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Intratendinous adipose-derived stromal vascular fraction (SVF) injection provides a safe, efficacious treatment for Achilles tendinopathy: results of a randomized controlled clinical trial at a 6-month follow-up

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Abstract
Purpose Although platelet-rich plasma (PRP) injection has shown controversial results for the treatment of Achilles tendinopathy, it remains the most used biological treatment. Recent findings seem to demonstrate that the stromal vascular fraction (SVF) within adipose tissue may counteract the impaired tendon homeostasis. The aim of this study was to prospectively compare the efficacy of PRP and SVF injection for the treatment of non-insertional Achilles tendinopathy.
Methods Fourty-four patients were recruited in the study; 23 of them were assigned to the PRP group whereas 21 to the SVF group, treated unilaterally or bilaterally for a total of 28 tendons per group. All patients (age 18–55 years) were clinically assessed pre-operatively and at 15, 30, 60, 120 and 180 days from treatment, using the VAS pain scale, the VISA-A, the AOFAS Ankle-Hindfoot Score and the SF-36 form. The patients were also evaluated by ultrasound and magnetic resonance before treatment and after 4 (US only) and 6 months.
Results Both treatments allowed for a significant improvement with respect to baseline. Comparing the two groups, VAS, AOFAS and VISA-A scored significantly better at 15 and 30 days in the SVF in comparison to PRP group ($p < 0.05$). At the following time points the scores were not significantly different between the two groups. No correlation has been found between clinical and radiological findings.
Conclusions Both PRP and SVF were safe, effective treatments for recalcitrant Achilles tendinopathy. The patients treated with SVF obtained faster results, thus suggesting that such a treatment should be taken into consideration for those patients who require an earlier return to daily activities or sport.
Level of evidence Randomized Controlled Clinical Trial, Level 1.

Keywords Achilles tendon · Achilles tendinopathy · PRP · Adipose-derived mesenchymal stem cells · Stromal vascular fraction (SVF)

Introduction

The Achilles tendon is one of the most vulnerable tendons of the human body [22, 37], often affected by tendinopathy, a multifactorial condition mainly related to overuse, degeneration and poor vascularization, representing 30–50% of all sports-related injuries [28]. In particular, non-insertional Achilles tendinopathies occur 2–6 cm proximal to the tendon insertion, which is an area characterized by a poorer vascularization. Modern research tools have contributed to creating convincing evidence that the inflammatory response is a key component of chronic tendinopathy. In particular, increased levels of macrophages, T and B lymphocytes, macrophage-derived interleukin-1 (IL-1), cyclo-oxygenase (COX)-1, COX-2, IL-6, isoforms

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of transforming growth factor-β (TGF-β) and substance P were demonstrated in chronic Achilles tendinopathy [32]. Given the pathophysiological factors involved in chronic tendinopathy, the prospective use of biologic agents to enhance or restore healing in this condition has shown great promise. Platelet-rich plasma (PRP) is an agent that holds considerable theoretical appeal as a means of contrasting some of the mechanisms responsible for the development or persistence of the tendon lesions [12, 25, 38]. Many in vitro and pre-clinical studies have demonstrated that PRP may help stimulating angiogenesis, epithelialisation, cell differentiation, replication, proliferation and formation of extracellular matrix [3] since it contains several different growth factors (GFs), cytokines, chemokines and other proteins. Nevertheless, despite this positive and encouraging evidence, very conflicting results about the clinical effectiveness of PRP in tendinopathy are reported in the literature, thus making it difficult to conclude whether PRP is an effective treatment [17]. However, the failure to demonstrate an evident therapeutic effect of PRP could be ascribed to an insufficient attention to patient selection, a lack of uniformity in term of tendinopathy severity and site of lesions and, above all, of univocal formulation of PRP [2, 3, 21]. Nevertheless, the scientific community still considers PRP as a potential useful tool to treat tendinopathy, as is demonstrated by the number of new ongoing investigations [40].

More recently, it has been demonstrated that many growth factors and molecules contained in PRP are produced and released by mesenchymal stem cells (MSCs) in response to a tissue injury or trauma. The recent discoveries indeed show that MSCs are associated to the perivascular district, they derive from pericytes [6] and they secrete considerable levels of both immunomodulatory and trophic agents, besides having the ability to differentiate into different end-stage cell types [1, 5, 31]. In this view, MSCs can participate to tendon regeneration both by direct differentiation into tendon cells and, more likely, by modulating the inflammatory response following an injury [36, 42]. Subcutaneous adipose tissue represents one of the most attractive sources to isolate MSCs due to a simple and less invasive method of harvesting, as well as the higher frequency of these cells within the stromal vascular fraction, if compared to bone marrow [9].

Pre-clinical studies have also demonstrated that adipose-derived stem cells (ASCs) provide significant improvements in the treatment of tendinopathy, suggesting the possibility to translate this approach to clinical applications [27, 29].

The aim of this prospective randomized controlled trial was to compare the effectiveness of the injection of a leucocyte-rich PRP formulation with the injection of adipose-derived SVF for the treatment of chronic Achilles tendinopathy. The quality and duration of clinical improvement as well as of radiological findings were assessed at different time points up to 6 months after treatment. Moreover, the immunomodulation mediated by the SVF was tested in an in vitro model of inflammation.

Materials and methods

All patients provided a written informed consent and agreed to comply with a strict follow-up program. Patients of both sexes affected by non-insertional Achilles tendinopathy referring to the senior author's institution Foot and Ankle Unit were assessed for eligibility and prospectively enrolled in the clinical study. The inclusion criteria were: unilateral or bilateral chronic tendinopathy of the Achilles tendon recalcitrant to traditional conservative treatments including non-steroidal anti-inflammatory drugs, eccentric loading exercises, stretching and biophysical therapy; symptoms lasting for at least 3 months; age between 18 and 55, VAS (visual analogue scale) pain at the first visit >5. Patients with clinical suspect of other musculoskeletal lesions of the Achilles tendon (insertional disorders, tendon rupture or tears), platelet count in whole blood $<150\times10^3$/μl, inflammatory disease or other conditions that affected the joints, immuno-mediated pathology, any conditions that could increase the interventional risk, use of tendon-detrimental drugs (i.e. fluoroquinolones), patients who received any previous injective treatment of the target Achilles tendon, patients pregnant or breast-feeding were not enrolled in the study.

A week before receiving the treatment the patients were asked to suspend any non-steroidal anti-inflammatory drugs intake to prevent an impaired platelet function that may result in lower PRP quality regarding the content of bioactive compounds [35]. A day before the treatment, the patients who satisfied the inclusion/exclusion criteria of the protocol were randomly assigned either to PRP ($n=28$ tendons) or adipose tissue SVF ($n=28$ tendons) injection group, using opaque sealed envelopes previously prepared.

The patients were evaluated clinically pre-operatively and at 15, 30, 60, 120 and 180 days from treatment, using the 0–10 Visual Analog Scale (VAS) for pain (0 points, no pain; 10 points, worst possible pain), the Victorian Institute of Sport Assessment-Achilles (VISA-A) questionnaire, [33] the American Orthopaedic Foot and Ankle Society (AOFAS) Ankle-Hindfoot Score [16] and the Short Form (36) Health Survey (SF-36) forms [4].

Preparation of PRP

PRP was prepared in the operating room using the GPS III System (Biomet Biology, Warsaw, IN, USA), a floating

buoy-based separator system. Briefly, 54 ml of peripheral blood were collected from the patients enrolled in the PRP group and added to 6 ml of anticoagulant (ACD-A, 1:10 ratio). The whole blood was transferred to a disposable separation tube that was centrifuged at 3200 rpm for 15 min in a customized centrifuge provided by the manufacturer. Platelet poor plasma (PPP) was removed and platelets were suspended by gently shaking the tube for 30 s. The resulting PRP (around 6 ml) was extracted from the tube using a 10-ml syringe.

The platelet count in peripheral blood and PRP was analyzed for all the patients of the PRP group.

Preparation of adipose tissue stromal vascular fraction (SVF)

In the operating room a small volume of subcutaneous adipose tissue (50 ml) was manually lipoaspirated from the patient's abdominal subcutaneous tissue by an expert plastic surgeon, to ensure the quality of the harvest. Two very thin patients required to have adipose tissue harvested from the internal side of the thigh.

The SVF was obtained processing the adipose tissue with the FastKit system (Corios, San Giuliano Milanese, Italy), following the instructions provided by the manufacturer. The adipose tissue was transferred to a soft plastic bag with a 120 μm internal filter. Adipose tissue was mechanically digested rubbing the tissue down until it passed through the filter. The disrupted portion of the tissue, including the SVF, was collected through a bottom connector and then centrifuged for 10 min at 400 g. The resulting bottom phase (around 10 ml) was then partially transferred to a new syringe of the volume required for the injection.

Platelet-rich plasma and SVF injection

In the operating room, a volume of 4 ml of either PRP or SVF was injected into the lesion location, intratendon and in the peritendon area, by the senior author utilizing ultrasonography scanner with high-frequency linear-array transducer (5.0–1.3 MHz), (Hitachi Hi Vision Preirus 14 MHz, Hitachi Medical Sysytem, Milan, Italy). After the treatment, the patients were asked to walk with crutches for the first 24 h and only paracetamol could be administered to control pain. No specific physical therapy was prescribed after the treatment and the patients were allowed to progressively resume their normal life and sport activities.

As per study protocol those patients who had presented a VAS > 3 and AOFAS < 70 at the 2-month follow-up visit were supposed to receive a second injection of the same product injected the first time.

MRI and US assessment

Patients were also evaluated by ultrasound (US) and magnetic resonance (MR) before the treatment and then after 120 days (US only) and 180 days. Both the radiologist and the clinical evaluator were blind to the allocation of the patients.

Radiologic examination included US images and MRI scans of Achilles tendon. All radiological measurements were made using the standard tools provided by the Institute Picture Archiving and Communication System (PACS).

US images were performed pre-injection, and then at 120- and 180-day follow-up, with a 1.5 T MR System (Avanto, Siemens Medical Solution, Milan, Italy). MRI scans of Achilles tendon were taken pre-injection and then at 6 months. In particular T1 (TR 400–750, TE 12–15) and T2 fat-saturated (TR 4000–5000, TE 70–85) images in the sagittal planes were evaluated. The area of the lesion was analyzed and measured observing the maximum diameter as a reliable parameter for the lesion size (Figs. 1, 2). US and MRI were evaluated by a musculoskeletal radiologist and an orthopaedic foot and ankle specialist, both blind to the patient's treatment.

In vitro characterization of SVF immunodulatory potential

In vitro analysis on adipose tissue samples before and after processing by FastKit system were performed on 7 patients. Unprocessed adipose tissue was digested by collagenase type I to isolate adipose-derived stem cells (ASCs) as previously reported [8]. Allogeneic peripheral blood leucocytes where isolated from 7 healthy donors not enrolled

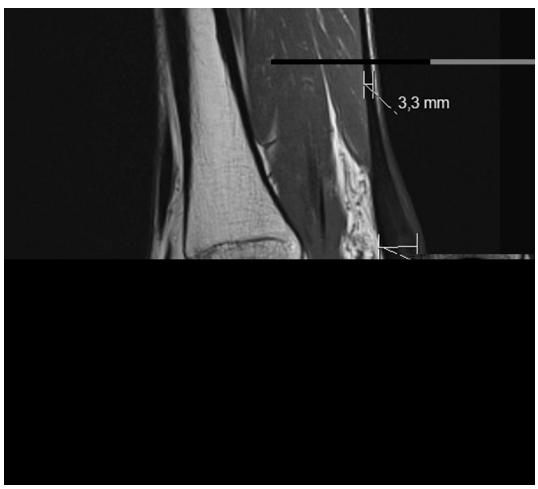

Fig. 1 Pre-op MRI showing measurement locations in the sagittal plane

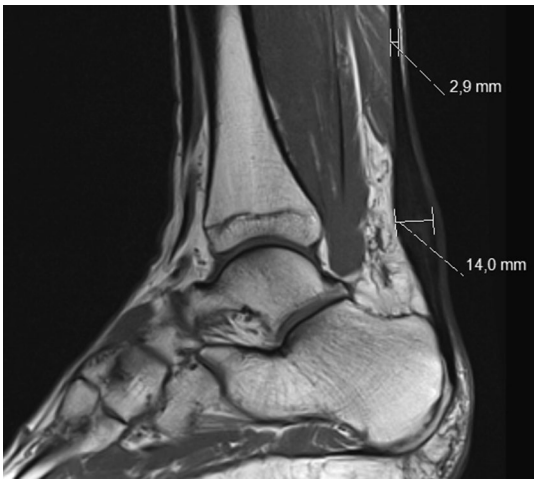

Fig. 2 Post-op MRI showing measurement locations in the sagittal plane

in the study under informed consent by Ficoll-isopaque technique (GE Healthcare, Little Chalfont UK). Briefly, 10 ml of peripheral blood was harvested from each donor, diluted 1:1 with sterile PBS and then centrifuged in falcon tube with 1:3 volume of Ficoll-isopaque. The lymphocyte layer was then harvested, washed twice with PBS and then counted and seeded in RPMI culture medium (Sigma Aldrich, St Louis, MO, USA).

Allogeneic PBLs were cultured in the bottom portion of a 96-well transwell incubated at the density of 50,000 cells/well, in presence or absence of 10 µg/ml Phytohemagglutinin (PHA; Sigma Aldrich), to stimulate the activation of the inflammatory response. A volume of 50 µl of either the output from FastKit or from collagenase digestion was added in the top portion of the transwell. The same volume of processed tissue and re-suspension between FastKit and collagenase isolation protocols were maintained to make the comparison reliable.

After 24 h of incubation, the production of IL-6 and IL-10, pro-infammatory and anti-inflammatory cytokines respectively, were analyzed in the supernatant by ELISA assay (Mabtech, Nacka Strand. Sweden). Limit of detection were respectively 10 pg/ml (intra-assay variation <4%) for IL-6 and 2 pg/ml (intra-assa variation <5%) for IL-10. The study was approved by an external Ethics Committee (Azienda Sanitaria Locale, Milan- Italy; protocol number 24 bis-12 MS).

Statistical analysis

The normality of data distribution was tested with the Kolmogorov–Smirnov test. The sample size of the study was calculated considering a difference of 12 points and a standard deviation of 15 in the VISA-A score as a clinically significant difference between treatment groups [10, 15]. Thus, accepting less than 5% probability of a type I error and a power of 80%, a total sample size of 25 tendons was required for each group. Predicting a 10% dropout rate, a total of 56 tendons were enrolled, equally divided in each group of treatment.

The statistical analysis was performed by use of Matlab statistical toolbox version 2008 (MathWorks, Natick, MA, USA) for 32-bit Windows. Differences between groups as well as within each group (preoperative vs postoperative in same group) were analyzed by use of Student t test for unpaired and paired data, respectively. The Fisher exact test and a χ^2 test were used to compare categorical data. A p value <0.05 was considered statistically significant.

Results

In vitro characterization of the immunomodulatory potential of SVF

The PBL expression of pro-inflammatory cytokine IL-6 was induced by PHA treatment. The addition of ASCs (from collagenase digestion) or SVF (from FastKit method) in the top portion of the transwell was able to significantly reduce the amount of IL-6 released in the supernatant by PBLs after 24 h of incubation, by 36% ($p<0.01$) and 15% ($p<0.05$), respectively (Fig. 3a). An increase in IL-10 release was also observed in the supernatant of the samples treated with either ASCs (+128%) or SVF (+40%). However, these increases were not statistically significant due to the high interdonor variability (Fig. 3b).

Clinical and functional results

Fourty-four patients were recruited into the study: 23 of them of them were assigned to the PRP group whereas 21 to the SVF group. A bilateral Achilles tendon injection was performed on 5 patients in the PRP group and in 7 patients in the SVF group, for a total of 28 tendons in each group (Fig. 4). All the patients were compliant with the study protocol with no loss during the study (Fig. 4).

Both the clinical and functional background data were not significantly different between the two groups, with the exception of the sex ratio, with a higher prevalence of males in the SVF group with regards to PRP ($p<0.05$) (Table 1).

Neither serious side effects nor adverse events were observed during the follow-up period. Five patients (25%) of the SVF groups also complained for hematoma and cutaneous discomfort at the adipose tissue harvest site for about a week after the procedure. At 2-month follow-up, all the patients had VAS and AOFAS score that met the study

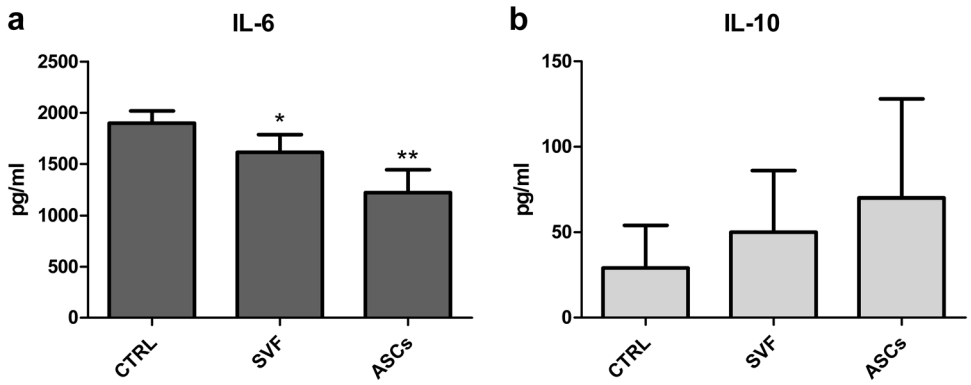

Fig. 3 Production of IL-6 (**a**) and IL-10 (**b**) in a culture of PHA-activated PBLs, in the presence or absence of ASCs (from collagenase digestion) and SVF (from Fastkit method) ($n=7$). *$p<0.05$ and **$p<0.01$ vs CTRL

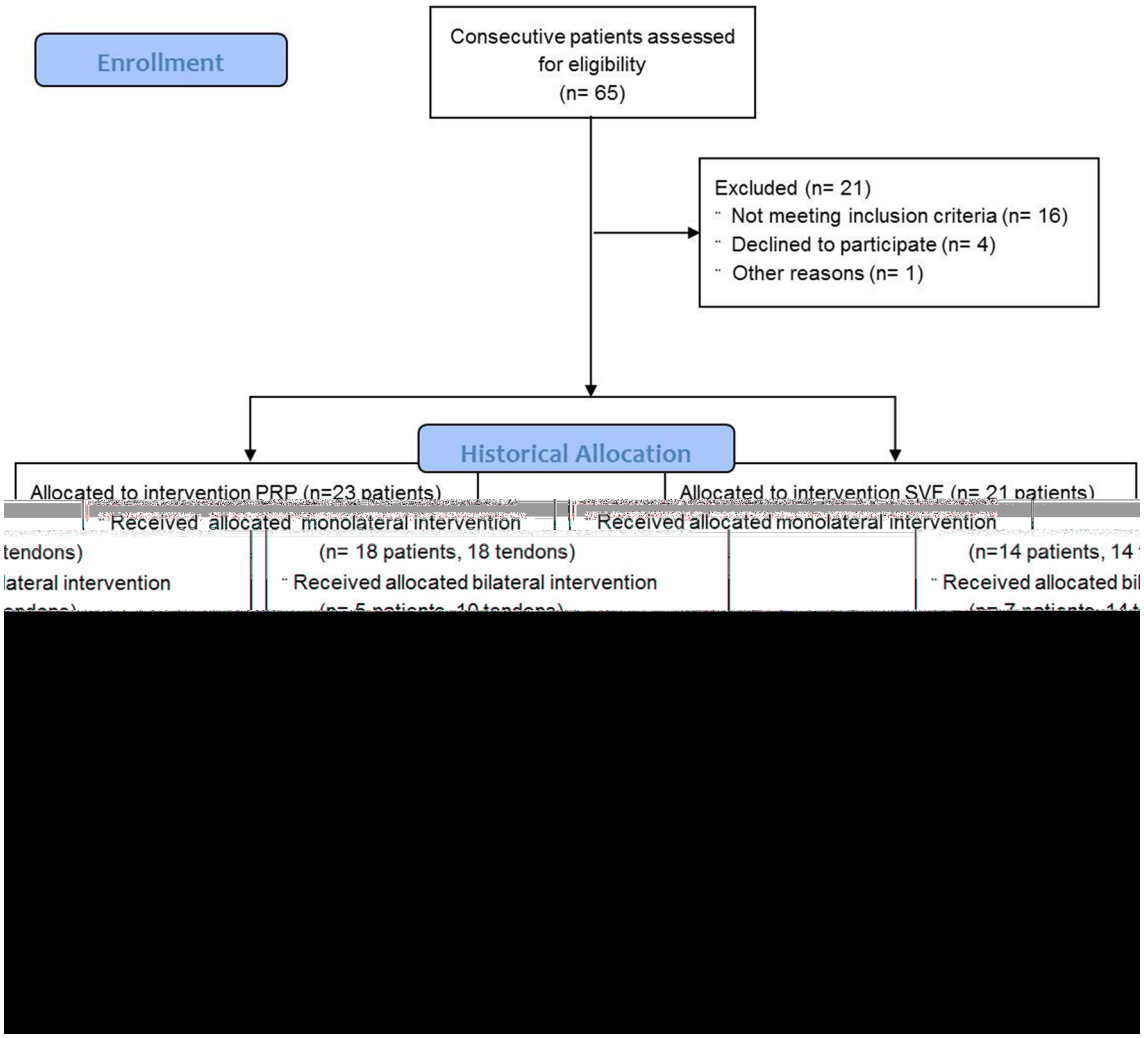

Fig. 4 Consolidated Standards of Reporting Trials (CONSORT) flow chart for patient enrolment in the study

protocol requirement (>3 and <70, respectively), so no one received a second injection at the Achilles tendon.

The mean basal platelet count in peripheral blood of the patients included in the PRP group was $240 \pm 55 \times 10^3$/µl; following the concentration procedure, the mean platelet count in PRP was $813 \pm 408 \times 10^3$/µl, with an average fold increase of 4.14 ± 1.9 with respect to the baseline.

During the follow-up, the patients of both groups had a significant improvement with respect to the pre-injection values. In particular, these improvements were

Table 1 Background data of the PRP and SVF patients

	PRP group	SVF group	*p*
VAS	6.3 ± 1.2	6.5 ± 1.6	NS
VISA	46.5 ± 23.6	41.6 ± 13.6	NS
AOFAS	63.2 ± 17.7	63.4 ± 20.1	NS
SF-36 P	38.5 ± 7.9	42.2 ± 5.5	NS
SF-36 M	51.21 ± 8	48.7 ± 5.7	NS
Age	46.6 ± 6.2	47.3 ± 3.8	<0.05
Sex M–F	8–15	14–7	<0.05

Data are expressed as mean ± standard deviation

faster in the SVF patients. Indeed, they showed significantly improvement for VAS and AOFAS scale already after 15 days from the injection (Figs. 5, 7) and VISA-A and SF-36 Score-Physical starting from 30 days (Figs. 6, 8). In the PRP group the VAS scale scored better results already after 15 days, whereas significantly better results were observed only at a later time, in particular after 30 and 60 days for VISA-A and AOFAS, respectively. No significant improvement with respect to the pre-injection value was observed for the SF-36 Score–Mental in neither group (Fig. 9). Consequently, a comparisonof the two groups showed that statistically significant differences in favor of the SVF group were just found at the earliest follow-up: VAS scored significantly better at both 15 and 30 days in the SVF patients in comparison to PRP ($p < 0.05$), as well as AOFAS and VISA-A at 15 and 30 days, respectively ($p < 0.05$) (Figs. 5, 6, 7). At the following time points the scores were not significantly different between the two groups anymore, even if SVF always scored slightly better than PRP.

US and MRI findings

Ultrasounds studies were a useful, reliable tool to identify tendon pathology and lesion site pre- and peri-operatively. However, this diagnostic procedure did not allow for any relevant information regarding the lesion evolution during the follow-up.

The mean preoperative lesion area assessed by MRI were 8.93 ± 2.10 ^{2} and 10.70 ± 3.38 mm^2 in the PRP and SVF group, respectively (NS). After 180 days from treatment, the lesion area was not significantly reduced in either PRP or SVF patients (8.67 ± 2.10 and 10.46 ± 3.37 mm^2, respectively, NS). These data demonstrated a lack of radiological improvement along the follow-up period, as well as any differences between groups at the final follow up.

No correlation between the pre- and post-injection lesion area and VAS, VISA-A, AOFAS, and SF-36 scores was found for both groups (NS).

Discussion

The main findings of this study showed that both the injection of a leucocyte-rich PRP and adipose-derived SVF have been able to provide a significant clinical improvement in term of pain relief and function restoration, with durable results for at least 6 months from treatment. Interestingly, the data yielded a faster recovery in

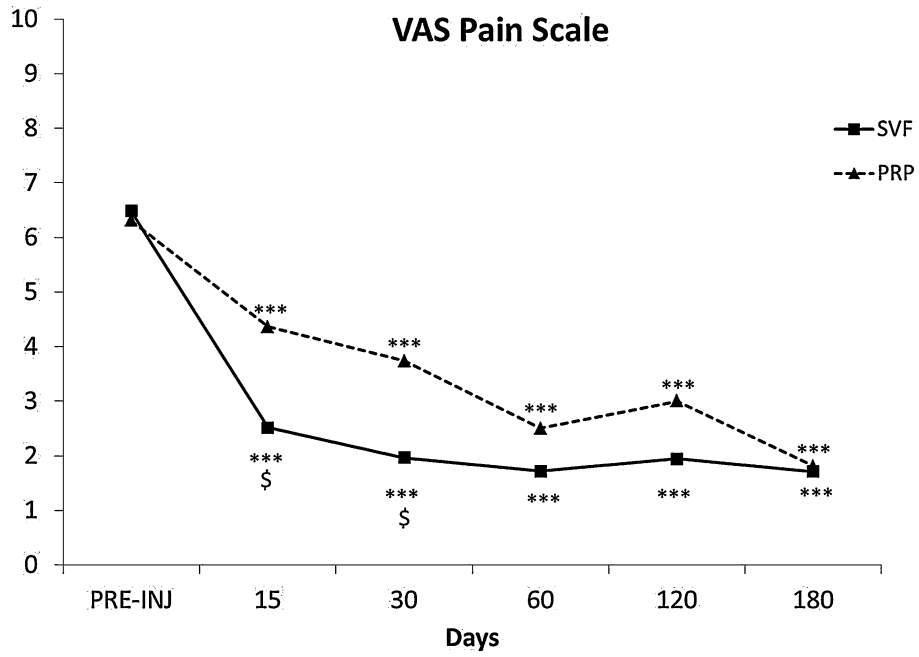

Fig. 5 Mean VAS score of patients treated with either PRP or SVF before injection and then during the follow up. ***$p < 0.001$ vs pre-injection, $^{\$}p < 0.05$ SVF vs PRP

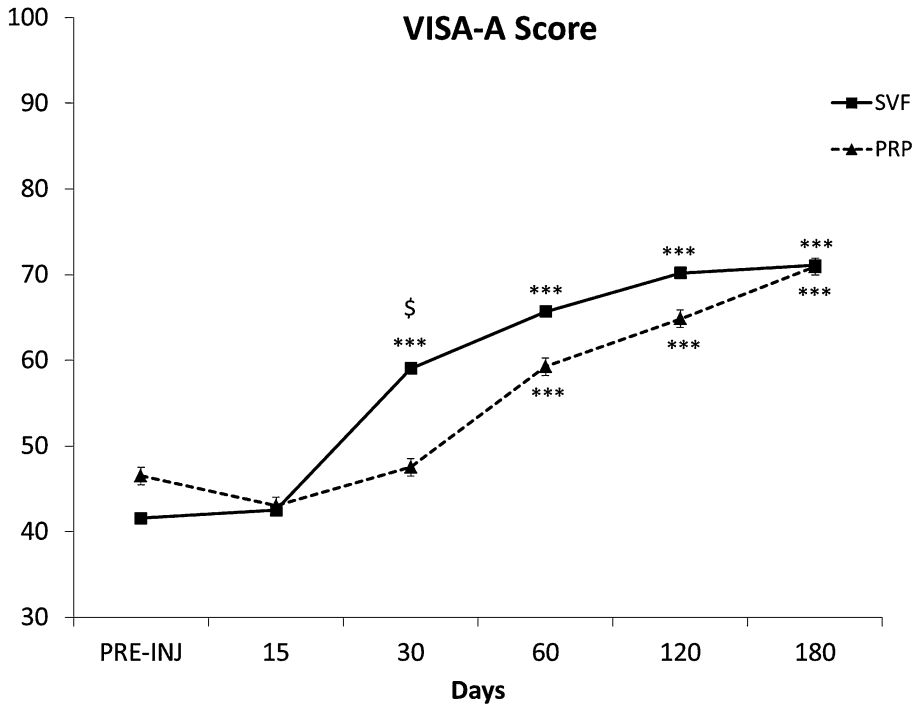

Fig. 6 Mean VISA-A score of patients treated with either PRP or SVF before injection and then during the follow up. ***$p < 0.001$ vs pre-injection, $^{\$}p < 0.05$ SVF vs PRP

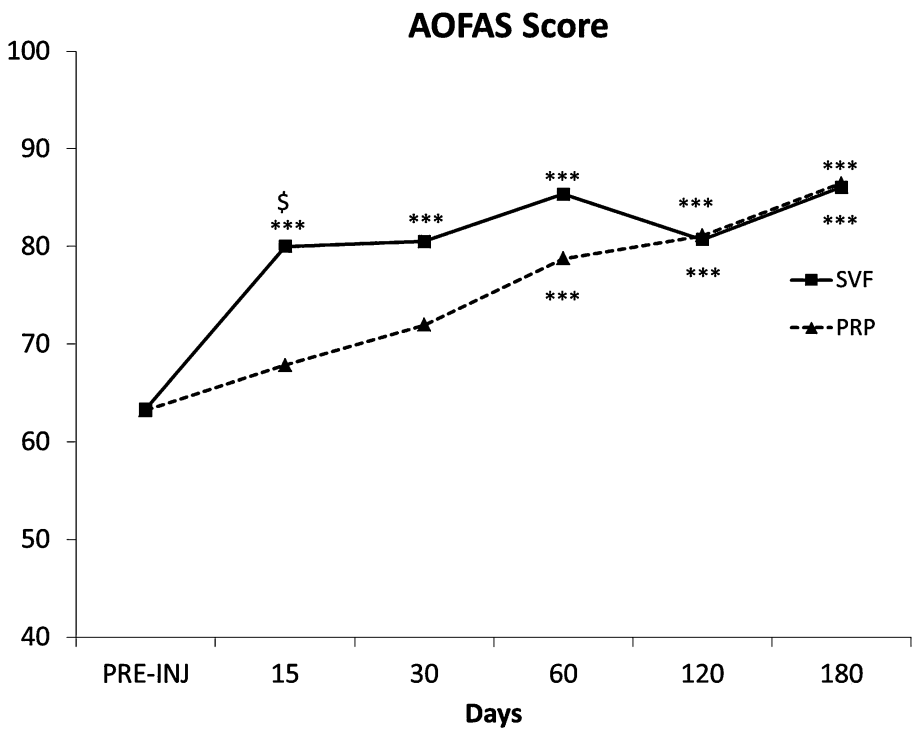

Fig. 7 Mean AOFAS score of patients treated with either PRP or SVF before injection and then during the follow up. ***$p < 0.001$ vs pre-injection, $^{\$}p < 0.05$ SVF vs PRP

the patients who had received the SVF injection, resulting in a significantly better outcome at 15- and 30 day-follow up in comparison to the patients of the PRP group. To our best knowledge, this is the first randomized clinical trial evaluating the effectiveness of adipose-derived SVF for the treatment of non-insertional Achilles tendinopathy. If indeed an increasing number of studies have demonstrated the potential of ASCs, either expanded or concentrated intraoperatively in form of SVF for the treatment of cartilage-related conditions [18, 23], very few data have been published to date to assess the effect of these cells for tendinopathy.

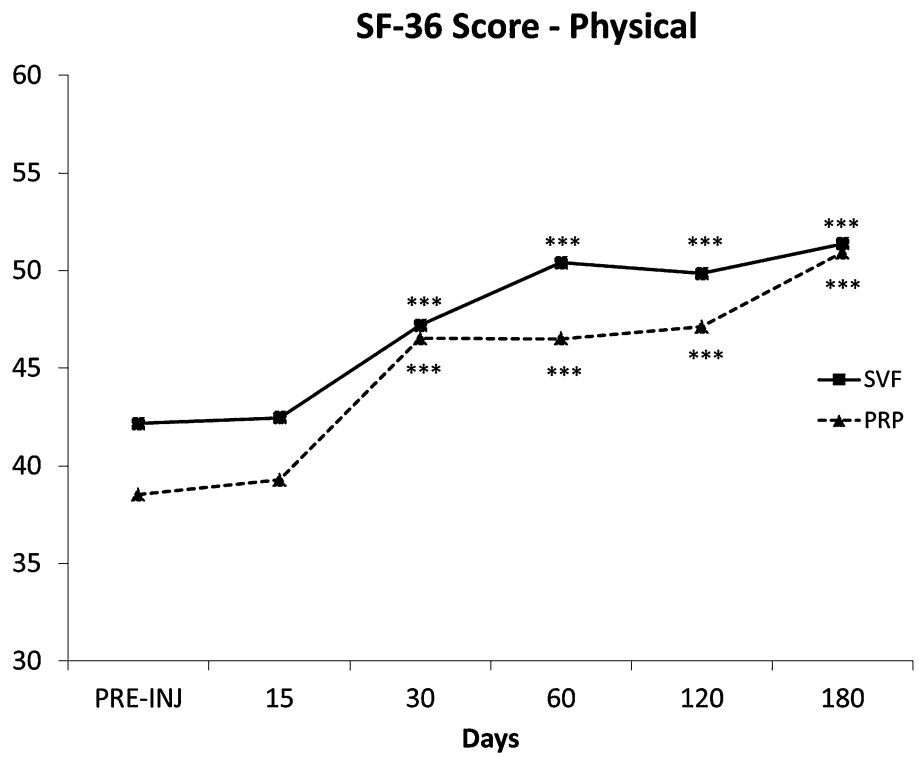

Fig. 8 Mean SF-36-Physical score of patients treated with either PRP or SVF before injection and then during the follow up. ***$p < 0.001$ vs pre-injection;

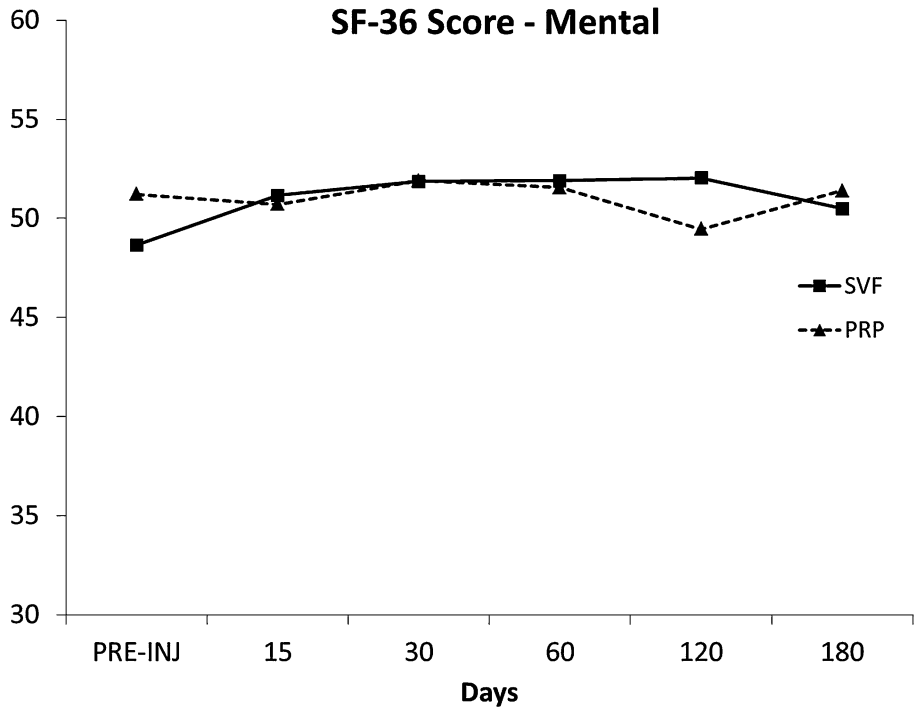

Fig. 9 Mean SF-36-Mental score of patients treated with either PRP or SVF before injection and then during the follow up

The immediate pain relief already after 15 days from the treatment and the subsequent functional improvement observed in the patients treated with SVF seem to indicate that the treatment was able to counteract more quickly the impaired tissue homeostasis. These findings may be explained by a higher and longer-lasting activity of the anti-inflammatory and immunomodulatory molecules released by the cells within the SVF compared to the molecules contained in the PRP. However, at the latest follow-up the two groups showed no significant difference, although the SVF patients always scored better than the ones treated with PRP over the entire study period.

Given the etiopathogenesis of tendinopathy, the wide anti-inflammatory and immunomodulatory properties of the molecules released by the cells within the SVF may provide alternative potential opportunities in treating chronic tendinopathies, replacing the traditional anti-inflammatory modalities (i.e. NSAIDs)., MSCs secrete, indeed, a plethora of growth factors and anti-inflammatory proteins in response to inflammatory molecules, including prostaglandin 2, TGF-β1, hepatocyte growth factor (HGF), stromal cell-derived factor-1 (SDF-1), nitrous oxide (NO), indoleamine 2,3-dioxygenase, IL-4, IL-6, IL-10 and IL-1 receptor antagonist (IL-1 RA) [1, 14, 26, 39, 41]. Moreover, MSCs prevent proliferation and function of many inflammatory immune cells, such as T cells, natural killer cells, B cells, monocytes, macrophages and dendritic cells [26]. Our in vitro findings confirmed these observations, showing a significant reduction in IL-6 production and an increase in IL-10 in an in vitro model of inflammation with peripheral blood leucocytes treated with SVF. The reason why the cells isolated with the traditional enzymatic method were able to further induce this effect may be probably ascribed to the higher number of mesenchymal stem cells in the samples. Indeed, the FastKit method usually allows to obtain about ten times fewer cells than using the collagenase digestion method (unpublished data): this is consistent with the characteristics of the kit, being a faster but obviously less accurate method to obtain MSCs than the traditional laboratory technique. However, the clinical findings seem to indicate that the cells contained in the SVF were sufficient to promote a fast pain relief and a consequent restoration of the Achilles tendon function.

Although the use of a homogenous MSC population has been traditionally considered the best approach, today the maintenance of the architecture of the so-called stem cell niche seems to represent a great advantage [13]. The adipose SVF, the native microenvironment that contains preadipocytes, vascular endothelial cells, smooth muscle cells, leucocytes, erythrocytes and pericytes, including ASCs, in a collagen scaffold within a vascular network, is considered to be the ASC niche. In this microenvironment ASCs may be able to work in a more physiological condition rather than after being purified from the rest of the other cell populations within the tissue. Moreover, the use of the SVF over purified ASCs represent a practical advantage since the use of autologous expanded cells would imply a two-step procedure, characterized by higher invasiveness and cost, and would be subjected to more rigorous regulatory requirements for their use in clinical practice since it is considered an advanced-therapy medicinal product (ATMPs).

This study design aimed to compare the effect of a single SVF injection with another injective treatment as it was considered the most proper control group. Since needling is indeed an accepted form of therapy to induce healing in tendinopathy of rotator cuff, Achilles tendon, patellar tendon as well as plantar fascia [19], if the SVF injection had been compared with a non-injective treatment, any positive effect derived from the SVF treatment might have been ascribed to the needling itself. Among the potentially effective injective alternatives, PRP was considered the best one since it has been used for about 10 years for the treatment of tendinopathies, with a very high safety profile, even if conflicting results are reported in literature [24]. No side or adverse effects were indeed recorded during the study, neither in PRP, nor in SVF patients. Moreover, in many studies PRP has been reported to positively influence health-relevant outcomes, such as pain and disability, as also reported by a review showing that PRP injections ameliorated pain in the intermediate-long term compared with control interventions by pooling pain outcomes over time and across different tendons [2]. Still, these findings cannot be applied to the management of individual patients since they are affected by low power and precision [2]. In particular very good results have been obtained using a high-platelet concentration and leucocyte-rich product, especially for the treatment of epicondylitis [24]. For these reasons the same PRP formulation was chosen for this study, which is in any case one of the best studied [10, 34]. Our results obtained in the PRP group confirmed the results obtained by other groups [7, 11]. However, at the same time, other Authors showed a lack of significant difference between a PRP and a saline injection for the treatment of Achilles tendinopathy [10, 20]. Such different outcomes can be ascribed to different inclusion criteria, evaluation methods, follow-up length, and poor number of patients as well as different study design. In a recent study, the Authors showed comparable results between an injection of PRP in Achilles tendons of 12 patients with respect to an injection of saline solution in the same number of patients [20]. Even if the Authors initially meant to evaluate the outcome at a 12-month follow-up, they met with a considerable dropout and were just able to analyze the data after 3 months from the injection. Since our data showed that the functional and clinical results after PRP injections continued to increase during the evaluation period, reaching the best scores after 6 months, an earlier evaluation might have partially masked the beneficial effects of the PRP effects. Moreover, the huge discontinuation from that study could be ascribed to its design: indeed, the participants, who were blind to the treatment received, were told that they could leave the study and receive other treatments at any time after 3 months, if unsatisfied. In any case, in our opinion the lack of clarity concerning the effectiveness of PRP in Achilles tendinopathy deserve further investigations in hopes to finally reach a wider consensus.

Interestingly, in the present study no patient felt the need for a second injection. Although the patients included in this study were all affected by chronic tendinopathy,

a single injective treatment, be it through PRP or SVF, indeed turned out to be very effective in relieving pain and quickly restoring functional properties.

Despite this clear positive clinical outcome, neither treatment group showed an improvement from a radiological point of view over the study duration, thus proving a complete lack of correlation between functional and radiological results. This finding is in line with most of the studies which failed to identify any morphological or histological change following the injection of biological agents in musculoskeletal disorders [23, 30], whereas just few evidence seem to support a correlation between clinical and radiological improvements [28]. This may find an explanation considering the strong anti-inflammatory and immunomodulatory properties of both treatments rather than a real regenerative action. However, they could also be due to a lack in sensitivity of MRI and US in monitoring the healing process. In this study, ultrasounds and MRI turned out to be useful instruments to support clinical diagnosis; further investigations are, therefore, needed to better analyze the real potential in the assessment of tissue regeneration.

One of the potential limitations of the study is the different sex distribution within the groups. Since the midportion Achilles tendinopathy equally affects women and men in the general population [9], this mismatch should, however, not have affected the results.

Since in many cases Achilles tendinopathy is bilateral, these patients were included in the trial too. To reduce the invasiveness of the treatment, both tendons were treated with the same products (PRP or SVF), so that patients just underwent either adipose or blood harvest. Another limitation could be represented by the lack of a control group treated with a saline injection. However, since the patients enrolled in this study were non-responders to the traditional conservative treatments and presented a moderate-high pain for some months (VAS > 5), the treatment with saline was not considered ethical by the Ethical Committee.

Although a gap still exists between pre-clinical investigations and clinical applications, not only did these results show the safety, feasibility and rapid efficacy of biologic treatments in curing tendinopathy, they also particularly fostered the use of SVF for those patients who require to come back to high-demanding daily activities or sports earlier.

Conclusions

Both PRP and SVF were safe, effective treatments to cure recalcitrant non-insertional Achilles tendinopathy, with SVF allowing for faster results, already after 15 days from treatment. The lack of any correlation between clinical and radiological findings would deserve further investigations, including the research of more reliable evaluation tools of tendon healing as well as longer follow up to reach firm conclusions and extend the use of this biological product to the clinical practice.

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