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ORIGINAL RESEARCH

Intensive training programme for ultrasound-guided minimally invasive synovial tissue biopsy on knees and wrists in different phases of inflammation

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ABSTRACT

Objectives To develop an intensive training programme for ultrasound (US)-guided synovial tissue (ST) biopsy on knees and wrists in inflammatory arthritis and to assess the learning curve, patient tolerability, sample quality and trainees' expectations.

Methods Active or remission rheumatoid arthritis patients were enrolled. Nine trainees joined the 4-month programme in a centre experienced in performing US-guided ST biopsies consisting of four sequential phases: (1) observation, (2) performance of guided step-by-step phases, (3) execution of the whole procedure on paired joints (knees or wrists) of the same patient in parallel with the trainer and (4) performance of the procedure autonomously. Sample representativity was assessed by histology, and procedure-related adverse events were recorded. Before and after the programme, trainees' expectations and perceptions were collected.

Results 328 ST biopsy procedures were included. The rate of trainees' informative samples was: (1) comparable to the trainers in active and remission knees, but lower in active wrists (70% for trainees vs 100% for trainers, p=0.06) in phase 3; (2) excellent on active knees and wrists (91.9% and 90.9% respectively) but lower (77.6%, p=0.0089) on remission knees in phase 4. Procedures performed by trainees did not affect patient tolerability. Trainees' expectations about procedure-related invasiveness and pain infliction decreased while the difficulty of procedure execution on active wrists and remission knees remained perceived as moderately difficult.

Conclusions This intensive training programme develops advanced skills in the performance of US-guided ST biopsy on knees and wrists, yielding high-quality specimens available for basic and translational studies on inflammatory joint diseases.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Synovial tissue (ST) research is reshaping the knowledge and possibly the management of chronic inflammatory arthritis in a rapidly expanding field. In the effort of narrow discrepancies during ST handling, European Alliance of Associations for Rheumatology recently drew up points to consider for minimal reporting requirements in ST research, including recommendations pertaining the biopsy procedure, the quality check and the experimental data. However, a minimal training programme for teaching ultrasound (US)-guided ST biopsy to rheumatologists remains a need and a priority.

INTRODUCTION

In the last decade, the study of synovial tissue (ST) has become an important aspect of rheumatological research.[1 2] This interest comes from the attempt of covering the knowledge gaps and unmet needs in understanding the processes that regulate inflammation and remission at the tissue level in chronic inflammatory arthritis, such as rheumatoid arthritis (RA) or psoriatic arthritis. Indeed, no definitive biomarkers are available to guide clinicians in the choice of the treatment throughout the clinical course,[3] nor in the tapering or cessation of the ongoing therapy when sustained remission is achieved.[4 5] ST sampling from peripheral joints has been successfully achieved using

WHAT THIS STUDY ADDS

⇒ This is the first intensive training programme developed to teach rheumatologists or rheumatology residents to perform US-guided minimally invasive ST biopsy on knees and wrists of rheumatoid arthritis patients across disease trajectory with excellent results in a unique setting. Regardless the different trainee's background, in a period of 4 months, they were able to retrieve gradable ST specimens in up to 91.9% of the procedures from joints of different size and inflammatory status, to understand the importance of sample representativity, with no negative impact on patient tolerability. The study highlights the need to adapt the intensity and timing of the training programme to reach comparable success rate when approaching the knee in remission patients.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The dissemination of a shared training programme among different centres could be the first step in promoting standardisation of ST-related research. The retrieval of high-quality samples in different disease phases of inflammation is the starting point in delineating ST atlas, where high-throughput technologies will allow addressing unmet needs in chronic inflammatory arthritis.

arthroscopy and ultrasound (US)-guided minimally invasive ST biopsy, the latter being able to easily access even medium and small joints.[6] Each procedure has proven to be safe, well tolerated and with excellent yield by robust data,[7–10] allowing real-life and clinical trial studies to be conducted.[3 11–15] To date, ST biopsy has still limited indications in clinical practice (eg, infections, neoplasms)[10 16] and is not included in the current recommendations for the management of RA.[17] However, the growing knowledge derived from real-life[12] and clinical trial ST biopsy-driven studies[3 18 19] in terms of diagnostic and prognostic insights[20] suggests that ST biopsy might have a future clinical and experimental expansion and will no longer be a prerogative of few highly specialised centres. In the effort of standardising ST handling, the European Alliance of Associations for Rheumatology (EULAR) recently proposed points to consider for minimal reporting requirements in ST research.[21] To ensure a biopsy sample to be informative, whether it is analysed by semiquantitative histology or in-depth molecular analysis, the operator must aim to obtain specimens in which all the anatomical areas of the synovium are present, hence representative. This is a crucial issue for a comprehensive analysis, as the lining and sublining layers composing ST are populated by heterogeneous cell clusters of resident and infiltrating cells (eg, macrophages and synovial fibroblasts) with distinct functions and location identities in different phases of tissue inflammation.[15 22 23]

While some standards have been addressed and established in the points to consider for ST research,[24 25] the EULAR research agenda identifies, as one of the first uncovered needs, the lack of a standardised training programme to teach physicians to perform US-guided ST biopsy, and how such training may affect patient tolerability and procedure performance.[21] So far, the procedure has been effectively taught to trainees by experienced peers in few clinical settings, in ways which are hardly reproducible and comparable among different centres. Here, no guidelines are available concerning the design of the training programme, the duration required to achieve an adequate success rate in terms of representative tissue sample, the possible adjustment of this timeframe according to the size and the inflammatory status of the target joints, and any trainees' prerequisites needed before joining the programme, such as experience in US or hands-on skills.

Based on these issues, the aims of the study were as follows: (1) to develop an intensive training programme for US-guided minimally invasive ST biopsy on knees and wrists across inflammatory arthritis phases in a specialised setting and (2) to assess the learning curve, patient tolerability, sample quality and trainees' expectations and perceptions.

METHODS

The intensive training programme took place at the SYNGem Synovial Tissue Biopsy Unit at the Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome, which is an academic hospital with a daily schedule of 5–7 patients with chronic inflammatory arthritis who are referred for a comprehensive rheumatological evaluation in a day hospital clinical setting, including US-guided minimally invasive ST biopsy.[12] Minimally invasive US-guided ST biopsies are routinely performed by senior rheumatologists with advanced expertise in US, henceforth the trainers. Junior rheumatologists or rheumatology residents at the host centre, regardless of their background in US, are regularly trained to perform this procedure as part of their educational programme. External rheumatologists or rheumatology residents, who have attended the Biopsy Unit for at least 4 months full time, were considered eligible to participate in the training programme. The training programme did not include additional training in musculoskeletal US. Patients fulfilling the 2010 EULAR/American College of Rheumatology classification criteria for RA with active disease[26] or in sustained clinical and US remission[4] were included in the study when biopsy was indicated either on the knee(s) or the wrist(s).

Intensive training programme for ST collection

As summarised in figure 1A–D, the intensive training programme consisted of the following four sequential phases:

i. Observation of the procedure performed by the trainer, during which each trainee observed the steps of the entire procedure, for example, US assessment for joint components identification, asepsis creation, arthrocentesis (if indicated), local anaesthesia (percutaneous), biopsy needle placement and ST retrieval, respectively. Each trainee repeated this phase at least five times for each joint type (knee and wrist

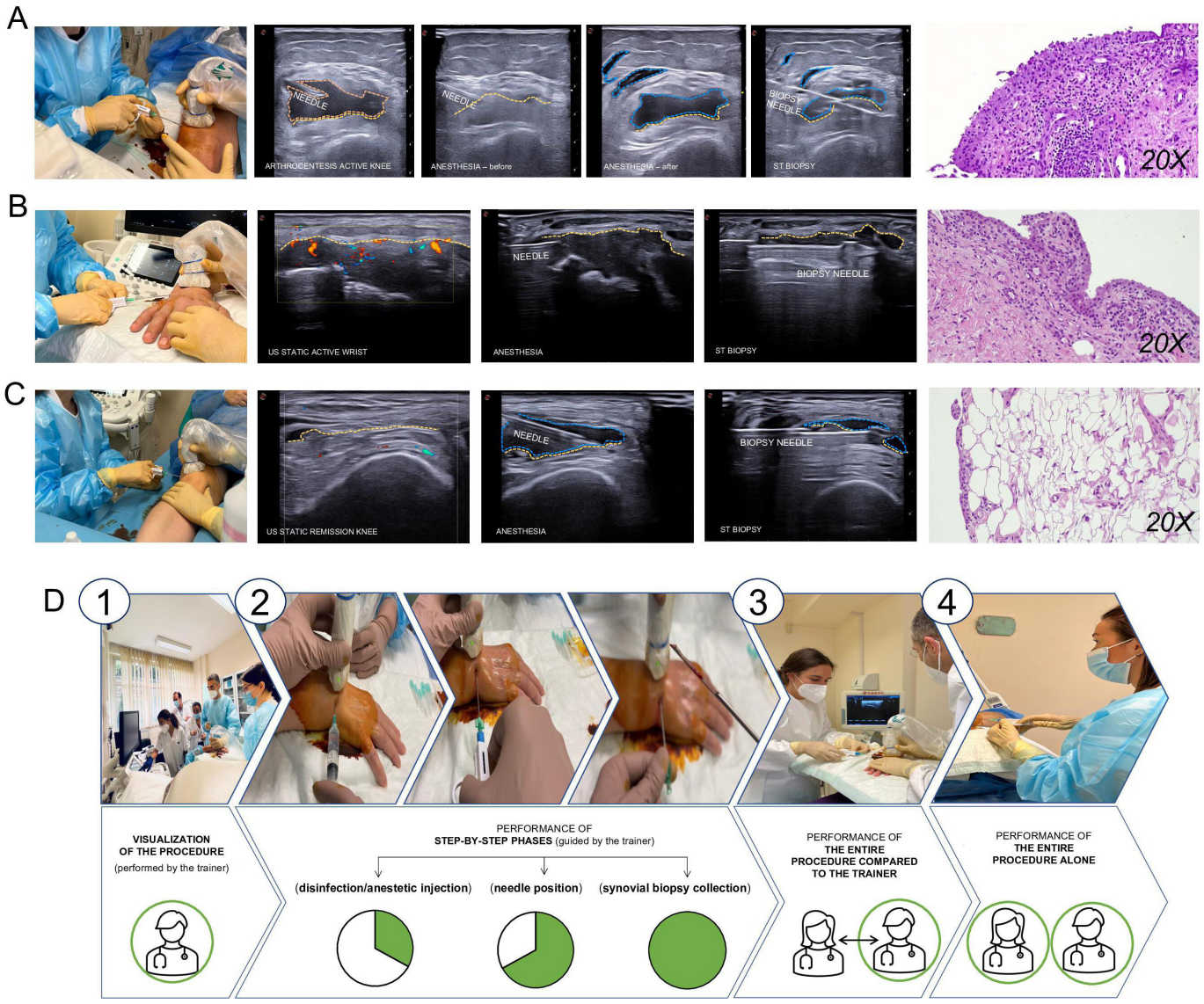

Figure 1 Intensive training programme for minimally invasive ultrasound-guided synovial tissue biopsy of active and remission joints. (A) Schematic of the different phases of minimally invasive ultrasound-guided synovial tissue (ST) biopsy on active knee; (B) Schematic of the different phases of minimally invasive ultrasound-guided ST biopsy on active wrist; (C) Schematic of the different phases of minimally invasive ultrasound-guided ST biopsy on remission knee; (D) Schematic of the steps of the intensive training programme for minimally invasive ultrasound-guided ST biopsy: (1) visualisation of the procedure performed by the trainer, (2) step-by-step performance of the procedure phases by the trainee guided by the trainer, (3) performance of the entire procedure by the trainee compared with the procedure performed by the trainer on the contralateral joint and (4) autonomous performance of the entire procedure by the trainee.

respectively) and disease condition (active and remission, respectively). For the knee synovial biopsy, the published standard operating procedure was used.[12] Briefly, the entry reference point of the biopsy needle was identified at the lateral margin of the suprapatellar recess for the knee (online supplemental figure 1), and at the ulnar side of the ulnocarpal joint for the wrist, between the IV and V extensor tendon compartments or between the V and the VI extensor tendon compartments depending on the patient's anatomy. Each patient was provided with a face mask and the procedure was performed under sterile conditions. The skin was disinfected with iodine solution, performed twice, starting from the point of needle entry to 25 cm (knee) or 15 cm (wrist) proximally and distally. The skin, subcutaneous tissue and joint capsule were anaesthetised with 20 mL (knee) or 10 mL (wrist) 2% lidocaine. For the knee joint in remission, lidocaine was used to expand the joint space and to improve visualisation of the synovial surface. A 14G needle (Precisa 1410-HS Hospital Service Spa, Italy) was then inserted into the joint (same needle's size for both knee and wrist). B-mode US assessment was performed, starting from the lateral side of the suprapatellar recess for the knee and from the ulnocarpal recess for the wrist, to identify

ST and visualise its surface, to ensure representative ST sampling (figure 1A–C). In the presence of synovial hypertrophy, power Doppler (PD) evaluation was performed to assess hypervascularisation. The operator then proceeded to sample different sites of US-detected ST using the portal-assisted needle device, changing the needle angles (online supplemental figure 1). Trainees were instructed on the minimum number of tissue fragments required for correct sampling,[24] the macroscopic appearance of ST and on how to distinguish it from other articular and periarticular structures, such as capsule, tendons, muscle and cartilage.

ii. Step-by-step execution of the procedure steps by the trainee under the guidance of the trainer until completion of the entire procedure: each trainee took part in the procedure sequentially stopping after disinfection/injection of anaesthetic or placement of the biopsy needle or completing the entire synovial biopsy. Each trainee repeated this step five times for each joint size and condition, as previously described.

iii. Performance of the entire synovial tissue biopsy procedure in comparison with the trainer on paired joints of the same patient: when a patient exhibits two contralateral joints with comparable clinical and US features (both in US Gray scale and PD) the entire ST biopsy is performed first by the trainer on one side, then immediately afterwards by the trainee on the contralateral side (in the different scenarios: active knees, active wrists, remission knees).

iv. Performance of the entire ST biopsy procedure autonomously by the trainee on active (knee and wrist) and n remission (knee) joints simulating the routine outpatient clinical setting. During this phase, the trainee was assisted by another trainee, who held the US probe to guide the procedure, with the trainer supervising the entire procedure without interfering unless strictly required.

ST specimens' quality assessment and synovitis degree scoring

To assess the quality of the sample and thus the success of the procedure, ST specimens were processed for histology (H&E) to determine first the presence of ST and then the anatomical representativeness of the specimen, given by the presence of lining and sublining layers in at least one fragment. Briefly, all ST specimens obtained (at least 6–8 fragments for the knee and 4–5 fragments for the wrist) were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 3 µm and stained for H&E as follows: sections were deparaffinised in xylene and rehydrated in a series of graded ethanol, stained in haematoxylin and counterstained in Eosin/Phloxine. Finally, sections were dehydrated, cleared in xylene and mounted with Bio Mount (Bio-Optica). Slides were examined under a light microscope (Leica DM 2000) by a trained pathologist (MG) who was blinded to the clinical and immunological characteristics of the patients and the identity of the operator. The anatomical structure of synovial membrane was examined (online supplemental figure 2A–C), and the severity of synovitis was graded according to three synovial features: synovial lining hyperplasia, stromal cell density and inflammatory infiltrate respectively, each scored on a semiquantitative scale from none (0), slight (1), moderate (2) to severe (3).[12 27] The analysis was done manually and included the assessment of the entire ST sections.

Trainees' perceptions of the ST biopsy procedure and educational expectations

Before and after the completion of the entire programme, each trainee was asked to fill a questionnaire regarding their expectations of the ST biopsy procedure, including the difficulty of its execution based on joint location (knee/wrist) or the disease activity (active/remission), as well as their perception of invasiveness and pain caused to the patient. In addition, at the end of the intensive programme, each trainee's personal feedback from each of the four training phases was recorded.

Monitoring and recording of procedure-related side effects

After the procedure, each enrolled patient was observed for 1 hour to monitor for any short-term adverse events. Prior to discharge, patients were instructed to contact the biopsy unit by telephone/email in the event of any post-procedure discomfort. In addition, each patient underwent a full rheumatological assessment in an outpatient clinic 28 days after the ST biopsy to record the occurrence of late-onset adverse events. At this time, any minor complaint that had not previously resulted in a phone call and/or email was recorded.

Statistical analysis

Statistical analysis was performed using SPSS V.20.0 and Prism software (GraphPad, San Diego, USA). Categorical and quantitative variables were described as frequencies, percentage and mean±SEM. Data on demographic and clinical features were compared between patients by the non-parametric Mann-Whitney U test or χ^2 test, as appropriate.

RESULTS

Intensive training programme participants' characteristics

A total of nine trainees (30.0±1.85 years) and two trainers (52 and 42 years, respectively) participated in the intensive training programme from September 2021 to December 2022. Table 1 summarises the demographic and professional characteristics of the trainees. Trainees were young rheumatology consultants or rheumatology residents who joined the programme for a period of 4 months. Specifically, 6 trainees (66.6%) were from Italian or foreign academic hospitals and 3 (32.3%) were rheumatology residents at the host institution. Considering pre-training skills, 5 (55.5%) trainees already had average or advanced skills in musculoskeletal US and 3 (32.3%) had sufficient experience in intra-articular injections.

Table 1 Demographic, educational and professional characteristics of the trainees joining the intensive training programme

Trainees' characteristics (n=9)	
Age (mean±SEM, years)	30.0±1.8
Female sex, n (%)	7 (77.7)
Professional title	
Rheumatology resident, n (%)	7 (77.7)
Rheumatologist consultant, n (%)	2 (22.3)
Hospital of origin	
Hosting hospital, n (%)	3 (32.3)
Other academic Italian hospital, n (%)	5 (55.6)
Other academic foreign hospital, n (%)	1 (11.1)
Frequency of US execution	
Never, n (%)	2 (22.2)
Infrequently, n (%)	2 (22.2)
Weekly, n (%)	4 (44.5)
Daily, n (%)	1 (11.1)
Frequency of intra-articular injection execution	
Never, n (%)	2 (22.2)
Infrequently, n (%)	4 (44.5)
Weekly, n (%)	3 (32.3)
Never seen an ST biopsy before	6 (66.6)

SEM, Standard Error of Mean; ST, Synovial Tissue; US, Ultrasonography.

Seven out of nine trainees completed the full intensive programme (knees and wrists), while two trainees completed only the training for knees (active and remission).

Procedure success for trainees is dependent on inflammation grade and joint location

A total of 328 US-guided minimally invasive ST biopsy procedures performed in 269 patients (185 with active disease and 84 in remission) were included. Considering the first aim, 100% of the procedures performed by the trainees, either in parallel with the trainer or alone, were successful, as the histological assessment showed evidence of ST in every specimen. However, the anatomical representativeness was not always persistent, as the lining layer could be absent (online supplemental figure 2A–C). Specifically, after completing the observational and guided step-by-step phases, the trainees performed 59 procedures in parallel with the trainers (n=32 on active knees, n=17 on knees in remission and n=10 on active wrists), each of them performing it at least 5 times (figure 2A–C, figure 3A,B). In this training phase, the sample representativeness, in terms of the presence of the lining layer, was comparable between trainees and trainers (93.8% and 94.1%, p>0.99) for active and remission knees (figure 2A,C), while lower for trainees (70%) than in trainers (100.0%, p=0.06) for active wrists (figure 2B). Based on this success rate, we identified five pooled procedures as necessary, at individual level, to progress from phase 3 to phase 4. However, when trainees performed the entire ST biopsy procedure autonomously, without trainers' guidance, simulating a real outpatient clinical setting (n=210 procedures with n=99 on active knees, n=44 on active wrists and n=67 on remission knees), the success rate of the procedure was significantly influenced by joint inflammation grade rather than joint location (figure 3A–D). Specifically, representative ST samples were obtained in 91.9% and 90.9% of active knees and active wrists, respectively, compared with 77.6% of knees in remission (p=0.0089) (figure 3E). Finally, the success rate was not influenced by previous trainee's expertise in musculoskeletal US and/or intra-articular injections (figure 3G).

Procedure-related adverse events

No serious adverse events were recorded during the procedures and only one presyncopal episode occurred (0.3%). Within 28 days after the procedure, one haemarthrosis (0.3%) occurred without sequelae while the most common minor complications were self-limiting arthralgia or local pain requiring analgesia (10.4%). No significant difference was observed in the rate of adverse events following the procedures performed by trainees compared with those performed by the trainers (p>0.05).

Pre-training and post-training perception of trainees on procedure invasiveness and performance difficulty

Before and after the completion of the training programme, each trainee was asked to complete a self-assessment questionnaire quantifying their perceptions of the procedural invasiveness and patient pain. As shown in figure 4, the completion of the intensive training programme resulted in a significant reduction in both the perception of the procedure invasiveness from moderate to low (p=0.0156) and the perception of pain caused to the patient from very high/moderate painful to slightly painful (p=0.0039) (figure 4A,B). When considering trainees' perceptions of difficulty in performing the procedure, the completion of the intensive training programme resulted in an overall reduction in perceived difficulty for active knee joints (p=0.0078). Conversely, performing the procedure on knees in remission remained more difficult than on active knees (p=0.0039), and approaching both active wrists and remission knees was still perceived as moderately difficult on average (figure 4C).

DISCUSSION

Our study was designed to address the unmet need of a uniform educational system, to reduce discrepancies between centres performing ST biopsies by promoting standardisation of ST collection and handling. The ultimate goal is to improve patient care by promoting ST-related research and incorporating ST analysis into patient workup.[21]

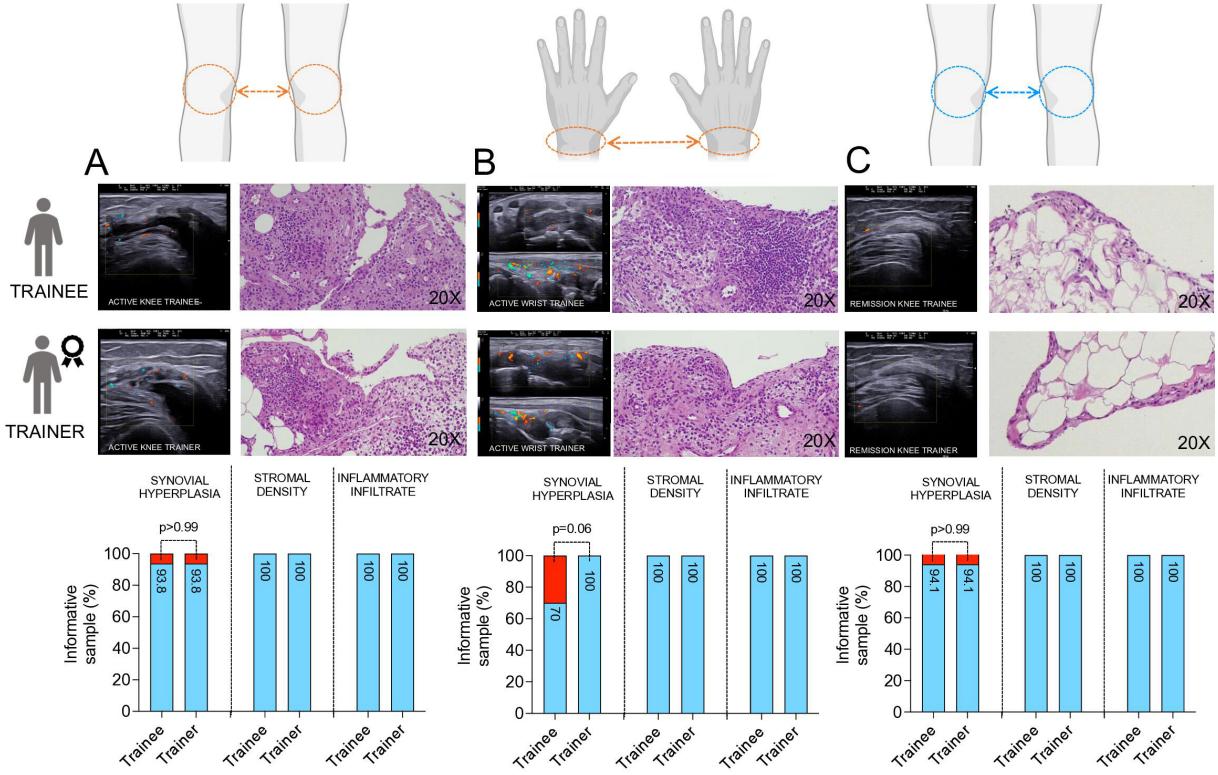

Figure 2 Comparative success rate of synovial tissue retrieval through minimally invasive ultrasound guided biopsy between trainee and trainer. Representative Ultrasound (Grey scale and power Doppler) images and histological (H&E staining) microphotographs and comparative success rate of informative sample retrieval between trainees and trainers on (A) paired active knees, (B) paired active wrists and (C) paired remission knees.

To achieve this goal, we developed the first intensive training programme, carried out in a unique setting to specifically train inexperienced rheumatologists and rheumatology residents to successfully perform minimally invasive US-guided ST biopsy on knees and wrists of RA patients across disease course.

Irrespective of differences in US background, in a period of 4 months, the trainees were able to retrieve ST in 100% of the procedures from joints of different size and inflammatory status. This unique stepwise approach allowed them to gradually gain confidence in a new environment, from assessing the patients in the context of their clinical disease state, to selecting the target joint by using US assessment, to performing the procedure and the specimen handling, to dialogue with the pathologist, up to interpreting the histological report by using a validated semiquantitative activity and severity score. The anatomical representativeness of the sample is a key point in the whole process, as retrieved ST samples must include all the anatomical areas of interest, that is, both lining and sublining layers, to allow a global assessment of tissue inflammation. In health, the synovial lining layer consists of a thin layer of ST macrophages (STMs) and fibroblast-like synoviocytes, both of which are also found in the sublining layer with scattered T cells, adipocytes and endothelial cells interspersed in a loose connective tissue.[1] Recent advances in omic analyses performed on ST specimens from patients with active and remission RA have shown that ST is enriched by distinct immune and stromal cell clusters, each of which has a precise anatomical position within the synovium (eg, lining layer, sublining layer, perivascular areas) and whose presence and proportions vary with disease activity.[15 20 22 23] For example, MerTK^{pos} STMs represent the main resident population in healthy ST, exhibit an important homoeostatic function and their proportion reflects disease activity, decreasing during inflammation, restoring during sustained remission and predicting maintenance of remission.[15 28] Conversely, MerTK^{neg} STMs exhibit a proinflammatory phenotype which contributes to the pathogenesis of synovitis.[15 28] Similar deep molecular heterogeneity applies to other cell populations in the synovium, as fibroblasts, where different cell clusters may interact with each other in a location-specific manner,[22 23] varying according to the disease status. This makes the development of minimally invasive biopsy procedures, differentiated by joint and grade of inflammation, essential to provide representative tissue specimens capturing all cells of interest.

Obtaining a representative tissue implies overcoming contextual technical challenges. Based on the success rates achieved by the trainees in phases 3 and 4, we identified a total of five pooled procedures as minimum to progress from phase 3 towards phase 4 of the training programme. Besides the joint size, also the inflammatory joint status proved to be a constraining factor. At the

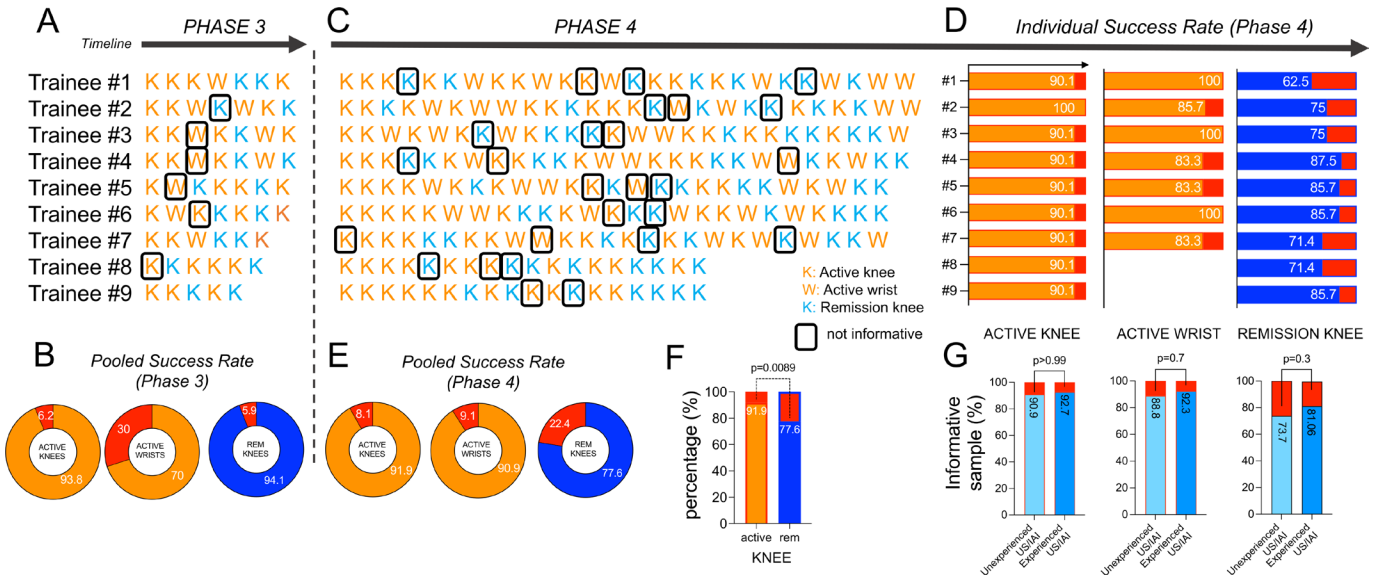

Figure 3 Trainee's individual path and pooled/individual success rate of the intensive training programme for minimally invasive ultrasound-guided synovial tissue (ST) biopsy of active and remission joints. Temporal schematic representation of each trainee's individual path during phase 3 (A, B) and phase 4 (C–G) of the intensive training programme for minimally invasive ultrasound-guided ST biopsy of active and remission joints. (A) Phase 3: the trainee performed the procedure immediately after the trainer on the contralateral joint with comparable imaging features of the same patient. Each letter (red K, red W, blue K) represents a procedure (on active knee, active wrist and remission knee, respectively). Black rectangles highlight non-representative procedures. (B) Donut graphs represent pooled success and unsuccess rate of biopsy retrieval for each joint condition of phase 3. (C) Phase 4: the trainee performed the entire procedure autonomously. (D) Phase 4 individual success rate per trainee for each joint condition. (E) Donut graphs represent pooled success and unsuccess rate of biopsy retrieval for each joint condition of phase 4. (F) Pooled success rate of representative ST specimens' retrieval by minimally invasive ultrasound guided ST biopsy autonomously performed by trainees on active and remission knees in phase 4. (G) Pooled success rate of informative samples autonomously collected by trainees based on their pre-training ultrasound and intra-articular injections' experience.

end of the training the representativeness of the sample obtained by performing the procedure autonomously was excellent, being both lining and sublining layers present in >90% of the samples of active knees and wrists, whereas a lower success rate was achieved in remission knee (77.6%). This discrepancy, also supported by the trainee's perception of the difficulty of performing the procedure in this condition, can be mainly ascribed to the need to successfully perform the first phase of the anaesthesia without the trainer support, in which the expansion of the joint cavity, by injecting the anaesthetic fluid, is critical to correctly visualise the ST surface (containing

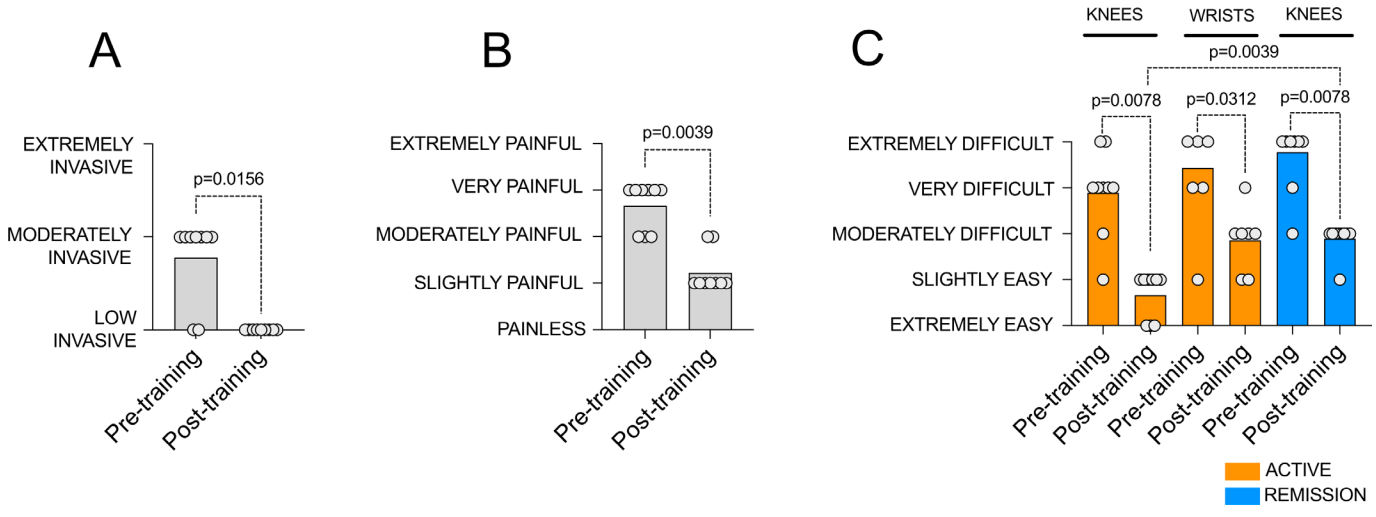

Figure 4 Pre-training and post-training perception of trainees on procedure invasiveness and performance difficulty on wrists and knees across disease activity. (A) Pre-training and post-training perception of trainees on procedure invasiveness; (B) Pre-training and post-training perception of trainees on procedure-related pain; (C) Pre-training and post-training perception of trainees on procedure performance difficulty on wrists and knees across diseases activity.

the lining layer) to be collected. In fact, when trainees performed the procedure on patients in remission in phase 3 with the trainer's support (ie, immediately after the trainer had shown it on the contralateral joint of the same patient), they felt more confident, and the success rate was excellent. If this approach is not feasible it could be envisioned that the trainee performs the procedure on a patient with comparable inflammation status immediately after the trainer has completed her/his own on the previous patient. This supports the need to adapt the intensity and timing of the training programme, possibly in more gradual steps, with a first round designed to allow the trainee to gain confidence in the approach of knees and wrists active joints, hence followed by a second round focusing on joints in remission.

Robust data have already highlighted the safety and tolerance of US-guided ST biopsies from a patient perspective,[8] which is further supported by our study, showing that patient tolerability, defined by the rate of procedure-related adverse events, was not affected by the learning setting. Also, from the clinician perspective, given some initial known scepticism, the evaluation of trainee's perceptions is also very promising and comforting. A daily attendance at the biopsy unit was sufficient to downsize both the expected invasiveness and pain caused to the patient, as well as the expected difficulty in performing the procedure. In conclusions, this intensive training programme lays the foundations for the dissemination of a useful minimally invasive procedure by harmonising the procedure-related teaching steps, and ultimately promoting the standardisation of ST sampling, ceasing to be the prerogative of well-selected centres. Providing high-quality ST specimens, valuable for high-throughput analyses from well characterised patients across different stages of inflammation, is an essential starting point for delineating ST phase-specific multiomics datasets that will pave the way for true personalised medicine in RA and other chronic inflammatory joint diseases.

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REFERENCES

1 Alivernini S, Firestein GS, McInnes IB. The pathogenesis of rheumatoid arthritis. *Immunity* 2022;55:2255–70.
2 Orr C, Vieira-Sousa E, Boyle DL, *et al*. Synovial tissue research: a state-of-the-art review. *Nat Rev Rheumatol* 2017;13:463–75.
3 Humby F, Durez P, Buch MH, *et al*. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. *The Lancet* 2021;397:305–17.
4 Alivernini S, Tolusso B, Petricca L, *et al*. Synovial features of patients with rheumatoid arthritis and psoriatic arthritis in clinical and ultrasound remission differ under anti-TNF therapy: a clue to interpret different chances of relapse after clinical remission? *Ann Rheum Dis* 2017;76:1228–36.
5 Alivernini S, Peluso G, Fedele AL, *et al*. Tapering and discontinuation of TNF-α blockers without disease relapse using ultrasonography as a tool to identify patients with rheumatoid arthritis in clinical and histological remission. *Arthritis Res Ther* 2016;18:39.
6 Johnsson H, Najm A. Synovial biopsies in clinical practice and research: current developments and perspectives. *Clin Rheumatol* 2021;40:2593–600.
7 Kelly S, Humby F, Filer A, *et al*. Ultrasound-guided synovial biopsy: a safe, well-tolerated and reliable technique for obtaining high-quality

synovial tissue from both large and small joints in early arthritis patients. *Ann Rheum Dis* 2015;74:611–7.
8 Just SA, Humby F, Lindegaard H, *et al*. Patient-reported outcomes and safety in patients undergoing synovial biopsy: comparison of ultrasound-guided needle biopsy, ultrasound-guided portal and forceps and arthroscopic-guided synovial biopsy techniques in five centres across Europe. *RMD Open* 2018;4:e000799.
9 Romão VC, Polido-Pereira J, Barros R, *et al*. Efficacy, Safety, and Sample Quality of Ultrasound-Guided Synovial Needle Biopsy in Clinical Practice and Research: A Prospective Observational Study. *Arthritis Care Res (Hoboken)* 2020;72:1497–505.
10 Najm A, Orr C, Heymann M-F, *et al*. Success Rate and Utility of Ultrasound-guided Synovial Biopsies in Clinical Practice. *J Rheumatol* 2016;43:2113–9.
11 Orr CK, Humby F, Fonseca JE. Editorial: Synovial Tissue: Turning the Page to Precision Medicine in Arthritis? *Front Med (Lausanne)* 2021;8:749062.
12 Alivernini S, Tolusso B, Gessi M, *et al*. Inclusion of Synovial Tissue-Derived Characteristics in a Nomogram for the Prediction of Treatment Response in Treatment-Naive Rheumatoid Arthritis Patients. *Arthritis Rheumatol* 2021;73:1601–13.
13 Humby F, Lewis M, Ramamoorthi N, *et al*. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. *Ann Rheum Dis* 2019;78:761–72.
14 Lliso-Ribera G, Humby F, Lewis M, *et al*. Synovial tissue signatures enhance clinical classification and prognostic/treatment response algorithms in early inflammatory arthritis and predict requirement for subsequent biological therapy: results from the pathobiology of early arthritis cohort (PEAC). *Ann Rheum Dis* 2019;78:1642–52.
15 Alivernini S, MacDonald L, Elmesmari A, *et al*. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat Med* 2020;26:1295–306.
16 Ingegnoli F, Coletto LA, Scotti I, *et al*. The Crucial Questions on Synovial Biopsy: When, Why, Who, What, Where, and How? *Front Med (Lausanne)* 2021;8:705382.
17 Smolen JS, Landewé RBM, Bergstra SA, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. *Ann Rheum Dis* 2023;82:3–18.
18 Rivellese F, Surace AEA, Goldmann K, *et al*. Rituximab versus tocilizumab in rheumatoid arthritis: synovial biopsy-based biomarker analysis of the phase 4 R4RA randomized trial. *Nat Med* 2022;28:1256–68.
19 Rivellese F, Nerviani A, Giorli G, *et al*. Stratification of biological therapies by pathobiology in biologic-naive patients with rheumatoid arthritis (STRAP and STRAP-EU): two parallel, open-label, biopsy-driven, randomised trials. *Lancet Rheumatol* 2023;5:e648–59.
20 Zhang F, Jonsson AH, Nathan A, *et al*. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. *Nature* 2023;623:616–24.
21 Najm A, Costantino F, Alivernini S, *et al*. EULAR points to consider for minimal reporting requirements in synovial tissue research in rheumatology. *Ann Rheum Dis* 2022;81:1640–6.
22 Croft AP, Campos J, Jansen K, *et al*. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* 2019;570:246–51.
23 Wei K, Korsunsky I, Marshall JL, *et al*. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* 2020;582:259–64.
24 Najm A, Le Goff B, Orr C, *et al*. Standardisation of synovial biopsy analyses in rheumatic diseases: a consensus of the EULAR Synovitis and OMERACT Synovial Tissue Biopsy Groups. *Arthritis Res Ther* 2018;20:265.
25 Wechalekar MD, Najm A, Veale DJ, *et al*. The 2018 OMERACT Synovial Tissue Biopsy Special Interest Group Report on Standardization of Synovial Biopsy Analysis. *J Rheumatol* 2019;46:1365–8.
26 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
27 Krenn V, Morawietz L, Burmester G-R, *et al*. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* 2006;49:358–64.
28 Kurowska-Stolarska M, Alivernini S. Synovial tissue macrophages in joint homeostasis, rheumatoid arthritis and disease remission. *Nat Rev Rheumatol* 2022;18:384–97.