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(Article begins on next page)

# **In Achilles tendinopathy, the neovascularization, detected by contrast-enhanced ultrasound (CEUS), is abundant but not related to symptoms**

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## **Abstract**

**Purpose and hypothesis:** Mid-portion Achilles tendinopathy is characterized by a proliferation of small vessels, called neovascularization, which can be demonstrated by power Doppler sonography (PD). Neovascularization can be correlated with diagnosis and consequent therapies focused on vascular supply. Published data regarding the relationship between neovascularisation and symptoms, such as pain and disability, are contradictory. The hypothesis that contrast-enhanced ultrasound (CEUS) could detect with more sensibility than PD the new vessel ingrowth in human degenerated Achilles tendons and therefore the correlation of neovascularization with pain and disability, was evaluated.

**Methods:** Thirty consecutive patients of recalcitrant Achilles tendinopathy were studied with ultrasound greyscale (US), PD, CEUS and magnetic resonance imaging. Neovascularization was recorded as percentage on the whole extension of examined area. The vascularization time was recorded as venous and arterial type. Imaging data were classified both concurrently with the examination and in a secondary blinded assessment; any difference in the subjective assessment was discussed and a consensus view formed. Pain and disability were assessed by Western Ontario McMaster Universities Arthritis Index (WOMAC) and EuroQuality of life 5-dimension-5-level questionnaire and visual analogue scale (EQ-VAS). All results were analysed with suitable statistical methods.

Results: 76.7% of cases were degenerated; 23.3% had also partial discontinuity of the fibres. PD detected vascularization in 54% of cases, whereas CEUS in 83% of cases: in 13 cases, PD did not detect vascularization. The vascularization time was rapid (< 20 s, arterial type) in 60% of cases. WOMAC pain mean value is 6.4 and SD 3.4; WOMAC total score mean value is 21.6 and SD 12.8. EQ-VAS mean value is 56 and SD 18.3. No statistically significant correlation emerged between vascularization and pain/disability.

Conclusions: CEUS showed a greater ability to detect neovessels than PD in chronic Achilles tendinopathies. Nevertheless in 30 consecutive tendinopathies, no correlation between pain/disability and neovascularization was found: the role of multiple neovessels continue to be unclear. The possibility to discriminate arterial from venous vessels ('vascularization time') could be useful to understand the pathophysiology of tendinopathies and its healing process.

Study type: Diagnostic study.

Level of evidence: II.

Keywords: Achilles tendon; Angiofibroblastic degeneration; Contrast-enhanced ultrasound (CEUS); Magnetic resonance imaging (MRI); Neovascularization; Power Doppler ultrasonography; Tendinopathy; Tendinosis.

#### [List of abbreviations](#)

US Ultrasound

AT Achilles tendon

CEUS Contrast-enhanced ultrasound

PD Power Doppler sonography

MRI Magnetic resonance imaging

ml millilitre

Hz Hertz

MHz Mega hertz

MI Mechanical index

T Tesla

SD Standard deviation

SE Standard error

STIR Short Tau inversion recovery

WOMAC Western Ontario and McMaster Universities

Arthritis Index

EQ-5D–5L EuroQuality of life 5-dimension–5-level questionnaire

EQ-VAS EuroQuality visual analogue scale

## Introduction

Mid-portion Achilles tendinopathy is a relatively common chronic disorder that occurs in athletic and occupational population [1]. The diagnosis is clinical with typical imaging. At ultrasound (US) evaluation, degenerated Achilles tendon (AT) is abnormally enlarged with altered inhomogeneous irregular echogenicity and contour defects [2–4]. Doppler sonography detects multiple microvessels, typical of angiofibroblastic degeneration. The neovessels and their accompanying nerves [5, 6] are usually considered the source of pain. This is why the neovascularization has relevance in terms of diagnosis, prognosis and therapies based on vascular supply [2, 4–21]. Reiter et al. [12] in twenty patients demonstrated a statistically significant association between neovascularization and pain and disability. Richards et al. [13, 14] demonstrated that there is a nonlinear relationship between microvessels and symptomatic tendinosis and AT size, but not with the duration of symptoms. Divani et al. [9] found that the site of maximum neovascularization correlates with the site of pain. Yang et al. [19] demonstrated in thirty-seven pathologic ATs that neovascularization, correlated with pain but not with disability, was present in 97.3% of cases. Other studies reported no correlation between blood flow detected with Doppler sonography and pain or functional results [7, 11, 15, 18]. In particular, Tol et al. [16] demonstrated in a prospective analysis on 556 paired measurements the absence of relationship between neovascularization and clinical severity [17]. Furthermore, De Junge et al. [1] demonstrated that there is no association between the tendon structure and symptoms. Many reasons could explain these contradictory data: first of all, the variance of performing Doppler ultrasound examination in terms of the US machine setting and transducer frequency [19]; furthermore, the neovascularization could be a dynamic finding that could disappear at inconstant rates [20]. Contrast-enhanced ultrasound (CEUS) is widely used to estimate in real time the focal perfusion, especially with the modern contrast medium [22]. Whereas power Doppler US and spectral wave analysis can evaluate vessels measuring at least 100  $\mu\text{m}$ , CEUS can visualize vessels measuring 40  $\mu\text{m}$ , namely the capillary network [22]. Until now, CEUS has been rarely used to study the tendon vascularization, although the efficiency of superficial multifrequency probes is optimal in evaluation of microbubbles. Chang et al. [22] in a review found no paper concerning the Achilles tendinopathy. Shen et al. [23] in an animal model demonstrated that sensitivity of CEUS in detecting hypervascularity was higher than that of Doppler ultrasonography. Genovese et al. [24] demonstrated in twenty-four athletes with previous AT surgical repair that CEUS was able to detect more vessels than PD. The research question of this study was whether CEUS could better detect neovascularisation than Doppler ultrasonography also in human degenerated Achilles tendons and whether the neovascularization detected by CEUS could be related to pain and disability, to give a contribute in the understanding of the real clinical role of neovessels in

tendinopathy. Only adult active patients affected by Achilles mid-portion tendinopathy, who did not profit from conservative therapy (recalcitrant tendinopathy), were investigated. This research assessed for the first time by CEUS the neovascularization of human chronic recalcitrant Achilles tendinopathies, never surgically treated before.

## Materials and methods

The study was conducted on patients referred at an interdisciplinary group between 2012 and 2014. Inclusion and exclusion criteria are reported in Table 1. To exclude any influence of muscular activity on blood flow, a fundamental inclusion criterion was rest, for at least 24 h, from any rehabilitation programme before imaging. Twenty-seven consecutive patients affected by midportion recalcitrant Achilles tendinopathy, three of them bilateral, for 30 total cases, were enrolled for this study: 19 males, 8 females, mean age 54.8 (SD 6.8) years for males and 55.2 (SD 9.7) for females. The right ankle was affected in 40% of cases. Five and 15 patients were, respectively, professional and recreational athletes; 7 patients did not perform routinely any physical activity. The recalcitrant symptoms following ineffective conservative therapy were present from 6 to 12 months in 13 cases, 1–2 years in 6 cases and more than 2 years in 11 cases. All patients gave their informed consent to perform the imaging and to utilize their clinical data for study purposes. The local institutional board approved the study, which was carried out in compliance with the Code of Ethics of the World Medical Association.

## Imaging

All cases were studied with ultrasound greyscale (US), power Doppler sonography (PD), contrast-enhanced ultrasound (CEUS) and magnetic resonance imaging (MRI). Imaging was performed after, at least, 24-h rest to exclude any influence of muscular activity on blood flow.

### *US*

The patients lay in prone position, with both feet and ankles hanging free with a small degree of plantar extension. The patients were instructed not to move throughout the examination. The US device was an Esaote MyLab Twice, equipped with multifrequency linear transducers, respectively, LA332 (3–11 MHz) for CEUS study and LA523 (4–13 MHz) for basal US and power Doppler studies (Esaote, Genoa, Italy). The panoramic study of the tendons was performed to visualize the complete tendon's structure; then, the study was focalized on the mid-portion involved in the tendon pathology. Whereas a normal tendon is characterized by fine fibrillar hyperechoic parallel bands (fibrillar pattern) with a thin adherent paratenon, a degenerated AT shows altered inhomogeneous irregular echogenicity and contour defects [2–4]. Three types of

ATs were defined: normal tendon, degenerated tendon without/with partial discontinuity of the degenerated fibers. The tendon thickness was defined as normal ( $\leq 6$  mm), enlarged (7–10 mm), moderately enlarged (11–15 mm), markedly enlarged ( $> 16$  mm) [4, 5, 25]. The margins and morphology were classified as normal or irregular; calcifications and fibrotic changes as present or absent. The echogenicity was assessed as percentage on the whole extension of examined area using the Connell method [26] and scored as normal fibrillar pattern, hypoechoic for  $< 30\%$ , hypoechoic 30–70%, hypoechoic for  $> 70\%$ , hyperechoic, hypoanechoic.

#### *Power Doppler sonography*

Power Doppler pulse repetition frequency was 500–1000 Hz and the colour gain adjusted so that no colour signal was present below the cortical bone on PD. In accordance with the appearance of vessels inside the tendons, subjective estimation (see Imaging assessment further on for criteria) was recorded as no visible vessels, one or two small vessels, several irregular vessels throughout the tendon [8, 21].

#### *CEUS*

CEUS scanning was performed with a low MI (mechanical index  $\leq 0.1$ ) technique and dedicated software (CnTI Contrast Tuned Imaging, Esaote, Genoa, Italy). Scanning time was at least 2 min. Each patient received a dose of 4.8 ml of ultrasound contrast agent consisting of microbubbles filled with sulphur hexafluoride (SonoVue™, Bracco, Milan, Italy) via a 20-gauge intravenous cannula, followed by a flush of 5 ml saline solution. No allergic reactions occurred. The assessment of neovessels presence was quantified subjectively and recorded as percentage on the whole extension of examined area and recorded as percentage on the whole extension of examined area:  $\leq 25$ , 26–50, 51–75 and 76–100% [26]. The time intercurrent between the end of the bolus injection into an antecubital vein and the contrast agent arrival, 'vascularization time', was tracked using a timer on the monitor during the transit of the microbubbles in the examined area and recorded as  $> 20$  and  $< 20$  s, respectively, venous and arterial type.

#### *MRI*

MRI was performed in order to identify any other possible source of pain, in particular bone marrow oedema, and avoid any possible misdiagnosis. MRI was performed with GE Optima 1.5T (General Electric Co, Fairfield, Connecticut, USA), sequences on sagittal plane (sequences FSE T1 weighted, thickness 3.5 mm, spacing 4 mm, DFOV  $14 \times 14$  cm, NEX 1, TR 786 ms, TE 9.4 ms; Dixon sequences with fat suppression, thickness 3.5 mm, spacing 4 mm, DFOV  $14 \times 14$  cm, NEX 2, FA  $90^\circ$ , TR 2200 ms, TE 36.90 ms) and axial plane (sequences FSE T2 weighted, thickness 4.5 mm, spacing 5 mm, DFOV  $14 \times 14$  cm, NEX 2, TR 2877 ms, TE 80 ms). The ATs were

assessed as tendinopathy when characterized by increased or intermediate signal in T1-weighted sequence and normal signal pattern on T2-weighted sequence [25, 27]. Bone marrow oedema, evaluated with STIR or fat-suppressed sequences, was recorded as present or absent.

### *Imaging assessment*

Imaging data (US, PD, CEUS, MRI) were classified both concurrently with the examination by a radiologists with at least 10 years of experience in musculoskeletal pathologies and in a blinded secondary assessment, carried out by two senior radiologists. Given the long-standing experience of the radiologists, intra- and inter-examiners variations were very few (2 or 3 cases); therefore, no specific assessment of the registered discordance was carried out.

### *Pain and disability*

The clinical data, in particular pain and disability, were assessed by means of:

1. the Western Ontario and McMaster Universities Arthritis Index (WOMAC) that evaluates pain (five items, score range 0–20), stiffness (two items, score range 0–8) and physical functioning of the ankle (seventeen items, score range 0–68); the total score is 0–96, where 96 is the best results;
2. EuroQuality of life 5-dimension–5-level questionnaire (EQ-5D–5L) consisting of two parts: the first one is descriptive and analyses 5 dimensions (mobility, selfcare, usual activities, pain/discomfort and anxiety/ depression) with 5 score levels (no problems, slight problems, moderate problems, severe problems and extreme problems); the second part utilizes a visual analogue scale (EQ-VAS), where the endpoints are labelled ‘Best imaginable health state’ and ‘Worst imaginable health state’.

The WOMAC total score, EQ-5D-5L total score and EQ-VAS were assumed as index of disability

### *Statistical analysis*

Data are presented as mean and standard deviation in case of continuous variables and as absolute and relative frequencies whether categorical (Tables 2, 3). The relationship between categorical variables (EQ pain with vascularization time and extension) has been tested by means of contingency tables and their significance assessed by Chi-square tests. The relationship between categorical (vascularization time and extension) and continuous variables (WOMAC pain, WOMAC total score, EQ-5D-5L total score and EQVAS) has been assessed by means of one-way ANOVA, comparing means across the different modalities of each categorical variable (Table 4). There was no 'a priori' sample size calculation, as all available patients presenting the requested inclusion criteria were included in the study. A 'post hoc' analysis of the sample size power was performed, taking as main outcome the WOMAC pain score in the three PD vascularization modalities. As one could foresee, the test power was quite low (14% probability of detecting a 0.72 difference among the means of the three groups) due to low numbers in the groups. Vascularization extension, originally a continuous variable, has been transformed in categorical for simplifying comparison among different groups: no vascularization, less than 50% and more than 50%. Significance has been taken at 0.5; all analyses have been performed with IBM SPSS version 23.

## Results

### *US*

The ATs were degenerated, enlarged, with irregular morphology: fusiform shaped in longitudinal scan and circular or oval shaped in axial scan (Table 2). Intratendinous calcifications and fibrotic changes were demonstrated in few patients.

### *Power Doppler sonography*

No visible vessels were detected in 18 cases (54%), one or two small vessels in 5 patients (16.7%), several irregular vessels were showed throughout the tendon in 7 patients (23.3%) (Fig. 1a). CEUS Vascularization was present in 25 cases (83%). The vessels extended for  $\leq 25\%$  in 4 cases (13.3%), for 26–50% in 6 cases (20%), for 51–75% in 5 cases (16.7%); in 10 cases (33.3%) vessels occupied the 100% of the tendons (Fig. 1b). The vascularization time was  $< 20$  s in 18 cases (60%) and  $> 20$  s in 7 cases (23.3%).

### *CEUS vs. power Doppler*

CEUS was able to detect blood vessels in 13 cases where PD did not detect any vascularization.



## *MRI*

MRI identified one case with a mild bone marrow oedema due to an insertional tendinitis and confirmed the clinical and US diagnosis.

## *Pain*

Pain ranged from 13 and 0, mean value 6.4 (SE: 0.6). WOMAC total score ranged from 6 to 46, mean value 21.6 (SE: 2.3) (Table 3). Two patients referred mild pain (6.7%), 18 patients referred moderate pain (60%), and 10 patients complained of severe pain (33.3%). EQ-5D-5L total score ranged from 5 and 11, mean value 8.37 (SE: 0.27).

EQ-VAS mean value was 56 (SE: 3.3).

## *Correlation of CEUS with pain and disability*

No statistically significant correlation emerged between vascularization detected by CEUS and pain and disability (Table 4).

## Discussion

The most important findings of the present study were the detection by CEUS of an abundant neovascularization in chronic recalcitrant Achilles tendinopathies and the absence of any correlation with symptoms. In comparison with the PD, CEUS demonstrated neovascular network in 83% of the patients (Fig. 1), but PD only in 54%, with 13 false negative, which implies that CEUS shows a higher standard in this domain. In accordance with Shen [23], these results can be related to the higher sensitivity of CEUS in comparison with traditional power Doppler ultrasound in assessing microvasculature in tendinopathies. In fact, whereas power Doppler can visualize, in optimal conditions, only vessels bigger than capillars, CEUS can show the real vascular map. These data confirm the presence of increased vascularization in chronic tendinopathies, as reported in many studies [5, 8, 9, 13–15, 23] and confirmed by gadolinium contrast enhancement in MRI study [27]. Rest from any rehabilitation programme for at least 24 h before CEUS examination was able to exclude any influence of muscular activity on blood flow and therefore any false positive [21]. Whereas the main part of patients referred severe and moderate pain (98%), and vascularization was detected in 83% of cases, in the studied sample no statistically significant correlation emerged between vascularization detected by CEUS and pain, neither with WOMAC and EQ-5D-5L total scores as indirect value of disability. These data are in accordance with Tol et al. [16, 17] and De Junge et al. [1] who emphasized that tendon structural abnormalities and neovessels cannot predict the severity of symptoms. In the study, 60% of cases showed rapid

time of vascularization at CEUS (< 40 s), probably new generated arterial vessels, whereas 40% of cases had venous time of vascularization (> 40 s). The assessment of the time between the bolus injection of US contrast agent, the arrival and the washout at site of investigation could be a new opportunity to evaluate the pathophysiology of tendinopathy and any reparative process [5, 16, 17, 23, 24, 28, 29]. This study has the following limits. First, US, PD and CEUS are subjective techniques and therefore the assessment of the variability between the examiners is a critical point. The imaging data were classified both concurrently with the examination and in a secondary blinded assessment, carried out by musculoskeletal radiology specialists. Furthermore, in the absence of a standard method to calculate the degree of vascularization, a semi-quantitative analysis was chosen, i.e. the assessment of the percentage of the tendon extension with neovessels, in accordance with the literature [26]. This method could be more precise than the other currently used methods, based on subjectively observed number of vessels [1, 2, 6, 16, 17], but less precise in comparison with the assessment with 3D power Doppler of volume of neovascularization and volume of Achilles tendon [19] or by MRI [29]. However, the semi-quantitative analysis could be used currently in everyday work. The restricted number of patients is a limitation; however, except for the perspective work of Tol et al. [16] on more than 500 cases, evaluated with PD sonography, all other published researches examined a comparable number of cases [1, 2, 5–15, 18–20, 22, 25]. Furthermore, in our study, the MRI excluded any possible misdiagnosis, where pain could be related not only to Achilles tendinopathy. Last limitation: when the study was performed, WOMAC and EuroQol scores were routinely used instead of VISA-A test; however, WOMAC pain generally coincides with joint function scales [30]. In the day-by-day clinical work, this study suggests that in chronic mid-portion Achilles tendinopathy, the referred pain and disability are not related to the neovascularization, also when CEUS detects neovessels with more sensibility than PD.

## Conclusions

In recalcitrant chronic Achilles tendinopathy, neovascularization, detected by CEUS, is abundant but not related to symptoms. In fact CEUS showed a greater ability to detect neovascular network than non-contrast ultrasound: PD detected neovessels only in 54% of patients, but CEUS in 83%. However, no correlation between pain/disability and abundant and well-detected neovascularization emerged. For the future, the CEUS ability to discriminate in real time between arterial and venous neovessels, assessing the time of vascularization, could be a new opportunity to evaluate the pathophysiological role of the neovessels in vivo.

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## Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

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Ethical approval: Appropriate ethical standards were followed in this study.

Informed consent: Informed consent was provided to all the patients participated in the study.

## References

1. De Jonge S, van den Berg C, de Vos RJ et al (2011) Incidence of midportion Achilles tendinopathy in the general population. *Br J Sports Med* 45:1026–10128
2. De Vos RJ, Weir A, Cobben LP, Tol JL (2007) The value of power Doppler ultrasonography in Achilles tendinopathy: a prospective study. *Am J Sports Med* 35:1696–1701
3. Yang X, Coleman DP, Pugh ND, Nokes LDM (2012) The volume of the neovascularity and its clinical implications in Achilles tendinopathy. *Ultrasound in Med Biol* 38:1887–1895
4. Öberg L, Alfredson H (2002) Ultrasound guided sclerosis of neovessels in painful chronic Achilles tendinosis: pilot study of a new treatment. *Br J Sports Med* 36:173–175 (Discussion 76–77)
5. Alfredson H, Öberg L, Forsgren S (2003) Is vasculo-neural ingrowth the cause of pain in chronic Achilles tendinosis? An investigation using ultrasonography and color Doppler, immunohistochemistry and diagnostic injections. *Knee Surg Sports Traumatol Arthrosc* 11:334–338
6. Öberg L, Alfredson H (2004) Effect of neovascularization behind the good results with eccentric training in chronic mid-portion Achilles tendinosis? *Knee Surg Sports Traumatol Arthrosc* 12:465–470
7. De Vos RJ, Weir A, Tol JL, Verhaar JAN, Weinans H, van Schie HTM (2011) No effect of PRP on ultrasonographic tendon structure and neovascularisation in chronic midportion Achilles tendinopathy. *Br J Sports Med* 45:387–392
8. Del Buono A, Chan O, Maffulli N (2013) Achilles tendon: functional anatomy and novel emerging models of imaging classification. *Int Orthop (SICOT)* 37:715–721
9. Divani K, Chan O, Padhiar N, Twycross-Lewis R, Maffulli N, Crisp T, Morrissey D (2010) Site of maximum neovascularisation correlates with the site of pain in recalcitrant mid-tendon Achilles tendinopathy. *Man Ther* 15:463–468
10. Hoksrud AF, Bahr R (2011) Injectable agents derived from or targeting vascularity: has clinical acceptance in managing tendon disorders superseded scientific evidence? *J Musculoskelet Neuronal Interact* 11:174–184
11. Peers KH, Brys PP, Lysen RJ (2003) Correlation between power Doppler ultrasonography and clinical severity in Achilles tendinopathy. *Int Orthop* 27:180–183
12. Reiter U, Ulreich N, Dirisamer A, Tscholakof D, Bucek RA (2004) Color and power Doppler sonography in symptomatic Achilles tendon disease. *Int J Sports Med* 25:301–305
13. Richards PJ, Win T, Jones PW (2005) The distribution of microvascular response in Achilles tendonopathy assessed by colour and power Doppler. *Skeletal Radiol* 34:336–342
14. Richards PJ, McCall IW, Day C, Belcher J, Maffulli N (2010) Longitudinal microvascularity in Achilles tendinopathy (power Doppler ultrasound, magnetic resonance imaging time-intensity curves and the Victorian Institute of Sport Assessment-Achilles questionnaire): a pilot study. *Skeletal Radiol* 39:509–521
15. Sengkerij PM, de Vos RJ, Weir A, van Weelde BJ, Tol JL (2009) Interobserver reliability of neovascularization score using power Doppler ultrasonography in midportion Achilles tendinopathy. *Am J Sports Med* 37:1627–1631
16. Tol J, de Jonge S, Weir A, de Vos RJ, Verhaar J (2012) Relationship between neovascularisation and clinical severity in Achilles tendinopathy: a prospective analysis of 556 paired measurements. *Knee Surg Traumatol Arthrosc* 20(suppl 1):S63
17. Tol JL, Spiezia F, Maffulli N (2012) Neovascularisation in Achilles tendonopathy: have we been chasing a red herring? *Knee Surg Sports Traumatol Arthrosc* 20:1891–1894
18. van Snellenberg W, Wiley JP, Brunet G (2007) Achilles tendon pain intensity and level of neovascularization in athletes as determined by color Doppler ultrasound. *Scand J Med Sci Sports* 17:530–534
19. Yang X, Pugh ND, Coleman DP, Nokes LDM (2010) Are Doppler studies a useful method of assessing neovascularization in human Achilles tendinopathy? A systematic review and suggestions for optimizing machine settings. *J Med Engineering Technology* 34:365–372

20. Zanetti M, Metzdorf A, Kundert HP, Zollinger H, Vienne P, Seifert B, Hodler J (2003) Achilles tendons: clinical relevance of neovascularisation diagnosed with power Doppler US. *Radiology* 227:556–560
21. Öberg L, Lorentzon R, Alfredson H (2011) Neovascularisation in Achilles tendons with painful tendonitis but not in normal tendons: an ultrasonographic investigation. *Knee Surg Sports Traumatol Arthrosc* 9:233–238
22. Chang KV, Lew HL, Wang TG, Chen WS (2012) Use of contrast-enhanced ultrasonography in musculoskeletal medicine. *Am J Phys Med Rehabil* 91:449–457
23. Shen HY, Chen SF, Wu CH, Chen WS, Wang TG, Chang KV (2012) Contrast-enhanced sonography for the evaluation of neovascularization in tendinopathic tissues. *J Med Ultrasound* 20:109–114
24. Genovese E, Ronga M, Recaldini C, Fontana F, Callegari L, Maffulli N, Fugazzola C (2011) Analysis of Achilles tendon vascularity with second-generation contrast-enhanced ultrasound. *Ultrasound* 39:141–145
25. Richards PJ, Dheer AK, McCall IM (2001) Achilles tendon size and power Doppler ultrasound changes compared to MRI: a preliminary observational study. *Clin Radiol* 56:843–850
26. Connell D, Burke F, Coombes P, McNealy S, Freeman D, Pryde D, Hoy G (2001) Sonographic examination of lateral epicondylitis. *AJR* 176:777–782
27. Shalabi A (2004) Magnetic resonance imaging in chronic Achilles tendinopathy. *Acta Radiol Suppl (Stockholm)* 432(432):1–45
28. Pingel J, Harrison A, Simonsen L, Suetta C, Bülow J, Langberg H (2013) The microvascular volume of the Achilles tendon is increased in patients with tendinopathy at rest and after a 1-hour treadmill run. *Am J Sports Med* 41:2400–2408
29. Syha R, Wurslin C, Ketelsen D, Martirosian P, Grosse U, Schick F, Claussen CD, Springer F (2012) Automated volumetric assessment of the Achilles tendon (AVAT) using a 3D T2 weighted SPACE sequence at 3T in healthy and pathologic cases. *Eur J Rad* 81:1612–1617
30. Gandek B (2015) Measurement properties of the Western Ontario and McMaster Universities Osteoarthritis Index: a systematic review. *Arthritis Care Res (Hoboken)* 67(2):216–229
31. De Jonge S, Tol JL, Weir A, Waarsing JH, Verhaar J, de Vos RJ (2015) The tendon structure returns to asymptomatic values in nonoperatively treated Achilles tendinopathy but is not associated with symptoms: a prospective study. *Am J Sports Med* 43(12):2950–2958

Inclusion criteria	Exclusion criteria
Healthy patients aged 18–75 years	Patients aged < 18 years, > 75 years, affected by peripheral vascular disease, diabetes mellitus, arthritis
Able to complete the WOMAC and EuroQuality of life questionnaire	Unable to complete the questionnaire for main psychiatric diseases
Exercise-associated symptoms in the Achilles tendon from more than 6 months with pain and swelling positioned at 2–6 cm proximal to the calcaneal insertion	Symptoms caused by insertional calcified or not calcified tendinitis/tendinosis and/or by retrocalcaneal bursitis
Recalcitrant to conservative therapies for at least 6 months	Previous surgical therapy and/or platelet-rich plasma injection
At US the presence of inhomogeneous hypo- or hyperechoic thickening and/or with loss of normal fibrillar pattern and/or irregular margins at mid-portion of the tendon	At US the presence of insertional calcified or not calcified tendinitis/tendinosis and/or pathological retrocalcaneal bursa
Rest for at least 24 h from any rehabilitation programme to exclude any influence of muscular activity on blood flow	Recent performance of a complete heavy local eccentric exercise programme

**Table 1**

Inclusion and exclusion criteria

N = 30	Sex		Baseline diagnosis		Tendon thickness				Tendon morphology		Calcification		
	Male	Female	Degenerated tendon	Partial rupture	Normal	Enlarged	Moderately enlarged	Markedly enlarged	Normal	Irregular	No calcification	Calcification	
N (%)	21 (70)	9 (30)	23 (76.7)	7 (23.3)	1 (3.3)	20 (66.7)	6 (20)	3 (10)	3 (10)	27 (90)	20 (66.7)	10 (33.3)	
Echostructure and % extension			Symptom duration			PD vascularization			CEUS vascular extension				
Hypoechoic < 30%	Hypoechoic < 30–70%	Hypoechoic > 70%	6 month–1 year	1–2 year	> 2 year	No vascularization	1–2 small vessels	> 2 vessels	0%	1–25%	26–50%	51–75%	76–100%
4 (13.3)	16 (53.3)	10 (33.3)	13 (43.3)	6 (20)	11 (36.7)	18 (60)	5 (16.7)	7 (23.3)	5 (16.7)	4 (13.3)	6 (20)	5 (16.7)	10 (33.3)
CEUS vascularization time							EQ pain						
No vascularization			< 20 s		> 20 s		1—better			2			3—worse
5 (16.7)			16 (60)		7 (23.3)		2 (6.7)			18 (60)			10 (33.3)

Sex, baseline diagnosis, tendon thickness and morphology, calcifications, echostructure and extension, duration of symptoms, vascularization at power Doppler and CEUS, vascularization time by CEUS and EQ pain are presented as absolute and relative frequencies (categorical variables)

**Table 2**

Clinical data and imaging results

N = 30	Range	Mean (SD)	SE
Age	43–73	54.93 (7.64)	1.39
WOMAC pain (0–20)	0–13	6.43 (3.42)	0.62
WOMAC total score (0–96)	6–46	21.63 (12.78)	2.33
EQ total score (0–20)	5–11	8.37 (1.47)	0.27
EQ-VAS (0–100)	20–90	56 (18.31)	3.34

**Table 3**

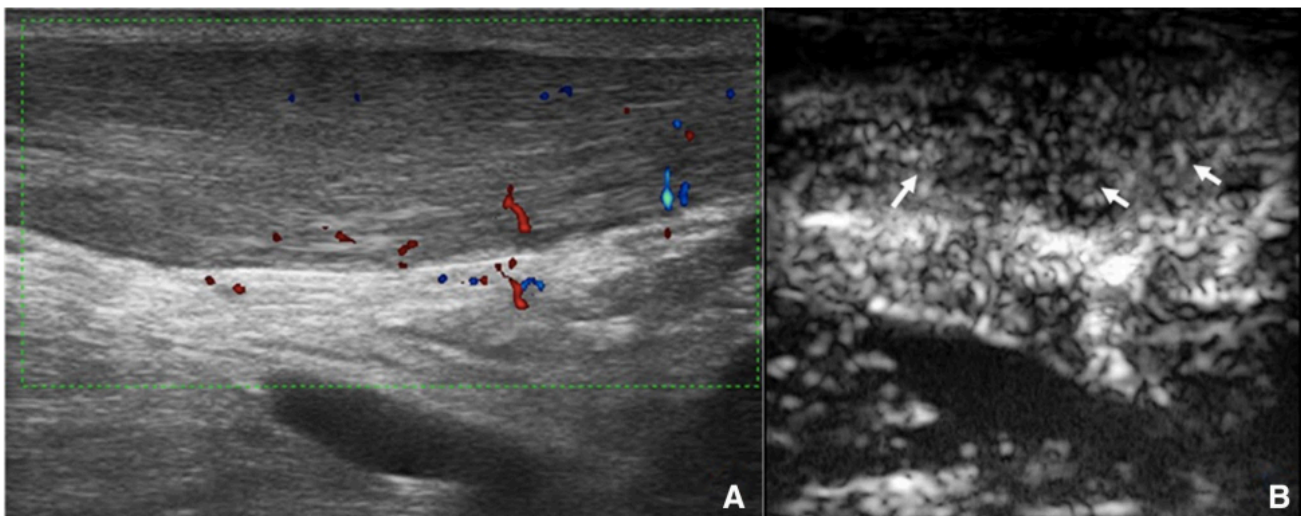
Clinical data: age, WOMAC pain and total score, EQ5D-5L pain and total score, and EQ-VAS presented as range, mean (standard deviation) and standard error

	N	WOMAC pain (0–20)		WOMAC Tot. (0–96)		EQ-Tot. (0–20)		EQ-VAS (0–100)	
		Mean (SD)	SE	Mean (SD)	SE	Mean (SD)	SE	Mean (SD)	SE
<i>Pd vascularisation</i>									
No vascularisation	18	6.7 (2.8)	0.7	22.3 (12.7)	3.0	8.4 (1.2)	0.3	54.7 (18.9)	4.5
1–2 small vessels	5	7.2 (3.7)	1.7	24.4 (12.8)	5.7	8.4 (2.1)	0.9	58.0 (11.0)	4.9
> 2 vessels	7	5.1 (4.7)	1.8	18.0 (14.1)	5.3	8.3 (1.9)	0.7	57.9 (22.7)	8.6
<i>CEUS vascular extension</i>									
0%	5	6.8 (3.1)	1.4	20.8 (13.1)	5.9	7.8 (0.8)	0.4	51.0 (20.7)	9.3
1–25%	4	7.0 (2.8)	1.4	24.0 (15.5)	7.7	9.0 (1.4)	0.7	53.7 (11.1)	5.5
26–50%	6	5.5 (2.3)	0.9	16.5 (6.7)	2.8	7.8 (1.2)	0.5	64.2 (13.6)	5.5
51–75%	5	8.8 (2.8)	1.2	33.0 (13.5)	6.0	9.0 (1.9)	0.8	58 (23.9)	10.7
76–100%	10	5.4 (4.4)	1.4	18.5 (12.7)	4.0	8.4 (1.7)	0.5	53.5 (20.6)	6.5
<i>CEUS vascularisation time</i>									
No vascularisation	5	6.8 (3.1)	1.4	20.8 (13.1)	5.9	7.8 (0.8)	0.4	51 (20.7)	9.3
< 20 s	18	7.1 (3.7)	0.9	24.1 (12.7)	3.0	8.6 (1.8)	0.4	53.6 (18.3)	4.3
> 20 s	7	4.6 (2.6)	1.0	16 (12.7)	4.8	8.3 (0.5)	0.2	65.7 (15.4)	5.8

**Table 4**

Means, SDs and SEs of WOMAC pain, WOMAC total score, EQ total score and EQ-VAS across the different categories of PD and CEUS vascularization variables.

The ANOVA test showed no significant differences among means of any of the analyzed variables



**Figure 1**

Power Doppler US and CEUS imaging (longitudinal plan) in the chronic mid-portion Achilles tendinopathy. Both methods detect neovessels (male, 60 years old, left Achilles tendon, recreational athlete, duration of symptoms > 2 years). **A.** Power Doppler shows few irregular vessels throughout the tendon. **B.** CEUS detects more vessels than power Doppler sonography: a rich amount of microbubbles (white arrows) occupies the tendon, CEUS vascularization extension almost 100%