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Platelet-Rich Plasma Injections for the Treatment of Hamstring Injuries

A Randomized Controlled Trial

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**Background:** A hamstring injury is one of the most common types of injury affecting athletes. Despite this, the optimal management of hamstring muscle injuries is not yet defined. The effect of autologous platelet-rich plasma (PRP) therapy on the recovery of hamstring injuries is unclear.

**Purpose:** To investigate the effect of a single PRP injection in the treatment of grade 2 hamstring muscle injuries.

**Study Design:** Randomized controlled trial; Level of evidence, 2.

**Methods:** Twenty-eight patients diagnosed with an acute hamstring injury were randomly allocated to autologous PRP therapy combined with a rehabilitation program or a rehabilitation program only. The primary outcome of this study was time to return to play. In addition, changes in pain severity and pain interference scores over time were examined.

**Results:** Patients in the PRP group achieved full recovery significantly earlier than controls ($P = .02$). The mean time to return to play was 42.5 ± 20.6 days in the control group and 26.7 ± 7.0 days in the PRP group. Significantly lower pain severity scores were observed in the PRP group throughout the study. However, no significant difference in the pain interference score was found between the 2 groups.

**Conclusion:** A single autologous PRP injection combined with a rehabilitation program was significantly more effective in treating hamstring injuries than a rehabilitation program alone.

**Keywords:** muscle injury; management; platelet-rich plasma (PRP); return to play

An acute hamstring injury is one of the most common types of muscle injury diagnosed in athletes.21,42 This injury usually results in loss of training and competition time.26,50 In the early stage after an injury, current injury management includes rest, ice, compression, and elevation.29,32 Other modalities include anti-inflammatory medications (painkillers), rehabilitation exercise programs, electrotherapeutic modalities, hyperbaric oxygen therapy, and prolotherapy injections.2,6,27,38 Nevertheless, clinical evidence to support these modalities is limited. The best treatment for hamstring injuries is yet to be identified.

More recently, autologous platelet-rich plasma (PRP) injections have gained much attention for treating soft tissue injuries.15,30,44 Patients with chronic lateral epicondylar tendinopathy treated with leukocyte-rich PRP showed significant improvement in pain scores and better functional outcomes than controls in a multicenter, double-blind randomized controlled trial (RCT).40 A positive effect of PRP on healing of patellar tendon harvest sites was also reported in a prospective RCT of 27 patients.16 In contrast, no beneficial effects of PRP on pain and functional outcomes were reported among patients with chronic Achilles
Complete tear of muscle as clinical evidence to support PRP use is lacking. In popularity, there is a growing debate about PRP efficacy trials with conflicting findings are available in the effect of PRP on muscle injuries. Only 2 case-control acute grade 2 hamstring injuries. autologous PRP injections on time to return to play after ment the natural healing process. Despite increasing popularity, there is a growing debate about PRP efficacy as clinical evidence to support PRP use is lacking. In addition, the best method for PRP therapy including the volume to be injected, delivery method (blind vs ultrasound guided), frequency of injections, and postinjection care have not been determined. We conducted a single-blind RCT to explore the effect of autologous PRP injections on time to return to play after acute grade 2 hamstring injuries.

MATERIALS AND METHODS

Study Design and Patients

This study was a single-blind (assessor blinded) RCT conducted at the Sports Medicine Clinic of the University of Malaya Medical Centre (UMMC). Patients aged ≥18 years who presented to the Sports Medicine Clinic with a suspected hamstring injury were screened for eligibility. The eligibility criteria for this study were as follows:

- **Inclusion criteria:** (1) age ≥18 years, (2) acute hamstring muscle injury (<7 days since injury onset), and (3) able to understand the study and follow the study protocol.
- **Exclusion criteria:** (1) had received any form of injection therapy for the current injury, (2) use of nonsteroidal anti-inflammatory drugs within 1 week before randomization, (3) unable to fulfill weekly follow-up appointments and comply with the rehabilitation program, and (4) significant cardiovascular, renal, or hepatic disease; malignancy; history of anemia; or previous muscle surgery.

Patients diagnosed with an acute grade 2 hamstring injury were invited to take part in the study. Hamstring injuries were classified by radiological (ultrasound) grading used at the UMMC (Table 1). This injury grading was based on the classification proposed by Peetrons. A radiologist trained in interventional musculoskeletal injections performed all diagnostic ultrasonography assessments. All eligible patients received detailed information about the trial and were required to complete the informed consent form before participation.

Patients' sociodemographic information including age, sex, type of sport, level of participation, and playing experience was documented on a standard clinical research form (CRF) (see the Appendix, available online at http://ajsm.sagepub.com/supplemental). Additionally, information pertaining to the current hamstring injury including date of injury and injury mechanism was also documented on the same CRF.

A computer-generated block randomization list was prepared by a colleague who had no clinical involvement in the trial (http://randomization.com). After patients' consent had been obtained, telephone contact was made with the same colleague for the allocation of treatment assignment.

Eligible patients were randomized to either receive a PRP injection combined with a rehabilitation program (PRP group) or undergo a rehabilitation program alone (control group). The rehabilitation program in this study focused on progressive agility and trunk stabilization (PATS) exercises. This program has been shown to be effective in promoting earlier return to play and preventing injury recurrence. Patients in both groups were followed up until full recovery or the end of the study period.

Study Interventions

Patients in both groups were prescribed a rehabilitation program by a sports physical therapist at enrollment. In addition, an instructional video and booklet on PATS exercises were distributed to each patient. All patients were asked to perform the home exercise program at least once a day and to record their session in the activity booklet provided. A placebo blood draw was not performed in the control group, owing to ethical concerns.

In addition to rehabilitation exercises, a single intraleision injection of autologous PRP was administered to patients in the PRP group. The PRP injection was given immediately after randomization, with a mean of 4.6 ± 1.94 days (range, 1-7 days) after injury onset. The autologous PRP was prepared using a commercially available kit (Biomet GPS III, Biomet Inc) in accordance with the manufacturer's guideline. A standard 60-mL PRP kit produced approximately 6 mL of PRP. Three milliliters of the PRP was delivered to the injured area under ultrasound guidance, 1 mL was sent to the UMMC laboratory for platelet and leukocyte counts, and the remaining 2 mL was stored at −20°C for analysis of transforming growth factor–β1 (TGF-β1) and basic fibroblast growth factor (bFGF), which was performed using enzyme-linked immunosorbent assay kits (Cusabio).

The current existing guideline on PRP therapy lacks evidence on the ideal PRP volume to administer, frequency of injection, and postinjection rehabilitation care. Our decision to use a single 3-mL PRP injection was based on the findings of existing clinical studies. The autologous PRP was delivered into the damaged area under

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**TABLE 1**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Ultrasound Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No ultrasound features seen</td>
</tr>
<tr>
<td>1</td>
<td>Muscle edema only</td>
</tr>
<tr>
<td>2a</td>
<td>Partial tears of muscle fibers, disruption involving &lt;33%</td>
</tr>
<tr>
<td>2b</td>
<td>Partial tears of muscle fibers, disruption involving ≥33%-66%</td>
</tr>
<tr>
<td>2c</td>
<td>Partial tears of muscle fibers, disruption involving ≥66%-99%</td>
</tr>
<tr>
<td>3</td>
<td>Complete tear of muscle</td>
</tr>
</tbody>
</table>

Grade Ultrasound Findings
ultrasound guidance. No activating agent was added to the PRP before the injection. Further, no local anesthetic was administered to the overlying skin before PRP administration. Immediately after the PRP injection, patients were kept supine for 10 to 15 minutes. Patients were asked to reduce their activities for the following 48 hours. Patients were allowed to take only acetaminophen (1000 mg) as required (maximum, 4 times a day) for pain control.

All patients were required to attend weekly follow-up assessment and rehabilitation sessions. A sports physical therapist with more than 5 years of experience in sports rehabilitation conducted these sessions. At each visit, patients were asked to complete the Brief Pain Inventory—Short Form (BPI-SF). The BPI-SF is a self-reported questionnaire that assesses the severity of pain (questions 2-6) and the effect of pain on daily function (pain interference; questions 9A-9G). The 2-factor structures of the BPI-SF have been validated: severity of pain and pain interference in daily activities. The Cronbach α reliability of the BPI-SF ranges from .77 to .91. A standard clinical examination to assess the patient’s readiness to return to play was then performed by a sports physical therapist blinded to the treatment allocation.

Outcome Measures

We used time to return to play as the primary outcome measure of this study. Time to return to play was defined as the time (in days) from the date of injury onset until the patient fulfilled the criteria for return to play. The determination of fitness for return to play was based on recent clinical sports medicine recommendations (Table 2).9,39 Patients who fulfilled the criteria for return to play were allowed to resume full activities and progressively increase their training load until reaching their preinjury levels.

The secondary outcomes of interest were changes in pain severity and pain interference scores between the 2 groups throughout the duration of the study. These were assessed using the BPI-SF, which participants completed after randomization (baseline) and at each follow-up visit.10

Statistical Analysis

Sample size was determined with the following formula:35

\[
N = \frac{2 \times \left[ z_{\frac{1-\alpha}{2}} + z_{\frac{1-\beta}{2}} \right]^2 \sigma^2}{\left( \mu_1 - \mu_2 \right)^2}
\]

where \( N \) is the sample size of each group, \( z_{\frac{1-\alpha}{2}} \) of 0.05 = 1.96 (percentage points of the normal distribution for a statistical significance level of .05), \( z_{\frac{1-\beta}{2}} \) of 80% = 0.84 (percentage points of the normal distribution for a statistical power of 80%), \( \mu_1 \) = population mean in treatment group 1, \( \mu_2 \) = population mean in treatment group 2, \( \mu_1 - \mu_2 \) = mean difference, and \( \sigma^2 \) = population SD.

Data from a 2004 case-control study by Wright-Carpenter et al.10 including the mean and SD time to return to play for control and autologous conditioned serum (ACS)-treated groups, were applied to the formula to estimate the sample size required for the current RCT. With a 30% estimation of the attrition rate, 14 patients in each group were required, giving a total of 28 patients for the study. As the main outcome variable for this study was time to return to play, survival analysis statistical procedures were chosen to assess the effectiveness of interventions. The Kaplan-Meier product limit estimator was performed as this allows the estimation of survival over time between treatment groups.52 The survival function between the 2 groups was compared using the log-rank (Mantel-Cox) test. The Cox proportional hazard model (Cox regression) was also performed to examine the effect of other covariates on time to return to play. Additionally, the time to return to play between groups was compared using either the \( t \) test or Kruskal-Wallis test depending on the data distribution (Shapiro-Wilk test).

Secondary outcome measures, including changes in pain severity and pain interference scores over time, were assessed using a linear mixed-model analysis.10 All statistical analyses conducted were 2-tailed, with the significance level set at \( P < .05 \).

The UMMC Medical Ethics Committee approved the study protocol (MEC No. 835.11). The trial was registered with the Current Controlled Trials registry (ISCRCT N66528592). The study protocol has been published elsewhere.1

RESULTS

Patient Characteristics

Patient enrollment began in January 2011 and was completed in May 2013. Thirty-four patients diagnosed with a hamstring muscle injury were approached and screened for eligibility. Two patients did not fulfill the inclusion criteria, and another 4 patients declined participation; thus, 28 patients underwent randomization (Figure 1). All patients were diagnosed with a grade 2a hamstring muscle injury at the time of screening. No significant difference in characteristics between patients who declined participation and patients assigned to a treatment group was noted (\( P > .05 \).

### TABLE 2

<table>
<thead>
<tr>
<th>Sign</th>
<th>General Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>Pain free (on direct palpation); pain free on hamstring contraction (resisted isometric hamstring muscle contraction)</td>
</tr>
<tr>
<td>Range of movement (active knee extension test)</td>
<td>Symmetrical with unaffected side (difference between affected and unaffected side of &lt;10°)</td>
</tr>
<tr>
<td>Hamstring strength</td>
<td>Concentric strength (60, 180, and 300 deg/s) within 10% of uninjured side</td>
</tr>
</tbody>
</table>

**Criteria for Return to Play**

- **Sign**: Symmetrical with unaffected side (difference between affected and unaffected side of <10°)
- **Range of movement (active knee extension test)**: Concentric strength (60, 180, and 300 deg/s) within 10% of uninjured side
- **Hamstring strength**: Concentric strength (60, 180, and 300 deg/s) within 10% of uninjured side
Four patients, 2 in each group, withdrew from the study. Two patients in the control group did not return for scheduled follow-ups and could not be reached. The other 2 patients in the PRP group recommenced activities without prior clearance from the study protocol. A total of 24 patients completed the study, representing 85.7% retention from baseline.

The median age of patients in the study was 21.00 years (interquartile range [IQR], 8.50; range, 17-49 years). More than two thirds (71.4%) of the patients were of Malay ethnicity, and the majority were men (85.7%). Most patients were national-level athletes (53.6%), while the rest were athletes at school (before university) or at state or club levels. The mean duration of injury before patient enrollment was 4.6 ± 2.15 days (range, 0-7 days). Most (64.3%) injuries occurred during training, especially while running (82%).

A significantly longer (length) area of injury ($P = .017$) was noted in the PRP group ($3.40 \pm 1.09$ cm) compared with controls ($2.30 \pm 1.04$ cm). However, when the other 2 dimensions (width and depth) were taken into account, the estimated median volume of the injured area between the 2 groups was comparable ($15.30 \text{[IQR, 34.24]} \text{cm}^3$ vs $19.50 \text{[IQR, 23.14]} \text{cm}^3$, respectively; $P = .713$). No other significant difference in baseline characteristics between the 2 groups was noted; however, there was a trend for more recurrent injuries in the PRP group ($P = .053$) (Table 3).

**Platelet-Rich Plasma**

The amount of platelets (1297 ± 3 x $10^3/\mu L$) and white blood cells (WBCs) (38.3 ± 3 x $10^3/\mu L$) in the PRP was significantly higher than that in the peripheral blood (234 ± 3 x $10^3/\mu L$ and 7.3 ± 3 x $10^3/\mu L$, respectively). The PRP produced and used in this study was classified as P4-x-A according to the PAW classification system.$^{18}$ The median level of TGF-$\beta$1 was 50.34 ng/mL (IQR, 54.09), representing a 90% to 260% increase compared with a previously reported level in plasma.$^{53}$ Meanwhile, the median bFGF level was 42.73 pg/mL (IQR, 25.51), representing a 22-fold increase compared with the level in plasma from a previous study.$^{33}$

**Primary Outcome**

A survival curve was plotted to illustrate the effect over time for patients in both intervention groups (Figure 2). Half of patients in the PRP group achieved full recovery at week 26 of follow-up, whereas 50% of patients in the control group achieved full recovery at week 39 of follow-up. Patients in the PRP group recovered earlier than those in the control group. The mean time to return to play was $26.7 \pm 7.0$ days and $42.5 \pm 20.6$ days for the PRP and control groups, respectively ($t(22) = 2.50, P = .20$). The log-rank (Mantel-Cox) test demonstrated a significant difference in survival function between the 2 groups ($\chi^2(1) [N = 14] = 7.528, P = .006$).

A Cox regression analysis was used to evaluate the effects of treatment and other covariates on time to return to play. Covariates other than treatment were entered first, followed by treatment, as this allowed a likelihood-ratio test of the effect of treatment after statistical adjustment for the other covariates.$^{52}$
Covariates including age, duration of injury, length of injured area, active knee range of movement deficit, and previous hamstring injury were selected based on the previous literature. Only PRP therapy demonstrated a statistically significant effect on time to return to play after taking into account other covariates ($G^2(1) = 5.688, P = .017$). None of the other covariates significantly predicted time to return to play after hamstring injuries (Table 4). The odds for patients in the PRP group to return earlier were 4.8 (95% CI, 1.3-19.3) times higher compared with that for patients in the control group. The time to return to play was well predicted by PRP therapy ($r^2 = 0.184$), with a moderate effect size of 0.23. No significant difference in the injured hamstring strength was noted between groups on return to play (Table 5).
Secondary Outcome

Changes in Pain Severity Scores (BPI-SF questions 2-6). No significant difference in baseline pain intensity scores was found between the 2 groups. Both groups showed gradual reduction in the pain severity scores over time (Figure 3). Patients in the PRP group had significantly lower pain severity scores than controls at all time points ($\beta = -0.390$; standard error [SE], $0.142$; 95% CI, $-0.67$ to $-0.11$; $P = .007$).

Changes in Pain Intensity Scores (BPI-SF questions 9A-9G). No significant difference in baseline pain intensity scores between groups was observed. A gradual reduction in the pain interference score was noted in both groups over time. Even though patients in the PRP group had lower pain intensity scores at all time points, the difference between the groups was not statistically significantly ($\beta = -0.185$; SE, $0.130$; 95% CI, $-0.44$ to $-0.07$; $P = .157$) (Figure 4).

Most patients complained of pain during blood withdrawing and PRP injections. No other adverse effect associated with PRP use was reported.

DISCUSSION

Despite increasingly being used for soft tissue injuries, evidence to support PRP therapy for muscle injuries is limited. To the best of our knowledge, this is the first RCT to assess the effectiveness of a PRP injection for hamstring injuries. Our results showed that patients with a grade 2a hamstring injury treated with a single autologous PRP injection combined with PATS rehabilitation recover significantly earlier than controls.

The potential effects of autologous biological substances to hasten muscle healing were reported in several case reports. Borrione et al. noted that athletes with grade 3 muscle strains treated with PRP showed earlier functional improvement and more complete recovery than those treated nonoperatively. Hamilton et al. successfully treated an athlete with a grade 2 semimembranosus muscle injury with a single 3-mL infiltration of platelet-enriched plasma under ultrasound guidance. The athlete was pain free and allowed to train at the preinjury intensity 21 days after treatment. The effect of a preparation rich in...
growth factors (PRGF) to hasten muscle recovery was reported in a 35-year-old professional bodybuilder diagnosed with a right adductor longus rupture. The athlete successfully returned to competitive training within 1 week after the third PRGF injection.54 The effect of PRP in lowering the pain intensity associated with a hamstring injury was also observed in the current study. Patients in the PRP group demonstrated significantly lower pain intensity scores at all time points throughout the study period.

An earlier recovery time was also reported among athletes with second-degree hamstring injuries treated with ACS.55 The ACS preparation method has been clearly described and involved a 24-hour incubation period during which monocyte activation occurred, leading to cytokine release.54 The ACS preparation contained a high concentration of several growth factors including TGF-β, FGF-2, and insulin-like growth factor–1, but the amount of platelets and WBCs present was not stated. Athletes in the ACS group received 2.5 mL of ACS administered into the injured area under palpation guidance every second day. Meanwhile, the retrospective control group was treated with 3 mL of Actovegin combined with 2 mL of Traumeel using similar injection techniques. Both groups started on a standard rehabilitation program and received an oral natural anti-inflammatory agent, bromelain.8 Athletes’ readiness to return to sports was based on the participant’s subjective readiness to resume exercise at a competition level. Athletes who received an ACS injection recovered significantly faster than controls (16.6 vs 22.3 days, respectively; \(P = .001\)). However, the earlier return to play observed in the previous study could have been confounded by the concurrent use of oral medication.

Contrary to the study by Wright-Carpenter et al,55 the current RCT evaluated the effect of PRP therapy on hamstring recovery. The PRP was prepared using a commercial kit and classified as P4-x-A according to the more recent PAW classification system.18 Additionally, the PRP produced in the study contained significantly higher levels of both TGF-β1 and bFGF compared with previously reported levels in plasma. Three milliliters of PRP was given once throughout the study period. To ensure the accurate delivery of PRP into the injured area, all injections were performed under ultrasound guidance. Further, no anti-inflammatory agents were prescribed to patients. Because no activating agents were used, the current study relies on endogenous platelet activation, which was shown to provide a more sustained release of anabolic cytokines.28

The decision on when to return to play in the current study was based on both the participant’s subjective assessment of pain (BPI-SF) and standardized objective physical and hamstring strength assessments (Biodex isokinetic machines). While the actual effect of PRP on soft tissue healing is not fully understood,22 our findings supported the possible role of higher growth factors (concentration level) in hastening recovery as postulated by previous researchers.23,24,37

Sanchez et al47 reported full functional recovery of hamstring and adductor muscle injuries 2 times faster in 20 professional athletes treated with a PRGF. The authors reported that smaller tears progressed well even after a single application of a PRGF, whereas medium- to large-sized tears needed 2 to 3 applications of a PRGF at 1-week intervals. However, the previous authors did not report objective assessments on the size of the tears. Our study demonstrated that a single PRP injection was effective in accelerating recovery for grade 2a (median volume of injury, 19.50 cm²; IQR, 23.14) hamstring injuries.

In contrast to the current findings, a recent study did not find any significant differences in time to return to play between athletes treated with a single PRP injection and controls.45 The study by Rettig et al145 was a retrospective case-control study that investigated the effects of an autologous PRP injection on time to return to play after acute hamstring injuries in professional National Football League (NFL) players. Ten professional players diagnosed with an acute hamstring injury were retrospectively divided into PRP (\(n = 5\)) and control (\(n = 5\)) groups. Patients in the PRP group were injected once with 6 mL of PRP under ultrasound guidance. Both groups went through the same rehabilitation program and were evaluated through a functional progression assessment by the same athletic trainer before returning to play.

Several differences were identified between the study by Rettig et al145 and the current study. First, the former was a retrospective case-control study of 10 professional football players from a single NFL team. The study included both grade 1 and 2 hamstring injuries. Contrary to this, the current study included only grade 2a hamstring injuries. While both studies used similar commercial kits in the PRP preparation, the concurrent use of an activating substance and a local anesthetic was not reported in the previous study. Also, the amount of sodium bicarbonate used in the previous study was higher than the amount recommended by the manufacturer, which may affect

### TABLE 5

| Injured Hamstring Strength on Return to Play in Both Intervention Groups\(^a\) |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Injured Hamstring Strength, N·m | Control | PRP | \(t\) Value | \(P\) Value |
| 60 deg/s | 101.9 ± 30.9 | 104.8 ± 31.0 | -0.227 | .823 |
| 180 deg/s | 87.9 ± 27.2 | 86.4 ± 29.0 | 0.134 | .895 |
| 300 deg/s | 77.6 ± 22.5 | 77.4 ± 27.6 | 0.015 | .988 |

\(^a\) \(P \geq .05\) = statistically significant difference between control and platelet-rich plasma (PRP) groups.
platelet function. The definition of time to return to play used in the current study was comprehensive and robust. Patients were required to fulfill several clinical criteria and display acceptable hamstring strength recovery (isokinetic strength assessment) before they were allowed to recommence their preinjury level of activities. In contrast to the study of Rettig et al., the assessor in the current study was blinded to patients’ treatment allocation.

Our study had several limitations. First, because ethical considerations prevented us from drawing blood from the controls and discarding it, the control patients were probably aware of their treatment allocation. This could have influenced their perception of their response to treatment. Second, it was unfortunate that most patients did not record their daily, unsupervised rehabilitation sessions at home in the activity diary. Nevertheless, at each follow-up appointment, patients did affirm that they performed the prescribed exercises as recommended. A home-based rehabilitation program combined with regularly scheduled telephone calls was shown to be associated with a higher adherence rate and is recommended for future studies.

Third, in our study, we did not assess the individual hamstring muscle (biceps femoris, semitendinosus, and semimembranosus muscles) because of limited resources. A previous study did report differences in recovery time between the specific muscles involved, but the differences were not statistically significant. Fourth, the current study assessed the short-term effects of PRP particularly on the recovery time. A future study with a longer follow-up period is recommended as this would enable an assessment of longer term effects of PRP therapy including the recurrence of hamstring injuries. In addition, such a study design would allow an assessment of long-term adverse effects associated with PRP therapy. Fifth, the lesions were longer (length) in the PRP group, and there was a trend for more revision patients in the PRP group. Finally, the small number of patients enrolled in the study was limited by the time and cost of PRP therapy. A larger sample size could have yielded better clinical significance with a higher effect size. However, the minimum sample size of 14 per group in this study was estimated based on 80% power and 5% type I error.

CONCLUSION

This study showed that a single 3-mL injection of autologous PRP (P4-x-A classification) combined with a PATS rehabilitation program was significantly more effective than a control in reducing the severity of pain and allowing a significantly shorter time to return to play after an acute grade 2a hamstring injury.

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REFERENCES


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