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ORIGINAL RESEARCH

Minimally invasive ultrasound-guided biopsy of the common extensor tendon enthesis: a cadaveric study to standardise the technique

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ABSTRACT

Objective To develop and validate a minimally invasive ultrasound (US)-guided biopsy technique to collect entheseal tissue from the common extensor tendon (CET) enthesis at the lateral humeral epicondyle.

Methods Seven sonographers performed a US examination of the CET on six human cadaveric upper limbs to locate the enthesis using an anatomical landmark-based approach. An adapted mini-arthroscopic system was introduced under US guidance to the target site for sample collection. At the end of the procedure, a dye was injected through the guide needle, followed by dissection, to confirm the sampling location. Histology and immunohistochemistry analyses were performed to assess the quality and representativeness of the samples. The reliability of the procedure among operators was evaluated by analysing the rate of successful sampling.

Results 24 samples were collected. The target site to be biopsied was identified as the insertion of the extensor carpi radialis brevis component of the CET. On dissection, the stain used to verify sampling accuracy was confirmed within the defined target area, with no damage to adjacent structures. Histology and immunohistochemistry indicated that most of the samples exhibited characteristics consistent with entheseal tissue (21 out of 24). All participants identified the CET and successfully completed the procedure, demonstrating reliable sample quality across operators.

Conclusion We developed a landmark-based approach to perform a minimally invasive full controlled US-guided biopsy of the CET enthesis that showed to be feasible and reproducible. We believe that this standardised, minimally invasive technique will widespread a reliable collection of entheseal tissue for future clinical and translational studies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Up to date, a percutaneous, ultrasound-guided entheseal biopsy procedure was not available, highlighting the need for standardising a reliable and safe technique to collect entheseal tissue.

WHAT THIS STUDY ADDS

⇒ We describe for the first time a standardised, fully controlled ultrasound-guided biopsy of the common extensor tendon, which is grounded in a robust sonoanatomical approach and has proven to be safe, feasible and capable of yielding informative tissue samples.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Rheumatologists can safely perform entheseal biopsy at the lateral humeral epicondyle following the described technique, facilitating translational research in spondyloarthritis and psoriatic arthritis.

INTRODUCTION

Entheses are unique anatomical structures that anchor tendons, ligaments, joint capsules and pulleys to bone.[1 2] Their function is mainly related to efficient motion through stabilisation of periarticular structures and transduction of mechanical forces. Entheseal biomechanics depends on the tissue architecture that is characterised by the presence of fibrous or fibrocartilaginous tissue at the interface between soft tissues and the periosteal surface.[3]

The microenvironment of entheses constitutes a distinctive anatomical complex, in which resident mesenchymal and immune cells are found. The inflammation of such structures, that is, enthesitis, is far more than a focal insertional disorder. The inflammatory response activated at the entheseal level by mechanical triggers, in genetically susceptible

individuals, recapitulates the dysregulated immune pathways of the spondyloarthritis (SpA) group, with an aberrant production of several pro-inflammatory mediators. Inflammatory changes prelude to structural damage at entheseal sites that characteristically feature new-bone formation, in the form of enthesophytes or syndesmophytes, but also to the inflammation of surrounding synovial niches determining the concept of 'synovio-entheseal organ'.[4] Indeed, enthesitis, at both axial and peripheral levels, is defined as the hallmark lesion of the SpA group, including psoriatic arthritis (PsA), and a pivotal early manifestation in their disease course.[5] The relevance of enthesitis justifies its inclusion in the classification criteria for SpA, in the PsA treatment recommendations, and in many disease activity scores.[6–9]

The diagnosis of peripheral enthesitis has dramatically changed with the introduction of imaging techniques. In particular, ultrasound (US) assessment of entheses, implemented with Doppler technologies (Power Doppler US (PDUS)), has been standardised and validated, becoming a reliable tool to identify both inflammatory and structural changes.[10–12] Especially for peripheral entheses, US is central in the early diagnostic process, and has been included as a powerful tool in clinical trials as well as in daily clinical practice.[13]

In addition, US is widely used to precisely guide interventional procedures on articular and periarticular structures, and has been recently used to assist a pioneering technique to perform entheseal biopsy of the common extensor tendon (CET) enthesis on the lateral epicondyle of the elbow.[14] Indeed, the in vivo study of entheseal tissue, retrieved from human peripheral entheses, may change the paradigm of enthesitis and deepen our knowledge of SpA/PsA development to foster new research and discover new molecular targets.

In this regard, the CET enthesis stands out as a valuable anatomical site to perform biopsies, particularly because of its anatomical properties that grant easy access to the enthesis in the absence of noble neighbouring structures, such as vessels and significant sensitive and motor nerve branches.[15] CET insertion takes part in the anatomical complex that stabilise the lateral elbow; it comprises four tendons deriving from the muscle extensors apparatus of the forearm. Namely, the extensor digitorum communis (EDC), the extensor carpi radialis brevis (ECRB), the extensor digiti minimi (EDM) and the extensor carpi ulnaris (ECU) compose the CET and intermingle their fibres to attach to the lateral epicondyle of the humerus. The lateral collateral ligament complex, including the radial collateral ligament (RCL), the annular ligament and lateral ulnar collateral ligament, completes the anatomical region of the lateral epicondyle.[16 17]

The CET enthesis displays clinical signs of enthesitis in up to one-third of PsA patients[18 19] and its assessment is included in major US scoring systems for enthesitis.[19] Up to date, a completely US-guided technique for entheseal biopsy of the CET has not been described. The previous description of the procedure included a US-assisted technique, performed by an orthopaedic surgeon, with the introduction of forceps via a classical skin incision.[14]

Hence, the aim of the present study was to develop and standardise a minimally invasive full US-guided biopsy technique for the retrieval of entheseal tissue of CET to define a safe procedure to collect samples from patients in a rheumatological setting.

METHODS

The study was designed in a stepwise manner: (1) establish a US-landmark-based approach to identify the region of interest; (2) define the technique and the proper instrument to perform the procedure; (3) perform the procedure on cadaveric specimens to collect CET enthesis samples; (4) evaluate the macroscopic status of the CET by anatomical dissection of elbow specimens undergoing the procedure; (5) validate the presence of entheseal tissue by histological analysis of the tissue samples; (6) compare the performance of different operators in obtaining valuable enthesis samples.

Definition of US landmarks

The US study was conducted on six upper arm specimens from three adult corpses (1 woman, two men; mean age 83.3±10 SD years old) cryopreserved at −20°C in the Dissection Laboratory of the Faculty of Medicine and Health Sciences at the University of Barcelona, Spain. All corpses were donations to the Faculty of Medicine and Health Science. They did not present evidence of traumatic injuries or surgical scars in the region of interest; no history of rheumatic diseases was recorded in the files. The samples were coded with a univocal number to allow identification according to the sonographic examination, the CET biopsy and the dissection order.

A LOGIQ P9 US unit (GE Ultrasound Korea, Seongnam, Republic of Korea) equipped with an 8–18 MHz linear stick transducer was used to identify the CET on cadaveric elbows and to subsequently guide the biopsy procedure. Depth and focus were adjusted to optimise the visualisation of the anatomical structures of interest.

ECRB enthesis identification by US

The identification of the CET was established after a consensus between an expert anatomist (MMP) and two musculoskeletal (MSK) sonographers experienced in sonoanatomy and investigational methodology (IM and MAD'A, respectively).

The systematic, landmark-based US approach comprised three consecutive steps:

- *Step 1.* The upper limb was positioned on a horizontal plane in a pronated position with an angle between the arm and forearm of approximately 90°–100° to ensure the correct visualisation of the epicondylar structures and to guarantee an appropriate manoeuvring area for the US transducer, the biopsy instruments and the operators.
- *Step 2.* A generous amount of gel was applied on the area of interest. Subsequently, the transducer

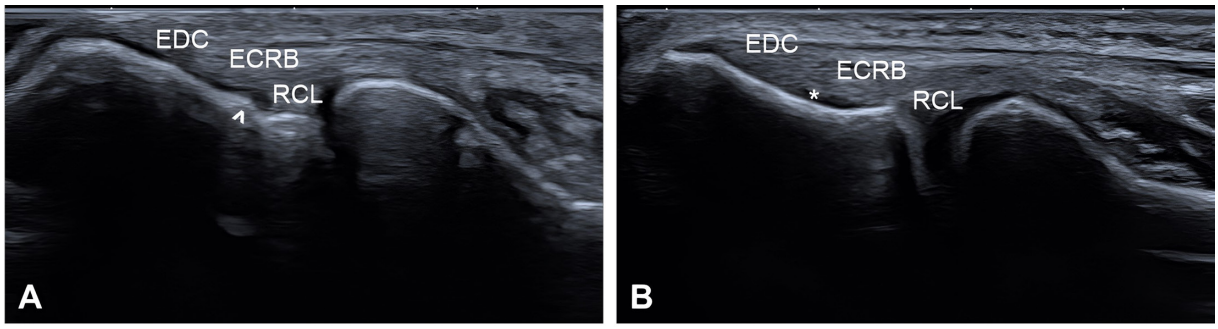

Figure 1 Longitudinal view of the CET enthesis to perform the ultrasound-guided biopsy. (A) Longitudinal view of the CET complex, evidencing the proposed landmark-based approach to identify the RCL. (B) Longitudinal view of the CET complex in the final position for CET enthesis biopsy. CET, common extensor tendon; ECRB, extensor carpi radialis brevis; EDC, extensor digitorum communis; RCL, radial collateral ligament. ˆAnterior tubercle; *fibrocartilage.

was positioned transversely in the mid-portion of the lateral forearm to identify the supinator muscle, which appeared as a broad, hypoechoic structure surrounding the proximal radius and lying deep to the extensor muscles. The four extensor muscles (EDC, ECRB, EDM and ECU), located superficially to the supinator, were identified and followed proximally to obtain the visualisation of their tendons up to the insertion on the lateral epicondyle, that appeared as a bright, curved hyperechoic line on the US image (online supplemental video 1A).

- *Step 3*. On the lateral epicondyle, the transducer was rotated 90° and aligned to the major radial axis to obtain the longitudinal view of the CET. The CET insertion appeared as a beak-shaped structure in which the ECRB constituted most of the deep anterior CET fibres, whereas the EDC contributed to the more superficial portion. The RCL was identified deeper to the ECRB with its superficial insertion marked by the anterior epicondylar tubercle. A hyperechoic interface separating the CET from the underlying ligament was visualised to clearly confirm the exact location of the two structures. The CET enthesis was identified as a hypoechoic zone adjacent to the bony cortex, just above the anterior epicondylar tubercle; the target zone, consisting in the portion of the CET where most of the insertional fibres belonged to the ECRB, was located above the clearly visualised RCL (figure 1 and online supplemental video 1B).

Biopsy technique

Among the CET enthesis components, the ECRB was selected as the optimal biopsy site due to its anatomical characteristics (ie, larger enthesis insertion) and strategic location (ie, anterior and deep location), which facilitate the clear visualisation of adjacent structures, providing an ergonomically favourable workspace for precise operators manoeuvring and ensuring the procedure safety in terms of capsule integrity.

Instrument

The instrument used to perform the biopsy procedure consisted of a coaxial system specifically adapted for an entheseal biopsy at the CET level (online supplemental figure 1).

A surgical forceps, derived from nano arthroscopy surgery, the NanoBiter straight (Arthtrex, Florida, USA) with a 130 mm length and a 2 mm diameter, was selected to sample the CET enthesis. The nano forceps are a sharp hand instrument designed to efficiently resect soft-tissue structures that, thanks to its reduced diameter, allows an atraumatic insertion and movement through tight spaces. The insertion kit was adapted from the Osteobell T kit (BPB MEDICA, Italy) for bone marrow biopsies. The trocar tip stylet guaranteed fast and non-traumatic penetration without the need for a skin incision. The cannula characteristics were 100 mm length×8G, with a 4 mm diameter.

Description of the US-guided procedure

Once the region of interest was identified, a simulation of the local anaesthesia was performed, releasing saline solution through a needle visualised with a US in-plane approach. The fluid granted the separation of anatomical planes, hydrodissection (online supplemental video 2A), and the optimal visualisation of the needle that was subsequently inserted up to the target zone to complete the simulation of local anaesthetic release (online supplemental video 2B). The biopsy instrument was finally inserted up to the target area, following the trajectory of the previous hydrodissection (online supplemental video 3). Once reached the CET enthesis and ensured the correct positioning in both short and long-axis views, a multiple sampling of the target tissue was performed (online supplemental video 4A,B).

Macroscopic evaluation

Following the US-guided procedure, the biopsy area was stained with a specific dye inserted through the cannula. MMP, blinded to the biopsy procedure, performed the anatomical dissection of specimens. To expose the area of interest, a 6 cm oblique incision of the skin situated anteriorly to the lateral epicondyle was performed. The cut extended up to the deep fascia to unveil the extensor carpi radialis longus (ECRL) and EDC tendons. Subsequently, a careful dissection between the ECRL and EDC

was performed to visualise the ECRB. The anterior aspect of the lateral epicondyle was then exposed, facilitating the identification of the ECRB enthesis and of the RCL deep to the ECRB, thereby ensuring the verification of its structural integrity.

The evaluation encompassed all components of the CET, including EDC, ECRB, EDM and ECU, as well as the verification of RCL and of the joint capsule integrity. This comprehensive assessment aimed at verifying any potential procedural-related damage and facilitating the identification of the biopsy site through the careful assessment of the positioning of the dye injected.

Microscopic evaluation of entheseal tissue

Histological study

The entheseal samples retrieved through the US-guided biopsies were fixed in 10% neutral-buffered formalin and paraffin-embedded. 4 µm thick tissue sections were H&E-stained for histological evaluation. Masson's trichrome and Safranin O-Fast green histochemical staining were performed to assess collagen deposition and cartilage matrix within the samples, respectively. Additionally, immunohistochemistry for collagen type II, collagen type X and aggrecan was performed (details are reported in online supplemental materials). Slides were examined under a light microscope (Axioscope A1) by a trained pathologist (BB) who was blinded to the procedure and to the identity of the operators. Microphotographs were collected using an Axiocam 503 Color with Zen V.2.0 Software (Zeiss).

Outcome inter-operators

During the same day of the biopsy, the participants attended a training session to learn how to identify the CET enthesis anatomy, under the guidance of IM, and specifically recognise the insertional site of the ECRB component as the target area and differentiate it from the RCL. All of them had previously performed interventional procedures on joints under US guidance, including synovial biopsies, although with a differential degree of experience in performing such procedures (table 1).

This allowed a dichotomic division into experienced operators (SA and MML), that were considered the reference in terms of procedural outcome, and newly trained operators (CR, PR, MGR, CT, LC). The procedure was performed on different elbows by operators, organised in pairs, to ensure the presence of a first operator performing the biopsy and a second operator, responsible for holding the transducer for continuous US guidance. The number of samples to collect was determined during the procedure, considering the size of the enthesis and the necessity of ensuring the integrity/functionality of the enthesis and surrounding structures. The pairs were organised as follows, with five newly trained operators and two experienced operators changing their role to ensure a balanced sample number (eg, two experienced operators or two newly trained operators or one experienced and one newly trained operator, respectively). The samples were categorised considering the operator that performed the biopsy as coming from the experienced or the newly trained ones. A comparison between groups (experienced vs newly trained) based on the quality of the retrieval, for example, informative samples of CET enthesis, was performed to assess the feasibility and reliability of the procedure.

Table 1 Characteristics of rheumatologists involved in the study divided into experienced and newly trained operators

	Experienced operators (n=2)	Newly trained operators (n=5)
Age, mean (SD), years old	48 (7)	34.2 (2.3)
Sex, M:F	2:0	3:2
Rheumatology consultant, years (SD)	19 (5.7)	3.2 (2.7)
MSK-US experience, n (%)		
Advanced	2 (100%)	2 (40%)
Intermediate	–	1 (20%)
Basic	–	2 (40%)
US-guided synovial biopsy experience		
Years, median (range)	16 (15–17)	1 (1–3)
Attendance of a training programme, n (%)	NA	4 (80%)

F, females; M, males; MSK-US, musculoskeletal ultrasound.

Statistical analysis

Categorical variables were presented as numbers and percentages, while continuous variables were reported as median and IQR or as mean and SD. Normality of the data was preliminarily assessed through the inspection of quantile–quantile plots. The comparison between groups was performed for categorical variables with Fisher's exact test and for continuous variables with the Mann-Whitney U test given the observed data distribution. Statistical significance was defined as a p value less than 0.05 for all analyses, and all tests were two-tailed. Data analysis was performed using RStudio (V.2024.04.02).

RESULTS

US-landmark based approach allowed a correct identification of the CET enthesis to perform the biopsy

Independently from the degree of experience in MSK-US and US-guided procedures, all participants were able to correctly visualise the target area and perform the interventional procedure, following the given instructions. In 100% of the procedures, the biopsy instruments were inserted up to the target area, with the double check of their position in the two orthogonal US planes, and a subsequent collection of tissue was performed (figure 2).

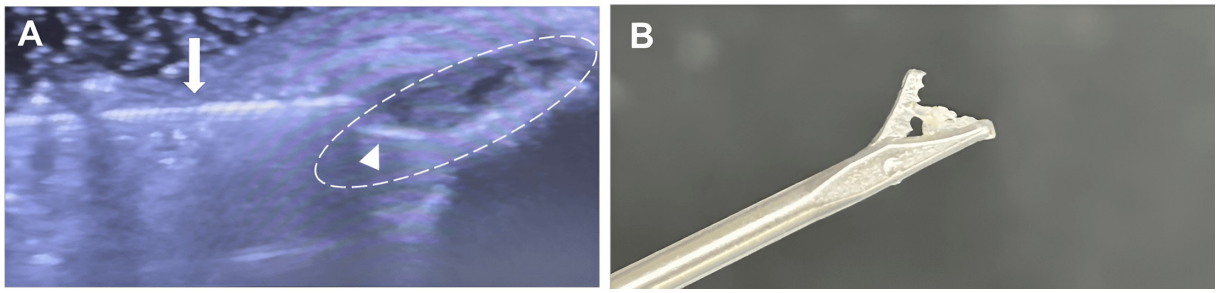

Figure 2 Minimally invasive US-guided biopsy technique of the CET enthesis. (A) US-guided biopsy technique, the guide needle (arrow) is inserted up to the target area of the CET enthesis (dashed line) where the tip of the nano-forceps is opened to collect tissue (arrowhead). (B) Nano-forceps tips with the entheseal sample collected. CET, common extensor tendon; US, ultrasound.

Minimally invasive US-guided entheseal biopsy was safe as revealed by the post-procedural dissection of specimens

Dissection of the elbows that underwent the biopsy was performed to look for the positioning of the green dye injected at the end of each sampling, and to assess the integrity of the structures surrounding the CET enthesis. The green dye was located inside the enthesis, in a position consistent with the insertion of the ECRB fibres (figure 3A). Notably, no damage to the articular capsule, verified by the absence of intra-articular staining, nor RCL alterations were identified. Macroscopically, the trajectory of the cannula did not determine tendon ruptures or muscle damage (figure 3B).

US-guided biopsy allows the retrieval of entheseal tissue from the CET enthesis

A total of 24 samples from six elbows were analysed. Specifically, four elbows each had four samples collected, while one had five samples collected and another had three. The reduced number of samples from the latter elbow was due to its anatomical characteristics, as the upper limb was notably thinner, due to sarcopenia and had a smaller enthesis size, which limited the feasibility of additional sampling. Overall median (IQR) sample length was 1.5 (1.0–2.0) mm. To evaluate the presence of entheseal tissue in relation to sample length, a comparison between the median length in positive versus negative samples was performed.

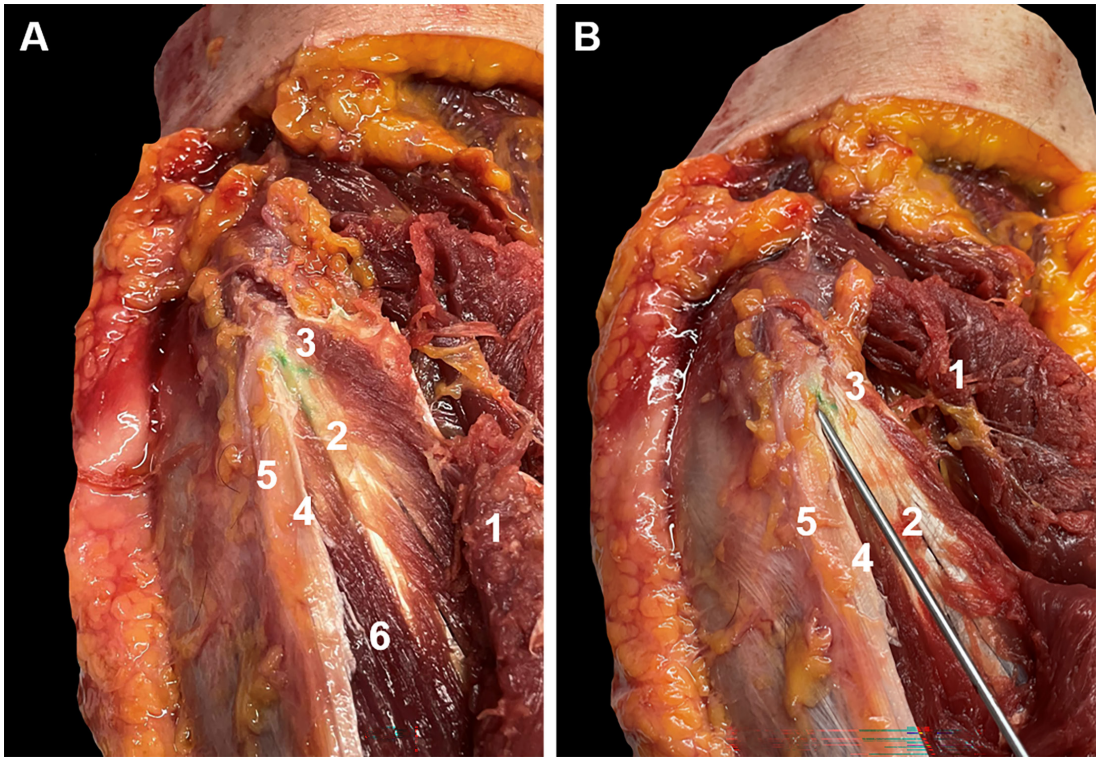

Figure 3 Post-procedural dissection of the lateral epicondyle. (A) Representative picture of the correct location of the green dye injected at the end of the procedure at the level of the CET insertion. (B) Representative picture showing the absence of muscle or tendon injuries in the trajectory of the biopsy instruments. CET, common extensor tendon; 1, extensor carpi radialis longus; 2, extensor carpi radialis brevis; 3, extensor digitorum communis; 4, extensor digiti minimi; 5, extensor carpi ulnaris; 6, supinator.

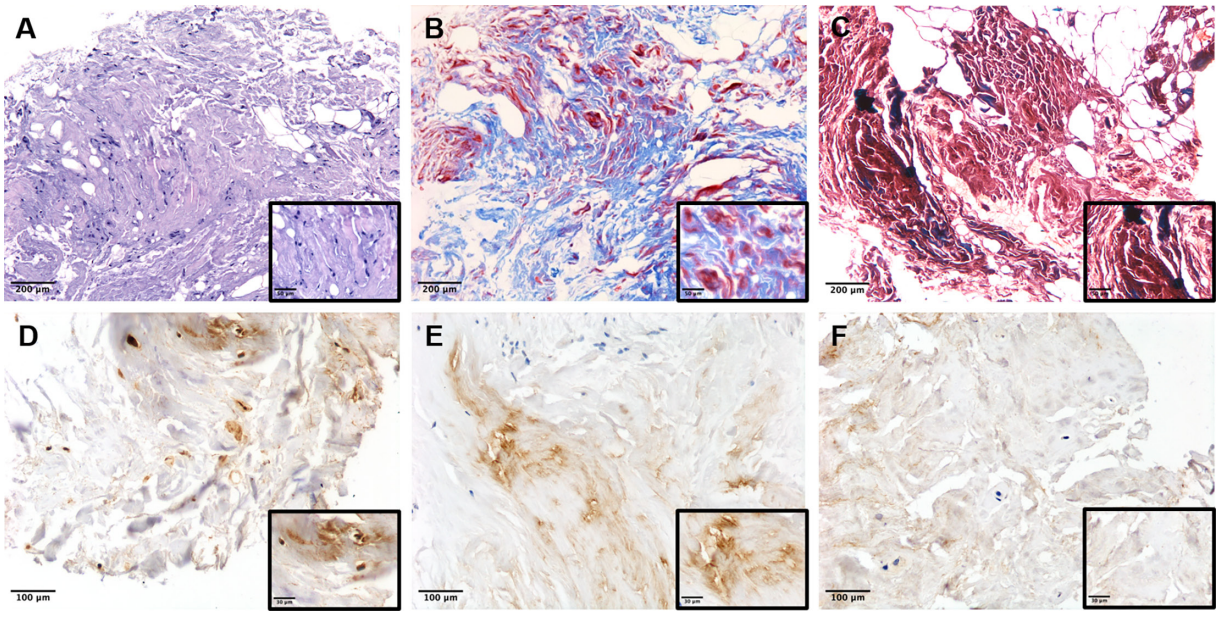

Figure 4 Histology of entheseal samples from the CET. (A–C) Representative pictures of entheseal tissue by H&E (A), MT (B) and Safranin O (C), respectively, with inset showing the area of fibrocartilage. Original magnification ×100, ×400 (insets). Scale bars, 200 µm and 50 µm. (D–F) Representative pictures of immunostaining for aggrecan (D), collagen type II (E) and collagen type X (F) with insets. Original magnification ×200, ×630 (insets). Scale bars, 100 µm and 30 µm. CET, common extensor tendon; H&E: haematoxylin and eosin; MT, Masson's trichrome.

Multiple tissue stainings were done to assess the congruity of the sampling site by identifying entheseal tissue (online supplemental table 1). In particular, H&E staining revealed that 87.5% (21/24) of specimens were compatible with entheseal tissue. The fibrocartilage area in the transition zone from tendon to bone was identified according to changes in the major orientation of collagen fibres, as well as to the presence of cells, that approaching the cortical bone display a round shape, in contrast with the elongated conformation found within the tendon part and are classically organised in doublets (figure 4A). Masson's trichrome staining distinguished tendon fibres (stained blue) and fibrocartilage with increased matrix density near the bone interface (stained red) (figure 4B) in 66.7% of samples. Subsequently, Safranin-O staining revealed strong proteoglycan content within the fibrocartilaginous zones (figure 4C), allowing the identification of entheseal tissue in 70.8% (17/24) of samples. The expression of type II collagen and aggrecan, markers of entheseal tissue described at the lateral epicondyle,[20] was investigated. The expression of the two proteins in the region of the fibrocartilage was evidenced in sequential slides, further validating the congruity of samples as enthesis (figure 4D,E). Finally, staining for type X collagen was carried out evidencing no expression of this molecule in our samples (figure 4F). This combination of histological and histochemical findings allowed a reliable characterisation of the enthesis structure.

In addition, the length of the entheseal tissue retrieved, although minimal, was considerately adequate. On H&E, median (IQR) lengths of samples showing entheseal tissue were 1.5 (1.0–2.0) mm versus 1 (0.6–1.0) mm for negative ones ($p<0.05$). On Masson's trichrome and on Safranin-O, there was not a statistically significant difference in terms of dimension between positive and negative samples (1.5 (1.0–2.0) mm vs 1.3 (0.9–1.6) mm, $p>0.05$ and 1.5 (1.0–2.0) vs 1.0 (0.8–1.5), $p>0.05$, respectively) (online supplemental table 2).

Rate of successful outcomes of entheseal biopsy was similar between operators with different experience in US-guided procedures

All operators were confident during the biopsy procedure; no technical issues nor evident lesions at the entrance site of the biopsy instruments were reported. The rate of successful entheseal sampling, as defined by the positive histology analysis, was comparable between experienced and newly trained operators ($p>0.05$) (table 2).

Parallelly, no statistically significant difference in terms of sample length was outlined between groups (online supplemental figure 2).

DISCUSSION

Entheses have been recognised as pivotal players in driving the inflammatory process underlying PsA and SpA. In PsA, the reported prevalence of enthesitis ranges between 27% and 65%, with the lateral epicondyle being one of the most commonly affected sites.[18] Additionally, enthesitis may occur in the very initial phases of disease, suggesting the importance of this immunological niche in fuelling systemic inflammation.[21] Literature data on entheseal tissue mainly derive from experimental models of SpA and from surgically derived entheses obtained during orthopaedics or neurosurgery procedures,[22–24] stressing the technical difficulties in safely retrieving

Table 2 Enthesis detection between experienced and newly trained operators

	H&E		MT		Safranin-O	
	Successful	Unsuccessful	Successful	Unsuccessful	Successful	Unsuccessful
Experienced operator, n (%)	10 (83)	2 (17)	8 (67)	4 (33)	9 (75)	3 (25)
Newly trained operator, n (%)	11 (92)	1 (8)	8 (67)	4 (33)	8 (67)	4 (33)

P>0.05 for comparisons between experienced and newly trained operators regarding successful and unsuccessful entheseal tissue detection on samples.
H&E, haematoxylin and eosin; MT, Masson's trichrome.

entheseal tissue from patients. Therefore, the development of a safe and effective procedure to collect entheseal tissue from patients may foster translational research and hopefully improve clinical management of patients. We demonstrated that this goal was achievable using a US multi-step approach based on a constant visualisation of the entheseal site. Our procedure can be considered almost atraumatic for soft tissues, as demonstrated by the macroscopical dissection. Moreover, the retrieval of entheseal samples was higher than previously reported, with more than 80% of samples showing entheseal features, which is probably due to the innovative technical approach we used.

Two previous enlightening studies described different techniques to perform enthesis biopsy with a mini-invasive approach. The first proposed the biopsy of the plantar fascia and the patellar tendon entheses after assessing local inflammation by MRI. Subsequently, the sampling was operated under US guidance with a 16G Jamshidi needle.[25] On the other hand, the paper published in 2022 described the biopsy of the CET enthesis with Blakesley forceps, a surgical instrument commonly used in nasal surgery, after localising the enthesis by US.[14] Main differences with our study encompass the biopsy tools and the application of US. The instrument used in our study derives from nano-arthroscopy surgery, being far smaller than the previous ones. Moreover, we adapted a cannula, used for bone marrow biopsy, as a trocar for the nano forceps, allowing a whole percutaneous procedure that did not require skin incision with scalpel nor post-procedural skin stitches. Additionally, the presence of a trocar avoids the risk of injuring tissues related to multiple sampling. In our case, differently from the other studies, the biopsy was performed with the constant visualisation of the target area and of the instruments in the lateral epicondyle, so that operators could continuously check for their position during the tissue collection.

To confirm the presence of entheseal tissue, several stainings were performed, including H&E, Masson's trichrome and Safranin-O. Of note, only in two samples there was evidence of adipose tissue and, in one, of muscular bundles. Additionally, immunohistochemical staining for type II collagen and aggrecan yielded positive results in our samples, confirming their identification as markers for typical fibrocartilage at the lateral epicondyle.[20] Our technique ensured that sufficient tissue was obtained for a comprehensive evaluation of all entheseal components, eliminating the need for a more aggressive collection of periosteal bone, which could risk injuring this highly innervated and vascularised region and causing pain in patients.

To our knowledge, no previous attempts of standardising entheseal biopsy by the comparison of outcome between operators, with different degrees of experience in interventional procedures and US, were performed. Our study demonstrates that a short anatomy and sonoanatomy training programme, performed by experts, is effective to grant the identification of the CET enthesis independently from the expertise in MSK-US.

Tissue collection was performed by rheumatologists categorised as experienced and newly trained in US-guided biopsy procedures. No significant differences in terms of entheseal tissue retrieval or sample dimension were outlined, meaning that the rate of successful outcomes of the procedure was comparable between groups. Taken together, such evidence confirms that the proposed technique is feasible, does not require advanced expertise in US while will probably benefit from a minimum experience in interventional procedures. Indeed, most of the participants in the newly trained group had previously attended a specific programme to learn how to perform US-guided synovial biopsies.[26] The entheseal biopsy described in this study mirrors the approach commonly used for synovial biopsy, in which US guides the procedure and allows the identification of the specific target tissue as well as the eventual grading of synovitis[27] during the entire procedure. Then, US is the most appropriate tool even for entheseal biopsy, since the Outcome Measures in Rheumatology US Working Group has provided a reliable definition and scoring system for enthesitis.[10] So, in vivo, the imaging features of the CET enthesis will be correlated to tissue data, allowing the comprehensive description of changes within the enthesis, reproducing the efforts undertaken in synovial tissue research.[28]

Some limitations of the present study should be acknowledged, mainly the sample size of available specimens that could have underpowered the reliability analysis, as well as the quality of the tissue that was impacted by the old age of donors and the cycles of thawing those specimens previously underwent. Another possible limitation is the lack of second-generation techniques for histology validation. The latter is mainly related to the elegant description in the paper by Pachowsky *et al*,[14] in

which second harmonic generation microscopy was used to assess the presence of entheseal tissue in the collected samples. Such techniques are not routinely available; however, the correct location of sampling, granted by the constant monitoring of the procedure by US, as well as the evaluation of samples performed by an experienced pathologist, and supported by multiple histological analysis, further ensured the quality of our samples. These aspects, together with the use of a completely percutaneous, mini-invasive biopsy tool, and the validation of the procedure across operators with different experience in both MSK-US and interventional procedures, stand out as the major strength points of the research. Importantly, the biopsy was performed by rheumatologists, and no orthopaedic surgeons were involved.

Future perspectives include transitioning to patients' studies to validate the technique in vivo and allow the evaluation of the local cellular and molecular milieu characterising the enthesitis process.

In conclusion, the present study standardises a minimally invasive US-guided procedure to perform entheseal biopsy from the CET enthesis at the lateral epicondyle. The procedure relies on a robust US landmark-based approach to identify the enthesis; the safety was proven by the absence of lesions to the important structures surrounding the CET; and the outcomes have been histologically validated.

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