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Stromal vascular fraction with platelet-rich plasma injection during surgery is feasible and safe in treatment-refractory perianal fistulising Crohn's disease: A pilot study

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Summary

Background: An unmet need remains for improved management in perianal fistulising Crohn's disease (pCD). Recently, local administration of adipose-derived cells has shown promising results.

Aims: To assess the safety and feasibility of injection of stromal vascular fraction (SVF) with platelet-rich plasma (PRP) in patients with pCD.

Methods: Patients ≥ 18 years with pCD were included and underwent fistula curettage, SVF with PRP injection, and closure of the internal opening. The primary endpoint was safety at 12 months. The secondary outcomes were complete radiological healing at 3 months (absence of fluid-containing tracts on MRI) and partial and complete clinical response at 3 and 12 months (closure of ≥1, respectively, all treated external opening(s)).

Results: Twenty-five patients were included (35 [IQR 25–40] years; 14 [56%] female); median CD duration 4 [IQR 2–8] years. Twenty-four (95%) patients had previously undergone fistula surgery. No adverse events were encountered at lipoharvesting sites. Two (8%) patients were readmitted to hospital and six (24%) underwent unplanned re-interventions. Post-operative MRI (n = 24) showed complete radiological healing in nine (37.5%) patients. Partial clinical response was present in 48% (12/25) at 3 months and in 68% (17/25) at 12 months, and complete clinical closure in five (20%) patients at 3 months and in 10 (40%) patients at 12 months.

Conclusion: Injection with autologous SVF with PRP is feasible and safe in patients with treatment-refractory pCD. Early complete radiological healing was observed in more than one-third of patients, and clinical response in two-thirds of patients at 12 months.

Clinical trial registration: Dutch trial registry NL8417.

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1 | INTRODUCTION

Perianal fistula represent a common and debilitating complication of Crohn's disease (CD) and affect up to 30% of CD patients.[1] Perianal fistulising CD (pCD) is associated with significant morbidity, resulting in reduced quality of life and increased healthcare costs.[2,3] The majority of CD perianal fistulas are complex, often resulting in refractory disease with long-term remission rates as low as 37%.[4,5] The current, most effective pCD treatment is a combined medical and surgical approach, including systemic therapy with anti-tumour necrosis factor (anti-TNF) agents.[6] Surgical procedures include curettage, primary closure, fistulotomy, mucosal advancement flap, ligation of the intersphincteric fistula tract (LIFT) and laser therapy.[7,8] Nevertheless, available options are limited by the risk of faecal incontinence, diminished wound healing and high failure rates.[8,9] To date, major surgical procedures such as colostomy, and even proctectomy, are inevitable in 10%–20% of patients with complex pCD.[10–12]

Novel therapies to improve fistula wound healing are under development, including the use of mesenchymal stromal cells derived from either bone-marrow or adipose tissue (adipose-derived stromal cells [ADSCs]).[12,13] More recently, ex vivo expanded allogenic ADSCs have shown to improve healing rates by approximately 17% when compared with surgery alone in patients with treatment-refractory pCD.[14] However, allogenic cell therapies are associated with potential risks, such as antibody development,[13] and can cost up to €70,500 per treatment.[15]

In addition to ADSCs, stromal vascular fraction (SVF) can be obtained from subcutaneous lipoaspirate through either enzymatic digestion or mechanical fractionation.[16,17] SVF consists of a heterogeneous population of cells including ADSCs, endothelial and adipogenic progenitor cells, leucocytes, macrophages and other stromal cells.[18] Like isolated, expanded ADSCs, SVF possesses immunomodulatory and wound-healing properties as shown in pre-clinical studies and has the ability to augment clinical dermal wounds.[19,20]

Platelet-rich plasma (PRP) is a well-known autologous product that promotes wound healing in various surgical defects.[21] It is derived from the centrifugation of peripheral blood and contains high concentrations of growth factors which promote tissue healing.[22]

A multimodal approach is needed to achieve effective pCD treatment, and treatment should be directed towards multiple pathophysiological factors, including obliterating the dead space, removal of epithelialisation and altering the pro-inflammatory environment which allow fistula to persist.[23] The combination of autologous non-cultured SVF with PRP may provide a unique treatment modality to suppress chronic inflammation and promote local tissue repair and has shown promising results in cryptoglandular fistulas.[24,25] Therefore, this study aimed to assess the safety and feasibility of local injection with adipose-derived SVF with PRP in patients with treatment-refractory pCD.

2 | MATERIALS AND METHODS

2.1 | Study design

Consecutive patients with pCD undergoing fistula surgery were enrolled in this prospective, non-randomised, pilot cohort study after informed consent between March 2019 and August 2020. Patients (≥18 years) were eligible if they had treatment-refractory pCD, which was defined as perianal fistulising disease with previously endoscopic or histological confirmed CD, and shown to be refractory to at least one of the following medical or surgical treatments: immunomodulators, anti-TNFα agents, and/or (re)operation. Exclusion criteria were rectovaginal fistula, active proctitis, presence of associated (not properly drained) pelvic abscess, severe active luminal CD at time of surgery, haematological disorders, and/or any oncological event in the past 5 years.

All patients underwent standardised fistula curettage, closure of the internal opening with sutures combined with SVF with PRP injection. At 3 months, postoperative magnetic resonance imaging (MRI) of the fistula was performed and centrally assessed by a blinded radiologist. From 3 months postoperative, repeated treatment with SVF with PRP was considered in patients with clinical and/or radiological suspicion of residual fistulising disease.

2.2 | Clinical data collection

Data were collected at a preoperative visit, at time of fistula surgery and at visits 6 weeks, 3, 6 and 12 months after surgery and included clinical data and physical examination. The collected data consisted of patient-related characteristics (e.g. age and smoking), disease-related characteristics (e.g. medication exposure prior to surgery and disease duration), and surgical characteristics (e.g. length of stay and postoperative complications).

2.3 | Endpoints

The primary endpoint was feasibility and safety. Safety was assessed by recording treatment-emergent (serious) adverse events through month 12. Fistula-related emergency room (ER) visits within 30 days (including fever, perianal pain and/or abscess), fistula-related readmissions within 30 days (including fever, perianal pain and/or abscess), unplanned re-interventions due to worsening of pCD (including clinically significant abnormalities at physical examination or development of perianal sepsis) and mortality were documented. Anticipated complications after standard surgical fistula closure include postoperative bleeding, infection, perianal pain, recurrent abscess formation and anal fistula recurrence.[26]

Secondary endpoints included *radiological treatment response* on 3 months postoperative MRI, determined as the presence of one or more of the following items on postoperative MRI: the presence of predominantly fibrous fistula tracts, reduction in T2w hyperintensity, or

reduction in the extension of the fluid-containing tracts. Furthermore, *complete radiological healing*, defined as the complete absence of fluid-containing fistula tracts with the absence of T2-weighted (T2w) hyperintense signal at the location of the treated fistula was assessed.

Furthermore, *clinical response* (defined as closure of ≥1 treated external opening(s) combined with the absence of discharge at physical examination) and *complete clinical closure* (defined as complete closure of all treated external opening(s) at physical examination) were recorded at 3 and 12 months. All data concerned the treated fistula tract(s), new localisations were not registered in this study.

2.4 | MRI evaluation

Pelvic MRI was performed prior to the operative procedure and at 3 months after surgery, using a 1.5T system with a four-channel phased array coil. Field of view consisted of the lower pelvis, perineum and skin area with full display of the anus and lower-mid rectum. The MRI protocol consisted of T2w sequences in three planes: axial with and without fat saturation, sagittal and coronal. Fistulas were identified based on hyperintense (white) signal. An experienced abdominal radiologist (R.S.D.) scored the pre- and postoperative MRIs in random order and was blinded for the clinical outcome.

Imaging results were evaluated using the modified van Assche Index, an MRI-based score for the severity of pCD, partially validated in previous studies.[27,28] The modified van Assche index includes the following items: fistula extension, hyperintensity on T2w images, rectal wall involvement, presence of inflammatory mass and the dominant feature of the fistula tract.[28]

2.5 | Operative technique

All patients underwent standardised fistula curettage with closure of the internal opening combined with harvesting of subcutaneous lipoaspirate and venous blood sampling to obtain SVF with PRP respectively.[17] All procedures were performed by four colorectal surgeons (O.v.R., M.B.v.O.A., E.J.D.G. and W.R.S.). Surgeons were trained by an experienced colorectal surgeon (W.R.S.) and followed the surgical protocol reference guide for the preparation and injection of SVF with PRP.[17] Patients underwent induction of general anaesthesia, endotracheal intubation and received metronidazole (500 mg) and cefuroxime (2000 mg) intravenously. A Lone Star retractor (Lone Star Retractor System, Lone Star Medical Products®, Inc.) was used to expose the internal opening(s) of the fistula. Setons were removed, if present. The external opening(s) of the fistula was enlarged by coring out to the exterior of the external anal sphincter. If fistula curettage and closure of the internal opening were considered feasible, lipoaspiration for the harvesting of SVF was initiated. Due to the hypothetical risk of subcutaneous infection, liposuction was performed prior to fistula curettage or by renewed sterilisation of the operating field and team.

A small paravertebral skin incision was made bilaterally approximately 5 cm cranial to the posterior superior iliac spine. The subcutaneous adipose tissue was infiltrated with 20–40 ml 0.9% saline solution, containing 20 mg/mL lidocaine and 5 μg/ml adrenaline. By aspiration, 15 ml of lipoaspirate was harvested bilaterally with a double syringe system (Arthrex GMBH). Obtained lipoaspirate was centrifuged (2500 rpm, 5 min) and the fistula procedure was initiated. The internal opening(s) and fistula tract(s) were cleaned and brushed. Centrifugation of the lipoaspirate resulted in three separate fractions: oil, condensed fatty tissue and aqueous infiltration fluid. The lipoaspirate was decanted, removing the oil and fluid fractions. Mechanical fractionation of cellular components was performed by vigorously passing the lipoaspirate forwards and backwards 30 times through a disposable fractionator (⌀1.4 mm, luer-to-luer transfer, Tulip). After mechanical fractionation, samples were centrifuged (2500 rpm, 5 min), after which the oily fraction was removed, leaving approximately 1 ml SVF.[19] Simultaneously, 15 ml of whole blood was centrifuged (1500 rpm, 4 min), after which 4–5 ml PRP was obtained from the upper layer (plasma). PRP, harvested this way, contains approximately 5×10^8 platelets/ml.[29] A total of approximately 1 ml SVF and 5 ml PRP were combined in one syringe to form platelet-rich stroma (PRS). PRS was injected into the tissue surrounding the curated internal opening(s) within approximately 2 mm, and into all quadrants of the fistula wall along the fistula tract(s) in several micro-blebs. Subsequently, the internal opening was closed full-thickness with absorbable sutures 2/0 Vicryl (Ethicon, Inc.).

2.6 | Statistical analysis

Categorical variables were described as frequencies and percentages, whereas continuous variables were described using medians and interquartile ranges (IQR) or ranges for non-normally distributed variables. Wilcoxon-rank test was performed to compare paired preoperative and postoperative modified van Assche Indexes. A p-value of 0.05 was considered statistically significant. Statistical analyses were performed with IBM Statistical Packages for Social Sciences (SPSS) for Windows (IBM Corp., Armonk).

2.7 | Ethical consideration

This study was performed in accordance with the 2008 Declaration of Helsinki. This study was reviewed and approved by the local Medical Ethical Committee (trial number NL8417). Written informed consent was obtained from all subjects before enrolment.

3 | RESULTS

3.1 | Study population

In total, 25 consecutive CD patients with treatment-refractory perianal fistulas were included. Fourteen (56%) patients were female, the median age was 35 (interquartile range [IQR] 25–40) years. The

median time of follow-up was 12 (IQR 6–18) months. One patient was diagnosed with Crohn's disease after LIFT procedure with additional injection of SVF combined with PRP. This patient was also included in this study. Four (16%) patients were active smokers. All patients were tertiary referral patients, with a median duration of fistulising disease of 4 (range 2–8) years and a median CD duration of 11 (range 3–19) years (Table 1). Fistula had infralevatoric extensions in 10 (40%) patients, horseshoe configuration in nine (38%) patients and supralevatoric extensions in 6 (25%) patients at baseline MRI.

All patients had persistent treatment-refractory fistula. Twenty-three (92%) patients had previously been exposed to antibiotics, 15 (605%) patients to corticosteroids, 21 (84%) patients to thiopurines and 23 (92%) patients to biologicals. At time of surgery, 1 (4%) patient used an immunomodulator for CD treatment, 20 (80%) patients used biological therapy (2 (4%) adalimumab, 5 (20%) infliximab, 1 (4%) vedolizumab, 2 (8%) ustekinumab) and 10 (40%) patients used immunomodulatory and biological combination therapy. Almost all patients underwent previous fistula surgery (24 [96%] patients), with a median number of prior procedures of 3 (range 1–12). One patient had been diverted by a colostomy prior to enrolment (Table 1).

3.2 | Surgical data

The median operating time was 70 (IQR 56–85) minutes. Some patients were enrolled with planned or unplanned staged repair as present fistula were considered too extensive to treat with 6 cc SVF with PRP. Additional subcutaneous seton placement was performed in four (16%) patients and malecot drain placement in one (4%) patient. At time of surgery, 10 (40%) patients had one external opening, 10 (40%) patients had two external openings and five (20%) had three external openings. The median length of hospital stay was 1 (range 0–3) days; 10 (40%) patients were treated in an outpatient surgery setting.

3.3 | Primary endpoint

3.3.1 | Feasibility

All patients had a successful liposuction procedure to harvest subcutaneous adipose tissue. The isolation of SVF from adipose tissue and PRP from whole blood samples was uneventful in all cases. Curettage of the fistula tract, closure of the internal opening and injection of the SVF with PRP mixture was successfully conducted in all patients.

3.3.2 | Safety

No adverse events were encountered at the lipoharvesting sites. The postoperative course was uneventful, except for three cases. One patient, who underwent LIFT procedure combined with SVF

TABLE 1 Baseline characteristics of patients with treatment-refractory perianal fistulising Crohn's disease who underwent SVF with PRP injection

	Study population ***N* = 25**
Age, years	35 (25–40)
Female	14 (66)
Smoking status at time of surgery	
Active	4 (16)
Previous	8 (32)
Never	13 (52)
CD duration years	11 [3–19]
Fistulising disease duration, year	4 [2–8]
Active luminal CD	
None	21 (84)
Ileum	1 (4)
Proximal colon	1 (4)
Sigmoid	2 (8)
Disease phenotype (montreal)	
Luminal (B1)	18 (72)
Stricturing (B2)	4 (16)
Penetrating (B3)	3 (12)
Disease localisation (montreal)	
Ileum (L1)	2 (8)
Colon (L2)	4 (16)
Ileocolonic (L3)	19 (76)
Previous fistula surgery	23 (92)
Number of previous fistula operations	3 [0–11]
Types of previous fistula surgery	
Fistulotomy	2 (8)
Drainage of abscess with seton or drain placement	9 (38)
Drainage of abscess without drain placement	15 (63)
Seton placement alone	17 (68)
Laser treatment	1 (4)
Diverting colostomy at time of surgery	1 (8)
Concomitant IBD medication at baseline	
Thiopurine	1 (4)
Anti-TNFα treatment[a]	7 (28)
Combination therapy[b]	10 (40)
Ustekinumab	2 (8)
Vedolizumab	1 (4)
None	4 (16)

Note: Values are *n* (%) or median (interquartile range). In case of missing data, valid percentages are presented.

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; anti-TNFα, anti-tumour necrosis factor alpha.

[a]Infliximab and/or adalimumab.

[b]Thiopurine combined with anti-TNFα medication.

with PRP injection, developed a wound infection. Two (8%) patients developed perianal haemorrhage needing additional wound dressing. Fistula drains were removed in all patients, only one subcutaneous seton resided for more than 3 months.

One (4%) patient was assessed at the ER for fistula-related complaints. Two (8%) patients were readmitted to the hospital and the number of readmissions ranged from 1 to 3. Six (24%) of patients underwent unplanned re-interventions within 12 months after surgery. The number of re-interventions per patient ranged from 1 to 4. The median time until the first re-intervention was 4.6 (IQR 3–9) months. In two (8%) patients, seton placement was performed. Incision and abscess drainage without seton placement was needed in four (16%) patients and incision and drainage with seton placement in three (12%) patients. No deaths occurred during the study.

3.4 | Secondary endpoints

3.4.1 | Radiological outcomes at 3 months postoperative MRI

Postoperative MRI was performed in 24 patients after a median time interval of 3.0 (IQR 2.4–5.3) months. In one patient, preoperative and postoperative imaging could only be obtained by CT scans due to contraindication for MRI. In total, 19 (79%) out of 24 patients showed imaging findings consistent with treatment response; 15 (63%) patients had a predominantly fibrous tract on postoperative MRI, 14 (58%) patients showed a marked reduction in T2w hyperintensity, and 10 (42%) patients showed a reduction in the degree of fistula extension (Figure 1). Complete radiological healing (absence of T2w hyperintensity) was seen in nine (38%) patients (Figure 2).

Postoperative MRI revealed fluid collections with a diameter >10 mm in only two out of 24 patients. In one of these patients, failure of treatment required faecal diversion by colostomy. The modified van Assche Index decreased significantly from a median of 11 (range 9–16) points at baseline to 6 (range 1–9) points on postoperative MRI ($p < 0.001$, Table 2 and Figure 3).

3.4.2 | Clinical outcome after SVF with PRP injection

Three months after the first SVF with PRP procedure, 12 (48%) patients showed clinical response (≥1 external opening closed), of which 5 (20%) patients had complete clinical closure. In all patients, clinical response and complete clinical closure were sustained until the end of follow-up of 12 months. One (4%) of the patients with clinical response at 3 months, achieved complete closure of the fistula tract at 12 months follow-up. Of the 13 patients without clinical response at 3 months, two (8%) additional patients achieved complete closure at 12 months without any further surgical intervention. This accumulates to an overall clinical response rate of 68% (17/25) at 12 months follow-up. The overall complete clinical closure rate was 40% (10/25). Comparing patients with and without anti-TNFα treatment, no difference was found in partial clinical closure rates (4/8 [50%] vs. 11/17 [64.7%], $p = 0.484$). Due to fistula healing, restoration of bowel continuity was performed in one patient with a diverting colostomy prior to inclusion.

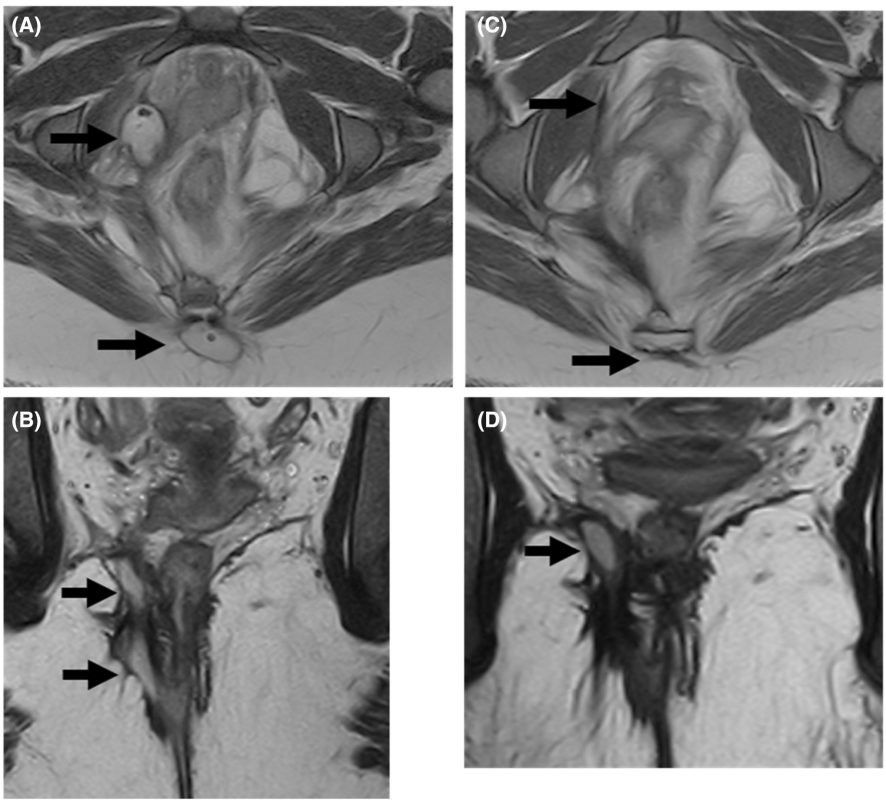

FIGURE 1 Treatment response on 3 months postoperative MRI after SVF combined with PRP treatment in one patient with treatment-refractory pCD with a high transsphincteric fistula. (A) Preoperative MRI, axial view showing two abscesses located supralevatoric and posterior of the coccygeal bone (arrows). (B) Preoperative MRI, coronal view showing a high-transsphincteric fistula on the right side. (C) Postoperative MRI, axial view showing complete resolution of both abscesses. (D) Postoperative MRI, coronal view showing remaining activity in the proximal part of the fistula tract.

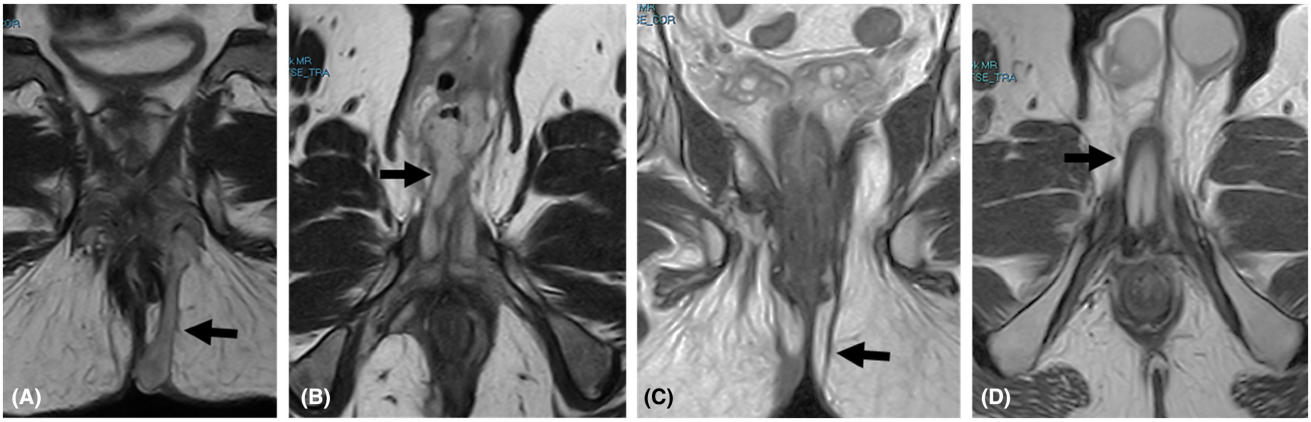

FIGURE 2 Complete radiological healing on 3 months postoperative MRI after SVF combined with PRP treatment in one patient with treatment-refractory pCD with a high transsphincteric fistula. (A, B) Preoperative MRI (coronal (A) and axial (B)) view showing a high transsphincteric fistula with perineal extensions (arrows). (C, D) postoperative MRI (coronal (C) and axial (D)) showing complete radiological healing with no residual fistula tracts.

3.4.3 | Clinical results after repeated SVF with PRP injection

During total follow-up, 11 (44%) patients underwent a second PRS procedure within 12 months. Of these patients, one (11%) patient showed partial clinical response and one (11%) patient showed complete clinical closure within 3–6 months after the repeated procedure and did not undergo further treatment. Four (55%) patients underwent a third PRS procedure, which resulted in partial clinical response in one (20%) patient 15 months after the primary PRS procedure. This patient refrained from further treatment. Two (40%) patients underwent a fourth procedure. Up to now, no additional clinical closure was observed after the fourth PRS treatment. The other patients refrained from repeated PRS treatment.

The one patient who underwent fistula surgery for the first time, did not show a better outcome after SVF with PRP injection, compared to patients who underwent fistula surgery before. This patient was previously exposed to multiple drugs, including immunomodulator/biological combination therapy, and had a suprasphincteric fistula tract. The fistula was only partially healed at 12 months, despite a second SVF with PRP procedure.

4 | DISCUSSION

Despite the current range of treatment options in pCD, an unmet need remains for improved management.[30–32] This pilot study shows that injection of autologous SVF with PRP in tertiary referral patients with treatment-refractory perianal fistulising CD is feasible and safe. No new type of adverse events occurred after autologous SVF with PRP treatment compared to the adverse events reported by Panes *et al.* for expanded allogenic ASCS treatment. Postoperative MRI showed a decrease in inflammatory features as well as an increase in fibrosis. Clinical response was seen in 68% at 12 months.

The use of autologous SVF with PRP obtained through mechanical fractionation of lipoaspirate, offers several advantages over recently developed, allogenic and autologous ADSCs treatments.[32,33] First, compared to off-the-shelf products such as allogenic expanded ADSCs, SVF with PRP is inexpensive.[14] Second, SVF with PRP has the advantage of harvesting and administration during one single procedure without the use of cell expansion, which could in theory affect cell viability and function.[32] It appears that survival of autologous MSC is superior compared to donor material[34] and that isolated ADSCs have a better differentiation potential as compared to culture-expanded cells.[35,36] Third, SVF with PRP provides an autologous product subsiding the potential risk of alloimmunity following MSC delivery, when considering repeated treatment.

Positioning of SVF and PRP in the treatment strategy for pCD remains to be determined. Compared to systemic therapies, such as anti-TNF agents, SVF with PRP has a favourable tolerability which could be due to the local application of the treatment. Despite this and above-mentioned advantages of the therapy, the clinical remission rates reported in this study seem somewhat lower than in other studies examining cell therapies in pCD.[30,37] It should be noted that all our patients were tertiary referred and presented with complex, treatment-refractory fistulas. For example, our study included more patients with ≥2 external openings (60%) compared to the landmark trial by Panes et al (44%). Furthermore, heterogeneity in study inclusion criteria, cell origin, dose and frequency of delivery, use of scaffolding and definition and time point of fistula healing, make it difficult to truly compare data between studies.[32] However, although definitions of clinical response were slightly different, more than two-thirds of patients showed clinical response after darvadstrocel treatment (66% reported by Panes et al, defined as ≥50% of all treated external openings), as well as after SVF and PRP injection (68% (17/25 patients)). All in all, this pilot study focused on feasibility and safety and head-to-head studies are needed to determine the absolute effect of SVF with PRP as compared to registered therapies.

TABLE 2 Comparison of the individual items of the modified van Assche index at baseline and 3 months postoperative in patients with treatment-refractory pCD who underwent SVF with PRP injection

	Baseline MRI	Postoperative MRI	*p*-value[a]
Extension			
Absent	0	9 (37.5)	0.003[b]
Infralevatoric	9 (37.5)	6 (25.0)	
Horseshoe	8 (33.3)	4 (16.7)	
Supralevatoric	7 (29.2)	5 (20.8)	
Hyperintensity on T2w			
None	0	9 (37.5)	0.001[b]
Mild	13 (54.2)	12 (50.0)	
Pronounced	11 (45.8)	3 (12.5)	
Rectal wall involvement			
Normal	11 (45.8)	9 (37.5)	0.739
Thickened	4 (16.7)	9 (37.5)	
Increased signal activity	9 (37.5)	6 (25.0)	
Inflammatory mass			
Absent	6 (25.0)	18 (75.0)	0.001[b]
Diffuse	2 (8.3)	4 (16.7)	
Focal	6 (25.0)	1 (4.2)	
Collection small	3 (12.5)	1 (4.2)	
Collection medium	4 (16.7)	0	
Collection large[c]	3 (12.5)	1 (4.2)	
Dominant feature			
Fibrous	6 (25.0)	15 (62.5)	0.011[b]
Granulation tissue	0	1 (4.2)	
Fluid/pus	18 (75)	8 (33.3)	

Note: Values are *n* (%).

[a]Wilcoxon Signed rank test.

[b]Statistically significant.

[c]Operative drainage of abscess was performed prior to the SVF with PRP procedure.

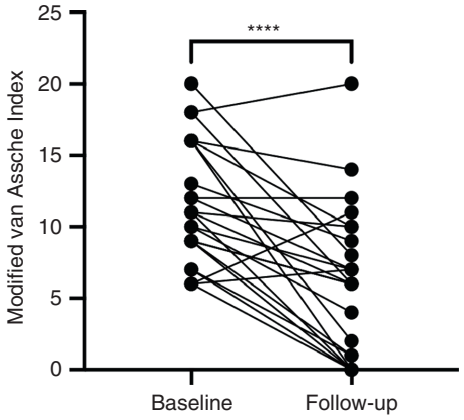

FIGURE 3 Comparison of the modified van Assche index at baseline and at 3 months postoperative follow-up MRI in treatment-refractory perianal fistulising Crohn's disease patients who underwent treatment with SVF combined with PRP. ****Statistically significant ($p < 0.05$).

Given previous observations that only the minority of complex perianal fistulas are in remission after a median follow-up of 10 years[5], the complete absence of active fistulas on postoperative MRI at 3 months in 38% of our patients is promising. In the recently published PISA-II trial, complete radiological healing in patients with anti-TNF therapy alone is merely 9% at 18 months, compared to 32% after anti-TNF treatment combined with surgical closure.[38] Furthermore, the PISA-II trial showed that clinical recurrence after initial healing only occurred in patients without radiological healing. However, the utility of MRI assessment in relation to clinical response remains to be determined.

The need for the addition of PRP to SVF remains unknown. The use of PRP alone as adjunct to fistula surgery appears to have limited additional value, as a randomised controlled trial examining this effect was prematurely ended due to insufficient results.[39] However, PRP is a rich source of growth factors, which promote wound healing and neovascularisation.[40] Furthermore, certain chemokines present in platelets (e.g. stromal-derived factor-1) are chemoattractant for ADSCs.[41–44] It is hypothesised that PRP might work as a cell scaffold to enhance and prolong the survival of stromal cells at the site of administration.[45,46] The additional value of PRP and the appropriate SVF to PRP ratio should be determined in future studies.

A few limitations of this study need consideration. First, as this is an explorative pilot study assessing feasibility and safety, no control group was used and the sample size was limited. Second, our study did not include patient reported outcomes. Third, MRI is the gold standard to identify perianal fistulas, but a validated tool to evaluate response to treatment is needed.[28] Furthermore, granulation tissue can have similar radiological appearance as pus, making it more difficult to assess healing rates on MRI. We overcame this issue by including clinical outcomes at physical examination in our study. It should be noted that not all hospitals have a dedicated radiologist available for fistula examination. Future studies are needed to determine whether deep-learning artificial intelligence technology is useful in determining clinical response. Lastly, it should be noted that the composition of autologous preparation of SVF with PRP might differ from donor to donor, similarly as all non-cultivated autologous MSCs, which potentially could in turn affect healing rates.[47] Basic research has confirmed the presence of stromal cells, endothelial-like cells, immune cells, T-cells and myeloid cells in SVF samples of CD patients; however, it does show interdonor variation in both IBD and non-IBD patients (manuscript in preparation).

In conclusion, this study shows that local injection with SVF with PRP during fistula surgery is feasible and safe in treatment-refractory perianal fistulising Crohn's disease. SVF with PRP is a promising therapy which could improve clinical and radiological healing rates. Further studies are required to establish the effectiveness of this therapy.

AUTHOR CONTRIBUTIONS

Oddeke van Ruler: Conceptualization (equal); data curation (equal); writing – original draft (equal); writing – review and editing (equal). **Roy S. Dwarkasing:** Data curation (equal); formal analysis (equal); writing – original draft (equal); writing – review and editing (equal). **Gwenny Fuhler:** Conceptualization (equal); writing – review and editing (equal). **W. Rudolph Schouten:** Conceptualization (equal); data curation (equal); writing – review and editing (equal). **Marjolein Blussé van Oud-Alblas:** Data curation (equal); writing – review and editing (equal). **Eelco de Graaf:** Data curation (equal); writing – review and editing (equal). **Annemarie C. De Vries:** Conceptualization (equal); data curation (equal); writing – review and editing (equal).

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None.

CONFLICT OF INTEREST

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