Biologics in the Treatment of Achilles Tendon Pathologies

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KEYWORDS

• Achilles • Biologics • Platelet-rich plasma • Stem cells • Imaging

KEY POINTS

- Biologics have a role in the management of midportion Achilles tendinopathy as a step between conservative and surgical treatment or as an augmentation.
- The level of treatment recommendation for biologics treatment of insertional tendinopathy is yet to be determined.
- The authors created an algorithm (FARG [Foot and Ankle Reconstruction Group] algorithm) for the use of biologics in midportion tendinopathy inspired by the MRI-based classification and for categorizing patients according to the sports activity.
- Combining imaging with patient's functional requests could be the way to reach a protocol for the use of biologics for the treatment of midportion tendinopathy.

INTRODUCTION

Among the human tendons, the Achilles tendon is one of the most prone to pathologic conditions.[1,2] Unfortunately, the ability of tendons to heal spontaneously is very inefficient and unreliable because of their hypocellularity and hypovascularity.[3] Unlike bone, tendon repair is incomplete and does not result in tissue homologous to its prepathologic state[4,5]: a fibrovascular scar is formed and it leads to a mechanically weaker tissue than the natural tendon.[6] The subsequent possible strength loss may increase risk for reinjury or other complications. Furthermore, surgical treatments are sometimes unsuccessful, in which case the main part of these patients develops a chronic condition that is susceptible to recur.[5] These considerations have stimulated the necessity to enhance the outcome of Achilles tendon tendinopathies treatment; in this perspective, biologics propose attractive solutions to be investigated.[5]

In the last decade, the use of biologics for improving repair and healing of Achilles tendon has gained growing interest.[4,5] At present, strategies using biological augmentation techniques are being investigated for potential benefits in tendon healing: they

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may enhance the healing process by improving the biomechanical quality of repairing tissue with the aim of simulating the native tissue.[7]

Among the several biological therapies that have been proposed and investigated, the use of growth factors, platelet-rich plasma (PRP), adipose-derived stem cells (ADSCs), bone marrow aspirate concentrate (BMAC), peripheral blood mononuclear cells (PBMNCs), and scaffolds is the focus of this article.

BIOLOGICS IN ACHILLES TENDON PATHOLOGIC CONDITIONS

Achilles tendon pathologic conditions embrace insertional Achilles tendinopathy and midportion Achilles tendinopathy. Insertional Achilles tendinopathy accounts for approximately 20% to 25% of Achilles tendon disorders, whereas midportion Achilles tendinopathy accounts for another 66%.[8,9]

Midportion Achilles Tendinopathy

Midportion Achilles tendinopathy refers to a tendon disorder located 2 to 7 cm from the posterior calcaneal tuberosity[9] (**Fig. 1**). The mainstay of initial management in midportion Achilles tendinopathy is conservative based on rest or modification of the activities and of the training regimens, eccentric exercises, and use of orthosis.[9,10] This pathologic condition is characterized by good responses to conservative treatment, but it fails to resolve symptoms and to allow sports continuation in 24.0% to 45.5% of cases.[11] Surgical treatment is usually considered when conservative therapies fail: the most frequently performed procedures are open release of adhesions with or without resection of the peritenon, multiple tenotomies with or without augmentation with plantaris tendon or tendon transfers (peroneus brevis, flexor digitorum longus [FDL], flexor hallucis longus [FHL]), or transfer of a soleus pedicle graft, stripping of the peritenon, endoscopic tendon debridement.[9] The main concern about Achilles tendon surgery is the nonnegligible risk of wound-healing complications and nerve and soft tissues damage.[9,12,13]

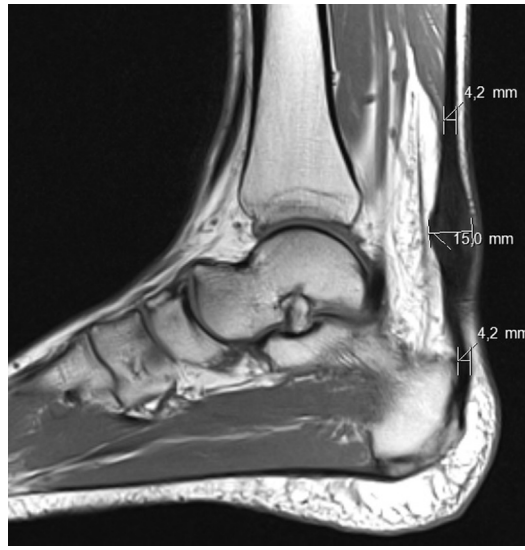

Fig. 1. MRI of midportion Achilles tendinopathy: T1 sagittal view shows midportion tendon thickening.

In this scenario, biologics therapies could have a role: they could support conservative strategies as biological augmentation; they could be considered a treatment option in patients recalcitrant to conservative strategies before considering surgery; and, finally, they can support surgery as biological augmentation, allowing a switch toward less invasive strategies with a lower rate of surgical complications.

Most studies focused on biological treatment of midportion Achilles tendinopathy and dealt with PRP injections, but many systematic reviews determined that high-level evidence does not support the use of PRP injections for a variety of clinical, sports, and instrumental outcomes in individuals with midportion Achilles tendinopathy[14–21] (**Fig. 2**). However, the variability of PRP formulations impedes the understanding of study data and the optimization of treatment protocols.[22]

Besides this, other biological therapies for midportion Achilles tendinopathy have been investigated. In randomized controlled clinical trials, PRP injections were prospectively compared with ADSCs injections (stromal vascular fraction) in a population of 44 patients affected by monolateral or bilateral midportion Achilles tendinopathy (28 tendons per group): both treatments were effective, but patients treated with ADSCs obtained faster results, and the investigators suggested that this treatment should be taken into consideration for those patients who require an earlier return to daily activities or sport.[23] Furthermore, in a review dealing with biologics in Achilles tendon healing, it appeared that ADSCs may be as effective as other mesenchymal stem cells (MSCs) as measured by their multipotency and proliferative efficiency and may have a higher concentration of the pluripotent stem cells compared with BMAC.[4]

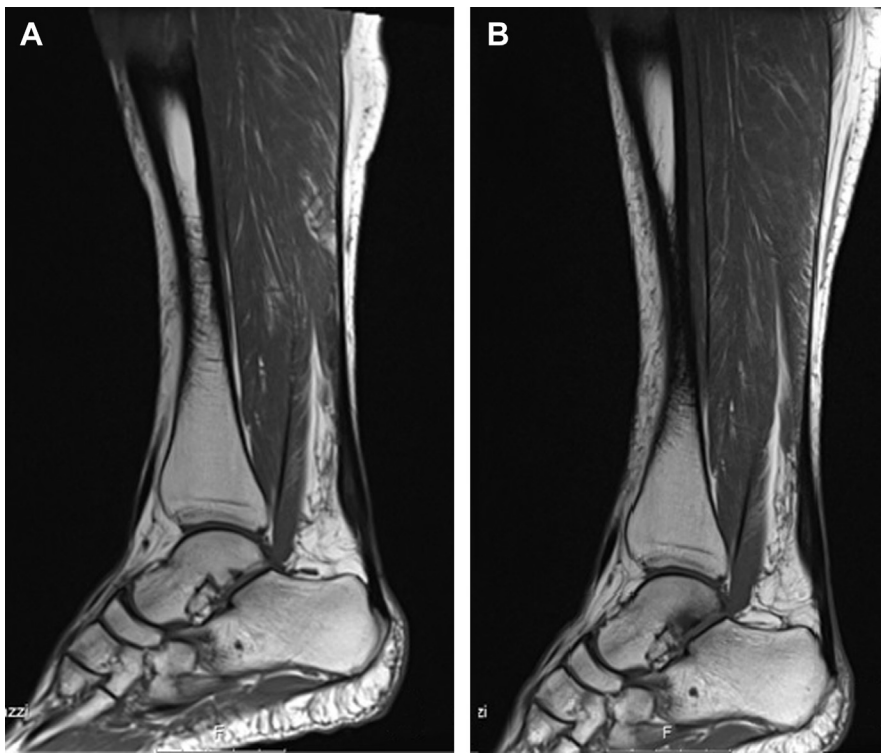

Fig. 2. (*A*) Pretreatment MRI appearance of midportion Achilles tendinopathy. (*B*) MRI appearance of the same patient 6 months after treatment with PRP injections.

Future investigation should focus on finding an ideal biological strategy for this pathologic condition, but it appears that biologics could play a role in the management of midportion Achilles tendinopathy as a step between conservative and surgical treatment or as an augmentation to optimize outcomes and lower complication rates of the treatment.

Insertional Achilles Tendinopathy

Insertional Achilles tendinopathy is situated at the insertion site of the Achilles tendon to the posterior calcaneal tuberosity, likely with the development of calcifications and bone spurs in the tendon at the bone insertion[9,24] (**Fig. 3**). For this pathologic condition, the good results obtained by conservative treatment of noninsertional disorders were not replicated: the success rate accounts for 28% to 32%.[25–27]

Currently, most studies focusing on biologics treatment of insertional Achilles tendon pathologic conditions deal with PRP in mixed cohorts of patients with insertional and noninsertional Achilles tendinopathies, and its use in this field remains controversial.[28] A prospective case series showed that PRP led to satisfaction in 28/30 patients of a mixed cohort with a 2-year follow-up (8 patients were affected by insertional Achilles tendinopathy), but both failures occurred in patients with insertional Achilles tendinopathy.[29] A retrospective case series compared 24 patients with insertional Achilles tendinopathy treated with 3 sessions of extracorporeal shock-wave therapy and 21 patients affected by insertional Achilles tendinopathy treated with 2 autologous PRP injections with a 6-month follow-up: a significant clinical and satisfaction improvement was detected in both groups without differences between the 2 groups, resulting in no better outcomes for PRP injections compared with extracorporeal shock-wave therapy.[30]

Higher-level studies with randomization and blinding are needed before determining a level of treatment recommendation for biologics strategies for insertional Achilles tendinopathy, whereas surgical treatment (debridement, calcifications and bone spur removal, tendon augmentation) is indicated in symptomatic patients who have failed conservative therapies.[27]

BIOLOGICS OPTIONS

Growth Factors

Growth factors, such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and insulin-like growth

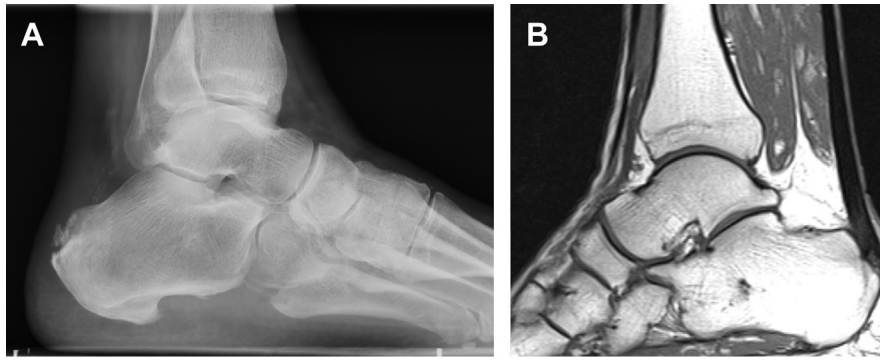

Fig. 3. Radiographs (*A*) and MRI (*B*) of a patient with insertional Achilles tendinopathy showing bone spurs and intratendinous calcifications at the bone insertion.

factor (IGF-I), are signal molecules involved in proliferation, differentiation cell chemotaxis, and synthesis of extracellular matrix. These molecules are produced by the tenocytes and white blood cells and are released from the platelets during the degranulation process. In the case of tendon injury, numerous growth factors are involved in activation and regulation of the cellular responses.[31]

Transforming growth factor–β

TGF-β is a family of proteins involved in many cellular processes and is present in 3 different isoforms (TGF-β1, -β2, and -β3) not always distinguishable regarding their effect on cell behavior. All 3 isoforms bind to cells via the same receptor, playing a key role in the healing process.[32]

TGF-β1 regulates cellular migration and proliferation and can increase the synthesis of collagen type I and III in tendon-derived cells; moreover, TGF-β1 is overexpressed in tendon in the early postinjury period but not in tendon sheath. Promising results have been reported using TGF-β1 complementary DNA-transduced BMSCs grafts, injections of TGF-β, and delivery of TGF-β by adenovirus-modified muscle grafts in rat Achilles tendon models.[33–35]

Maeda and colleagues[36] demonstrated that in acute injury the interruption to tendon continuity can cause a loss of tensile loading, resulting in the destabilization of the extracellular matrix and releasing too high levels of active TGF-β leading to tenocyte death. Furthermore, several in vivo and in vitro studies have shown that if the production of TGF-β1 is stopped, this can lead to a reduction in adhesion formation and increased range of motion in injured tendons. In an Achilles tendon model, mannose-6-phosphate has been shown to reduce activation of latent TGF-β, resulting in an increase in elastin production and increased strain and peak stress failure.[37,38] Recently, Potter and colleagues[39] evaluated the role of TGF-β1 in regulating tendon extracellular matrix after acute exercise in rats, showing that TGF-β1 signaling is necessary for the regulation of tendon cross-link formation as well as collagen and lysyl-oxidase gene transcription in an exercise-dependent manner. TGF-β therapy can increase mechanical strength of the healing Achilles tendon by the regulation of collagen synthesis, upregulation of cross-link formation, and enhanced matrix remodeling.[39]

Vascular endothelial growth factor

The VEGF family consists of several isoforms (VEGF-A, -B, -C, -D, and -E, and placenta growth factor) due to different messenger RNA (mRNA) splicing. VEGF isoforms exert their biological activity through 3 tyrosine kinase receptors, but the bioavailability depends on the isoform binding to the receptor.[40]

VEGF-A is known to be a regulator of neovascularization that is a prerequisite for tissue healing; however, it has been shown that excessive production of VEGF also can result in excessive scar formation.[41] In vitro studies showed that during tendon repair VEGF mRNA is increased, peaking at days 7 to 10, but returned to baseline by day 14, which suggests its function in neovascularization around the repair site.[42] In an Achilles tendon model, VEGF gene therapy increased TGF-β gene expression, and exogenous VEGF appears to increase tensile strength.[43] In a recent study, Tempfer and colleagues[44] blocked VEGF-A signaling using local injection of Bevacizumab, a monoclonal antibody, in a rat model with complete Achilles tendon rupture. After the treatment, angiogenesis was found to be significantly reduced in the Bevacizumab-treated repair tissue, accompanied by significantly reduced cross-sectional area, improved matrix organization, increased stiffness and Young's modulus, and maximum load and stress.[44]

Platelet-Derived Growth Factor

PDGF is a basic protein composed of 2 subunits, an A and a B chain, that exists in 3 different isoforms (PDGF-AA, PDGF-BB, and PDGF-AB). These isoforms act as chemotactic agents for inflammatory cells and help to increase type I collagen synthesis and induce TGF-β1 expression and IGF-I.[45]

Tenocytes can increase the expression of type I collagen with the addition of exogenous PDGF.[46] In vivo, a sustained delivery of PDGF-BB via a fibrin matrix led to an increase in cell density, cell proliferation, and type I collagen mRNA expression, and a fibrin/heparin delivery system demonstrated that PDGF-BB improved tendon function but not tendon structure.[47,48]

Insulin-like growth factor

IGF-I is one of the 3 single-chain polypeptides belonging to the IGF family (IGF-I, IGF-II, and insulin). Its expression increases during wound healing, and its absence is thought to impair dermal repair.[49] IGF-I has been successfully used by Kurtz and colleagues[50] to increase the rate of healing in the transacted rat Achilles tendon. Following transection, each tendon was treated with 25 μg of a recombinant variant form of IGF-I and showed a positive effect on healing within 24 hours after the transection and addition of IGF-I; this effect continued up until the tenth and last measurement, on day 15. Lyras and colleagues[51] have shown in an Achilles tendon rupture model treated with PRP that IGF-I increased expression in both epitenon and endotenon throughout the healing phases.

Platelet-Rich Plasma

PRP is the plasma fraction of the blood containing concentrated platelets and, in most cases, white blood cells. Because of the autologous nature, PRP is inherently safe, providing a natural conductive scaffold and containing many growth factors (eg, PDGF, TGF-β, VEGF, and hepatocyte growth factor), supposing to enhance tendon healing in this way.[52]

In 2009, a new classification was proposed to avoid the debates regarding the contents and the role of different preparations.[53] The classification separates the products following the presence of a cell's content (mostly leukocytes) and the fibrin architecture. Four main families of PRP have been proposed:

- Pure platelet-rich plasma, or leukocyte-poor platelet-rich plasma, is a preparation without leukocytes and with a low-density fibrin network after activation. It can be used as a liquid solution or in an activated gel form. Gel form is often used during surgery and can be injected. Many methods of preparation exist, particularly using cell separators (continuous flow plasmapheresis), even if this method is too heavy to be used easily in daily practice.
- Leukocyte- and platelet-rich plasma is a preparation with leukocytes and with a low-density fibrin network after activation. It can be used as a liquid solution or in an activated gel form. Most commercial systems belong to this family, and several protocols have been developed in the last few years, requiring the use of specific kits that allow minimum handling of the blood samples and maximum standardization of the preparations.
- Pure platelet-rich fibrin, or leukocyte-poor platelet-rich fibrin, is a preparation without leukocytes and with a high-density fibrin network. This product only exists in a strongly activated gel form and cannot be injected or used like traditional fibrin glues. However, because of its strong fibrin matrix, it can be handled like a

real solid material for other applications. Its main inconvenience remains its cost and relative complexity in comparison to the other forms of platelet-rich fibrin.
- Leukocyte- and platelet-rich fibrin products are preparations with leukocytes and with a high-density fibrin network, and these products only exist in a strongly activated gel form and cannot be injected or used like traditional fibrin glues. However, because of their strong fibrin matrix, they can be handled like a real solid material for other applications.

The use of PRP in Achilles tendon pathologic condition is still debated. A recent review aimed to compare the effectiveness of autologous blood-derived products (ABP) injection with that of placebo (sham injection or no injection or physiotherapy alone) in patients with Achilles tendinopathy.[54] Seven articles were included in the meta-analysis. The ABP injection and placebo revealed equal effectiveness in Victorian Institute of Sports Assessment - Achilles questionnaire (VISA-A) score improvement at 4 to 6 weeks, 12 weeks, 24 weeks, and 48 weeks. In meta-regression, there was no association between change in VISA-A score and duration of symptoms at 4 to 6 weeks (short term), 12 weeks (medium term), and 24 weeks (long term). The investigators concluded that ABP injection was not more effective than placebo (sham injection, no injection, or physiotherapy alone) in Achilles tendinopathy and that no association was found between therapeutic effects and duration of symptoms.[54]

A recent review identified 4 papers dealing with the use of PRP for Achilles tendon rupture[17]: no beneficial effects of PRP administration during and/or immediately after tendon suturing were reported and, in particular, Schepull and colleagues[55] hypothesized that PRP addition could even be detrimental in tissue healing because no biomechanical advantages and lower performance were reported in PRP patients with respect to the “suture-alone” group.

Adipose-Derived Stem Cells

In last several years, ADSCs have been the focus of numerous in vitro and in vivo studies for tendon regeneration. All this interest is mainly due to their high numbers in the human body (ADSCs are 5% of nucleated cells in adipose tissue), the simplicity of harvesting, and their rapid expansion and high proliferative potential[56,57] (**Fig. 4**). They can differentiate into different cellular lines, such as adipocytes, chondrocytes, osteoblasts, hepatocytes, pancreatic cells, muscle cells, and neuron-like cells both in vitro and in vivo.[56,57]

In tendon tissue, ADSCs can enhance the gene expression profile of cartilage oligomeric matrix protein (COMP), an extracellular matrix protein primarily present in cartilage. COMP is crucial to bind and organize collagen fibrils.[56,58] The use of ADSCs for the treatment of tendon pathologic conditions has been widely investigated in experimental animal models, with encouraging mechanical and histologic results.[57] ADSCs can induce tenocytes differentiation overexpressing the bone morphogenetic protein 12 gene.[57,59,60] Usuelli and colleagues[23] described the use of ADSCs to treat human noninsertional Achilles tendinopathy compared with PRP injection (28 patients in ADSCs group and 28 in PRP group). At final follow-up, there were no clinical (Visual Analog Scale pain, the VISA-A, the American Orthopaedic Foot and Ankle Society Ankle-Hindfoot Score, and the Short Form-36 [SF-36]) or imaging (MRI and ultrasonography [US]) differences between the 2 groups, and neither serious side effects nor adverse events were observed during the follow-up period: both treatments were effective, but patients treated with ADSCs obtained faster results and they should be taken into consideration for patients who require an earlier return to daily activities (**Fig. 5**).

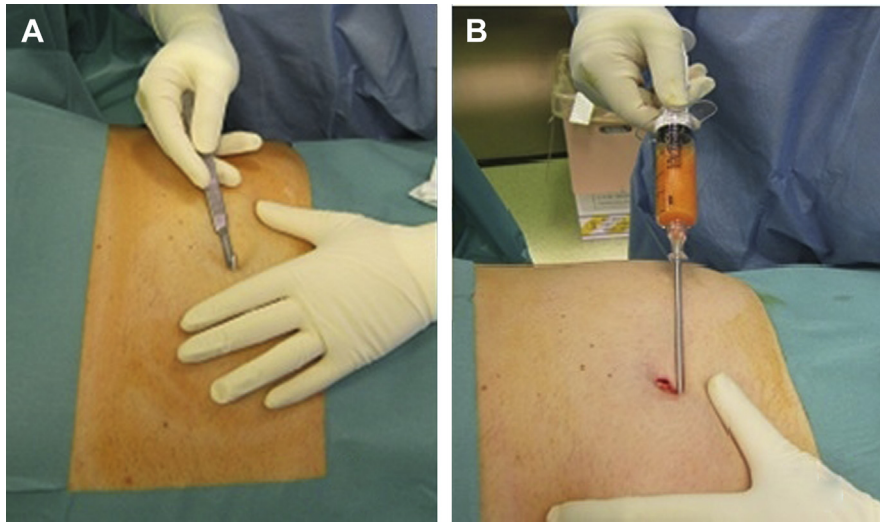

Fig. 4. Two phases of adipose tissue harvest to collect ADSCs. (*A*) Periumbilical incision. (*B*) Vacuum-syringe aspiration of abdominal adipose tissue.

Peripheral Blood Mononuclear Cells

Recently, several studies indicated PBMNCs (monocytes/macrophages and lymphocytes) as a new generation of regenerative autologous cell concentrate[61,62]: monocytes and macrophages promote tissue repair and guide regeneration.[62,63]

In fact, monocytes and macrophages have a plasticity comparable to marrow stem cells (they are able to differentiate and interact into the tissues depending on the surrounding microenvironment) and have multiple action mechanisms[64]: an angiogenic action thanks to the release of VEGF[65–67]; a regenerative action through the release of growth factors, cytokines, and messenger molecules[68–70]; and, furthermore, a recent study affirmed that osteo-inductive action is characteristic of monocyte population rather than stem cells population[71]; they are able to active resident MSCs through a paracrine effect and the release of exosomes[70,72–74]; they have an anti-inflammatory and immune-modulatory action through the polarization of macrophages M1 in M2[75–80]: in injured tissues with a healing delay, there is a majority of macrophages activated in M1 state (degenerative inflammatory), whereas polarization in M2 (macrophages activated in anti-inflammatory regenerative state) allows the regeneration of the injured or inflamed tissues.

Immunohistochemical studies have confirmed that inflammatory cells, such as mast cells, T cells, and macrophages, are present in the early stages of tendinopathies in humans.[81,82] Inflammation in the injured tendons is characterized by the infiltration of immune cells, such as neutrophils and macrophages. Initially, proinflammatory macrophages release cytokines at the repair site and promote degradation of the extracellular tendon matrix, inflammation, and apoptosis. In the later stages of tendon healing, macrophages repair tissues and release anti-inflammatory cytokines to alleviate inflammation and promote tendon remodeling.[83–85] Therefore, the balance between proinflammatory and anti-inflammatory cells (M1/M2) and soluble factors in the tendon-healing process has a major impact on the successful resolution of inflammation.

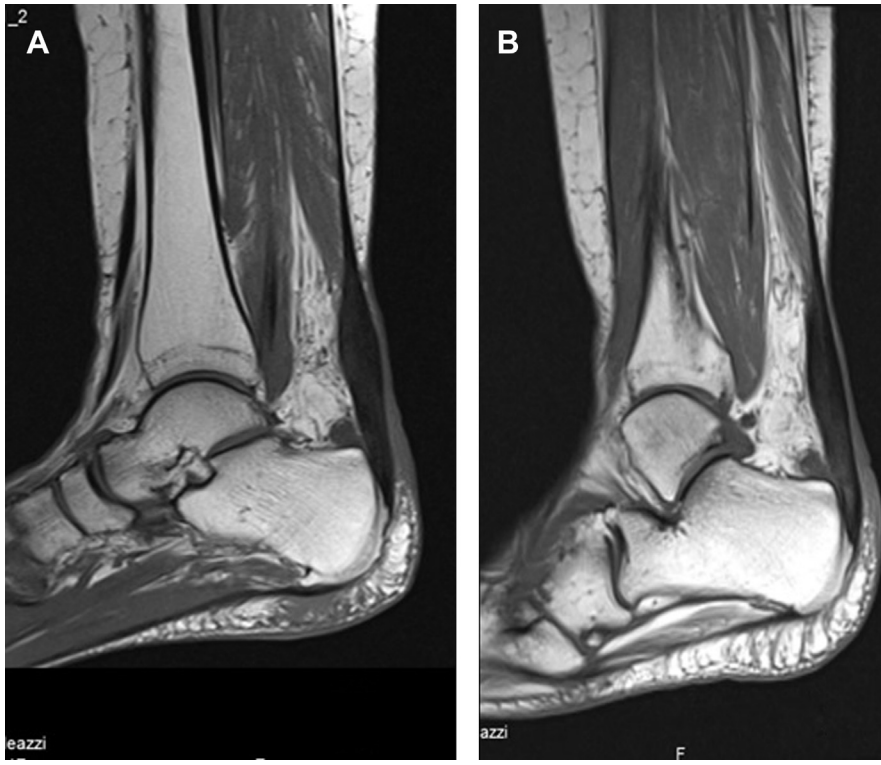

Fig. 5. (*A*) Pretreatment MRI appearance of midportion Achilles tendinopathy. (*B*) MRI appearance of the same patient 6 months after treatment with ADSCs injections.

Most tendons are surrounded by a layer of epithelial cells that could provide a source of fibroblasts to repair injured tendons. Epithelial cells can transdifferentiate into fibroblasts, which can then regenerate the damaged extracellular matrix. This process is triggered by the activation of a signal pathway called epithelial-mesenchymal transition. Sugg and colleagues[86] performed a tenotomy and a repair of the Achilles tendons of adult rats and studied and monitored changes in the macrophage phenotype (M1/M2) and related genes of both the extracellular matrix and the epithelial-mesenchymal transition pathways for a period of 4 weeks. The results suggest that changes in the phenotype of macrophages and the activation of epithelial-mesenchymal transition-related programs probably contribute first to the degradation of the injured tissue and then to the subsequent repair of the tendon tissue. The results also confirmed that the sequential transition between the M1 and M2 phenotypes supports the dual function of macrophages in the degradation and repair of damaged tendon tissue.

Moreover, Stolk and colleagues[87] stimulated tenocytes isolated from injured supraspinatus tendons with PBMNC. They demonstrated that tenocytes respond to an inflammatory microenvironment by altering surface receptors and release of cytokines, influencing the state polarization of macrophages and regulating the expression of collagen.[87] Furthermore, all these changes occurred both by direct cell-cell contact (macrophage-tenocytes) and by the release of endogenous factors (paracrine effect).

The role of monocytes-derived macrophages in tissue remodeling and repair is known for many tissues and pathologic conditions, and it is probably important in

tendinopathy because infiltration of $CD14^+$ myeloid cells (monocytes) and $CD68^+$ (macrophages) has been extensively demonstrated, mainly by immunohistochemistry, cytofluorimetry, or mRNA expression analysis.[87–93]

Macrophages are activated and polarized under the influence of the surrounding matrix and the environment.[94] The balancing of the M1/M2 polarization influences the outcome of the repair, and an inadequate or unregulated resolution of the inflammation would lead to chronic inflammation and fibrosis.[95] Despite stem cells having been considered a promising solution for the treatment of degenerative disease by "disseminating" them inside damaged tissues, recently it has been observed that regenerative capacity of stem cells is influenced and regulated by local immune response to tissue damage and particularly, by monocytes/macrophages that represent a central component of response to tissue damage and are coordinators of tissue repairing and regeneration.[63,96]

It has recently been affirmed that monocytes from peripheral blood are able to induce the polarization of M1 macrophages in M2 in muscle tissue.[97] Given the fundamental inflammatory component of the tendon lesions and the imbalance in the relationship between macrophages M1 and M2 in both acute and chronic tendinitis, the injections of autologous circulating monocytes could represent a valid cell therapy for supporting injured or degenerated tendons.[82]

Even though there are no studies focusing exclusively on the treatment of human Achilles tendinopathies with PBMNCs, autologous cell therapy with the injection into the tissues of monocytes simply harvested from peripheral blood could represent a new therapeutic option with low invasiveness and a solid scientific rationale in the treatment of these pathologic conditions. Moreover, it could be associated with Achilles tendon surgical procedures as biological augmentation to enhance healing process, improve surgical outcomes, and reduce complication rates, allowing for less invasive strategies.

Bone Marrow Aspirate Concentrate

BMAC is the result of different density gradient centrifugations of bone marrow aspirated from the iliac crest.[98,99] This aspirate has a concentration of nucleated cells less than 0.01%, and its role is to deliver MSCs to the injured tendon.[100] This procedure concentrates the mononucleated cells, hematopoietic stem cells, and platelets in 1 layer and the red blood cells in another. The efficacy of cells contained in BMAC is to modulate the healing response of pathologic tendon by controlling inflammation, reducing fibrosis, and recruiting other cells, including tenocytes and MSCs.[101] An in vitro study demonstrated an increase in cell proliferation in Achilles tendon scaffolds seeded with bone marrow aspirate.[102] In the only in vivo study reporting outcomes in patients with sport-related Achilles tendon ruptures treated via open repair augmented with BMAC injection, a total of 27 patients treated with open repair and BMAC injection were reevaluated at a mean follow-up of 29.7 ± 6.1 months and no reruptures were noted. Of the patients, 92% returned to their sport at 5.9 ± 1.8 months. No soft tissue masses, bone formation, or tumors were observed in the operative extremity.[103]

Scaffolds

In recent years, several natural and synthetic materials, such as collagen, silk, or synthetic polymers, have been examined, and in some cases also hybrid materials with the aim to promote cellular growth and provide mechanical support for tendon repair.[104]

The ideal scaffold for Achilles tendon should allow a natural and fast bridging of tendinous defect as well as organized collagen-rich tissue with complete incorporation of the material within 8 weeks. Moreover, the scaffold should release chemotactic factors to promote the recruitment of progenitor cells.[105]

Polyhydroxyalkanoates is a material that possesses several of the above qualities. It is part of a family of biopolymers consisting of polyesters produced in nature by microorganisms to store energy and carbon. These materials, in particular, poly-3-hydroxybutyrate-*co*-3-hydroxyhexanoate (PHBHHx), are known to be compatible with many mesenchyme-derived cell types and have adaptable mechanical properties along with delayed biodegradability.[104] A study by Webb and colleagues[104] reported how tendons repair using PHBHHx scaffold was mechanically and histologically superior in comparison to controls.

Another treatment regards the use of decellularized tendon tissue as a scaffold, which maintains the native characteristics (ultrastructure, biochemical composition, and tensile strength of the tendon extracellular matrix) and preserves more than 90% of the proteoglycans and growth factors.[106] In vitro, the decellularized tendon slices were able to facilitate repopulation and attachment of fibroblasts. Farnebo and colleagues,[107] analyzing the use of decellularized grafts in rats, demonstrated an enhancement of mechanical properties and reduced immune response. Decellularized porcine tendon can also be recellularized with human tenocytes.[108]

An acellular human dermal allograft (GraftJacket; Wright Medical Technology, Inc, Arlington, TN, USA) reported significant improvement in mechanical strength and stiffness in biomechanical test. In in vivo studies, patients treated with GraftJacket showed a desirable return-to-activity time without complications.[109,110] Recently, interest has also increased about scaffolds of animal origin; in fact, the use of xenograft, in addition to suture repair, seems to improve tendon strength compared with isolated repair. The most used tissue is porcine small intestinal submucosa (SIS).[111] Preclinical studies demonstrated the ability of SIS to remodel tendon: SIS retains several biologically active growth factors, including VEGF, TGF-β, and fibroblast growth factor, which likely contribute to the behavior and migration of cells into the scaffold.[112,113] Moreover, SIS is subject to a rapid degradation, with 60% of the mass lost after 30 days and complete degradation within 90 days. After complete degradation, the extracellular matrix looks very similar to native tissue for vascularity and organization. A strength of SIS is its ability to recruit marrow-derived cells involved in the remodeling and repair process.[112,113]

IMAGING IN BIOLOGICS TREATMENT OF ACHILLES TENDON PATHOLOGIC CONDITIONS

Ultrasonography

Because of its easy accessibility, large tissue volume, and straight course, US is an ideal technique to evaluate the Achilles tendon. Moreover, the contralateral assessment results in a very easy and dynamic evaluation, which is easily accomplished with active and passive ankle movement. Color/power Doppler imaging can theoretically demonstrate hyperemia, increased vascularity, and varicosities.[114]

A recent systematic review confirms and recommends US's diagnostic role in patients with Achilles tendon pain. US can determine the type (full, partial, or even plantaris tendon) and level of rupture, define the extent of tissue damage and prognosis as well as aid in providing an indication for treatment selection (surgery or conservative treatment). Literature regarding US after regenerative treatment is scarce.[115] Recently, Albano and colleagues[116] assessed the correlation between US findings

and clinical outcome after intratendinous injection of leucocyte-rich PRP or ADSCs in patients with noninsertional Achilles tendinopathy. Significant increase of tendon thickness measured using US ($P = .012$) and power Doppler signal ($P = .027$) was seen. There was no significant difference between pretreatment and posttreatment cross-sectional area, signal intensity, and echotexture ($P > .217$). None of the pretreatment parameters was a predictor of treatment outcome ($P > .104$).

MRI

MRI is an excellent technique for imaging the internal morphology of the Achilles tendon. It can easily distinguish between paratenonitis and tendinosis. MRI is not user-dependent and can provide multiplanar images of the Achilles. It is also useful in determining the extent of degeneration in the tendon, which is useful for preoperative planning. In last several years, dynamic MRI has been developed, and this procedure provides relevant images, particularly at the myotendinous junction level of the Achilles tendon.[114,115]

Few studies analyzed MRI changing after regenerative treatment. The study of Albano and colleagues[116] highlighted how, after ADSCs or PRP treatment, Achilles tendon increased its thickness, as measured using MRI ($P = .013$).

Oloff and colleagues[117] evaluated 13 individuals who underwent Achilles tendon surgery and PRP treatment and 13 individuals who underwent PRP treatment alone, correlating clinical outcomes pretreatment and posttreatment with MRI. At final follow-up, the MRI score did not correlate with the VISA-A questionnaire score ($P = .13$), whereas the Pearson's correlation test suggested a linear trend between the difference in MRI score and VISA-A questionnaire.

MRI is useful not only for assessing effects of biologic treatment of Achilles tendon pathologic conditions but also for creating an imaging-based classification. The classification of Achilles tendinopathy proposed by Oloff and colleagues[117] was based on MRI appearance and distinguished 5 grades of Achilles tendinopathy depending on the thickness of the tendon (ranging from hypertrophy to severe thickness) and on the signal changes of the tendon (ranging from homogeneous signal to greater than 50% of tendon with abnormal signal or partial tendon tear).

Sonoelastography

Sonoelastography (SE) is an image method based on US that analyzes the viscoelastic behavior of a tissue subjected to a deformation applied by the examiner during the execution of the diagnostic examination.[118] The SE uses the real-time Doppler US technique to render in images the vibration resulting from the propagation of a low-frequency wave (<1 kHz) through the examined tissue. This low frequency is generated by an external source, such as the pressure of the operator and propagated by means of a probe. The SE allows a conventional-type evaluation (B-mode) contextually to the evaluation of the elasticity of the investigated structures. The software returns the elastogram as a scale of colors that turn from blue, an indicator of maximum stiffness, to green and yellow, which indicate intermediate stiffness, up to red, indicating the greater elasticity.[118]

There is still a lack of consensus in the application of SE to detect tendon pathologic condition. In normal Achilles tendon, tissue can be classified into 3 grades: grade 1, blue (hardest tissue) to green (hard tissue); grade 2, yellow (intermediate tissue); or grade 3, red (soft tissue). During aging, Achilles tendon exhibited an increased stiffness when compared with young adults.[119]

In those patients with symptomatic Achilles tendons but normal US appearance, SE was able to highlight very early changes in tissue elasticity, due to initial edema and

inflammation, usually missed at conventional US. SE demonstrated high to excellent sensitivity, specificity, and accuracy, and also a high agreement with US (k = 0.81) and clinical examination (k = 0.91).[119]

THE AUTHORS' ALGORITHM FOR USE OF BIOLOGICS IN MIDPORTION ACHILLES TENDINOPATHY (FOOT AND ANKLE RECONSTRUCTION GROUP ALGORITHM)

Over the years, numerous types of treatment have been proposed for chronic Achilles tendinopathies, but with the advent of regenerative medicine, the therapeutic possibilities have multiplied without a precise treatment algorithm. The authors created an algorithm for the use of biologics in midportion Achilles tendinopathy based on their clinical experience. Developing the FARG (Foot and Ankle Reconstruction Group) algorithm for midportion Achilles tendinopathy, the authors have been inspired by the MRI-based classification of tendinopathy described by Oloff and colleagues,[117] and they have categorized symptomatic patients according to their level of sporting activity:

- Sport-active patients (sports activity at least 2 times a week)
- Nonathletic patients (sports activity <2 times a week)

The treatment scheme is shown in **Table 1**. It is noteworthy that the major differences concern grades 1 and 2 of tendinopathy in which, considering the lower functional request, the nonathletic patient can more probably benefit from less invasive treatments.

BIOLOGICS IN ACUTE AND CHRONIC ACHILLES RUPTURES

In recent years, the use of regenerative medicine for the treatment of acute Achilles tendon rupture has been widely described. Zou and colleagues[120] hypothesized that PRP can be used as biological augmentation for surgical treatment of acute Achilles tendon rupture. Patients with acute Achilles tendon rupture were randomly assigned to either control group (isolated end-to-end Krackow suture) or PRP group (suture plus PRP). At 3 months, the PRP group had better isokinetic muscle. The PRP group also achieved higher SF-36 and Leppilahti scores at 6 and 12 months.

Table 1
Foot and Ankle Reconstruction Group algorithm for midportion Achilles tendinopathy

Sport-Active Patients	Achilles Tendinopathy Grade	Nonathletic Patients
Conservative treatment	Grade 0: Hypertrophy, with homogeneous signal	Conservative treatment
Biologic treatment[b]	Grade 1: Hypertrophy, with isolated signal changes in <25% of tendon	Conservative treatment
Stripping[a] + biologic treatment[b]	Grade 2: Hypertrophy, with signal changes in >1 area, or diffuse changes in >25% of tendon	Biologic[a] treatment[b]
Stripping[a] + biologic treatment[b]	Grade 3: Severe hypertrophy, <50% tendon signal changes, with interstitial tear	Stripping[a] + biologic treatment[b]
FHL transfer ± biologic treatment[b]	Grade 4: Severe thickening, >50% of tendon with abnormal signal, partial tendon tear	FHL transfer ± biologic treatment[b]

[a] Stripping technique as described by Maffulli and colleagues.[121]
[b] Authors' preferred biologic treatments are ADSCs and PBMNCs injections.

At 24 months, the PRP group had an improved ankle range of motion compared with the control group.[120]

In contrast, Schepull and colleagues[55] evaluated 30 patients with acute Achilles tendon rupture, 16 of whom were injected with 10 mL PRP at time of surgery. No significant group differences in elasticity modulus could be shown. There was no significant difference in heel raise index. The Achilles Tendon Total Rupture Score was lower in the PRP group, suggesting a detrimental effect. There was a correlation between the elasticity modulus at 7 and 19 weeks and the heel raise index at 52 weeks.

The use of PRP was analyzed also in an athlete population. Sánchez and colleagues[122] evaluated 12 athletes who underwent open suture repair after complete Achilles tendon tear.[122] Open suture repair in conjunction with a preparation rich in growth factors (PRGF) was performed in 6 athletes. Athletes receiving PRGF recovered their range of motion earlier, showed no wound complication, and took less time to take up gentle running and to resume training activities.

An immunohistochemistry study, moreover, showed that local application of PRP, in acute Achilles tendon rupture, can enhance the healing capacity by promoting better collagen I deposition, decreasing cellularity and vascularity, and increasing glycosaminoglycan content when compared with control samples.[123]

Concerning BMAC, only 1 study evaluated clinical effects in primary Achilles tendon repair. Stein and colleagues[103] reviewed patients with sport-related Achilles tendon ruptures treated via open repair augmented with BMAC injection. A total of 28 tendons were identified with a mean age of 38.3. At mean follow-up of 29.7 months, there were no reruptures. Walking without a boot was achieved at 1.8 months' participation, in light activity at 3.4 months, and 92% of patients returned to their sport at 5.9 months.

A recent meta-analysis aimed to determine if augmented surgical repair with gastrocnemius (3 studies) or plantaris tendon (1 study) of an acute Achilles tendon rupture improved subjective patient satisfaction without an increase in rerupture rates.[124] In 4 studies, 169 patients were analyzed, of which 83 participants were treated with augmented repair and 86 were treated with nonaugmented repair. Augmented repair led to similar responses when compared with nonaugmented repair for acute Achilles tendon rupture. The rerupture rates showed no significant difference for augmented versus nonaugmented repair. No differences in superficial and deep infections occurred in augmented (7 infections) and nonaugmented (8 infections) repair groups during postoperative follow-up. No significant differences in other complications were found between augmented (7.2%) and nonaugmented (8.1%) repair.[124]

The treatment of chronic Achilles tendon ruptures is still a challenge for foot and ankle surgeons, and given the lack of prospective randomized trials and the small number of the studies, a gold standard treatment has not been described. The key role in this pathologic condition is the size of tendon defects. Most of surgical repair involves tendon transfer or the use of an allograft or scaffold to cover the defect.[125] Several tendon transfer techniques have been described, including the peroneus brevis, FDL, and FHL.[125] Each of these techniques has its own pertinent anatomy, advantages, and disadvantages that should be taken into consideration before surgery. By using the peroneus brevis, it is possible to report a loss of eversion strength.[126] Concerning FDL, the risk is linked with a weakened toe flexion and development of lesser toe deformities. Moreover, the possibility of damaging the adjacent neurovascular bundle has been reported in the literature.[125]

One transfer technique described for chronic Achilles tendon rupture is the use FHL tendon: in addition to the mechanical function, comes into play also a biological mechanism due to the muscular proximity with the consequent contribution of blood and growth factors[127] (**Fig. 6**). Literature reports no functional deficits related to the FHL

tendon harvest, but doubts remain regarding the potential to develop hallux claw deformity, decreased great toe push-off strength, and transfer metatarsalgia following FHL tendon harvest.[128,129]

Another strategy to fill the tendon defect involves the use of autograft or allograft tendon for augmentation. The use of fascia lata or hamstrings tendon has been widely described with excellent clinical results and with a high return to preinjury sport activity.[130–134]

Otherwise synthetic materials avoid the risks and the comorbidities related to tendon harvesting. In contrast, the literature reported a higher risk of wound complications, infection, and inflammatory reactions. Several published articles reported the use of different scaffolds, such as polymer-carbon fiber, Marlex mesh, or polyester tape, reporting a complication rate ranging from 17.3% to 31.3%.[135–139]

Another alternative for the treatment of neglected Achilles tendon rupture regards the use of acellular dermal human matrix. This patch can be used in the "burrito" technique or to augment a direct end-to-end repair in which the goal is early mobilization. There are similar xenograft options commercially available. These grafts have acellular collagen matrix derived from sources, such as equine pericardium or porcine urinary bladder matrix.[109,140,141]

SUMMARY AND FUTURE PERSPECTIVES

Achilles tendinopathies are challenging pathologic conditions considering the scarce tendency to healing and the average high-functional requests of the patients that are often young and active in sports activities. Conservative treatments for insertional Achilles tendinopathies have shown low success rate and biologics, and presently, have not shown better outcomes: surgery remains the main indication in patients resistant to conservative treatment.

In contrast, several studies have focused on biologics for the treatment of midportion Achilles tendinopathy with good outcomes. Despite this, because of the variability of the treatments evaluated and the wide spectrum of techniques and technologies available, standardized protocols have still not been created. Combining imaging appearance with patients' functional requests could be the way to reach a protocol for the use of biologics for the treatment of midportion Achilles tendinopathy and, for this perspective, the authors have described their actual protocol in this field.

Regenerative medicine has demonstrated the in vitro and in vivo ability to enhance tendon-healing processes, and biologics will gain more and more space as

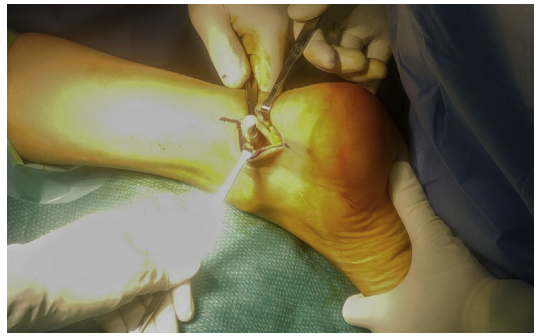

Fig. 6. FHL tendon harvesting through the posteromedial approach in a patient with chronic Achilles tendon rupture.

augmentation or support in surgical procedures for Achilles pathologic conditions and acute or chronic Achilles tears. Despite this, further scientific evaluation is needed to reach 3 goals that will widen the general use of biologics for Achilles tendon pathologic conditions:

1. To identify the most appropriate biologic tools to address these pathologic conditions
2. To achieve a strong level of treatment recommendation
3. To create evidence-based and personalized protocols of treatment.

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