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Comparison of Postinjection Protocols After Intratendinous Achilles Platelet-Rich Plasma Injections: A Cadaveric Study

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ABSTRACT

The purpose of the present investigation was to evaluate the distribution of intratendinous injected platelet-rich plasma (PRP) after 15 minutes of prone resting versus immediate manipulation simulating weightbearing. Ten cadaveric lower limbs were injected under ultrasound guidance with PRP dyed with India blue ink. The dyed PRP was injected into the mid-portion of the Achilles tendon, after which 5 specimens were placed in the prone position for 15 minutes (simulating rest) and the remaining 5 specimens were manipulated through 100 cycles of ankle dorsiflexion and plantarflexion (simulating walking). Thereafter, the specimens were dissected, and the distribution of the India blue dye was ascertained. In the simulated rest group, every specimen showed dyed PRP in the Achilles tendon and in the space between the paratenon and tendon. The median craniocaudal spread of the PRP was 140 (range 125 to 190) mm. In 4 of the simulated rest tendons (80%), the distribution of PRP extended across the entire transverse plane width of the tendon. In the simulated motion group, every specimen showed dyed PRP extending across the entire transverse plane width of the tendon and in the space between the paratenon and tendon. The median craniocaudal spread was 135 (range 115 to 117) mm. No statistically significant difference was found in the amount of craniocaudal spread between the simulated motion and rest groups. In conclusion, it does not appear to matter whether the ankle has been moved through its range of motion or maintained stationary during the first 15 minutes after PRP injection into the mid-portion of the Achilles tendon. The precise meaning of this information in the clinical realm remains to be discerned.

The possible healing effect of platelet-rich plasma (PRP) on Achilles tendinopathy depends on the influence of the platelets on the damaged tenocytes and adequate delivery of the PRP at the lesion site (1–5). A previous study has confirmed that PRP reaches the designated anatomic location after ultrasound-guided injection into and around the Achilles tendon (AT) (6). In addition to the role of the specific injection location, the postinjection protocol could be of importance (6). Little is known about the distribution of the fluid after injection into the AT (6). The PRP, injected at the desired location, might gradually disperse to other locations under the influence of gravity or ankle movement after injection. Different postinjection protocols have been described in clinical studies reporting on the effects of PRP in tendinopathy (7–12). One postinjection protocol advocated immediate weightbearing. In contrast, others (7) have advised maintaining a prone position for at least 10 to 15 minutes after injection (11,12). Finally, partial weightbearing for the first few days after the injection has also been recommended (8–10). These considerable differences could cause variation in the clinical outcomes after PRP therapy for Achilles tendinopathy (1,12).

The primary purpose of the present study was to evaluate the effect of 2 different postinjection protocols after intratendinous, mid-portion AT PRP injection on the spread of PRP in and around the AT. We measured the spread of the PRP after simulated ankle motion and compared it with the spread after a 15-minute postinjection period of rest in a cadaveric model. Our secondary aim was to compare the results of our investigation with those described in a previously published report (6).

Materials and Methods

In the present cadaveric study, 10 lower limbs were injected with India blue-dyed PRP. The PRP was injected into the AT. The duration of each injection was timed, with

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the time starting as the radiologist (M.J.) received the syringe with dyed PRP and ending as the radiologist signaled the injection had finished. After injection, 5 limbs (50%) were placed in the prone position for 15 minutes. The other 5 (50%) were manipulated manually for 5 minutes through 100 cycles of ankle dorsiflexion and plantarflexion in an effort to simulate the motion associated with immediate weightbearing ambulation in the clinical setting. The specimens were randomly assigned in equal size groups using a computerized randomization program to either the simulated motion or rest group. Specifically, the postinjection cadaveric ankle was maximally dorsiflexed from the neutral position (foot at 90° to the leg) and maximally plantarflexed to the soft tissue end range of motion in each direction, with each excursion from the neutral position to maximum dorsiflexion and then to maximum plantarflexion and back to the neutral position, accounting for 1 cycle. Thereafter, an orthopedic surgeon (G.K., C.D.) carefully dissected each specimen, and the presence of the dyed PRP was documented in regard to its gross anatomic distribution in and about the AT. Also, the extent of the spread from the site of the injection in the mid-portion of the AT was measured.

Specimen Demographics

The present study included 10 fresh frozen cadaveric lower limbs (4 [40%] male and 6 [60%] female specimens). The median age of the specimens was 74 (range 58 to 83) years. None of the specimens displayed evidence of previous surgery on any part of the extremity, and we did not have any information regarding any specific AT pathologic features. To be included in the present study, inspection of the cadaveric specimen had to reveal the absence of any apparent scarring in the AT region.

PRP Production, Coloring, and Injection Technique

For meaningful comparison with previous studies (6,13), we chose to use a PRP production, coloring (dyeing), and injection technique that has been previously described (6). The PRP was prepared at the clinical chemistry laboratory of the Academic Medical Center (Amsterdam, The Netherlands). The process involved the use of 210 mL of donor citrate blood (0.0109 M), which was used to make 50 mL of PRP. The blood was retrieved using an open system without storing the whole blood, after which the blood was centrifuged at 180*g* for 15 minutes at 20°C (Rotina 46 RS Hettich Zentrifugen, Tuttlingen, Germany). After centrifuging, the PRP was removed using a pipette, and 1.25 mL of India blue dye was added to the 50-mL aliquot of PRP, binding directly with the plasma, enabling a thorough detection of PRP with the bound dye in the tissues after injection. The ankles were placed in the prone position (Fig. 1). The designated injection location was recorded before the injection. A medial approach to the mid-portion of the AT was used, because this is commonly used in clinical practice to avoid damage to the sural nerve. A Philips iU22 ultrasound machine with a 17.5-mHz transducer (Philips Healthcare, Philips Medical Systems, Eindhoven, The Netherlands), tuned to the musculoskeletal presetting, was used to visualize the tendon for accurate placement of the injection into the substance of the AT and to determine whether any of the specimens displayed a defect identifiable by ultrasonography. Each of the cadavers was injected intratendinously at the mid-portion level (2 to 6 cm proximal to the AT insertion into the calcaneus) (14). The injections were not specifically directed toward a tendon lesion, because hypoechogenicity (irregular or few internal echo patterns) of the AT was not found in any of the specimens. The total injection, consisting of 5 mL of dyed PRP, was administered in 3 separate portions of approximately 1.5 mL, placed approximately 1.5 cm apart using a peppering technique, as previously described for the in vivo treatment of Achilles tendinopathy (7). The same 16-gauge needle and 5-mL syringe were used for each of the 3 injections, and the ultrasound transducer was used to guide the transverse plane injection of the dyed PRP. After positioning the tip of the needle in the desired intratendinous location, the dyed PRP was injected. Thereafter, the specimen was either manipulated to simulate motion or allowed to rest in the prone position, similar to the clinical setting (7), for 15 minutes.

Anatomic Dissection

Just as with the PRP production and injection technique, the anatomic dissection of the specimens was performed using a previously described technique (6). The technique preserves the anatomic relationship among the paratenon, AT, and surrounding tissues during dissection (Fig. 2). The dissection entailed a longitudinal skin incision that extended from the distal margin of the gastrocnemius muscle to the calcaneal insertion of the AT (Fig. 3). The most proximal and most distal grossly visible extent was measured from the point of injection of the dyed PRP using a ruler and confirmed by 2 observers. To fully appreciate the spread of the dyed PRP, the AT was transected proximally, distally, and at the level of the injection point (Fig. 4).

Results

A statistical description of the results is presented in Table. In the simulated rest group, the median duration of the injection of the dyed PRP was 111 (range 99 to 164) seconds. Moreover, in the rest group, the median craniocaudal gross visible spread of the dyed PRP after 15 minutes in the prone position was 140 (range 125 to 190) mm. All the specimens displayed dyed PRP in the AT at the tendon's insertion into the calcaneus and proximal to the mid-portion injection site and in the area between the paratenon and AT (Fig. 3). In 4 (80%) of the 5 tendons, the dyed PRP was distributed throughout the entire coronal plane of the AT (Fig. 4). Three of the tendons (60%) showed grossly visible traces of the dyed PRP in the pre-Achilles fat pad, and none of the tendons in the rest group showed infiltration of the dyed PRP into the plantaris tendon. In the simulated motion group, the median duration of the injection of the dyed PRP was 124 (range 99 to 131)

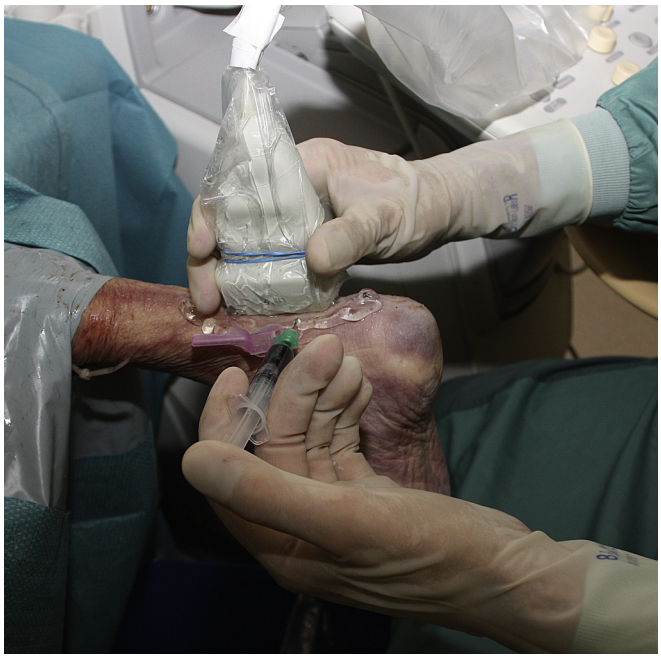

Fig. 1. Placement of cadaveric ankles for injection procedure and location of ultrasound-guided injections.

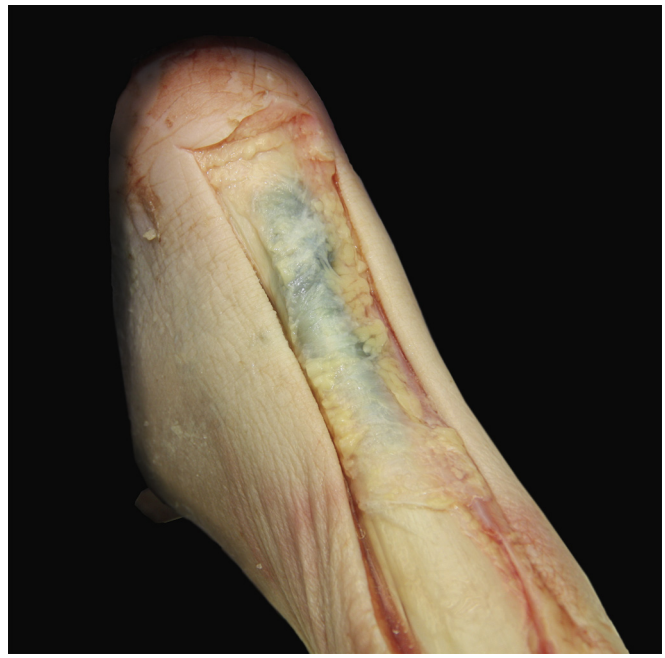

Fig. 2. Anatomic dissection of the cadaveric lower leg showing infiltration of the Achilles tendon with India blue-dyed platelet-rich plasma.

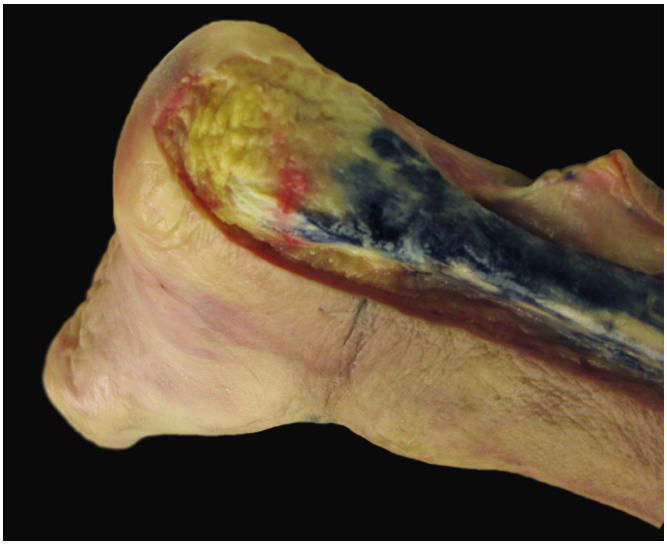

Fig. 3. Anatomic dissection of the cadaveric lower leg showing infiltration of the Achilles tendon with India blue-dyed platelet-rich plasma spreading up to the insertion of the Achilles tendon onto the calcaneus.

seconds. Moreover, in the simulated motion group, the median craniocaudal gross visible spread of the dyed PRP after 100 cycles of dorsiflexion and plantarflexion motion was 135 (range 115 to 170) mm. In all 5 of these tendons, the dyed PRP was distributed throughout the entire coronal plane of the AT and in the area between the paratenon and AT. Four of the simulated motion tendons (80%) showed grossly visible traces of the dyed PRP in the pre-Achilles fat pad (Fig. 5), and none showed infiltration of the dyed PRP into the plantaris tendon.

Discussion

The present study evaluated the effect of 2 different postinjection protocols after injection of PRP, dyed with India ink, into the midportion of cadaveric ATs. In 1 group of ATs, motion was simulated using ankle 100 cycles of dorsiflexion and plantarflexion; in the other group, the specimen was maintained stationary in the prone position for 15 minutes before dissection and identification of the spread of the dyed PRP. Previous studies have evaluated the feasibility of fluid injections into and around different ankle ligament and the feasibility and accuracy of PRP injections into and around the AT (6,13). To our knowledge, this is the first study comparing postinjection protocols on the spread of PRP after intratendinous injection. As a consequence of this absence, the postinjection protocols have differed widely among therapeutic studies on the effect of PRP (7–12). With the varying results from these studies and the accuracy of the injections in mind, the postinjection protocol could have significant influence on the outcomes of these studies (4,7–9,12,15–19). Because most intratendinous injection methods are currently comparable, it would be interesting to examine the influence of the postinjection protocols on the outcomes of PRP injection.

From the results of the present study, no relation seems to be present between a specific postinjection protocol and the spread of PRP. Hence, when extrapolating the results of our study to clinical scenarios, no benefit seems to exist for 1 compared with the other postinjection protocol. The clinical use of different postinjection protocols therefore remains debatable.

Compared with direct dissection, a substantially more expanded spread of PRP was found with any postinjection protocol (6). The direct dissection injections were placed identical to the injections used in the present study, with only the postinjection method as the variable. The different results are obvious. Wiegerinck et al (6) published a craniocaudal spread of 95 mm directly after injection (6). Compared with the current median of 135 mm in the simulated rest

Table
Comparison of the results from simulated rest and manipulation groups after injection with India blue-dyed platelet-rich plasma (n = 10 cadaveric lower extremities)

Variable	Rest (n = 5)	Manipulation (n = 5)	Overall
Craniocaudal spread (cm)			
Median	140	135	145
Range	125 to 190	115 to 170	115 to 190
Dyed PRP between paratenon and AT	5 (100)	5 (100)	10 (100)
Dyed PRP evident in AT	5 (100)	5 (100)	10 (100)
Dyed PRP in plantaris tendon	0 (0)	0 (0)	0 (0)
Dyed PRP in pre-Achilles fat space	3 (60)	4 (80)	7 (70)
Duration of injection (s)			
Median	111	124	115
Range	99 to 164	99 to 131	99 to 164

Abbreviations: AT, Achilles tendon; PRP, platelet-rich plasma.

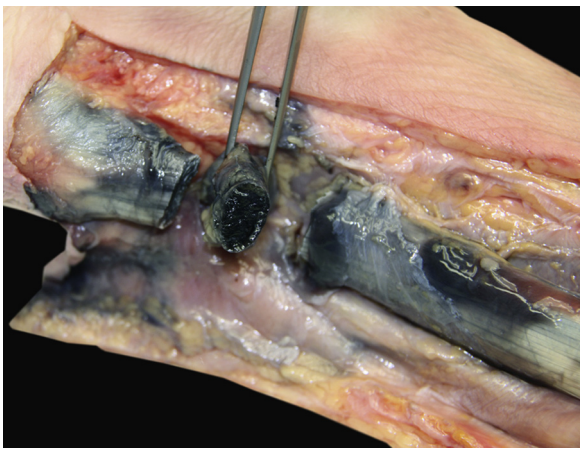

Fig. 4. Anatomic dissection of the cadaveric lower leg showing infiltration of India blue-dyed platelet-rich plasma throughout the transverse plane of the Achilles tendon.

Fig. 5. Anatomic dissection of the cadaveric lower leg showing infiltration of India blue-dyed platelet-rich plasma throughout the Achilles tendon. The plantaris tendon was not infiltrated with platelet-rich plasma because it has a separate tendon sheath. Note how the platelet-rich plasma spreads throughout the insertion of the Achilles tendon onto the calcaneus.

group and 140 mm in the simulated movement group, substantially expanded spread was noted. Although it might seem irrelevant and prone to bias to compare the spread of PRP directly after dissection (this was not a clinical situation), with any postinjection protocol, the findings were highly interesting. Also, from this comparison, one can conclude that PRP will spread further than was observed directly after the injection (6). Hence, it might not be necessary to locate the PRP exactly at the location of the pathologic lesion, knowing the PRP will spread substantially throughout the entire AT (and its near surrounding tissue).

One of the obvious limitations of the present study was the cadaveric setting in which our study was performed. Logically, the effect of blood flow, microcirculation, muscle pumps, and diffusion at the cellular level was not possible to test in the present study (17,18). However, the results of our study have shown a large spread of PRP after injection. The spread seen in the present study would easily reach over the preferred designated location of the PRP to treat Achilles tendinopathy. When taking the possible beneficial effects of blood flow and muscle pumps into account, one can hypothesize that the in vitro spread would be even larger than that in the cadaveric setting (17,18).

We realize that our conclusions could be threatened by a number of methodologic shortcomings. As previously stated, we were not informed about any AT-related pathologic features of the limbs. However, the fresh frozen limbs were selected to not have any macroscopic scarring in the AT region, and no hypoechoic zones were observed during the ultrasound-guided injection. Another possible limitation with the use of any fresh frozen cadaveric specimens is contracture, specimen age, and method of freezing and defrosting. This was countered by using the hospital's standard fresh frozen limb freezing and prolonged defrosting protocol. After dissection, we did not perform any histologic testing of the state of the AT. As mentioned, the results might be different in a noncadaveric setting. Also, the total group (power) of the present study was low ($n = 5$ in each group). No proper statistical analyses could be performed with such a small group owing to the presence of a type 2 statistical error. A full analysis would suggest the proper power of the study, creating an unjustifiable suggestion that the results, regardless of their outcome, were based on proper power. The postinjection protocols used were chosen in accordance with results from previous clinical studies; however, postinjection placement in the patients differed substantially among the studies. Some have advised supine placement, which was not evaluated in our study; others have advised a prolonged prone position. The results of the present cadaveric study should not be used to represent these postinjection protocols. Furthermore, because these protocols have differed slightly or substantially, the effect of gravity should not be underestimated; this was not evaluated in the present study. Finally, the comparison with the previous study in this field was prone to bias because methodologic differences and interpretation could easily occur (6). Additional evaluation of the spread of PRP could be of interest, because all current studies regarding this matter have been cadaveric. Future studies should thus focus on the evaluation of the spread of PRP in vivo.

In conclusion, we found every AT to have been gradually infiltrated with PRP after mid-portion AT injection. No difference was found in the craniocaudal spread between 15 minutes of rest after injection and 100 manipulations after injection. The plantaris tendon was never infiltrated with PRP in either subgroup. Our findings showed no relation between the spread of PRP (both intratendinously and peritendinously) and a specific postinjection protocol after mid-portion AT PRP injection.

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