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# Efficacy, safety and sample quality of ultrasound-guided synovial needle biopsy in clinical practice and research: a prospective observational study

*Running head:* **US-guided synovial needle biopsies in clinical practice and research**

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**Abstract**

**Objective**: To study the efficacy, tolerability, safety and sampling variation of ultrasound (US)-guided synovial biopsies performed in clinical practice and research.

**Methods**: We included all patients having an US-guided synovial needle biopsy from November 2013 to January 2018. Patients were evaluated for procedure safety and tolerability. Usefulness of synovial biopsy was considered based on contribution for achieving the proposed aims. We analyzed samples for presence and quality of synovial tissue, synovitis score/grade and pathotype. Variation across patients, samples, section levels and sampling order was assessed.

**Results**: Sixty-four US-guided synovial biopsies were performed (clinical practice n=52, research n=12). Patient tolerability (70% no/mild discomfort) was remarkably high. There was no significant aggravation of biopsied joint symptoms or US synovitis. Procedures were overall safe, with few minor, two moderate and no major adverse events. Usefulness of US-

guided synovial biopsies was high, both in clinical practice (37% direct diagnostic impact, 100%/95% positive/negative predictive values for infection) and in research (92% success). Synovial tissue was retrieved in 88% of biopsies, with a median of 75% gradable samples. There was significant variation in sample quality and synovitis features across patients and samples, but not between different section levels. Samples collected later in the procedure had a lower frequency of synovial tissue and were poorly concordant in pathotype with those collected earlier.

**Conclusion**: US-guided synovial needle biopsy is an effective, safe, well-tolerated mean to collect good quality synovial tissue for clinical and research purposes. Samples collected for different aims should be retrieved in parallel, rather than sequentially.

**Significance and Innovations**

- Synovial biopsy is an important tool with high utility for the study of synovitis in clinical practice and research.
- Ultrasound (US)-guided synovial needle biopsy is a safe, well-tolerated technique that allows for effective collection of good quality synovial tissue in all types of joints, which can be performed by rheumatologists with experience in US-guided procedures.
- Sample quality and synovitis features vary greatly across patients, individual samples and sampling order.
- When collecting synovial tissue for different purposes, clinicians and researchers should aim to do so simultaneously throughout the procedure, rather than sequentially, in order to avoid heterogeneity in quality and other characteristics, to uniformize sample collection and to provide a full picture of the synovitis process.

## Introduction

Despite significant advances in the diagnosis and management of inflammatory joint diseases, synovial tissue study still retains a fundamental role for better understanding synovitis in both clinical practice and research (1–3). In the context of undifferentiated arthritis (UA) or unexplained synovitis in patients with an established rheumatic disease, when arthrocentesis and other standard imaging and laboratory exams are inconclusive, analysis of the synovial tissue may help to clarify the diagnosis, rule out infection and other serious joint diseases (e.g., tumors) (4–6). Moreover, in recent years synovial pathobiology has contributed to deep cellular and molecular characterization of heterogeneous diseases such as rheumatoid arthritis (RA) and efforts are in progress to identify synovium-based biomarkers of prognosis and treatment response, aiming at patient stratification (2,7,8).

Synovial biopsies have facilitated synovial tissue sampling for several decades now, but most techniques are considered too invasive and imprecise (e.g., blind needle biopsies using Parker-Pearson needles (9)) or too complex to be performed widely (e.g., arthroscopy) (10,11). With the incorporation of ultrasound (US) and US-guided procedures into routine clinical care, US-guided synovial biopsies have recently emerged as an attractive alternative to allow collection of synovial tissue from a variety of joints in a simple, minimally invasive, precise way (12,13).

US-guided synovial biopsies have been shown to be well tolerated and generate good quality tissue in the context of clinical trials (14–17). Recently, Humby *et al* have shown that US-guided synovial biopsies performed in a research setting were superior to blind needle biopsies and as successful as arthroscopy in reliably retrieving synovial tissue from large and small joints (18). Given the importance of expanding this technique to a wider scale, the need for more data on the outcomes of US-guided synovial biopsies has been advocated (12,19). More specifically, studies performed in routine clinical practice are limited and mainly

focused on efficacy and safety (20–25). Thus, tolerability and sample quality data are mostly missing in this context. Finally, a number of questions are still a matter of debate, including: (i) the minimum and optimal number of samples to be retrieved and analyzed for each aim and setting; (ii) the variation of synovitis features across synovial samples and within separate sections of the same sample; (iii) the changes in tissue characteristics according to timing of collection during the procedure (i.e., later or earlier into the biopsy).

In this study we report our experience with US-guided synovial biopsies performed in clinical practice and research, focusing on several important aspects that contribute to better understanding the application and importance of this technique. Our main goals were to assess (1) patient tolerability; (2) procedure safety; (3) synovial biopsy usefulness and impact; (4) efficacy in obtaining good quality synovial tissue; (5) and synovitis features across biopsies, samples and sections, including those collected at different stages during the procedure.

## Materials and Methods

### *Patients inclusion and synovial biopsy indication*

We prospectively included all patients undergoing an US-guided synovial biopsy in the Rheumatology Department of Hospital de Santa Maria, for clinical or research purposes, from November 2013 to January 2018. Synovial biopsies were performed in patients with clinically unspecified forms of synovitis or patients with an established diagnosis and an unexplained monoarthritis, where infection or other etiologies were suspected but could not be confirmed through arthrocentesis and other routine imaging or laboratory methods. Patients with active synovitis participating in synovial tissue-based research studies were also evaluated for procedure success, safety and tolerability. Patient informed consent was obtained for the US-guided synovial biopsy procedure and for the collection of additional

synovial tissue samples for the synovial tissue biobank collection at Biobanco-IMM, Lisbon Academic Medical Centre. Ethics approval was obtained from the local ethics committee for each research study.

*US-guided synovial biopsy procedure and evaluation of tolerability and safety*

The synovial biopsy was performed under US guidance by four different operators with extensive musculoskeletal US and US-guided procedures experience (FS, JPP, RB) or specific training on US-guided synovial biopsies (VCR). We used the technique previously described by Kelly *et al* (14), with a GE Logiq E9 (ML6-15-D and L8-18i-D probe) or GE Logiq e (12L-RS probe) machine (GE Healthcare, Chicago, Illinois, United States) and a 16G Speedybell semiautomatic guillotine-type biopsy needle (Biopsybell, Mirandola, Italy) without outer coaxial needle. Greyscale (GS) and power Doppler (PD) synovitis of the biopsied joint were classified on a semi-quantitative scale of 0-3, in accordance with EULAR-OMERACT definition (26,27), prior to and after the biopsy.

In clinical practice we collected a minimum of 4-5 and 5-6 samples for microbiological analysis and histological examination, respectively. If the patient consented, we further retrieved samples for biobank storage including 4-6 samples for paraffin embedment, RNA extraction and freezing, in this order. The total number of samples collected was registered.

To evaluate biopsy tolerability and safety patients underwent clinical and ultrasound examination and a standardized questionnaire was applied prior to and 5-10 days after the biopsy. Patient-reported outcomes (PRO) were obtained for biopsy joint symptoms, immediate tolerability, discomfort/pain during the procedure and likelihood to repeat it. Analgesic/anti-inflammatory intake following the biopsy was collected. We recorded immediate adverse events occurring during the biopsy and others of specific interest,

including joint or skin infection, haemarthrosis, deep vein thrombosis and neurovascular or tendinous-ligament damage.

*Usefulness and clinical impact of synovial biopsies*

Synovial biopsy samples performed in clinical practice were independently analyzed by experienced pathologists in the assessment of synovial tissue (EV, RL). We reviewed the findings reported, including specific aspects (e.g., crystal deposition or intense neutrophil-rich acute synovitis suggestive of septic arthritis), and assessed the impact of these results in clarifying diagnosis. The same was performed for microbiology results. We also reviewed the outcome of all patients concerning the final diagnosis established by the attending rheumatologist. Finally, research biopsies were considered to be useful if study goals related to synovial tissue were met.

*Histological evaluation of synovial tissue*

We retrieved the H&E-stained synovial tissue slides from the Pathology Department and Biobanco-IMM and scanned them for sample quality analysis (using NDP.view 2.6.13, Hamamatsu Photonics, Hamamatsu, Japan). Samples were assessed for the presence of synovial tissue, which was considered gradable if the lining layer was present. We evaluated the frequency of biopsies with at least one gradable sample. Additionally, we identified those with four or more gradable synovial samples, a number reported to represent the overall degree of synovitis (17). We measured the area of synovial tissue, which was compared with the total area of retrieved tissue. We calculated the synovitis score (0 to 9) using a previously published semi-quantitative score (28), and graded each synovial sample as ‘no synovitis’ (0-1), ‘low-grade synovitis’ (2-4) or ‘high-grade synovitis’ (5-9). The mean and maximum synovitis score/grade were calculated for each biopsy. Furthermore, we classified H&E-

stained samples according to pathotype, previously described as follicular (with formation of lymphoid follicle-like aggregates), diffuse (widespread lymphocyte and macrophage infiltration, without lymphoid aggregates) and pauci-immune (few inflammatory cells, fibroblast-rich) (7,8). Each biopsy was classified according to the sample with the highest degree of organized inflammation (i.e., follicular, diffuse and pauci-immune, in this order). In order to assess the variation in sample quality and characteristics across tissue sections we analyzed biopsies with additional sections at a deeper level (at least 25-35µm apart) and compared different sections of the same sample. Finally, we were able to assess the impact of sampling order on tissue quality by comparing samples collected later in the procedure for the biobank with the ones retrieved earlier for the Pathology Department.

*Statistical analysis*

Patient and biopsy characteristics were represented as mean ± standard deviation or median (interquartile range), as applicable. Comparison of pre and post-biopsy assessments and of different tissue sections was performed using paired T-test or Wilcoxon signed-ranks test, based on normality assessment. To study the variation of synovitis features across patients, samples and section levels, we performed nested analysis of variance (ANOVA) and estimated variance for each of these parameters. All statistical analyses were performed using Stata 12.1 for Mac (StataCorp, College Station, TX, USA) and GraphPad Prism 7 for Mac OS X (GraphPad Software, San Diego, USA). P-value was considered significant at $p<0.05$.

## Results

*Biopsy and patient characteristics*

Fifty-eight patients (64% female, mean age 59 ± 16 years; Supplementary Table 1) underwent 64 US-guided synovial biopsies: 52 for clinical reasons (infection exclusion and diagnosis clarification) and 12 for research studies (Table 1). Thirty-one patients had a defined rheumatic disease prior to the biopsy, although a significant proportion of patients had a diagnosis of UA (n=24). As expected in a clinical practice setting, monoarthritis was the main indication for synovial biopsy (66%). Disease flare was the reason for biopsy in the 12 patients undergoing a research biopsy and in 2 RA patients with poor response to therapy and atypical course. All types of joints were biopsied, mostly medium sized (wrist in 41% of cases), but also bursae and tendon sheaths (Table 1). Four patients had 2 US-guided synovial biopsies (2 clinical, 2 research) and one patient with septic arthritis underwent 3 biopsies.

*Procedure tolerability and safety*

Synovial biopsies were well tolerated. Two thirds of patients classified the procedure as easy or very easy and 70% stated to have felt no or only mild discomfort (Figure 1A). Importantly, willingness to repeat an US-guided synovial biopsy was high, with 74% of patients considering it to be likely or very likely (Figure 1A). After a median of 8 days following the procedure, there was a significant decrease in visual analogue score (VAS) of pain, swelling and stiffness of the biopsied joint (Figure 1B), while US synovitis scores remained similar (Figure 1C). None of the patients received a local glucocorticoid injection during the biopsy. Thirty-six percent (20/56) of patients reported increased intake of analgesics in the days following the procedure. There were no differences in tolerability outcomes according to joint, degree of US synovitis or operator.

Biopsies were overall safe, with 6 minor immediate adverse events and 7 adverse events identified on post-biopsy evaluation (Table 2). There were no cases of haemarthrosis, joint infection, periarticular infection or major neurovascular damage. The transient wrist extensor paresis due to local anesthesia seen in a patient undergoing an elbow biopsy resolved after 3 hours. The cases of local hematomas and mild local inflammation all resolved after a few days. Two of the post-biopsy adverse events were considered of moderate severity and included 2 patients reporting mild limitation of digit extension, with no detectable tendinous rupture on US evaluation, which persisted during follow-up. Given the close relation to tendon structures in both cases, we consider lesion of tendinous microfibers beyond the resolution of the US scan to be the most likely cause for this event, although we cannot completely exclude a micro-neurovascular injury.

*Clinical and research usefulness of synovial biopsy*

The usefulness of US-guided synovial biopsies in clinical practice and research was high. In 19 (37%) patients having a synovial biopsy for clinical reasons there was a direct impact of the biopsy findings in diagnosis, prognosis and therapeutic guidance (Table 3; Supplementary Table 2; Supplementary Figure 1).

The differential diagnosis of septic arthritis/bursitis was confirmed in 8 cases, through identification of intense acute neutrophilic synovitis on histological examination and/or isolation of bacteria in culture (Table 3). All of these cases had a high clinical suspicion of infection. Two septic patients with acute synovitis of the shoulder/subacromial bursa had technically difficult biopsies that did not add significant value (insufficient tissue for histological analysis and negative bacteriological exams), but based on the clinical context the diagnosis of septic arthritis was, nonetheless, assumed. None of the patients deemed not to have infection subsequently required antibiotic therapy or had a late diagnosis of septic

synovitis, after a follow-up of 5 months to 4.5 years. This leads to excellent positive (100%) and negative (95%) predictive values for infection of combined histological and microbiological analysis of US-guided synovial biopsies.

Thirty-one biopsies (Supplementary Table 2), did not have specific findings but allowed to rule out infection and to safely adjust treatment, including glucocorticoid joint injection in patients with persistent synovitis. Fifteen out of the 20 patients with UA retained this diagnosis after follow-up (2.0 ± 1.1 years, 5 months-3.5 years). Consequently, only 9 of all the UA patients biopsied (n=24) evolved to a concrete diagnosis (RA n=5, microcrystalline arthritis n=2, synovial chondromatosis n=1, peripheral spondyloarthritis n=1; mean follow-up 2.1 ± 1.1 years, 9.4 months-4.3 years), and in 4 of these (sensitivity=44%) the biopsy helped in establishing diagnosis.

Concerning the 12 patients having a synovial biopsy for research purposes, all but one (92%) generated good quality tissue that allowed research goals to be pursued. This patient, with longstanding RA, had a wrist biopsy that was technically complicated and terminated early due to dense fibrous pannus proliferation.

*Efficacy of ultrasound-guided synovial biopsies and sample quality*

US-guided synovial biopsies were an effective mean for collecting substantial amounts of synovial tissue (17 ± 7 samples (range 3-32) per procedure).

A mean of 6 ± 2 samples per procedure were available for analysis and a total of 386 samples were independently analyzed. Synovial tissue was obtained in 56 (88%) procedures, of which 53 (95%) generated at least one gradable biopsy sample. A median of 75% (50-100%) of synovial tissue samples were considered gradable, with a median number of gradable samples of 4 (2-6) per biopsy. Importantly, a significant proportion of biopsies (57% of biopsies with at least one synovial sample and 48% of all biopsies) generated at least 4 gradable samples. Mean cumulative total sample and synovial tissue areas were 18.4 ± 9.7mm$^2$ and 11.6 ±

$7.2mm^2$, respectively. Synovial tissue area corresponded on average to 66% (median 73%) of the total sample area in biopsies with at least one synovium sample. Mean synovitis score per sample and per biopsy was 3.4 ± 1.3 and 4.1 ± 1.6, respectively. This corresponded to a frequency of low-grade/high-grade synovitis of 77%/23% and 65%/35%, respectively. Synovial pathotype was classified as follicular, diffuse or pauci-immune in 22%, 51% and 27% of biopsies, respectively.

There was significant variation across patients and between individual samples from the same patient in terms of presence of synovial tissue and gradable synovial tissue (Table 4). Synovitis score, grade and pathotype also varied significantly between not only patients but also samples. In contrast, the variance between different section levels of the same sample was much lower for all synovitis features analyzed, compared to that observed between patients and samples (Table 4). Indeed, in 35 biopsies with available in-depth sections, the mean number and percentage of synovium or gradable samples did not differ significantly between levels of analysis (Supplementary Figure 2A). Moreover, mean synovitis score and pathotype were also similar, with only one biopsy reclassified from diffuse to follicular due to a large lymphoid aggregate not identified in the previous level (Supplementary Figure 2B,C).

Regarding the differences of samples collected at an earlier or later stage of the biopsy procedure, in 19 biopsies with available data the frequency of synovial tissue samples per total samples collected was lower in samples retrieved later in the procedure, whereas the frequency of gradable samples per synovial tissue samples was similar (Figure 2A). However, a smaller number of samples were collected for biobank compared to those sent to pathology (4 ± 1 vs. 6 ± 2, respectively, p=0.005), which might limit comparisons. Of note, in two biopsies, synovial tissue was not identified in the samples collected earlier but was present and gradable in those retrieved later. In line with this, there was some variability at

the individual level in mean synovitis score of samples collected earlier or later in the procedure, although overall it was not statistically significant (Figure 2B). Moreover, pathotype classification per biopsy was poorly concordant between samples collected at different stages of the procedure (Figure 2C). These aspects are important to account for when samples from the same patient are collected for separate purposes at different stages of the biopsy.

**Discussion**

As noted in the Kelly *et al* landmark study (14), more data from multiple sites is needed to confirm the performance of US-guided synovial needle biopsy and our study is the first to report a comprehensive assessment of this procedure in both clinical practice and research, including evaluation of efficacy, PRO and sample quality.

In clinical practice, synovial biopsy proved to be a valuable resource for clinicians, who frequently requested it in the setting of unexplained synovitis. This included most frequently UA (24/52), but also a significant number of refractory monoarthritis in otherwise stable established rheumatic patients (19/52). It should be emphasized that in all these cases arthrocentesis had been inconclusive or was technically impossible, as in small joints (e.g., interphalangeal, naviculocuneiform) or in those without synovial fluid (e.g., sternoclavicular, chronic synovitis with profuse synovial proliferation). Biopsy indication and aims were consistent with published data and recommendations (1,3,20). Importantly, the US-guided approach allowed the study of every type of joint, synovial bursae and tendon sheaths, highlighting its added value in comparison with other techniques, that are more dependent on the localization of synovitis.

The tolerability profile of US-guided synovial biopsies was excellent, in accordance with previous studies that formally assessed this technique in a research context (14–16). We provide evidence of the remarkable tolerability of this procedure in a clinical setting, in several joints and in a different patient population.

There was no aggravation of joint symptoms or US synovitis following the procedure, similarly to what was previously reported in research-driven biopsies (14,16). We hypothesize that the slight improvement in symptoms observed, also described in a different context (16), may be due to: (i) patient satisfaction with the biopsy procedure (as discussed above) and with the overall care and attention paid by the attending physician and biopsy operator, as it has been shown that PRO are affected by both the clinical outcome and patient satisfaction with the healthcare experience (29,30); (ii) enhanced placebo effect related to the biopsy *per se*, as this tends to be proportional to the invasiveness of a clinical intervention (31,32).

We reported mild adverse events, although slightly more frequent than previously published (14,16,20,21,25), and 2 moderate adverse events. Different definitions of reportable adverse events may explain these discrepancies and we stress that in our experience most adverse events were of little clinical relevance (e.g., minor local bleeding) and there were no severe complications (e.g., hemarthrosis, infection). However, we did have two cases of tendon-related minor injury, that deserve emphasis. Caution should be taken with tendon sheath and wrist biopsies ($5^{th}$ extensor compartment) and if retrieving a high number of samples, when an outer coaxial needle may be considered. Although we cannot entirely exclude damage to the tendon microvasculature and/or microinnervation, this would unlikely lead to clinically-evident motor deficit, as motor innervation occurs more proximally and collateral microcirculation would probably suffice for the low tendon vascular demands (33,34). The aims of US-guided synovial biopsies were frequently met. It contributed directly for

clarification of diagnosis in a considerable proportion (37%) of patients, highlighting its value for the study of synovitis (1,4). Synovial biopsy was particularly relevant for confirmation of infection (19%, similar to other series (21)), through bacterial isolation and histological identification of intense synovial neutrophil infiltration (35). Sensitivity (80%) and specificity (100%) for confirming or excluding septic synovitis were high and enabled treatment adjustment in a quicker and safer way. Other specific diagnoses were established based on histological analysis, most notably microcrystalline disease, which is in accordance with previous series where a coaxial needle was used (20,21). Synovial biopsy contributed to diagnosis in 4/24 (17%) of all UA patients in whom standard clinical procedures had been inconclusive. Moreover, it had a sensitivity of 44% and a negative predictive value of 75% for the identification of UA patients who were eventually diagnosed with a specific rheumatic disease, which is relevant given the paucity of predictors of progression of UA (36,37). Finally, research goals were also met, as good quality synovial tissue was collected in 11/12 cases (92%), confirming the value of this technique in observational and interventional studies (17,19).

We were able to harvest a higher number of samples than most previous studies (20,21,23,25), with excellent tolerability and a similar success rate (14,15,20,21,24,25,38), which is in accordance with recommendations (17). This allowed collection of samples for different purposes and that a sufficient number of specimens were analyzed and gradable synovial tissue retrieved.

We have shown that sample quality and synovitis features, including synovitis score and pathotype, greatly vary across patients and samples, but not section levels of the same block, confirming in a different setting and population what had earlier been reported for RA and immune-cell infiltration (17,38). This implies that in order to get a full picture of the synovitis process a sufficient number of samples should be assessed.

Furthermore, to our knowledge we are the first to describe that sampling order is an important factor to consider, as the frequency of synovial tissue was lower in samples collected later in the procedure, mean synovitis score varied and pathotype classification was discordant. This may be explained by the fact that as samples are collected, available synovial tissue in a given area decreases, forcing the operator to sample other sites, which may prove technically difficult, especially in smaller joints. This decreases the chance of retrieving good quality samples, that may also differ in their overall characteristics later in the procedure. The direct implication of this is that collecting synovial tissue for different purposes may be done better simultaneously throughout the procedure, rather than sequentially, in order to avoid obtaining various sets of samples with discordant information.

Our study has several limitations. Despite being conducted in standard clinical practice or specific research setting, we retrospectively retrieved some missing information. Also, in a few clinical practice biopsies, follow-up was not always possible (e.g., inpatients with more severe complications), resulting in incomplete tolerability and safety outcomes assessment. There may be limitations to generalization of biopsies usefulness, considering the heterogeneity of the population and small representation of some joints. Quality and efficacy measures could be influenced by the slight variation in number of samples collected across biopsies, of which only part had different section levels or biobank samples.

In conclusion, synovial biopsy is an important tool for clinical practice and research. US-guided synovial needle biopsies are an effective, safe, well-tolerated mean of collecting good quality synovial tissue that can be performed by rheumatologists with experience in US-guided procedures. Considering the wide access to US in current rheumatology practice, this is an attractive mean to allow synovial tissue collection at a global scale for clinical and research efforts.

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**Author Contributions**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Fonseca had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Romão, Polido-Pereira, Luís, Vieira-Sousa, Humby, Kelly, Pitzalis, Saraiva, Fonseca.

**Acquisition of data.** Romão, Polido-Pereira, Barros, Luís, Vidal, Vitorino, Saraiva, Fonseca.

**Analysis and interpretation of data.** Romão, Fonseca.

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**Tables**

**Table 1** – Baseline diagnosis and biopsy indication and site of patients who had an ultrasound-guided synovial biopsy.

| Baseline diagnosis | N (%) | Biopsy indication | N (%) | Biopsy site | N (%) |
|---|---|---|---|---|---|
| UA | 24 (38) | Chronic monoarthritis | 26 (41) | *Large joint* | 17 (27) |
| *Monoarthritis* | 20 | Acute monoarthritis | 16 (25) | Shoulder | 2 |
| *Oligoarthritis* | 2 | Chronic oligoarthritis | 1 (2) | Elbow | 7 |
| *Polyarthritis* | 2 | Chronic polyarthritis | 3 (5) | Hip | 3 |
| RA | 19 (30) | Flare | 14 (22) | Knee | 5 |
| PsA | 4 (6) | Chronic bursitis | 2 (3) | *Medium joint* | 35 (55) |
| SpA | 2 (3) | Acute bursitis | 1 (2) | Wrist | 26 |
| OA | 4 (6) | Acute tenosynovitis | 1 (2) | Ankle | 9 |
| SLE | 1 (2) | | | *Small joint* | 7 (11) |
| CPPD | 1(2) | | | MCP | 1 |
| Other | 9 (16) | | | MTP | 2 |
| *Sepsis* | 4 | | | PIP | 2 |

| | | | |
|---|---|---|---|
| *Previous SA* | 2 | Naviculocuneiform | 1 |
| *Psoriasis* | 1 | Sternoclavicular | 1 |
| *Femoral AVN* | 1 | *Bursa* | 4 (6) |
| *HCV* | 1 | Subacromial | 4 |
| | | *Tendon sheath* | 1 (2) |
| | | 1st EC wrist | 1 |

AVN, avascular necrosis; CPPD, calcium pyrophosphate deposition disease; Dx, diagnosis; EC, extensor compartment; HCV, hepatitis C virus; MCP, metacarpophalangeal; MTP, metatarsophalangeal; OA, osteoarthritis; PIP, proximal interphalangeal; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SA, septic arthritis; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; UA, undifferentiated arthritis.

**Table 2 –** Adverse events identified during and after ultrasound-guided synovial needle biopsies.

| Adverse event | N (%) |
|---|---|
| **Immediate adverse events** | **6 (9)** |
| - Minor local bleeding | 5 |
| - Transient wrist extensor paresis (radial nerve block from local anaesthesia)[a] | 1 |
| **Other adverse events** | **7 (11)** |
| - Local haematoma[b] | 3 |
| - Mild local inflammation on puncture site[c] | 2 |
| - Mild digit extension limitation | 2 |
| Hand digit 5[c] | 1 |
| Hand digit 1[d] | 1 |

[a]Elbow biopsy; [b]Elbow (2) and ankle biopsies; [c]Wrist biopsy; [d]1st extensor compartment of the wrist tendon sheath.

**Table 3** – Specific biopsy findings and diagnosis established after US-guided biopsy of 19 patients.

| Specific biopsy findings | N (%) | Post-biopsy diagnosis | N (%) |
|---|---|---|---|
| Intense acute synovitis (neutrophil inf.) | 5 (26) | Septic | 8 (42) |
| Crystals | 6 (32) | *Arthritis* | 7 |
| *Calcium pyrophosphate* | 3 | *Bursitis* | 1 |
| *Sodium monourate* | 1 | Microcrystalline | 6 (32) |
| *Basic calcium phosphate* | 2 | *CPPD* | 3 |
| Rheumatoid nodule | 1 (5) | *Gout* | 1 |
| Synovial chondromatosis | 1 (5) | *Basic calcium phosphate*[a] | 2 |
| Foreign body synovitis | 1 (5) | RA | 1 (5) |
| Intense chronic lymphoid synovitis | 1 (5) | PsA | 1 (5) |
| Chronic synovitis compatible with PsA | 1 (5) | Rheumatoid nodule | 1 (5) |
| Positive microbiology culture | 4 (21) | Synovial chondromatosis | 1 (5) |
| *MSSA* | 3 | Foreign body synovitis | 1 (5) |
| *Streptococcus mitis* | 1 | | |

[a]Milwaukee shoulder (n=1) and RA with microcrystalline component (n=1). CPPD, calcium pyrophosphate deposition disease; inf, infiltrate; MSSA, methicillin-sensitive *Staphylococcus aureus*; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

**Table 4** – Analysis of variance of synovium and synovitis features across patients, samples and section levels.

| | | F-statistic | P-value | Variance estimate (95% CI) |
|---|---|---|---|---|
| Synovium | Patient | 4.81 | <0.001 | 0.09 (0.06-0.15) |
| | Sample | 14.39 | <0.001 | 0.09 (0.07-0.11) |
| | Level | - | - | 0.01 (0.01-0.01) |
| Gradable synovium | Patient | 2.48 | <0.001 | 0.05 (0.02-0.10) |
| | Sample | 8.04 | <0.001 | 0.13 (0.10-0.16) |
| | Level | - | - | 0.03 (0.02-0.04) |
| Synovitis score | Patient | 5.99 | <0.001 | 1.40 (0.83-2.33) |
| | Sample | 9.16 | <0.001 | 0.87 (0.66-1.16) |
| | Level | - | - | 0.20 (0.15-0.25) |
| Synovitis grade | Patient | 4.93 | <0.001 | 0.11 (0.07-0.20) |
| | Sample | 4.88 | <0.001 | 0.09 (0.07-0.13) |
| | Level | - | - | 0.04 (0.03-0.06) |
| Pathotype | Patient | 6.49 | <0.001 | 0.49 (0.30-0.80) |
| | Sample | 5.75 | <0.001 | 0.28 (0.21-0.37) |
| | Level | - | - | 0.11 (0.09-0.13) |

F-statistic, p-value and variance estimation of nested analysis of variance (ANOVA) models with presence of synovium/gradable samples (0-1), synovitis score (0-9), synovitis grade (0-2) and pathotype (0-2) as the dependent variables and patient, sample and section levels as independent variables.

**Figures Legends**

**Figure 1** – Tolerability and variation of joint symptoms and US-synovitis following ultrasound-guided synovial biopsies.

(A) Relative frequency (percentage) of patients' classification of immediate post-biopsy tolerability (n=54), discomfort felt during the biopsy (n=54) and likeliness to repeat an ultrasound-guided synovial biopsy (n=51). (B) Patient visual analogue scale (VAS) classification of pain, swelling and stiffness of the biopsied joint before and after the ultrasound-guided synovial biopsy (n=46). **p < 0.01; ***p < 0.001. (C) Ultrasound greyscale and power Doppler synovitis grade of the biopsied joint before and after the ultrasound-guided synovial biopsy (n=41).

**Figure 2 -** Sample quality, synovitis score and pathotype according to timing of sample collection.

(A) Mean (standard deviation) relative frequency of synovial (per total) and gradable (per synovium) samples according to timing of retrieval (earlier or later in the procedure; n=19). *p < 0.05. (B) Mean synovitis score of each individual patient in samples collected earlier or later in the procedure (n=11). (C) Pathotype classification of each biopsy/patient according to timing of retrieval (earlier or later in the procedure; n=19). Each dot represents a biopsy/patient. Green and red squares correspond to concordant and discordant classification between sampling timing, respectively. Kappa coefficient for classification agreement.