The Achilles heel of adults and children
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INJECTION TECHNIQUES OF PLATELET-RICH PLASMA INTO AND AROUND THE ACHILLES TENDON: A CADAVERIC STUDY

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ABSTRACT

Background: Platelet Rich Plasma (PRP) injections are used to treat (Achilles) tendinopathies. It has been injected at different locations, but the feasibility of PRP injections and distribution after injection has not been studied.

Purpose: to evaluate (1) the feasibility of ultrasound guided PRP injections into the Achilles tendon (AT), and in the area between the paratenon and the AT (2) the distribution of PRP after injection into the AT and in the area between the paratenon and AT.

Study design: Descriptive laboratory study.

Methods: 15 cadaveric lower limbs were injected under ultrasound guidance with Indian blue-dyed PRP. Five injections were placed into the AT at the midportion level; five injections were located anterior between the paratenon and AT and five posterior between the paratenon and AT. The limbs were anatomically dissected and evaluated on presence and distribution of PRP.

Results: All injections into the AT showed PRP infiltration in the AT as well as in the area between the paratenon and AT (median craniocaudal spread: 100 mm (75-110)); one of five limbs showed PRP leakage into the Kager fat pad after AT injection. All anterior and posterior injections showed PRP infiltration in the area between the paratenon and AT (median 100 mm (75-150)). The AT was infiltrated with PRP after three of ten paratenon injections.

Conclusions: The “AT” and “Paratenon” injections under ultrasound guidance proved to be accurate. Injections into the AT showed distribution of PRP into the AT as well as in the area between the paratenon and AT. All injections between the paratenon and AT showed PRP distribution in that area, as well as in the Kager fat. pad
INTRODUCTION

A number of articles have been published on the possible healing effects of Platelet Rich Plasma (PRP) in patients with Achilles tendinopathy\(^7,9,12,23,27\). The injection technique in Achilles tendons (AT) and the distribution of PRP after injection has not been studied. PRP is currently injected at different locations in and/or around the AT. It has been injected in the AT\(^9\); “at the hypoechogenic lesion of the AT on ultrasonography”\(^12\); “at the intratendinous and peritendinous lesion”\(^10\) or “at the site of pain and any bulbous mass”\(^28\) and “at the site of injury”\(^27\). These ‘locations’ however lack a precise anatomic description of the injection site. It seems fair to choose the injection site based on the anatomical location of pathology (eg, in the tendon). The symptoms in patients with Achilles tendinopathy may result however, from pathology between the AT and the paratenon, not the tendon itself\(^2,4,8,15,17,18,24,32\). This justifies an injection between the tendon and the paratenon, in addition to or instead of an intratendinous injection. Hence, the location of PRP after injection is important: if PRP is not located at the site of pathology, it may be of no help just because of its wrong location. Reach et al. described the approach to tendons and joint spaces of the foot using an ultrasound guided needle. However they did not study the distribution of the injected substance\(^25\).

The purpose of this study was to evaluate (1) the feasibility of ultrasound guided PRP injections into AT, and the area between the AT and paratenon, (2) the distribution of PRP after injecting it into the AT and in the area between the AT and paratenon. Additionally, we were interested if multiple injections into the AT differ from a single injection into the AT.

METHODS

In this cadaveric study 15 fifteen lower limbs were used to inject Indian blue-dyed PRP. The PRP was injected into the AT (five times), anterior between the paratenon and the AT (five times), and posterior between the paratenon and the AT (five times) (Fig. 1). The duration of each injection was timed, starting as the radiologist received the syringe with PRP, ending as the radiologist signaled he had finished the injection. After injection, the lower limbs were carefully dissected by one orthopedic surgeon. During dissection the different structures were evaluated on the presence and distribution of dyed PRP.
Specimen demographics

This study was undertaken on 15 fresh frozen cadaveric lower limbs, six male, and nine female specimens. Age of the specimens ranged from 68 to 89 years (median, 84.5 years). The feet had not been operated on. Information regarding tendinopathy or other pathology of the AT was not known to the authors.

PRP production and colouring

We chose to use 4.5 mL PRP as major clinical studies used this amount of PRP for their injections. The PRP was prepared at the laboratory of “clinical chemistry”, 300 mL donor citrate blood (0.0109M) was used to make 70 mL PRP. The blood was retrieved by means of an open system without stowing, hereafter the blood was centrifuged at 180 G for 15 minutes at 20 degrees Celsius (Rotina 46 RS Hettich Zentrifugen, Tuttlingen Germany). After centrifuging, the PRP was removed using a pipette. A small amount, 0.3 mL injection, of the colouring agent Indian blue was added to the PRP to simplify the detection of PRP during the dissection.

Injection Technique

The ankles were placed in prone position by the preference of the radiologist (Fig. 2). The designated injection location was recorded prior to the injection. A medial approach to the AT was chosen. This is the same approach as used in vivo to avoid damage to the sural nerve. We used a Philips iU22 ultrasound machine (Philips Healthcare, Philips Medical Systems, Eindhoven, the Netherlands) with a 17.5 MHZ transducer for all ankles. The musculoskeletal presetting of the machine was used. Five cadavers were injected at the midportion level into the AT. No hypoechogenicity of the tendon was recorded, therefore

Fig. 1: Axial T1-weighted MIR study of the ankle. A, the location of the injection needle anterior between the paratenon and the Achilles tendon (AT). B, the location of the injection needle into the AT. C, the location of the injection needle posterior between the paratenon and the AT.
the injection were not specifically directed towards tendon lesions. In 2 of the 5 ankles, the injection was given in 3 separate locations using a peppering technique, as described in the literature in vivo. The final three tendons were injected through a single PRP injection. As the paratenon itself is not always clearly visible with ultrasound, the only way to inject PRP between the paratenon and the AT is to stay as close to the AT as possible with the tip of the needle. In five cadavers the PRP was injected at the interface between the paratenon and the AT (anterior paratenon injection). Five cadavers were injected dorsally between the AT and the paratenon (posterior paratenon injection). The needle, always 16G, was introduced with the US transducer in the transverse plane. In this direction the needle tip is visualized as a small, very hyperechoic structure with acoustic shadowing (Fig. 3). After
positioning of the tip in the desired area, a total 4.5 mL of PRP/Indian blue mixture was injected. The ankle was dissected directly after the injection.

**Anatomical dissection**

Similar to the clinical setting, the foot stayed in prone position throughout the injection and dissection procedure\(^\text{27}\). Anatomical dissection of the specimens was performed using a standard technique by one orthopaedic surgeon. This consisted of a longitudinal skin dissection from the gastrocnemius muscle to the calcaneus insertion. The injection location was marked by a small skin incision or by keeping the needle in place during the dissection. We considered this approach would preserve the anatomical relationship between the paratenon and AT during dissection. The proximal and distal distance from the PRP-injection fluid to the needle was measured using a ruler and confirmed by two observers. Hereafter the AT was transected proximally, distally and at the level of the needle.

**RESULTS**

The results are divided in three groups, based on injection technique: an AT group; an anterior paratenon group and a posterior paratenon group (Table 1).

**Table 1.** Table showing the number of injections, craniocaudal spread (median, min and max), presence of PRP in the AT, in the area between the AT and the paratenon and in Kager’s fat as well as the injection time (median, min and max). Results are shown, in total and divided in subgroups of the different injection techniques.

<table>
<thead>
<tr>
<th>Injection Technique</th>
<th>Number of injections</th>
<th>Craniocaudal spread</th>
<th>PRP traces in AT</th>
<th>PRP traces between the AT and paratenon</th>
<th>PRP traces in Kager’s fat</th>
<th>Injection time (per injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Paratenon injection</td>
<td>5</td>
<td>95 mm (70-110)</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>130 sec (105-197)</td>
</tr>
<tr>
<td>Posterior Paratenon injection</td>
<td>5</td>
<td>100 mm (80-150)</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>121 sec (110-140)</td>
</tr>
<tr>
<td>AT Multiple injections</td>
<td>2</td>
<td>90 mm (80-100)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>118.5 sec (105-132)</td>
</tr>
<tr>
<td>AT Single injection</td>
<td>3</td>
<td>100 mm (70-110)</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>56 sec (55-73)</td>
</tr>
<tr>
<td>All injections</td>
<td>15</td>
<td>100 mm (70-150)</td>
<td>8</td>
<td>15</td>
<td>11</td>
<td>115 sec (55-197)</td>
</tr>
</tbody>
</table>
AT injections

The median craniocaudal spread of PRP after injection into the tendon was 100 mm (range, 70-110 mm). All AT injection resulted in PRP infiltration of the area between the paratenon and AT (Fig. 4A). On dissection, five out of five AT injections showed infiltration of PRP in the AT (Fig. 4B). Regarding the involvement of the Kager fat pad after an AT injection, only one out of five showed traces of PRP after injection. This was seen in a case where the multiple injection technique was used. Furthermore, no differences were found in distribution of PRP between a single injection and multiple injections. The radiologist noted that a single injection requires more pressure from the injector than multiple separate injections. The amount of necessary pressure was not measured. The median duration for an AT injection was: 73 seconds (range 55-132 seconds).

Fig. 4: A, right cadaveric ankle after injecting platelet-rich plasma (PRP) into the Achilles tendon (AT). A gradual spread of PRP between the paratenon and AT was seen. B, distribution of PRP was seen in the AT and between the paratenon and AT, mainly in the dorsal direction.

Fig. 5: A, left cadaveric ankle after injecting platelet-rich plasma (PRP) anterior between the paratenon and the Achilles tendon (AT). A gradual spread of PRP between the paratenon and AT was seen. However, the posteromedial part of the area between the paratenon and the AT did not show infiltration of PRP. Fig. 5B: Distribution of PRP was seen between the paratenon and AT mainly in the ventral direction, including Kager fat pad. The AT was not infiltrated with PRP.
Anterior paratenon injections

Injecting PRP using the anterior paratenon technique gave a mean craniocaudal spread of 95 mm (range, 70-110mm). All five injections showed an infiltration of the area between paratenon and AT. Furthermore, we found that the injection caused a gradual spread of PRP around the AT, but never in the posteromedial part of the area between the paratenon and AT (Fig. 5A). One specimen showed PRP infiltrations in the AT. All five specimens in this group showed involvement of the Kager fat pad after PRP injection (Fig. 5B). The median duration for an anterior paratenon injection was 130 seconds (range, 105-197 seconds).

Posterior paratenon injections

Injecting PRP using the posterior paratenon technique gave a median craniocaudal spread of 100 mm (range, 80-150mm) (Fig. 6A). All five injections showed an infiltration of the area between paratenon and AT. A posterior paratenon injection resulted in a full and gradual spread of PRP on the dorsal side of the AT (Fig. 6B). Two specimens showed PRP infiltrations in the AT, with a spread of 80 and 90 mm craniocaudally. Only the posterior half of the tendon’s transversal surface was infiltrated. All five specimens in this group showed involvement of the Kager fat pad after PRP injection. However, compared to anterior paratenon injections the involvement of PRP in the Kager fat pad was evidently less. The median duration for a posterior paratenon injection was 121 seconds (range, 110-140 seconds).

Fig. 6: A, right cadaveric ankle after injecting platelet-rich plasma (PRP) posterior between the paratenon and the Achilles tendon (AT). A gradual spread of PRP between the paratenon and AT was seen. Fig. 6B: Distribution of PRP was seen between the paratenon and AT, mainly in the dorsal direction. The AT was partially infiltrated with PRP.
DISCUSSION

Our study has shown it is feasible to inject PRP under ultrasound guidance into the AT as well as in the area between the AT and paratenon. All injections into the midportion of the AT showed PRP infiltration in the AT as well as in the area between the paratenon and AT. The injections between the paratenon and AT showed PRP infiltration in that area, as well as in the Kager fat pad. We found evident differences between intratendinous injections and paratenon injections regarding the distribution of PRP after injection.

Two different intratendinous injection techniques were studied: a single AT midportion injection versus multiple AT midportion injections using a peppering technique. In both techniques the same total amount of PRP was injected. The difference in craniocaudal spread of PRP between the two techniques was marginal (Table 1). This suggests, regarding the objective spread of PRP, there is no benefit of the more invasive peppering, multi-injection technique over the single injection technique. With the peppering technique multiple punctures of the tendon are created, leading to more damage and possible more leakage of PRP. We found PRP to be present in the area between the AT and the paratenon after every intratendinous injection. This can be explained by basic physics: PRP takes the path of least resistance. As the AT is relative dense, pressure builds during the injection process. At a certain moment it is easier for PRP to spread back along the length of the needle towards the injection site. After leaving the AT and spreading according the path of least resistance, the first area for the PRP to spread out (easily) is the area between the AT and paratenon. Only one of five dissected specimen carried (minor) traces of PRP in the Kager fat pad. It should be noted specifically these were minor. These traces may have been caused by a retraction of the needle, giving PRP the opportunity to follow the path of the needle outside the tendon and into the Kager fat pad. Another option is the puncture of the paratenon during the injection procedure. Hereby creating a small defect causing the PRP to leak into the Kager fat pad. An intratendinous injection took less time when compared to a paratenon injection (posterior or anterior). This is probably a result of the easy detectable AT, compared to the more difficult to find area between the paratenon and the AT, speeding the process of proper needle placement prior to injection. Although injections between the paratenon and the AT proved to be feasible, it should be noted other structures also contained traces of PRP. We found PRP to be present in the AT in 3 out of 10 dissected specimens. Additionally, the Kager fat pad contained PRP in every specimen. The large spread of PRP into the Kager triangle after paratenon injections may be explained by the following: a paratenon injection, both posterior and anterior, located precise and only between the paratenon and the AT is a difficult procedure. Even under US control, the chances of double puncturing the paratenon are real: for injection a medial puncture of the paratenon is necessary, however if the needle is inserted too far/deep the paratenon is also punctured at the lateral side (Fig. 7). The radiologist may correct the location of the needle...
by retracting the needle into the area between the paratenon and the AT, the paratenon however is damaged. Hereby a passage outward the paratenon and into the Kager fat pad is created. If one injects after puncturing and correcting the needle, PRP will not only be injected in the area between the paratenon and AT, but also in the Kager fat pad. Furthermore, after the injection the needle is retrieved, a small defect is left behind: this is another defect of the paratenon, possibly leading to additional PRP leakage. The findings of PRP in the AT of three specimens may also be explained by the difficulty of this particular injection. In addition, if the AT is merely touched by the needle a passage is created, giving PRP the opportunity to spread into the AT. The posteromedial part of the area between the paratenon and the AT did not contain any PRP after anterior paratenon injections. This may be the result of the necessary pressure PRP needs to reach around the entire AT. The surrounding tissues’ pressure was probably larger than the volemic pressure of the injected PRP, hereby inhibiting a more dorsal spread of PRP. The imbalance of pressures may have been amplified by gravity as the specimens were in a prone position. This implies that a posterior paratenon injection would fill the midline with PRP. Our results confirmed this.

Treatment protocols have focused on the disruption of possible neo-vascularisation and neo-innervation in the area between the AT and the paratenon. Along with the possible healing effects, PRP injections may be beneficial in Achilles tendinopathy by means of another mechanism: located between the paratenon and the AT, PRP may cause a disruption of the aforementioned neo-structures in that area. Either an intratendinous injection or a posterior paratenon injection may provide this healing effect, as all specimens showed infiltration of the entire area between the AT and the paratenon. However, none of the anterior paratenon injections resulted in a full and gradual spread of PRP around the entire AT (Fig 4, Fig 5). A disruption of the entire area around the AT after anterior paratenon injections is therefore highly unlikely.

Steroids have been injected into the AT to treat tendinopathies, complications hereof, specifically AT rupture, have been discussed thoroughly. As a reaction to these complications, steroids are also injected around the AT. In 1992 Mahler and Fritschy questioned the difference in spread after injection of steroids between “peritendinous” injections and “intratendinous” injections throughout the AT. We found traces of PRP in the AT in 30% of the injections between the paratenon and AT. This may not only apply to PRP but also to other injected substances (eg, steroids). Steroids therefore may be present in the tendon, with its complication risk, even if they are not injected into the AT.

As previously stated, we did not have information regarding tendinopathy or other pathology of the AT, nor did we test for this histologically. The question remains whether the same results are found in Achilles tendons with diagnosed Achilles tendinopathy or other pathology. Further research is necessary to study the results of different injections techniques in specimens with diagnosed Achilles tendinopathy.
In conclusion we found PRP injections to be accurate both in the AT as well as in the area between paratenon and the AT. Both injection techniques cause a spread of PRP throughout the area between the paratenon and the AT. Due to the level of difficulty of this technique, PRP may leak into the Kager fat pad after paratenon injections. Keeping in mind that the healing effect of PRP injections for midportion Achilles tendinopathy is strongly debated, if PRP is injected, we advice the following: the best PRP injection for midportion Achilles tendinopathy would be a single posterior ultrasound guided paratenon injection. A posterior paratenon injection gives the best chance for a proper distribution of PRP at the presumed location of pathology. In addition to the anatomic location, one should keep the difficulty of this injection and the loss of PRP in mind.

![Fig. 7](image.png)

A, the injection needle between the paratenon and Achilles tendon (AT) is inserted too far, hereby puncturing the paratenon. B, a small defect (arrow) is created in the paratenon after the needle is retrieved. C, during the injection procedure platelet-rich plasma (PRP) may leak outside the paratenon and AT.
REFERENCES