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EULAR Points to consider for minimal reporting requirements in synovial tissue research in rheumatology.

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## **Abstract (248 words)**

**Background:** Synovial tissue research has become widely developed in several rheumatology centers, however large discrepancies exist in the way synovial tissue is handled and, more specifically, how data pertaining to biopsy procedure, quality check and experimental results are reported in the literature. This heterogeneity hampers the progress of research in this rapidly expanding field. In that context, under the umbrella of EULAR, we aimed at proposing Points to Consider (PtC) for minimal reporting requirements in synovial tissue research.

**Methods:** Twenty-five members from 10 countries across Europe and USA met virtually to define the key areas needing evaluation and formulating the research questions to inform a systematic literature review (SLR). The results were presented during a 2<sup>nd</sup> virtual meeting where PtC were formulated and agreed.

**Results:** Study design, biopsy procedures, tissue handling, tissue quality control and tissue outcomes (imaging, DNA/RNA analysis and disaggregation) were identified as important aspects for the quality of synovial tissue research. The SLR interrogated 4 databases, retrieved 7654 abstracts and included 26 manuscripts. Three overarching principles and 9 PtC were formulated covering the following areas: description of biopsy procedure, overarching clinical design, patient characteristics, tissue handling and processing, quality control, histopathology, transcriptomic analyses and single-cell technologies.

**Conclusions:** These PtC provide guidance on how research involving synovial tissue should be reported to ensure a better evaluation of results by readers, reviewers and the broader scientific community. We anticipate that these PtC will enable the field to progress in a robust and transparent manner over the coming years.

**KEYWORDS:** synovial tissue, synovial biopsy, translational research, synovium, ultrasound guided synovial biopsy, arthroscopic biopsy, recommendation, task force

## **KEY MESSAGES**

### What is already known about this subject?

- Synovial tissue research is increasing in the Rheumatology community.
- Significant heterogeneity in patient recruitment, biopsy procedure, tissue sampling, handling, analysis and methods of reporting exists in published articles.

### What does this study add?

- These are the first EULAR–endorsed ‘points to consider’ (PtC) providing a framework to optimise the way research involving synovial tissue is reported in the scientific literature.
- This article highlights areas for improvements and suggests items that should be reported by authors for every study involving synovial tissue, including histopathological analysis, transcriptomic analysis and single-cell analysis.

### How might this impact on research practice?

- It is anticipated that researchers in the field of synovial tissue, reviewers and journals editors will use these PtC while writing and reviewing scientific manuscripts.
- These PtC will also serve the broader scientific community while assessing the reliability and generalisability of literature in the field of synovial tissue research.

**WORD COUNT: 3001**

## **Introduction**

Analyses of synovial tissue (ST) at both cellular and molecular levels offer a promising approach for personalized therapy in rheumatic diseases. ST analysis may also advance understanding of disease pathophysiological mechanisms and permit identification of potential therapeutic targets.[1–3] Moreover, new developments in single cell methodologies are driving innovation and demand for ST-based studies.[4–6] Methods to obtain ST, namely synovial biopsy (SB) procedures, are becoming more acceptable to patients and have been performed with increasing frequency over recent years for both clinical and translational research purposes. This is due in part to the introduction of ultrasound guidance enabling minimally invasive approaches that have now been extensively validated in terms of safety, tolerability and tissue yield.[7–11]

However, the recent increase in numbers of studies using ST as a source of scientific material also raises questions in terms of interpretability and generalizability. Previous efforts have been initiated by the Outcome Measures in Rheumatology (OMERACT) group and the European Alliance of Associations for Rheumatology (EULAR) Synovitis Study Group (ESSG) in providing guidance on harmonization of synovial tissue analysis procedures across centers for both clinical practice and research.[12–16] Nevertheless, minimal requirements for reporting of SB procedures and handling methods of ST remain to be defined. Both reliability and validity of results in the field rely critically on tissue quality and processing. Moreover, selection of patients, methods of retrieval (as well as location within the joint), experience of the operator, handling and analysis methods, and quality of the tissue have potential to affect the final research outcome. Therefore, there is an unmet need for evidence and consensus-based points to consider defining minimum reporting requirements that could ensure interpretability of the research. Complete and accurate reporting will allow the reader to detect potential biases in the study (internal validity) and to assess the generalizability and applicability of the results (external validity). In this context, the aim of this work was to formulate the EULAR Points to Consider (PtC) for minimal reporting requirements in synovial tissue clinical practice and research in rheumatology.

## **Methods**

From December 2020 to May 2021, a steering committee composed of the conveners (AN - also fellow - and AF) and the senior and junior methodologists (MADA and FC) led a multidisciplinary task force

following the 2014 updated EULAR standardized operating procedures (SOPs).[17] The task force included in total 25 members (including the steering committee) from 10 countries, composed of 19 rheumatologists (two of them also representing the Emerging EULAR Network [EMEUNET]), 1 translational immunologist and 2 pathologists alongside one allied health professional and two patient representatives. Two virtual meetings of the task force were held, one in December 2020 and one in May 2021. During the first task force meeting, research questions pertaining to the project were formulated. The fellow (AN), guided by the methodologists, performed an SLR, gathering articles on ST biopsy procedures, their tolerance and outcomes, tissue handling and randomization, tissue quality control and tissue outcomes. The SLR is published separately, and it forms an integral part of the project.

During the second task force meeting, the results of the SLR were presented and discussed, leading to the formulation of PtC based on evidence and expert opinion. Every statement was presented, iteratively discussed and voted on (informal voting). The level of evidence (LoE) supporting each statement was assigned according to the Oxford Centre for Evidence Based Medicine 2011 Levels of Evidence.[18] Of note, a level of evidence of 5 corresponds to Expert opinion and a level of evidence of 4 corresponds to case-control studies. Finally, each task force member anonymously indicated their level of agreement (LoA) with each PtC online (numerical rating scale ranging from 0=“completely disagree” to 10=“completely agree”). The aspects emerging during discussion that required further evidence were integrated in the research agenda. All these steps are summarized in Figure 1. The final manuscript was reviewed and approved by all task force members, followed by verification by the EULAR Executive Committee.

## **Results**

Three overarching principles (OP) and 9 PtC were formulated (Table 1). All OP and PtC were approved after one round of hand raised voting during the task force meeting and one round of online voting after the task force meeting. The mean LoAs were higher or equal to 9, with a percentage of votes above 8/10 of 100% for most of the OP and PtC. LoA are reported in Table 1. The LoE was 4 or 5 for all PtCs. The PtC are intended to provide guidance on how research involving ST should be reported in the following areas: biopsy procedures, study design, patients and disease characteristics, handling and processing methods of tissue, quality control, histological analysis, molecular analysis and single cell technologies. The target population was identified as rheumatologists, pathologists and scientists (for example, computational biologists, translational immunologists,



molecular scientists), using or involved in research on ST. The target users were defined as physicians and allied health professionals (eg., physiotherapists and specialist nurses), patient research partners and patient charities and organizations, reviewers, journal editors, scientific societies and OMERACT, pharmaceutical industry, biopsy device manufacturers, and the enhancing the quality and transparency of health research (EQUATOR) network.

### ***Overarching principles***

*OP-1) Synovial biopsies (single and sequential), performed in aseptic conditions, are safe, well-tolerated and can be performed for both clinical and research purposes.*

SB are performed in both clinical and research settings across numerous centers in Europe. The body of evidence suggesting that the technique is safe and well-tolerated has grown over the years, and the safety of the procedure is now well-established.[7,8,10,11,19–23] The task force emphasized that this applies to both single and sequential biopsies.[11,20]

*OP-2) In both clinical and research settings, synovial biopsies should be guided by imaging techniques. Arthroscopy and ultrasonography are the preferred techniques to guide synovial biopsies.*

The task force strongly felt that SB should no longer be performed without imaging guidance. This is justified by the fact that blind needle biopsy procedures retrieve less graded tissue than guided techniques.[24] The most commonly used imaging techniques to guide synovial biopsies are ultrasound-guided needle biopsy (US-NB), ultrasound-guided portal and forceps (US-P&F) and arthroscopy. Computed tomography (CT) and Magnetic Resonance Imaging (MRI) guidance are not used commonly and therefore cannot be recommended.

*OP-3) Ultrasound or arthroscopy can be used to guide the synovial biopsy without affecting the tolerability of the procedure or the minimal required tissue for meaningful analysis.*

While the number of graded ST fragments/total number of ST fragments does not differ with ultrasound and arthroscopic guided biopsy, the quantity and quality of RNA retrieved was superior with arthroscopy in a study comparing the tissue outputs of different techniques.[24] Nevertheless, all techniques allow retrieval of a sufficient

quantity of ST for meaningful analysis. Short- and long-term tolerance is satisfactory with all guided techniques in terms of Visual Analog Scale (VAS) pain, swelling and stiffness for both small and large joints, with no difference reported between techniques in a study of over 500 procedures.[11]

***Points to consider***

*PtC-1) The details of the biopsy procedure should be reported in every study. This should include non-exhaustively:*

*-Exclusion criteria for biopsy*

*-Target joint(s) and recess*

*-Intra-articular steroids in the previous 4 weeks or during the procedure*

*-Technique used (type and size of biopsy retrieval device)*

*-Machine/probe for ultrasound guided biopsies, arthroscopic equipment*

*-Adverse events*

*-Operator's experience and training.*

Among the 26 manuscripts retrieved by the supporting SLR reporting on biopsy procedures, details of the procedure were very heterogeneously reported. For instance, exclusion criteria for biopsy and intra-articular treatments in the previous 4 weeks or during the procedure (intra-articular steroids) were reported in less than 10% of the manuscripts, while the target joint(s) and recess, technique used, and equipment were more frequently reported (>75% of included studies). Adverse events were reported in only 20% of the manuscripts and none reported operator's experience.

Based on these results, the task force developed a non-exhaustive list of elements pertaining to the procedure itself that should be mentioned in every study involving SB. Although minimal training requirements for SB are not yet defined, operator's experience and training should be reported in every study. Of note, a standardized training model for ultrasound-guided, minimally-invasive SB procedures in large and small joints constitutes another EULAR task force initiative.[25] In addition, depending on the study design, a description of patient tolerability of the procedure is desirable.

*PtC-2). Overarching clinical study design, including aspects related to participant disease characteristics and treatments, must be defined in order to evaluate the generalisability and validity of the outcome.*

This point refers to the study design, defined *a priori* when the study framework is elaborated by authors. It is known that treatments and disease phenotype can affect ST outcomes in terms of histopathology and transcriptomics especially in inflammatory arthritis.[1,4,26–32] Therefore, the task force recommends that aspects pertaining to study design, including participants, disease characteristics (including fulfillment of classification criteria) and treatments, should always be reported in manuscripts.

*PtC-3). Conventional patient disease activity measures, disease stage and treatment should be described in order to evaluate the generalisability and validity of the outcome.*

This point refers specifically to outcome measures and characteristics of the patients included in the study that should always be reported. In the SLR, 100% of studies reported patient demographics and diagnosis, but only 62% reported clinical data, such as disease activity and current therapy. More specifically, disease activity measures should be outlined, including disease activity score 28 (DAS 28) CRP or ESR, clinical disease activity index (CDAI) or simple disease activity index (SDAI) for rheumatoid arthritis or other measures depending on the rheumatic disease under evaluation. Disease duration and conventional synthetic, targeted synthetic or biologic disease-modifying anti-rheumatic drugs (DMARDs) should be reported.

*PtC-4). Clinical and contemporary imaging characteristics of the biopsied joints should be described in order to evaluate the generalisability and validity of the outcome.*

Firstly, the task force felt that clinical assessment of the biopsied joint including swelling should be reported. In the context of ultrasound-guided SB, the ultrasound synovitis grade of the target joint is typically assessed. Surprisingly, these data were described in only 36% of the manuscripts describing ultrasound-guided SB in the SLR. The task force emphasized that the ultrasound grade of the synovitis in B mode and Doppler can affect tissue quality and outcomes [7,33,34] and therefore recommends that contemporary imaging characteristics of the biopsied joint are described. In addition, when available, radiographic aspects should be described and when the erosive status of the biopsied joint is known, this information should be provided.

*PtC-5). Tissue handling and processing methods must be described in order to ensure reproducibility, including numbers and size of fragments allocated to each analytic.*

Our SLR retrieved numerous studies looking into intra-articular variability of tissue outcomes and sampling error.[34–40] More specifically, immune cell infiltrate, immunohistochemistry, cytokine mRNA and T cell repertoire displayed little or no difference when retrieved in different parts of large joints.[37–39]

Of interest, studies assessing sampling error showed that a minimum of 4 tissue fragments provided a reliable sample with 10% sampling error for small joint histopathological analysis [34], while 4 to 7 tissue fragments were required to detect a 2 fold change with a 25% sampling error in PCR in large joints [35] and the percentage mean difference for the staining of immunohistochemical cellular markers decreases below  $\pm 10\%$  when a minimum of eight samples are considered in the evaluation.[40] In addition, a minimum of 6 fragments and the assessment of an area of tissue of minimum  $2.5\text{mm}^2$  were deemed necessary to ensure representativity of histological analysis.[41,42] In this context, the task force recommends that authors report data pertaining to tissue handling and processing, including numbers and size of fragments allocated to each analytic.

*PtC-6). Method and results of tissue quality assessment should be reported, including the percentage of graded tissue.*

Among the 26 studies included in the SLR, only 17 reported having controlled the tissue quality during their study (65%). The task force felt it was absolutely necessary for a quality control to be performed and reported by authors in manuscripts in order to ensure reliability and reproducibility of the results. When histopathological analysis is performed, the percentage of tissue presenting with a typical ST structure and an intact lining layer or positive CD68 staining should be reported.[7,15,16]

*PtC-7). When histological or immunohistological analysis is performed, the scoring or analysis system should be defined including:*

- Representative images*
- Reference to original publication for validated scoring systems only*
- Digital analysis software, including version numbers of platforms used.*
- Immunohistological staining protocol, including antibody sources and clones*
- Area assessed and sampling strategy*
- Number of observers and intra- and inter-observer variability.*

Since numerous studies assess histological aspects of the tissue, it was felt important by the task force to formulate a PtC related to histological scoring. More specifically, several aspects were deemed mandatory by the task force, such as describing staining protocols and antibodies sources and clones, providing representative images illustrating the findings and describing area assessed and sampling strategy. In the manuscripts included in the SLR, scoring systems were rarely described and chains of references to previous publications, but not the original scoring system, were often observed. Subsequently, the task force recommended that only the original publication describing the scoring system should be cited. The inter- and intra- observer variability for histological analysis was similarly rarely described (n=4/26 publications, 15%) and should always be reported for studies using scoring by observers.

*PtC-8). Methods of extraction and quantification should be defined and purity, quantity and quality of DNA/RNA should be reported.*

Although no study specifically assessed the difference in outcomes arising from tissues yielding DNA or RNA of high versus poor quality (measured by the RNA integrity number (RIN) for RNA), it was considered important by the task force members that such information should be reported in every manuscript. It is indeed anticipated that poor quality RNA, if used for RNA sequencing, will provide unreliable results. In addition, it has been noted in the SLR that such information was very rarely reported in the analyzed publications (2 out 8 publications looking at molecular aspects of ST (25%)).

*PtC-9). In case of single cell analysis, methods used and quality outcomes should be detailed, including:*

*-Methods of tissue or cell preservation*

*-Methods of tissue dissociation*

*-Percentage of viable cells recovered or analyzed*

*-Percentage of mitochondrial gene expression seen in the sequenced cells and the threshold chosen for analysis*

*-If sorting is used, the strategy used and purity of sorted cells*

The recent development of single cell technologies has also raised methodological challenges. Of interest, the methods of tissue conservation or dissociation can influence the tissue outcome. In a study from Donlin et al., mechanical versus mechanical and enzymatic ST dissociation methods have been compared, showing that the

latter retrieved a higher total cell count per gram of tissue, a higher viable cell count and a more representative number of cell subpopulations. In addition, they compared methods of tissue preservation, showing that cryopreserved samples retrieved similar numbers of viable cells and a similar variety of cell subpopulations to fresh samples.[36] Therefore, the task force recommends that these elements are reported in every publication, in addition to other aspects related to quality, such as the percentage of viable cells and percentage of mitochondrial gene expression seen in the sequenced cells alongside parameters related to cell sorting methods, including flow cytometric staining protocols.

## **Discussion**

These are the first EULAR-endorsed PtC on ST research in rheumatology with the aim of them serving as a reference and checklist for clinicians and scientists involved in publishing, reviewing and reading manuscripts reporting ST research. They have been proposed by a multidisciplinary team of international experts in the field involving rheumatologists, translational researchers, methodologists and pathologists.

Our SLR retrieved several manuscripts, which were analyzed from different perspectives. With respect to the overarching principles, we emphasized that the body of evidence on SB tolerability and safety is very reassuring for both single and sequential biopsies. In addition, the task force stated that SB should no longer be performed without imaging guidance, more specifically blind needle biopsies are no longer recommended. There is no preferred guiding technique between ultrasound or arthroscopy, since both allow retrieval of a sufficient quantity of tissue for meaningful analysis and are well tolerated.[11,24] Of interest, we assessed how comprehensively data relevant to study design, patients' characteristics, biopsy procedures, tissue handling, quality control and tissue outcomes were reported in the publications. In this regard, these PtC focus on specific areas requiring attention from authors when reporting their study results in order to ensure internal and external validity of the studies and generalizability. These PtCs are also proposed in an editor friendly document appearing as a checklist and provided in Supplementary Material 1.

While conducting this work, we realized that the paucity of literature on clinical applications of tissue analysis did not allow the formulation of PtC dedicated to the clinical aspects. Indeed, although recent publications propose encouraging data for the use of SB for diagnosis, outcome prediction or disease management in clinical practice, the task force felt that these aspects should be included in the research agenda.[2,4,43–45] One major limitation encountered in the development of these PtC was the scarcity of literature appraising the practical aspects of tissue

retrieval, handling and analysis. Due to the paucity of evidence comparing methods or outcomes based on tissue handling, quality or analysis, most of these PtC rely on expert opinion. In this respect, it is noteworthy that the members of this task force acted as representatives of the most prominent centers working in the field of translational research in ST, including EULAR centers of excellence. Based on the SLR results and the inputs and discussion arising from the second task force meeting, other relevant items were incorporated in the research agenda (Table 2).

In conclusion, these EULAR PtCs provide relevant guidance on minimal reporting requirements in ST research in Rheumatology. These first EULAR PtCs are intended to be disseminated and used by the broad research community, adding to previous initiatives from OMERACT and ESSG in order to allow the field of ST research to evolve in a robust and transparent manner in the future.

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**Table 1. Overarching principles and points to consider for minimal reporting requirements in synovial tissue clinical practice and research in rheumatology, with levels of evidence (LoE) and levels of agreement (LoA).**

<b>Overarching principles</b>	<b>LoA mean (SD); % of votes ≥8/10</b>
1. Synovial biopsies (single and sequential), performed in aseptic conditions, are safe, well-tolerated and can be performed for both clinical and research purposes.	<b>9.77 (0.53), 100%</b>
2. In both clinical and research settings, synovial biopsies should be guided by imaging techniques. Arthroscopy and ultrasound are the preferred techniques to guide synovial biopsies.	<b>9.71 (0.56), 100%</b>
3. Ultrasound or arthroscopy can be used to guide the synovial biopsy without affecting the tolerability of the procedure or the minimal required tissue for meaningful analysis.	<b>9.14 (0.96), 83.6%</b>
<b>Points to consider</b>	
1. The details of the biopsy procedure should be reported in every study. This should include non-exhaustively: -Exclusion criteria for biopsy -Target joint(s) and recess -Intra-articular steroids in the previous 4 weeks or during the procedure -Technique used (type and size of biopsy retrieval device) -Machine/probe for ultrasound guided biopsies and arthroscopic equipment -Adverse events -Operator’s experience and training (noting that no minimal training requirements are yet defined). (LoE 5)	<b>9.38 (0.80), 100%</b>
2. Overarching clinical study design, including aspects related to participant disease characteristics and treatments, must be defined in order to evaluate the generalizability and validity of the outcome. (LoE 5)	<b>9.81 (0.51), 100%</b>
3. Conventional patient disease activity measures, disease stage and treatment should be described in order to evaluate the generalizability and validity of the outcome. (LoE 5)	<b>9.45 (1.19), 95%</b>
4. Clinical and contemporary imaging characteristics of the biopsied joints should be described in order to evaluate the generalizability and validity of the outcome. (LoE 4)	<b>8.95 (1.28), 90.5%</b>

5. Tissue handling and processing methods must be described in order to ensure reproducibility, including numbers and size of fragments allocated randomly to each analytic. (LoE 4)	<b>9.10 (1.64), 90.5%</b>
6. Method and results of tissue quality assessment should be reported, including the percentage of graded tissue. (LoE 5)	<b>9.33 (1.06), 90.5%</b>
7. When histological or immunohistological analysis is performed, the scoring or analysis system should be defined including: -Representative images -Reference to original publication for validated scoring systems only. -Digital analysis software used, including version numbers of platforms. -Immunohistological staining protocol, including antibody sources and clones -Area assessed and sampling strategy -Number of observers and intra- and inter-observer variability. (LoE 5)	<b>9.48 (0.75), 100%</b>
8. Methods of extraction and quantification should be defined, and purity, quantity and quality of DNA/RNA should be reported (LoE 5).	<b>9.67 (0.58), 100%</b>
9. In case of single cell analysis, methods used and quality outcomes should be detailed, including: -Methods of tissue or cell preservation -Methods of tissue dissociation -Percentage of viable cells -Percentage of mitochondrial gene expression seen in the sequenced cells and the threshold chosen for analysis -If sorting is used, the strategy used and purity of sorted cells (LoE 4)	<b>9.71 (0.56), 100%</b>

**Table 2. Research agenda**

<b>Research agenda</b>
Minimal training requirement for ultrasound guided SB for physicians and allied health professionals
Impact of training on patient tolerability; tissue yield/quality/outcomes.
Influence of tissue handling and processing (fixation, SOPs for freezing in optimal cutting temperature (OCT), fixation, and freezing (and time to freezing) for subsequent live tissue/cell analysis) on tissue quality/outcome.
Clinical practice: supportive data for diagnosis or prognosis or disease management
Risk of Bias tools for translational research
Tissues considered as best “non-inflammatory” controls (e.g., healthy subjects, meniscectomy surgery, traumatic arthritis, cadavers).

## LEGENDS

Figure 1: Project framework

Table 1: Overarching principles and points to consider for minimal reporting requirements in synovial tissue clinical practice and research in rheumatology, with levels of evidence (LoE) and levels of agreement (LoA).

Table 2: Research agenda

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