Efficacy of second intra-tendinous platelet-rich-plasma injection in case of incomplete response of the first injection: Three-year follow up experience

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Tendinopathy; Tear; Platelet; PRP; US

Abstract
Purpose: Tendinopathy is a frequent and ubiquitous disease developing early disorganized collagen fibers with neo-angiogenesis on histology. Peritendinous injection of corticosteroid is the commonly accepted strategy despite the absence of inflammation in tendinopathy. Platelet-rich plasma (PRP) might be a useful strategy to rapidly accelerate healing of the tendinopathy but there is a lack of knowledge about the amount of PRP to be injected and the opportunity of a second injection in case of partial pain relief. The aim of our study was to assess the potential therapeutic effect of early second PRP intra-tendinous to treat persistent painful tendon tear.
Tendon tear and tendinosis is a very common and disabling condition, resulting in impairment of quality of life. Indeed, tendinopathy of the rotator cuff affects $3-20\%$ of the general population, mainly women between 40- and 65-year-old whereas Achilles’ tendinopathy affects $5-6\%$ of the general population, especially young men. In most cases, it progresses to a disabling pain and cessation of activities [1,2].

Healthy tendon is made up of type 1 collagen and elastin fibers, within an extracellular matrix formed by tenocytes, tenoblastes and water. In case of tendinopathy, histology shows thinned and disorganized collagen fibers, mucoid and/or lipid degeneration and increased inter-fibrillar glycosaminoglycan deposition [3,4]. In addition to these lesions, neo-angiogenesis associating neo-vessels and nerve fibers development are constantly reported at the beginning of tendinopathy and during tendon healing [5–10]. Several lines of research have been explored for the treatment of tendon tear or tendinosis, including ultrasound (US)-guided fenestration or tenotomy [11–14], hyperosmolar solutions [14], bone morphogenic protein [15], or platelet-rich plasma (PRP) intra-tendinous injections, with varying efficiencies [16–23].

Despite these potential treatments, peritendinous injection of corticosteroid remains the commonly accepted strategy to treat tendinopathy [24], despite the absence of inflammation in tendinopathy, and proven tendon damage due to intra-tendinous injection [25,26].

PRP is defined as serum with three to eight more platelet concentration than blood, which allows disposing of more important concentration of active growth factors (PDGF, TGF-β, VEGF…) stimulating healing. PRP promotes stem cells recruitment and stimulates directly collagen production by the fibroblast of tendon with proliferation and differentiation of human tenocytes and plays a central role in tendon regeneration. To our knowledge, there have been only few studies so far evaluating the clinical efficacy of PRP injections with long-term follow-up. Strategies of treatment often vary and less is known about the dose to be injected and the interest of a second PRP injection in case of partial pain relief.

The aim of our study was to assess the potential therapeutic effect of early second PRP intra-tendinous injection to treat tendon tear and tendinosis in case of persistent pain despite a first PRP intratendinous injection.

Materials and methods

Patients

This monocentric retrospective study was performed from January 2010 to September 2012. Twenty-four consecutive ambulatory patients with degenerative, micro-traumatic or traumatic tendinopathy (T+) as tendon tear or tendinosis were referred to our institution for US-guided PRP injection. Pain history and clinical data using Quick Dash test (QD) for upper limbs and Western Ontario and Mac Master Universities Osteoarthritis Index (WOMAC) test for lower limbs and Visual Analogic Score (VAS) score were noted. US assessment of tendinopathy was performed at Day 0 (D0) and was followed by PRP intra-tendinous injection in tear or tendinosis area under US guidance (PRPT+).

The same US and clinical control was systematically performed 6 weeks later (W6). Patients who had no pain relief or persistent pain over 3 on VAS score and remaining tendon abnormalities on US at W6, independently of tendon lesion size or functional score, benefited of a second PRP injection under US guidance (PRPT2+).

The same US and clinical control was also performed 6 weeks later (W12). All these procedures were performed by a senior musculoskeletal radiologist (LP, PM, AS, BD). The exclusion criteria were in contraindication to injection

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under US guidance (pregnancy, infectious arthritis, previous corticosteroid local injection), acquired or induced immuno-deficiency. There were no age or sex inclusion criteria. Among the eligible patients, none had exclusion criteria. All patients were informed of the study procedure and gave their informed consent.

Platelet-rich plasma preparation

For PRP preparation, the patient was referred to a biologist doctor (AP). A sample of 27 mL of venous blood was collected in a syringe containing 3 mL of ACD-A anticoagulant and was centrifuged at 620 g during 15 min. A final volume of 3 mL of PRP was recuperated in the middle part of plasma. The obtained PRP had a platelet concentration equal to about three times the concentration measured in the blood as verified counting platelets under an optical microscope for 40 patients (900,000 ± 15,000 platelets per mm$^3$). Leukocytes were also measured (255 ± 35 per mm$^3$). PRP (without activator) was injected within 30 min at the end of centrifugation.

Ultrasonography

All patients had US evaluation with a 17 MHz linear probe using mode B-mode (IU 22 Philips Medical Healthcare Amsterdam, Netherlands) to confirm tendon tear or tendinosis. Loco-regional anesthesia was performed using 1 mL of 1% Xylocaine® within the subcutaneous fat. Tendon puncture were performed under US guidance, with a 21 G intramuscular needle (LP, PM, AS, BD). After confirmation of intra-tendinosis or tear position of the needle, we injected 3 cm$^3$ of PRP mixture in first and second PRP treatment (PRPT+ and PRPT2+) (Fig. 1).

Image interpretation and clinical data

All the images were anonymized and the date of examination was hidden. All pre- and post-therapeutic US were interpreted by a senior musculoskeletal radiologist (BD) in random patient order. We used a pre-written reading grid, recording for each anatomic compartment lesion type (tear or tendinosis), size of the lesion on T+ at D0 (baseline), on PRPT+ at W6 and on PRPT2+ at W12.

Clinical data assessment at D0 before PRP treatment, W6, W12 and on long-term 32-month used QD [27] for upper limbs and WOMAC test [28] for lower limbs to calculate a global score, VAS score and only one binary (yes or not) subjective index satisfaction for both are collected. Others treatments, such as infiltration or surgery, were noted.

Statistical methods

Statistical analysis was performed using the SAS software (Cary, NC). Binary variables were tested with the Mac Nemar test. Differences on lesion size in tear or tendinosis were compared using the Wilcoxon test. Then, the evolution of PRPT+ was assessed using a Friedman test. A $P$ value less than 0.05 was considered as significant.

Results

Population data

Twenty-four consecutive patients (20 men and 4 women) were included in this study from January 2010 to September 2012 with an average follow-up of 21.5 months. Mean age was 47.2 ± 12.4 years and median age was 46 years. Seventeen lesions out of the 17 patients (14 men and 3 women) involved the upper limb [lateral epicondylar tendons: $n=15$ (88%), medial epicondylar tendons: $n=2$ (12%)] and seven out of the seven patients (6 men and 1 woman) involved the lower limb [Achilles tendon: $n=3$ (43%), patellar tendon: $n=2$ (29%), peroneal tendons: $n=2$ (29%)].

Before first PRP injection, the average length of time for symptoms was 5.3 ± 1.7 months.

Previous treatments consisted in rehabilitation using analgesic physiotherapy and eccentric work. Corticosteroid injection was an exclusion criteria.

No additional treatment was used between first and second PRP injection. Besides transitory local pain, no major side effects or complications were encountered.

Efficacy of PRP2+ to treat PRPT+: US data

The mean US size of lesions for upper and lower member were not significantly lower at W12 after PRPT2+ as compared to W6 ($P=0.86$ in upper and $P=NS$ in lower member), respectively for lateral epicondylar tendon lesions ($P=0.78$), for medial epicondylar tendon lesions ($P=NS$); for patellar tendon ($P=0.5$) and for the others anatomic compartments ($P=NS$) in the lower limb (Fig. 2).

Nevertheless, comparison between size of lesions at D0 to W6, and D0 to W12 were statistically significant ($P<0.001$).

Figure 1. Ultrasound image of lateral epicondylar tendon (long axis) shows longitudinal tear (white arrows) before (a) and during (b) PRP injection using a 25 G needle (arrowheads).
in upper and lower member). All these results were independent of age (P=0.22), gender (P=0.97) and type of tendinopathy (P=NS).

Table 1 summarizes the mean and standard deviation (SD) of tendon lesions for each anatomic compartment.

**Efficacy of PRP to treat PRPT+: clinical data**

Patients do not recover a significant ability to mobilize pathologic tendons when comparing PRPT2+ at W12 with PRPT+ at W6 (P=0.69 for upper and P=0.66 for lower limb) and with long-term follow-up (P=0.86 for upper and P=0.75 for lower limb).

In upper member, QD is 39.8 ± 9.4 at D0, 17.2 ± 9.1 at W6, 14.7 ± 3.2 at W12, 14.2 ± 4.3 at 21.5 months of average follow-up. In lower member, WOMAC is 34 ± 12.1 at D0, 9.4 ± 2.7 at W6, 7.3 ± 2.2 at W12, 6.7 ± 4.9 at 21.5 months of average follow-up.

Table 2 summarizes the mean and standard deviation (SD) of functional evaluation in tendon lesions and each anatomic compartment.

Nevertheless, comparison between baseline and long-term follow-up functional score were statistically significant (P<0.001 in upper and lower member) and patients recover finally a significant ability to mobilize pathologic tendons.

All these clinical results are independent of age (P=0.39), gender (P=0.63) and kind of tendinopathy (P=NS) at W12 and on long-term follow-up.

VAS score were also no statistically significant (P>0.27) between W6 and W12: 5.8 ± 1.7 at D0, 3.8 ± 2.2 at W6, 2 ± 0.7 at W12, 1.4 ± 1.9 at long-term follow-up.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean and SD (millimeters) values of US lesions in each anatomic compartment in upper and lower limb at day 0 (D0), week 6 (W6) and week 12 (W12).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper limb</td>
<td>Lower limb</td>
</tr>
<tr>
<td></td>
<td>DO</td>
</tr>
<tr>
<td>Lateral epicondylar tendons</td>
<td>8.4 ± 2.6</td>
</tr>
<tr>
<td>Medial epicondylar tendons</td>
<td>8 ± 1.4</td>
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</table>
However, there were 22 (92%) patients who were satisfied of the procedure and PRP treatment on long-term follow-up. None of the patients got corticosteroid injections or surgery after second PRP injection.

**Discussion**

Our study suggests that second early intra tendinous PRP injection under US guidance does not permit rapid decrease of tendinopathy area in US nor does it rapidly improve clinical pain and functional data in case of incomplete efficiency of a first PRP injection. However, our clinical data also suggest that protocol based on 2 PRP injections is efficient in long term follow up as patients recovered a significant ability to mobilize pathologic tendons after PRP injections.

Many therapeutic strategies are currently being evaluated clinically. Reports assessing autologous blood, PRP, sclerotic drugs administrated in different injection sites (intra-, peritendinous) under different conditions (clinically-guided, imaging-guided) on small populations have been published. However, these studies involved biases, such as lack of long-term follow-up and no histological examination [25]; hence, resulting in contradicting conclusions. Studies assessing the efficacy of intra- or peritendinous [29–32] or intra-articular PRP injections [33,34] on clinical patients have also provided discordant results, with no consensus on whether PRP injection should be used or not. Consequently, there is no consensus regarding which drug to use in this indication. By following patients treated by PRP with long-term follow-up, including a systematic clinical and US examination, our study provides strong evidence that PRP might be a useful strategy.

Physiologically, a tendon is formed by collagen fibers, associated with striated muscle cells in smaller quantities than in muscle. Histology shows early anarchic misalignment of muscle cells and collagen fibers, resulting in important fibrillar disorganization after induction of tendinosis [2]. Reports show no sign of inflammation. Regarding neo-angiogenesis, it has been shown that neo-vessels, even early after the beginning of tendinosis, carry proteolytic enzymes, nitric oxide and deleterious prostaglandines that may be responsible for tendon degeneration [35–37]. Conversely, in the healing process, these neo-vessels provide active growth factors, which stimulate scarring, and leucocytes recruitment for antibacterial effect. Growth factors promote stem cells and stimulate directly fibroblast-mediated collagen production [37]. Intra-tendinous injection of PRP, by providing important concentration of active growth factors (PDGF, TGF-β, VEGF...), might promote stem cells recruitment and fibroblast collagen production, and therefore, stimulate tendon scarring [38,39].

We acknowledge that our study have several limitations. One should note that our injection protocol was based on an early second intra-tendinous injection of PRP [19,29] and used a limited platelet concentration. Our results might have been different if we had used lower or higher platelet concentration and/or repeated intra-tendinous injections. Such strategies will have to be evaluated in further studies.

Second, US was only use to assess the pathological status of tendons at D0, W6 and W12, but not on long-term follow-up. None surgical evaluation or MRI were used to assess the pathological status of tendons. However, 17MHz US transducers provide excellent spatial resolution and allow precise measurement of stretched tendons as well as needle guidance. We also voluntary choose US rather than MRI as it is a faster, more available, cheaper and as efficient for tendon assessment. Third, our study is retrospective with only 24 patients and suffers from the lack of a control group and therefore requires future confirmation and further studies. According to our experience, we are confident that our study that includes 24 patients offers sufficient evidence to conclude that an early second PRP injection is useless in case of incomplete inefficiency of first injection.

However, to our knowledge, this is the most important retrospective study about second PRP injection efficacy with an exact knowledge of PRP composition concerning platelets and leukocytes and reproducible clinical scores. PRP therapies are more complicated than previously acknowledged, and an understanding of the fundamental processes and pivotal molecules involved will hopefully be elucidated soon. Therefore, major issues, including standardization of formulations and application procedures, need to be clarified to inform clinical studies before recommending best practice guidelines [40–43].

**Conclusion**

Our study suggests that second early intra tendinous PRP injection under US guidance does not permit rapid decrease of tendinopathy area in US nor does it rapidly improve clinical pain and functional data in case of incomplete efficiency of a first PRP injection. However, our clinical data also suggest that protocol based on 2 PRP injections is efficient in long term follow up as patients recovered a significant ability to mobilize pathologic tendons after PRP injections.
of tendinopathy area in US, nor does it quickly improve clinical pain and functional data in case of incomplete efficiency of a first PRP injection.

However, our clinical data also suggest that protocol based on 2 PRP injections is efficient in long-term follow-up as patients recovered a significant ability to mobilize pathologic tendons after PRP injections. Additional pre-clinical and clinical studies comparing PRP to currently used methods might be of high interest to consolidate clinical practice in human [44–48].

Disclosure of interest
The authors declare that they have no conflicts of interest concerning this article.

References


