International Journal of Colorectal Disease (2019) 34:1113–1119
https://doi.org/10.1007/s00384-019-03290-6

ORIGINAL ARTICLE

# Platelet-rich plasma (PRP) versus fibrin glue in cryptogenic fistula-in-ano: a phase III single-center, randomized, double-blind trial

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Accepted: 3 April 2019 / Published online: 29 April 2019


## Abstract

**Purpose** To compare the clinical outcome of autologous platelet-rich growth factor (PRP) with commercial fibrin glue in the management of high cryptogenic fistulae-in-ano.

**Method** The study was conducted at a single center between July 2012 and July 2015 and performed as a phase III, randomized, double-blind comparison of autologously prepared PRP versus fibrin glue for cryptoglandular anal fistulae without active sepsis. Patients were assessed with clinical and endosonographic follow-up. Patients were followed up at 1 week and then at 3, 6, and 12 postoperative months. The primary outcome measure was the fistula healing rate (complete, partial, and non-healing) with secondary outcome measures assessing fistula recurrence, continence status, quality of life, and visual analog pain scores.

**Results** Of the 56 enrolled patients, 32 were PRP-treated and 24 were fibrin-treated. The groups were well matched for fistula type with an improved overall healing rate for PRP-treated over fibrin-treated cases (71% vs. 58.3%, respectively; $P = 0.608$); a complete healing rate of 48.4% vs. 41.7%, respectively; and a partial healing rate of 22.6% vs. 16.7%, respectively. The median pain scores of PRP-treated patients were lower at the first visit with a greater initial pain decrease early during follow-up. Improvements in pain reduction impacted the quality of life measures ($P = 0.035$). All adverse events were minor and no patient experienced a negative impact on continence.

**Conclusion** Treatment of complex cryptoglandular anal fistula with autologous PRP is as effective as fibrin glue with less cost and no adverse effect on continence.

**Keywords** Cryptogenic fistula-in-ano · Fibrin glue · Platelet-rich plasma

## Introduction

Cryptoglandular fistula-in-ano is one of the commonest anorectal conditions with a prevalence ranging between 8.6–10 per 100,000 of the population per annum [1] and between 10 and 30% of interventions in most coloproctology units performed for this particular problem [2]. The majority of these fistulae are successfully treated by fistulotomy [3]; however, there are a group of complex and high anal fistulae referred to dedicated colorectal practices which require specialized treatment [4, 5]. In these cases, the balance in successful management is between durable cure and the risk of significant postoperative functional disturbance [6]. Over recent years, either as alternatives to standard surgery or as adjunctive therapies, a range of fistular sealants has been used in management. These have been utilized either alone or in combination with fistulotomy, fistulectomy, and advancement anoplasty, with the bulk of reports describing outcomes using fibrin glues [7, 8].

Autologous platelet-rich plasma (PRP) has been used to promote healing in a range of tissues, most notably in maxillofacial [9] and plastic surgery, [10] with recent application in cryptogenic fistula-in-ano either alone [11, 12] or in

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combination with mucosal advancement anoplasty [13, 14]. Some additional success has been reported in selected cases with perianal Crohn's disease [15]. The production of autologous PRP has been considerably improved with automated microprocessor-controlled kits capable of rapidly generating standardized levels of polymerized fibrin clot almost independently of the initial fibrinogen concentration within the blood [16]. The resultant PRP is a rich source of growth factors implicated in tissue healing and regeneration [17]. In preliminary clinical studies, durably successful closure of anal fistulae has been variably reported in small numbers of recruited patients as between 62 and 83% of treated cases [11, 13, 14]. We present the initial results of a phase III single-center, randomized, double-blind trial comparing the safety and efficacy of autologous PRP versus a commercial fibrin glue (Tissucol Duo®; Baxter, Valencia) in the management of patients with high cryptoglandular fistula-in-ano.

## Method

The study was conducted as a phase III, randomized, double-blind parallel group design which was approved by the local University Ethics Committee, with each patient included providing written informed consent for participation. Patients with a complex cryptoglandular fistula-in-ano were recruited between July 2012 and July 2015 where there was a maximum of external fistular openings but with a single internal opening. Fistulae were considered "high" in type if they traversed > one-third of the coronal length of the anal sphincter as measured endosonographically or if there was a trans- or supralevator extension detected on imaging. Excluded from the analysis were those patients < 18 years of age, those with secondary perianal fistulae (inflammatory bowel disease, post-radiation, carcinoma), those with active sepsis, and patients with a coincident collection (> 2 cm in diameter). Patients with a rectovaginal fistula, anal stenosis, immunosuppression, or a coagulopathy were also excluded from consideration. Those with simple fistulae (a single internal and external opening where the fistula lay below one-third of the coronal length of the anal sphincter) were also excluded as were those patients on mandatory anti-platelet therapy. A flowchart of the study design is shown in Fig. 1 with patients randomized by assignment of computer-generated numbers either for treatment with PRP or with Tissucol Duo®.

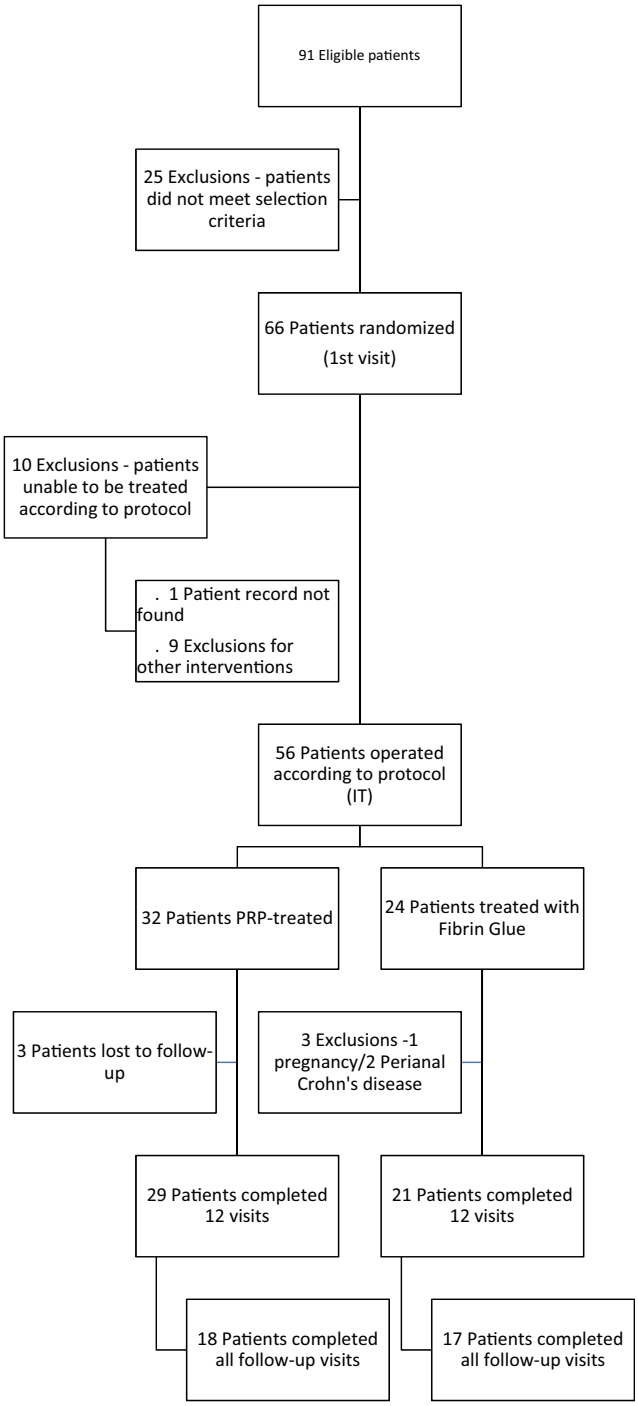


**Fig. 1** Flow chart of study patient recruitment and follow-up outcome

## PRP preparation

Autologous PRP was prepared on a 40-mL peripheral venous blood sample collected in a sterile container coated with 3.8% sodium citrate (Venoject® Terumo Corp, Madrid) according to the method previously described by Anitúa et al. [9]. In brief, blood was centrifuged at 1800 rpm for 8 min with separation of the upper plasma fraction from the leukocyte and erythrocyte fractions and with further separation of the platelet-poor from the platelet-rich components. The addition of a 10% $CaCl_2$ solution resulted in the production of a stable, cross-linked fibrin 2 polymer with entrapped platelets as a source of growth factors after endogenous activation of thrombin and factor XIII. The platelet-rich fibrin polymer formed is

produced as a stable clot which is relatively resistant to proteolysis and which slowly releases a multitude of growth factors including platelet-derived GF, transforming GF-β, insulin-like GF-1, fibroblast GF, and epidermal GF [18].

## Patient preparation, surgical intervention, and follow-up assessment

During the preoperative phase, all patients underwent perianal examination by a consultant with the location of the external and internal fistular openings, routine anoscopy, and extended imaging (endosonography and MR imaging). Randomization was performed approximately 12 weeks before sealant treatment, with the prior insertion of a temporary seton where indicated for drainage of any small collection (within protocol). Patients received a phosphate enema the night before treatment with routine perioperative antibiotic prophylaxis with amoxicillin-clavulanic acid. Sealants were instilled according to a protocol checklist with each procedure inspected by an independent study auditor. The fistula tract was then cannulated and cleaned extensively to remove the epithelial lining using different-sized gauze.

The intraoperative use of hydrogen peroxide in fistula identification was prohibited and all internal openings were closed with a single 3/0 PDS suture. Half of the PRP solution was injected into the internal opening at the submucosal level with the other half injected in small aliquots along the wall of the fistula tract. The tract was then sealed with instillation of between 2 and 4 mL of the gelatinous platelet-poor fraction via the external opening and along the length of the fistula tract. In the fibrin group, the fistula tract was sealed with glue via the external opening with syringe withdrawal and removal of the skin surrounding the external opening. All patients were discharged on the same day with oral analgesics and a 10-day course of ciprofloxacin/metronidazole. Patients were examined by a specialist coloproctologist at 1 week and then at 3, 6, and 12 postoperative months. Table 1 shows the demographic data of the different treatment groups.

A clinical complete cure was defined as those patients where at 1 year after treatment, clinical evidence of closure and epithelialization of the fistula without any reported or evident discharge was there. Partial healing was deemed to have occurred when there was no suppurative discharge or staining from the external opening but where that opening had not fully epithelialized**.** Partial healing was also recorded when multiple external fistula openings were present at the commencement and where at least one (but not all) fistula openings had closed without discharge. This could, however, still be accompanied by persistent discharge from other openings and be considered a partial treatment response. The time until either partial or total healing was recorded as was the percentage of patients with scarring evident on endoanal sonography. Anal endosonography was performed by FDLP, MLR, and JVJM, each of whom was blinded to the clinical management groups. Ultrasonographic cure was considered to have occurred if, on examination, there was no fistula opening where hydrogen peroxide might be able to be instilled and there was no coincident abscess cavity identifiable. Continence was assessed using the Wexner Cleveland Clinic Florida (CCF) Scale [19] with determination of quality of life (QoL) parameters using the SF 36-V2 questionnaire at the screening and final visits. All adverse events with each treatment were recorded and defined as mild, moderate, or severe. A visual analog scale (VAS) was utilized at each visit to record patient pain where 0 = no pain and 10 = the most severe pain experienced.

Patients were separated into discrete assessment groups. The IT (intention to treat) group included those with an intention to treat and with at least one post-treatment assessment. The ITM (modified intention to treat) group included with an intention to treat with a variable follow-up but an ability to assess patients at their final visit. The PP (protocol patients) group included those patients with no deviation whatsoever from the management protocol. This latter group includes those patients leaving the study due to a lack of efficacy or due to an adverse event.

## Statistical analysis

The analysis was performed using the SPSS Version 19 software (Chicago, IL). Quantitative variables are presented as means and standard deviations or as medians and interquartile ranges where appropriate. Qualitative variables are presented as percentages with comparisons made by the chi-square test and Fisher's exact test where indicated. The normality of data was tested with the Shapiro-Wilks test, and the equality of variances (the homoscedasticity) was determined by Levene's test. The Mann-Whitney U test was used for non-normal data with the Wilcoxon test used to assess the pre- and post-treatment variations in each of the groups (intra- and inter-group testing of QoL parameters). When confidence intervals were set at 95% with a power for the study of 80%, the total sample size was calculated using a predicted difference in efficacy between the groups of 35% or more. Given an expectation of 5% loss per group, the total patient number needed for recruitment was defined at 66 (33 per group). For the purposes of analysis, $P$ values $< 0.05$ were considered statistically significant.

## Results

Of the 91 eligible patients who attended the screening visit, 66 met the selection criteria for inclusion in the study. Of these 66

**Table 1** Patient demographic data

| | | Patient population | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | IT | | | ITm | | | PP | | |
| | | (*n* = 56) | | | (*n* = 50) | | | (*n* = 35) | | |
| | | Group | | | Group | | | Group | | |
| | | Fibrin | PRP | *P* | Fibrin | PRP | *P* | Fibrin | PRP | *P* |
| Gender (%) male/female | | 54.2/45.8 | 81.2/18.8 | 0.029 | 52.2/47.8 | 78.6/ 21.4 | 0.047 | 50/50 | 71.4/28.6 | 0.199 |
| Age* (± SD) | | 47.96 (13.46) | 50.28 (13.22) | 0.502 | 47.39 (13.47) | 50.46 (13.85) | 0.394 | 48.71 (14.26) | 48.19 (12.59) | 0.960 |
| BMI* (± SD) (kg/m$^2$) | | 28.66 (7.37) | 30.3 (6.72) | 0.312 | 28.87 (7.46) | 30.47 (17.1) | 0.394 | 30.21 (8.79) | 30.06 (7.89) | 0.987 |
| ASA (%) | I | 25 | 37.5 | 0.322 | 26.1 | 35.7 | 0,416 | 21.4 | 33.3 | 0.521 |
| | II | 66.7 | 46.9 | 0.140 | 65.2 | 46.4 | 0,196 | 64.3 | 47.6 | 0.380 |
| | III | 8.3 | 15.6 | 0.414 | 8.7 | 17.9 | 0,438 | 14.3 | 17.1 | 0.679 |
| Smoker (%) | | 41.7 | 56.3 | 0.280 | 43.5 | 52.6 | 0.473 | 42.9 | 61.9 | 0.268 |
| Anti-coagulants (no.) | | 0 | 3 | 0.252 | 0 | 2 | 0.495 | 0 | 2 | 0.506 |

*IT*, intention to treat; *ITm*, modified intention to treat; *PP*, protocol patients

*Age (years) and BMI are both presented as means

patients, 10 withdrew from the study as they were unable to complete the protocol (no external fistula opening was evident in 1 with the remaining 9 undergoing additional procedures deemed necessary by the surgeon). This left 32 PRP-treated and 24 fibrin glue–treated cases for analysis. During the conduct of the study, there were 6 subjects who withdrew but who still completed the follow-up. Two of these patients had exclusion criterion discovered after intervention (both with Crohn's disease), and 2 patients withdrew for various reasons (one patient became pregnant during the study and one further patient was removed from the study by the principal investigator because of repeated hospitalizations for acute exacerbations of comorbid illnesses). All patients were monitorized for 1 year.

The patient groups were well matched with more PRP-treated male patients in the IT and ITM groups (Table 1). In the IT group, the median duration of symptoms for fibrin-treated cases was 24 months (range 7–60 months) and for the PRP-treated cases was 24 months (range 5–120 months; $P = 0.154$). Overall, there were 18 patients (32.1%) with a recurrent fistula (33.3% fibrin-treated vs. 31.3% PRP-treated cases, respectively; $P = 0.869$). Thirty-one patients had indwelling setons at the time of surgery including 13 (54.2%) in the fibrin-treated group and 18 (56.3%) in the PRP-treated group ($P = 0.877$). There were 2 fibrin-treated cases (8.7%) and one PRP-treated patient (3.6%) who presented with 2 external openings. At the initial assessment, 42 patients (82.4%) in the IT group had a trans-sphincteric fistula on endosonography including 19 (82.6%) in the fibrin-treated group and 23 (82.1%) in the PRP-treated group. Overall, 5 patients (9.8%) had an intersphincteric fistula, i.e., 3 (13%) from the fibrin-treated group and 2 (7.1%) from the PRP-treated group. Three patients had a suprasphincteric fistula (all derived from the PRP-treated group) with one suprasphincteric fistula in the fibrin-treated group. Regarding this aspect, no relationship was found between healing and fistula type.

The median overall preoperative Wexner CCF score for the fibrin-treated patients was 0 (range 0–4) and 0 (range 0–6.75) for the PRP-treated group ($P = 0.927$). The mean operative time in the fibrin-treated group was 31 min (± 13.1 min), and 26.3 min (± 10 min) for the PRP-treated group. Table 2 provides a summary of follow-up compliance showing a high level of compliance at all times between treated groups. There were no differences in the healing rates (overall, complete, partial, and non-healing) between groups, within the IT group, with a cure rate of 41.7% overall for the fibrin-treated cases vs. 48.4% for the PRP-treated cases. Of these, 16.7% of the fibrin-treated patients had a complete cure compared with 22.6% of the PRP-treated cases. There was a partial healing rate of 58.3% in the fibrin-treated cases and 71% in the PRP-treated patients (Table 3). We are known that by the third month, there was the greatest response to both types of treatments, with a similar timing of the greatest effect but with a difference in cure rates (near 60% vs. 30%, respectively). Despite this, over time PRP treatment is associated with enhanced healing alter the sixth month (increasing up to 50%) whereas, at this time in the fibrin-treated group, healing remains static. Ultrasonographic diagnosis of a cure for the PRP- and fibrin-treated cases was comparable with the clinical findings (14 patients; 48.3% vs. 9 patients; 42.9%, respectively).

**Table 2** Monitored follow-up compliance between groups

| | Initial assessment | 1st visit (V1) (intervention) | 2nd visit 1 week | 3rd visit 3 months | 4th visit 6 months | 5th visit 12 months |
|---|---|---|---|---|---|---|
| Fibrin(%) | 24 (100%) | 24 (100%) | 23 (95.83%) | 21 (87.5%) | 21 (87.5%) | 21 (87.5%) |
| PRP (%) | 32 (100%) | 32 (100%) | 26 (81.25%) | 25 (78.13%) | 27 (84.38%) | 29 (90.63%) |

In the analysis of pain, there was a decrease in recorded pain in the first review visit (V2) where the IT and ITM groups reported a greater decrease in the median pain scores in the PRP-treated compared with the fibrin-treated group (0, range 0–4 vs. 3, range 1.75–5.25, respectively; $P = 0.023$). The remainder of the visits revealed a progressive decrease in pain scores for each treatment group but without significant differences demonstrated. There were no differences recorded between groups in the median comparative Wexner CCF scores (0, range 0–0 vs. 0, range 0–7.5). There were no significant differences observed in the comparisons of QoL domains for the treatment groups. The changes in QoL between the initial assessment (V0) and the final visit (V5) with only demonstrable effects are from PRP treatment but not from fibrin treatment for reported pain as an expression in the QoL questionnaire. These differences were noted in the PRP-treated cases in the IT, ITM, and PP groups ($P = 0.035$; 0.049 and 0.045, respectively).

There were 60 adverse events recorded with only 4 related to treatment (2 moderate and 2 mild) and 9 reported in 8 patients (5 in the PRP-treated group and 4 in the fibrin-treated group). Overall, 33 of the registered adverse events were moderate (17 PRP-treated cases vs. 16 fibrin-treated cases). The moderate adverse events included one case of severe proctalgia and one perianal abscess which drained spontaneously. Eighteen mild adverse events were recorded in 11 patients (8 PRP-treated vs. 10 fibrin-treated cases). No patient required surgical intervention for an adverse event.

## Discussion

This preliminary randomized trial shows that autologous platelet-rich plasma (PRP) is as effective over a medium-term follow-up in the management of complex cryptoglandular fistula-in-ano as fibrin blue, and it is less expansive.

In spite of the fact that results following the use of sealant and plugs for anal fistula are scarce and costly, this therapy should be still offered to patients, as it is considered a non-invasive technique and does not limit other treatments in case of fistula recurrence. This is the main message of our study, as outcomes of PRP are similar to fibrin glue and results in minor cost and less possibility of rejection related to its autologous source.

We are demonstrating in our study that treatment with autologous PRP is safe with the bulk of fistula healing occurring early by the second post-procedural visit. There was a greater decrease in the early postoperative reported pain scores and in the impact of perceived pain on quality of life measures. These results reflect earlier work by our group in a phase II study designed to evaluate the safety and initial efficacy of autologous PRP injection where a fibrin plug derived from the

**Table 3** Healing rates (overall, complete, partial, and non-healing) between groups

| Healing rates, *n*/% | Patient population | | | | | |
|---|---|---|---|---|---|---|
| | IT | | ITm | | PP | |
| | (*n* = 56) | | (*n* = 50) | | (*n* = 35) | |
| | Fibrin | PRP | Fibrin | PRP | Fibrin | PRP |
| Overall | 10 (41.7%) | 15 (48.4%) | 10 (43.5%) | 12 (44.4%) | 7 (50%) | 8 (40%) |
| Complete | 4 (16.7%) | 7 (22.6%) | 3 (13%) | 6 (22.2%) | 2 (14.3%) | 4 (20%) |
| Partial | 14 (58.3%) | 21 (71%) | 13 (56.5%) | 18 (66.7%) | 9 (64.3%) | 12 (60%) |
| Non-healing | 10 (41.7%) | 9 (29%) | 10 (43.5%) | 9 (33.3%) | 5 (35.7%) | 8 (40%) |
| *P* | 0.608 | | 0.432 | | 0.568 | |

*IT*, intention to treat; *ITm*, modified intention to treat; *PP*, protocol patients

activated platelet-poor fraction was used as an additional fistula sealant [20].

Most of the studies previously published concerning the use of PRP are heterogeneous and present a small sample size. The combination of PRP and other curative techniques, as the mucosal flap, can lead to a biased interpretation of the results (Table 4).

The theoretical advantage of PRP over fibrin glue is the capacity of the polymerized clot with its entrapped platelets to release a sustained local concentrate of growth factors involved in soft-tissue healing of the fistula. Although most treatment failures tend to occur relatively early, there are some medium-term and even long-term recurrences. This finding is akin to other reported data concerning the use of fibrin glue [21]; however, in our study, there appears to be a differential effect in the PRP-treated cases concerning the timing of healing. This will likely affect the acceptable period of waiting prior to a consideration of re-intervention. In this respect, larger numbers of patients are needed in order to make a better judgment where it initially seems that if a fibrin-treated case has not healed by 3 months, it is unlikely to do so. By contrast, based on our preliminary results, a PRP-treated patient might be able to be observed for at least a year before considering re-intervention (3 patients healed at the third visit vs. 14 at the fifth visit). We have not been able to find publications with PRP that allow us to establish comparisons about this topic. In a publication we looked, in which collagen paste was used as a sealant, we found that half of the treated patients were cured at 6 months, remaining until the year without other new cures [22].

From our experience between the phase II and phase III studies, the improvement in outcome with PRP is probably a result of the learning curve both in the indication for and conditions of its use, either alone or as an adjunct to other fistula treatments. In general, PRP use importantly has both a low complication rate and minimal impact on continence. In this regard, the comparative study by Pérez-Lara and colleagues [11] assessing 60 patients with PRP instillation and closure of the internal opening reported a 0% incidence of incontinence following the combined procedure. Further in their study, 10/15 recurrent fistulae healed with a second instillation of preserved autologous PRP so that re-treatment with PRP which has been stored for future use can be valuable in the management of recurrent cases. The effect of repeat PRP use in recurrent fistula-in-ano on continence remains, however, to be determined.

In this respect, there is a specific association reported between improvement in formal continence scoring after treatment of complex fistulae with adipose-derived stem cell therapy and ultimate fistula healing [23] suggesting that a responsive Wexner CCF score may predict outcome when local intrafistular treatments are used. In the current study, adverse events were uncommon and none were serious, representing a reduction in the number recorded in our prior phase II study [20].

There are several limitations of our study, most notably that the patient numbers are currently small with the need for multi-institutional patient recruitment over a more prolonged follow-up. Comparisons with other reported studies using PRP fractions are limited by differences in fibrin clot preparation, variability in the use of preliminary draining setons, the volume of autologous polymer instilled, the suture material used in the closure of the internal opening, the extent of curettage of the primary fistula tract, the use of bowel preparation and coincident patient comorbidity (smoking, BMI, etc.), or the consideration of partial healing as an outcome variable recently evaluated in perianal fistulas in Crohn's disease studies.

**Table 4** Summary of studies with platelet-rich fibrin for treating of anal fistula

| | No. patients | Study type | Intervention | Healing rates (%) | Follow-up (months) |
|---|---|---|---|---|---|
| Pérez-Lara FJ et al. 2015 [11] | 60 | Prospective, longitudinal, multileft, non-randomized | Platelet-rich fibrin and closing the internal orifice | 66.6 | 24 |
| Moreno-Serrano et al. 2016 [12] | 23 | Prospective, longitudinal, unileft, non-randomized | Platelet-rich fibrin and closing the internal orifice | 62 | 12 |
| Van der Hagen et al. 2011 [13] | 10 | Prospective, longitudinal, unileft, non-randomized | Autologous platelet-rich plasma and mucosal advancement flap | 90 | 26 |
| Göttgens at al. 2014 [14] | 25 | Retrospective | Autologous platelet-rich plasma and mucosal advancement flap | 80 | 27 |
| Göttgens et al. 2015 [15] | 10 | Prospective, longitudinal, unileft, non-randomized (patients with Crohn's disease) | Autologous platelet-rich plasma and mucosal advancement flap | 70 | 23.3 |
| de la Portilla et al. 2017 [20] | 36 | Prospective, phase II clinical trial, non-randomized | Platelet-rich fibrin and closing the internal orifice | 33.3 complete healing<br>11 partial healing | 12 |

Whereas previously, commercially available fibrin (which contains the bovine-derived proteolytic inhibitor aprotinin) had the potential for significant selective allergic reactivity, the use of autologous PRP eliminates this risk. Cost is also an important consideration where per patient PRP is less expensive than fibrin glue (€50 vs. €280, respectively).

## Conclusion

Autologous PRP as a definitive treatment in selected complex cryptogenic fistula-in-ano appears safe and equivalent to commercial fibrin glue with less cost. PRP-treated patients have no disturbance of continence and their fistulae may continue to heal over the first year, with less-reported postoperative pain or pain during follow-up and with a better-reported quality of life.

**Acknowledgments** The authors thank Andrew Zbar for the critical review of the manuscript.

**Author contribution** FDP and VDMC: Study design, interpreting results, drafting of the manuscript.

MVM: Data acquisition.

JMVM, ANGC, MLR, JMDP, and RMJR: Surgical protocol, study design, interpreting results.

FJP: Critical review of the manuscript.

**Funding information** A Grant for this work was provided by the Fundación Española de Coloproctología.

### Compliance with ethical standards

The study was conducted as a phase III, randomized, double-blind parallel group design which was approved by the local University Ethics Committee, with each patient included providing written informed consent for participation.

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