in patients who did not respond to conventional and/or biological treatment. Panés et al showed a 50% rate of combined remission at 24 weeks (clinical assessment of closure of all treated external openings and absence of collections >2 cm of the treated perianal fistulas confirmed by masked central MRI) after a single local administration of allogenic expanded ASCs (Cx601), combined with the closure of the internal opening with polyglactin absorbable 2/0 stitches.[43]

Despite the encouraging results, the technique adopted in all of these trials required several weeks for in vitro cell expansion with a portion of cultures lost due to infection. This represents a costly and time-consuming approach that causes limitations in daily clinical practice. Compared with ASCs, microfragmented adipose tissue does not require any enzymatic treatment that inevitably destroys the stromal vascular niches. Moreover, the use of ASCs implies complex restrictions related to cell expansion and extensive manipulation.[18, 19] In this context, the technique we selected in this study potentially overcomes all the aforementioned issues because it is 1-step, minimally invasive, and compliant with the regulatory panorama. The injected microfragmented adipose tissue has been extensively studied and characterized in vitro by other authors.[22, 44] The findings show that it contains an abundant number of cells in their intact native niche able to secrete a variety of bioactive molecules that act through a paracrine mechanism to prime and sustain angiogenic, anti-fibrotic, anti-apoptotic, and immunomodulatory responses in the target tissue.

The occurrence of treatment-related abscesses, due to a "false" closure of the fistula tract, is a concern among patients with PCD receiving short- or long-term infliximab treatment, when the definition of "fistula healing" is based only on the clinical examination and defined as "absence of drainage upon gentle finger compression at the external orifice;" recurrences were reported in 12%[45] and 11%[46] of cases, respectively. Similarly, Rasul et al demonstrated that while 5 mg/kg of infliximab produced clinical remission in 49% of the patients at 8 weeks, complete radiological healing occurred in only 6% of the patients.[47] In the long-term retrospective evaluation of MSC and fibrin glue,[41] no correspondence between MRI findings and the clinical fistula status was detected independently from the treatment, most likely because of a closure limited only to the cutaneous opening but not to the entire fistula tract. Although the PDAI provides an assessment of the severity of PCD, it does not specifically assess the severity of perianal fistulas. Additionally, a limitation of the PDAI is that it can be influenced by luminal symptoms because 2 out of its 5 domains are not specifically perianal. For these reasons, we assessed the healing combining robust clinical[31] and radiological MRI evaluation to have a 100% accuracy.[43, 48]

The fact that no patient reported treatment-related abscesses is important and confirms the effectiveness of the combination of surgical drainage with fistulectomy and the regenerative therapy approach. The injection was performed after sanitization of the perineum in the mucosa surrounding the internal orifice and along the residual fistula tract. The very fluid fat obtained with the Lipogems device, which can easily pass through fine sharp needles (21 up to 25 G), can be distributed uniformly in the target tissues promoting the repair of the internal opening, which is the real source of the fistula, with likely subsequent closure of the entire tract.

Most importantly, no surgical repair technique at the internal opening was performed in combination with microfragmented fat injection; thus, the results are awarded only to the tested procedure. Due to the small sample size, it was not possible to identify any factor associated with an unsuccessful outcome. The presence of a diverting stoma, mild proctitis, or jejuno-ileitis did not significantly influence the outcome. Only anal stenosis, due to postoperative scarring or inflammation, resulted in the significant decrease in the possibility of healing; indeed, 3 of the 5 patients who did not obtain complete closure presented with anal substenosis at enrollment. This finding is consistent with previous studies defining anal-canal stricture, particularly when associated with suppurative disease, as a bad prognostic indicator of the likelihood of preserving intestinal continuity.[4]

Furthermore, because complex fistulizing PCD has a negative impact on health-related quality of life, we used 2

validated questionnaires to address personal implications, one disease-specific (IBDQ) and the other generic (SF-36). Anal pain and discomfort are unfavorable factors compromising the quality of life, and self-reported depressive symptoms are frequently concomitantly observed, underlining the negative impact on quality of life.[49] Furthermore, patients with active perianal fistula report a higher rate of work disability because of their disease, which may result in great economic burden.[50] We have demonstrated here that, after microfragmented fat injection, the IBDQ scores improved in the healed patients when compared with pre-operative condition, even though the change was not dramatic because of the small sample size and possibly because the questionnaires were administered too early after treatment. Perhaps the administration also at 12 and 24 months could have shown a more pronounced positive impact on the quality of life due to a stable remission of the perianal disease.

Finally, yet of no less importance, there were no side effects related to adipose tissue harvesting and injection and/or surgical treatment. We recorded only minor bleeding from the surgical wound within 12 hours post-op in 1 patient, which needed sutures placement.

This is certainly only a preliminary experience dealing with a small number of patients without a control group. Nevertheless, based on our clinical experience, these patients had no alternative treatment other than maintaining a seton with significant impairment in their quality of life or undergoing proctectomy with permanent oostomy.

CONCLUSIONS

The local injection of autologous microfragmented adipose tissue for the treatment of complex fistulizing PCD is simple, feasible, and safe. The routine employment of this technique could have major implications in those patients where recurrent, refractory and complex perianal fistulas usually require repeated surgery with the risk of sphincteric damage and fecal incontinence and, eventually, the subsequent need of proctectomy and permanent oostomy. By contrast, the injection of microfragmented adipose tissue is minimally invasive with less risk of sphincteric damage and can be carried out in day-surgery setting.

Although there are certainly limitations of small sample size and the lack of a control group, these results confirm that the technique is feasible and safe. This approach represents a promising treatment option for patients with complex fistulizing PCD. Confirmation of these data can lead to a decrease of costs of long-term treatment and eventual need for subsequent major surgery, work absenteeism, hygiene products, and psychological counselling. However, further research is required on a larger number of patients, which would allow confirmation of clinical efficacy and could possibly widen the range of indications.

REFERENCES

1. Gionchetti P, Dignass A, Danese S, et al.; ECCO. 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: part 2: surgical management and special situations. *J Crohns Colitis.* 2017;11:135–149.
2. Peyrin-Biroulet L, Loftus EV Jr, Colombel JF, et al. The natural history of adult Crohn's disease in population-based cohorts. *Am J Gastroenterol.* 2010;105:289–297.
3. Mueller MH, Geis M, Glatzle J, et al. Risk of fecal diversion in complicated perianal Crohn's disease. *J Gastrointest Surg.* 2007;11:529–537.
4. Galandiuk S, Kimberling J, Al-Mishlab TG, et al. Perianal Crohn disease: predictors of need for permanent diversion. *Ann Surg.* 2005;241:796–801; discussion 801.
5. Bouguen G, Siproudhis L, Gizard E, et al. Long-term outcome of perianal fistulizing Crohn's disease treated with infliximab. *Clin Gastroenterol Hepatol.* 2013;11:975–981.e1.
6. Senéjoux A, Siproudhis L, Abramowitz L, et al.; Groupe d'Etude Thérapeutique des Affections Inflammatoires du tube Digestif [GETAID]. Fistula plug in fistulising ano-perineal Crohn's disease: a randomised controlled trial. *J Crohns Colitis.* 2016;10:141–148.
7. Marchesa P, Hull TL, Fazio VW. Advancement sleeve flaps for treatment of severe perianal Crohn's disease. *Br J Surg.* 1998;85:1695–1698.
8. Cho YB, Lee WY, Park KJ, et al. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transplant.* 2013;22:279–285.
9. Ciccocioppo R, Bernardo ME, Sgarella A, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut.* 2011;60:788–798.
10. De la Portilla F, Alba F, Garcia-Olmo D, et al. 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.
11. Garcia-Olmo D, Herreros D, Pascual I, 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.
12. Lee WY, Park KJ, Cho YB, 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.
13. Caplan AI, Correa D. The MSC: an injury drugstore. *Cell Stem Cell.* 2011;9:11–15.
14. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem.* 2006;98:1076–1084.
15. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002;13:4279–4295.
16. Aronowitz JA, Lockhart RA, Hakakian CS. Mechanical versus enzymatic isolation of stromal vascular fraction cells from adipose tissue. *Springerplus.* 2015;4:713.
17. Bora P, Majumdar AS. Adipose tissue-derived stromal vascular fraction in regenerative medicine: a brief review on biology and translation. *Stem Cell Res Ther.* 2017;8:145.
18. Ahrlund-Richter L, De Luca M, Marshak DR, et al. Isolation and production of cells suitable for human therapy: challenges ahead. *Cell Stem Cell.* 2009;4:20–26.
19. Sensebé L, Bourin P, Tarte K. Good manufacturing practices production of mesenchymal stem/stromal cells. *Hum Gene Ther.* 2011;22:19–26.
20. Oberbauer E, Steffenhagen C, Wurzer C, et al. Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: current state of the art. *Cell Regen (Lond).* 2015;4:7.
21. Gutowski KA; ASPS Fat Graft Task Force. Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. *Plast Reconstr Surg.* 2009;124:272–280.
22. Bianchi F, Maioli M, Leonardi E, et al. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. *Cell Transplant.* 2013;22:2063–2077.
23. Benzi R, Marfia G, Bosetti M, et al. Microfractured lipoaspirate may help oral bone and soft tissue regeneration: a case report. *CellR4.* 2015;3:e1583.
24. Cattaneo G, De Caro A, Napoli F, et al. Micro-fragmented adipose tissue injection associated with arthroscopic procedures in patients with symptomatic knee osteoarthritis. *BMC Musculoskelet Disord.* 2018;19:176.
25. Giori A, Tremolada C, Vailati R, et al. Recovery of function in anal incontinence after micro-fragmented fat graft (Lipogems) injection: two years follow up of the first 5 cases. *CellR4.* 2015;3:e1544.
26. Naldini G, Sturiale A, Fabiani B, et al. Micro-fragmented adipose tissue injection for the treatment of complex anal fistula: a pilot study accessing safety and feasibility. *Tech Coloproctol.* 2018;22:107–113.
27. Raffaini M, Tremolada C. Micro fractured and purified adipose tissue graft (Lipogems) can improve the orthognathic surgery outcomes both aesthetically and in postoperative healing. *CellR4.* 2014;2:e1118.
28. Russo A, Screpis D, Di Donato SL, et al. Autologous micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis: an update at 3 year follow-up. *J Exp Orthop.* 2018;5:52.

29. Zeira O, Scaccia S, Pettinari L, et al. Intra-articular administration of autologous micro-fragmented adipose tissue in dogs with spontaneous osteoarthritis: safety, feasibility, and clinical outcomes. *Stem Cells Transl Med.* 2018;7:819–828.

30. Sandborn WJ, Fazio VW, Feagan BG, et al.; American Gastroenterological Association Clinical Practice Committee. AGA technical review on perianal Crohn's disease. *Gastroenterology.* 2003;125:1508–1530.

31. Poggioli G, Laureti S, Pierangeli F, et al. Local injection of infliximab for the treatment of perianal Crohn's disease. *Dis Colon Rectum.* 2005;48:768–774.

32. Van Assche G, Vanbeckevoort D, Bielen D, et al. Magnetic resonance imaging of the effects of infliximab on perianal fistulizing Crohn's disease. *Am J Gastroenterol.* 2003;98:332–339.

33. Asteria CR, Ficari F, Bagnoli S, et al. 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.

34. El-Gazzaz G, Hull T, Church J. Biological immunomodulators improve the healing rate in surgically treated perianal Crohn's fistulas. *Colorectal Dis.* 2012;14:1217–1223.

35. Haennig A, Staumont G, Lepage B, et al. The results of seton drainage combined with anti-tnfα therapy for anal fistula in Crohn's disease. *Colorectal Dis.* 2015;17:311–319.

36. Poggioli G, Laureti S, Pierangeli F, et al. Local injection of adalimumab for perianal Crohn's disease: better than infliximab? *Inflamm Bowel Dis.* 2010;16:1631.

37. 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.

38. Ciccocioppo R, Gallia A, Sgarella A, et al. *Long-Term Follow-Up of Crohn Disease Fistulas After Local Injections of Bone Marrow–Derived Mesenchymal Stem Cells.* Mayo Clinic Proceedings, Vol. 90: Philadelphia, Pennsylvania: Elsevier, 2015, 747–755.

39. García-Olmo D, García-Arranz M, García LG, et al. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int J Colorectal Dis.* 2003;18:451–454.

40. García-Olmo D, García-Arranz M, Herreros D, et al. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum.* 2005;48:1416–1423.

41. Guadalajara H, Herreros D, De-La-Quintana P, et al. Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. *Int J Colorectal Dis.* 2012;27:595–600.

42. Herreros MD, Garcia-Arranz M, Guadalajara H, et al.; FATT Collaborative Group. Autologous expanded adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1: fistula advanced therapy trial 1) and long-term evaluation. *Dis Colon Rectum.* 2012;55:762–772.

43. Panés J, García-Olmo D, Van Assche G, et al. Long-term efficacy and safety ofstem cell therapy (Cx601) for complex perianal fistulas in patients with Crohn's disease. *Gastroenterology*. 2017;154:1334–1342.

44. Ceserani V, Ferri A, Berenzi A, et al. Angiogenic and anti-inflammatory properties of micro-fragmented fat tissue and its derived mesenchymal stromal cells. *Vasc Cell.* 2016;8:3.

45. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med.* 2004;350:876–885.

46. Present DH, Rutgeerts P, Targan S, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med.* 1999;340:1398–1405.

47. Rasul I, Wilson SR, MacRae H, et al. Clinical and radiological responses after infliximab treatment for perianal fistulizing Crohn's disease. *Am J Gastroenterol.* 2004;99:82–88.

48. Schwartz DA, Wiersema MJ, Dudiak KM, et al. A comparison of endoscopic ultrasound, magnetic resonance imaging, and exam under anesthesia for evaluation of Crohn's perianal fistulas. *Gastroenterology.* 2001;121:1064–1072.

49. Mahadev S, Young JM, Selby W, et al. Self-reported depressive symptoms and suicidal feelings in perianal Crohn's disease. *Colorectal Dis.* 2012;14:331–335.

50. van der Valk ME, Mangen MJ, Severs M, et al.; COIN study group and the Dutch Initiative on Crohn and Colitis. Evolution of costs of inflammatory bowel disease over two years of follow-up. *PLoS One.* 2016;11:e0142481.