Treatment of Achilles tendon partial injuries with injection of peripheral blood mononuclear cells (PB-MNCs): a case series

Francesco Caravaggio (1,2), Fabio Depalmi (1,2), Michele Antonelli (3)

(1) Foot and Ankle Surgery Center, "Casa di Cura Città di Parma" (Parma, Italy); (2) "Associazione Ex Alumni G. Pisani" (Cuneo, Italy); (3) Public Health Service, AUSL-IRCCS di Reggio Emilia, Reggio Emilia, Italy.

Abstract

Three patients with Achilles tendon partial injury were treated with local injection of peripheral blood mononuclear cells (PB-MNCs). All subjects were evaluated both clinically (American Orthopedic Foot & Ankle Society - AOFAS scale) and radiologically (MRI examination) at 2 months, and a clinical reassessment with the AOFAS scale was performed at 6 months. Functional and radiological signs of tendon healing processes were detected as early as 2 months after the procedure and the AOFAS scale rose from an initial average value of 37.0 to 82.7. Even though this study only involved a limited number of participants, our preliminary results indicate that regenerative therapies with PB-MNCs may be a valid alternative to surgical options for Achilles tendon partial injuries, especially in patients with contraindications to surgery, when other conservative approaches (exercises, physical therapies, sclerosing treatment) have failed. Further investigations on the subject seem rationally supported and advisable.

Key Words: Monocytes; macrophages; Achilles tendon; regenerative medicine; case series.

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Achilles tendinopathy, a degenerative condition of the Achilles tendon with traumatic and/or inflammatory etiology, is more prevalent among athletes, even though as many as 30% of all cases actually occur in patients with a sedentary lifestyle.[1] Achilles tendinopathy has a multifactorial origin, with both extrinsic and intrinsic factors playing a role in its pathogenesis. Extrinsic factors include physical training errors with excessive workload and inadequate recovery, lipid and glucose dysmetabolism, thyroid hormone imbalances, rheumatic diseases, corticosteroid drugs, and quinolone antibiotics.[2,3] Instead, intrinsic factors, often conditioned by genetic predisposition, include tendon vascular problems, aging processes, as well as flat or hollow foot, which can cause tendon torsion or traction, respectively.[4] Some authors have also hypothesized a pathogenic role of plantar fascia alterations, especially if we consider the Achilles-calcaneal-plantar system as a functional unit and the fascia-peritenon anatomical relationship.[5]

Prolonged tendon inflammation is characterized by infiltration of immune cells such as neutrophils and macrophages, which can regulate tissue homeostasis and develop different functions depending on micro-environmental signals.[6] In particular, M1 (type 1) macrophages intervene in early stages of tissue damage and have a pro-inflammatory function, whereas M2 (type 2) macrophages (subsequently divided into different subspecies) share anti-inflammatory properties and play a key role in the reconstructive-regenerative phase.[7] For this reason, injection of autologous peripheral blood mononuclear cells (PB-MNC) can cause M1-to-M2 macrophage polarization and may represent a valid cell therapy for improving injured or degenerating tendons.

The aim of this study was to understand whether PB-MNC injections can be an effective conservative approach for Achilles tendon partial injuries.

Materials and Methods

Full informed consent, provided on a free and voluntary basis, was obtained from all patients involved in this study. Clinical data were completely anonymized as per Italian and European laws.[8] The patients were free to withdraw from the study at any time, but none of them did so. This research was conducted in accordance with the Declaration of Helsinki and its subsequent modifications.[9] Ethics approval was waived because of national regulations and the observational design of the

Table 1. *Baseline characteristics of the patients examined.*

Case	Age and gender	Symptom onset	Treatments tried for the AT lesion without success	Comorbidities and medicinal drugs
A	32 M	4 months Right AT	TECAR therapy YAG laser therapy 3 local injections of hyaluronic acid	None
B	45 F	8 months Right AT	TECAR therapy Ultrasound physical therapy Shockwave therapy	None
C	62 M	3 months Left AT	TECAR therapy	Hypertension, gastroesophageal reflux disease Verapamil 120 mg q.d., hydrochlorothiazide 25 mg q.d., omeprazole 40 mg q.d.

Case	Smoking habit	Past surgery or traumas	Job	Sporting activities
A	No	Right knee sprain and anterior cruciate ligament injury Right knee medial meniscectomy (arthroscopic)	Professional athlete	Soccer (every day)
B	No	Haglund's deformity Appendicectomy Cesarean delivery	Lawyer	Running, cycling, swimming (3-to-4 times a week)
C	No	Left knee medial meniscectomy (arthroscopic) Inguinal hernia repair	Office employee	Tennis, cycling (twice a week)

Legends. AT=Achilles Tendon. F=Female. M=Male. Q.D.=Quaque Die (a capsule once a day).

study ("Regolamento CE AVEN - versione 4 del 22.09.2020"). This single-center study was designed as a non-consecutive series of case reports. Data were collected throughout 2021 in the orthopedics outpatient clinic of an Italian private hospital ("Casa di Cura Città di Parma", Athos Maestri square, 5, 43123 Parma - Italy). Three patients (2 males and 1 female, aged between 32 and 62 years) with non-traumatic Achilles tendon partial injury were recruited for this study. One patient also had secondary tallodinia due to Haglund's deformity. The symptom onset was dated from 2 to 6 months prior to enrollment and all patients had already undergone other conservative treatments (i.e. physical therapies) before getting PC-MNC injections. Preliminary clinical assessment was carried out following the American Orthopedic Foot and Ankle

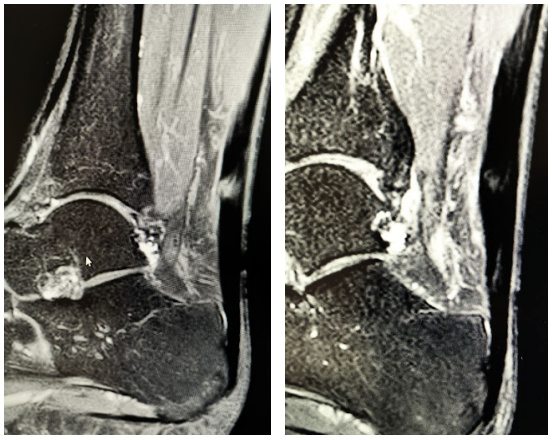

Fig 1A **Fig 1B**

Fig 1. *Case A (MRI, sagittal plane): Achilles Tendon Lesion before (1A) and 2 months after (1B) the regenerative intervention (the images are published with the patient's consent).*

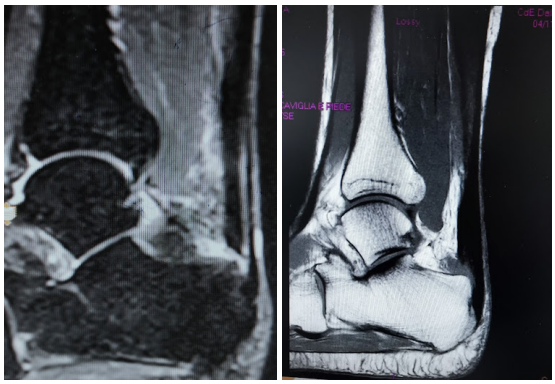

Fig 2A **Fig 2B**

Fig 2. *Case B (MRI, sagittal plane): Achilles Tendon Lesion before (2A) and 2 months after (2B) the regenerative intervention (the images are published with the patient's consent).*

Society (AOFAS) scale.[10] Ultrasound examination and MRI showed the Achilles tendon injury and a widespread tendinosis. Additionally, the patient affected by Haglund's disease presented some intra-spongious oedema of the posterior calcaneal tuberosity. When clinically examined, patients reported pain in the Achilles tendon, so intense that it limited their walking autonomy. Patients who used to practice sports were nearly unable to exercise. Tendon pain was elicited by manual palpation, which revealed an increased thickness due to tendinosis. The patient with Haglund's disease also reported pain in the posterior calcaneal tuberosity which appeared slightly swollen. Pre-

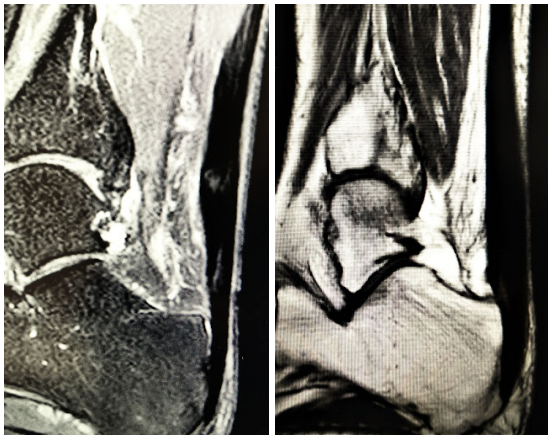

Fig 3A **Fig 3B**

Fig 3. *Case C (MRI): sagittal plane): Achilles Tendon Lesion before (3A) and 2 months after (3B) the regenerative intervention (the images are published with the patient's consent).*

treatment values on the AOFAS scale ranged from 31 to 45, with a mean value of 37.8. The patients' baseline characteristics and comorbidities were reported in Table 1. The PB-MNC concentrate was obtained with the Hematrate Blood Filtration System (HemaTrate® - Cook Regentec), a selective point-of-care filtration system used for autologous human cell therapy. Following the manufacturer's instructions, 100-120 cc of each patient's blood was anticoagulated with a 10% citrate dextrose solution (ACDA) and then stored into the upper bag of the system. Filtration was driven by gravity and the procedure took around 10 min. PB-MNCs remained trapped in the filter and collected in a syringe through backwash with 10 cc of physiological solution. The cell concentrate was injected immediately after sampling. The patients were asked to uncover their lower limbs and lie in a prone position in order to perform the injections. The leg where PB-MNCs had to be injected was disinfected with an alcohol-based chlorhexidine product, and then a sterile field was prepared. Local anesthetics were not used because they could induce cell necrosis and apoptosis,[11] thus hindering the benefits of the regenerative therapy. Around 60% of the PB-MNC concentrate was injected directly into the injured area, while the remaining 40% was injected in smaller amounts (0.1-0.2 cc for each injection) into the surrounding parts of the Achilles tendon affected by tendinosis. Optimal sites of injection were located with the help of clinical and radiological findings available for each patient (in cases of doubts, the entire procedure can be ultrasound-guided). The whole procedure (from PB-MNC sampling to completing the injection) took between 45 and 60 min, and it was fully performed by an orthopedic physician (F.C. or F.D.) in the abovementioned outpatient clinic.

The post-treatment rehabilitation and follow-up protocol was as follows:

- Day 0: intervention.
- 3-4 days after intervention: light physical exercises (cycling and swimming).
- 10 days after intervention: eccentric exercises and stretching of the posterior muscle chain.
- 15-20 days after intervention: assisted physio-kinesiotherapy.
- 30 days after intervention: clinical check performed by an orthopedic physician.
- 2 months after intervention: clinical check performed by an orthopedic physician (AOFAS scale measurement) and MRI assessment. Then, depending on individual health conditions, resumption of specific sports activities (running, tennis, soccer), if practiced by the patient prior to the symptom onset.
- 6 months after intervention: clinical check performed by an orthopedic physician (AOFAS scale measurement).

Results

The three patients were discharged soon after the procedure with full weight-bearing. All of them reported transient mild pain in the site of injection with full resolution within 24 hours after taking 30 mg of Ketorolac. Table 2 reports baseline and follow-up measures of the AOFAS scale for each patient. The AOFAS scale increased from the initial average value of 37 (minimum: 35; maximum: 39) to a first endpoint value of 76 (minimum: 54; maximum: 94), collected 2 months after treatment. The AOFAS scale values remained roughly the same 6 months after the procedure (mean: 82.7; minimum: 60; maximum: 97). In all patients, clinical and radiological signs showed complete healing of the tendon injury 2 months after the procedure (Figures 1, 2 and 3). Tallonitis persisted only in the patient affected by Haglund's disease (case B), probably because of the underlying anatomical conflict between the calcaneus deformity and the Achilles tendon insertion. In this patient, the MRI showed persistent radiological signs of intra-spongious edema in the calcaneal tuberosity (Figure 2) and the clinical examination revealed a more limited post-intervention improvement in the AOFAS scale, which only rose from 31 to 58 (Table 2).

This patient agreed to schedule a surgical intervention to correct Haglund's deformity.

Thanks to their full recovery, the two patients who used to practice amateur aerobic sports activities (triathlon, marathon) prior to the symptom onset, started again with their physical exercises without problems.

Discussion

Chronic tendinopathy is characterized by extracellular matrix alterations, with an increased production of amorphous substance and type 3 collagen, and a decrease in the number of tenocytes, usually associated with a loss of their normal alignment. Therefore, collagen fibers and tendon tissue disarray can be detected microscopically, with signs of intratendinous angiogenesis due to lack of endostatin inhibition and evident onset of degenerative processes.[12] When M1 macrophages prevail over M2 macrophages, subacute inflammation persists and inhibits the regenerative activity of resident mesenchymal stem cells (MSCs). Conversely, M1-to-M2 macrophage polarization can increase the regenerative activity of resident MSCs, thus promoting tendon healing.[13,14] Most tendons are surrounded by a layer of epithelial cells which can provide a source of fibroblasts capable of repairing local injuries. Epithelial cells can trans-differentiate into fibroblasts and regenerate the extracellular matrix.[15] This process begins with the activation of a signal pathway called epithelial-to-mesenchymal transition (EMT): changes in macrophage phenotype and activation of EMT-related pathways can contribute to the injured tissue degradation and subsequent tendon repair.[15] In addition to this, the peritenon is often the main cause of pain because, in contrast with the inner part of the tendon, it is rich in nerves and blood vessels.[16] As such, M2 macrophages may have an anti-inflammatory effect both within the tendon and in the peritenon, and this double action can be useful to favor tendon healing and reduce inflammation-related painful symptoms. In fact, among the potential mechanisms of action of regenerative therapies for musculoskeletal disorders, two are deemed crucial: inflammation modulation and peritenon neovascularization, especially in early stages of tendon injury repair.[17]

The filtering system used to trap PB-MNCs is highly selective for mononuclear cells (monocytes / macrophages, lymphocytes, CD34+ hematopoietic stem

Table 2. *Baseline and follow-up measures (AOFAS scale).*

Case	Baseline	2 months	6 months
A	39	94	97
B	35	54	60
C	37	80	91

cells), if compared with the Stromal Vascular Fraction obtained from adipose tissue, which consists of more heterogeneous cell populations, including pericytes, smooth muscle cells, endothelial cells, fibroblasts, macrophages, and stem cells.[18,19] Moreover, local injections of PB-MNCs have been demonstrated to be effective in polarizing macrophages towards the M2 phenotype in several tissues.[20] The indirect regenerative role played by macrophages explains why the PB-MNC concentrate can hinder sub-acute inflammation and trigger tendon healing processes, thus potentially explaining our positive results.

Interestingly, it has previously been shown in coculturing myoblasts and macrophages that myotube formation is strongly increased *in vitro* by the presence of acid stable, heat-labile, soluble growth factors secreted by macrophages.[21-23] In another research, the same authors obtained macrophages from peritoneal washing and demonstrated that an ED2-positive (ED2+) macrophage subpopulation is responsible for myoblast enhanced proliferation.[24] ED2+ macrophages were separated by a magnetic-activated cell sorter (MACS) using a monoclonal antibody against ED2, a membrane antigen peculiar to macrophages. Both ED2+ macrophages and their conditioned medium increased myotube formation when added to primary muscle cultures. Furthermore, it was demonstrated that muscle growth induced by macrophages is mainly the consequence of an increased myoblast proliferation, as shown by the presence of an increased number of MyoD-positive (MyoD+) myonuclei.[24]

The PB-MNCs technique differs from plasma rich platelet injections, characterized by a more prevalent anti-inflammatory (rather than regenerative) therapeutic effect.[25] Additionally, PB-MNC injections were quite well-tolerated by the patients described in this report (the only side effect was a mild and transient local pain), and they can be performed in elderly subjects, even because mononuclear cell functionality does not fail with age. In fact, this regenerative technique was first developed for elderly patients with no-other-option critical limb ischemia or diabetic foot.[26]

In conclusion, even though this study only involved a limited number of participants, our preliminary results indicate that regenerative therapies with autologous PB-MNCs may be a valid alternative to surgical options for Achilles tendon partial injuries. In particular, this type of treatment can be useful for patients with contraindications to surgery, when other conservative approaches (exercises, physical therapies, sclerosing treatment) have failed. Advantages of cell therapies with PB-MNCs include minimal invasiveness, fast recovery time, a good degree of reproducibility (cell therapies are usually less "operator-dependent" than other modalities), and the opportunity, if necessary, to be easily repeated over time.

Further investigations on the topic are advised, involving more patients and a control group, in order to evaluate long-term clinical outcomes of regenerative therapies and to identify any moderators of their therapeutic effect.

List of acronyms

ACDA - anticoagulation citrate dextrose solution
AOFAS - American Orthopedic Foot and Ankle Society
AT - Achilles Tendon.
EMT - epithelial-to-mesenchymal transition
F - Female.
YAG - neodymium-doped yttrium aluminum garnet
M - Male.
MRI - magnetic resonance imaging
MSCs - resident mesenchymal stem cells
PB-MNCs - peripheral blood mononuclear cells
Q.D. - Quaque Die (once a day)
TECAR - Capacitive and Resistive Energy Transfer

Contributions of Authors

All authors have read and approved the final edited typescript.

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Conflict of Interest

The authors declare no financial, personal, or other conflicts of interest.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Corresponding Author

Foot and Ankle Surgery Center, "Casa di Cura Città di Parma", Athos Maestri Square, 5, 43123 Parma, Italy.
ORCID ID: 0000-0001-7366-5749
E-mail: dott.caravaggio@gmail.com

E-mails and ORCID iD of co-authors

Fabio Depalmi: depa73@inwind.it
ORCID iD: 0000-0002-2288-229X
Michele Antonelli: michele.antonelli@ausl.re.it
ORCID iD: 0000-0002-5941-6604

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