SYNOVIAL TISSUE: TURNING THE PAGE TO PRECISION MEDICINE IN ARTHRITIS?

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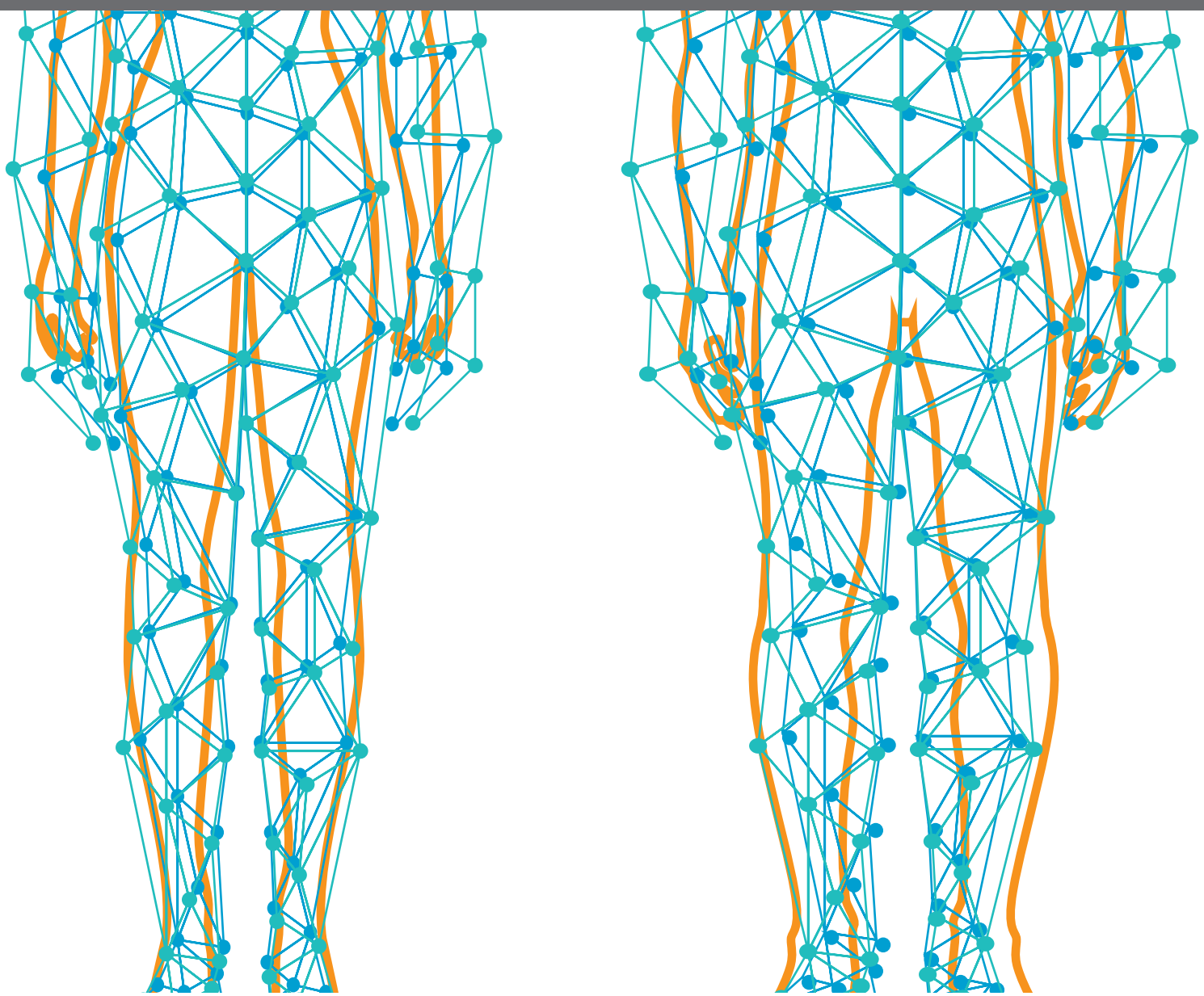

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SYNOVIAL TISSUE: TURNING THE PAGE TO PRECISION MEDICINE IN ARTHRITIS?

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Editorial: Synovial Tissue: Turning the Page to Precision Medicine in Arthritis?

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Editorial on the Research Topic

Synovial Tissue: Turning the Page to Precision Medicine in Arthritis?

It is with great pleasure that we present in this article collection, a timely overview of the rapidly developing field of synovial tissue analysis. Some of the most prominent protagonists in the field have contributed, and the collection walks the reader through everything from the history of the field's development, to technical aspects of sampling, providing an update on the science and clinical applications, as well as discussing potential future perspectives.

A broad consensus exists amongst clinicians and scientists, that a patient-centred, precision medicine approach holds the most promise to improve patient outcomes. The relevance of synovial biopsies in achieving this end is a major theme of this article collection. We are currently at an exciting juncture in this important field. This collection not only discusses the enormous potential of synovial tissue as a research and clinical tool, but also the many challenges in advancing its role in translational and clinical applications. Several key advancements concerning synovial biopsies over the last number of years have together contributed to the rheumatology community discussing in earnest how such sampling can contribute to precision medicine in arthritis.

In the first instance, the technical feasibility, the safety, and the patient tolerability of the procedures used to retrieve the tissue have been extensively studied, and there is now wide acknowledgement that these procedures carry minimal risk and are well-tolerated. Veale provides a historical perspective and overview of synovial biopsy research to date. Four key points emerge, of which arguably the most important concerns the safety of the procedures utilised to sample the synovium; whether ultrasound (US) or arthroscopically guided. Complication rates

ranging from 0.4 to 0.9% are reported, and most are not serious. The author outlines the overarching objectives of synovial biopsy research- namely to identify predictors of the development of arthritis, predictors of response to treatment, as well as to objectively measure disease activity and response to treatment. It is widely recognised that realising these objectives will depend on the identification of reliable biomarkers, which will allow for better patient stratification, improved therapies, and the development of new therapeutic targets. Together, these developments will lead to a more precise approach to treatment.

Ingegnoli et al. provide a comprehensive and practical guide to selecting candidates for synovial biopsy. The authors contextualise the current and potential future role for tissue analysis in both the research and the clinical setting. In their contribution, De Bellefon and Lazarou outline the principles underlying US guided biopsy, many of which are immediately relevant to other modalities of synovial biopsy. They provide a comprehensive overview of the two commonly performed US guided techniques, portal and forceps and semi-automatic guillotine biopsy needle. Specific emphasis is placed on peri-procedural anti-sepsis, and they present a step-by step guide, including helpful graphics, for these approaches to sampling. They also present data on some long-debated questions such as assuring the safety of intra-articular steroid after the procedure and the association of lignocaine with chondrolysis.

The technique for US guided biopsy is expanded upon by Polido-Pereira, with a specific focus on medium and large joints, as well as discussing the differences between the two sonographic methodologies. The author points out that it is possible to biopsy nearly all synovial joints. In this review, techniques for biopsying the shoulder, elbow, hip, knee and tibiotalar joint, are described using descriptive anatomy and appropriate visual aids. The author notes that the grade of grayscale synovitis is the most important determinant for synovial tissue yield. It is interesting to note that this review advises that 12 biopsies should be the minimum number to be taken to ensure representative sampling. Others have contested that 6 to 8 might suffice, pointing to evidence that this allows for reliable scores for T-cell infiltration with a variance of $<$10%, as well as a less than two-fold difference in gene expression as quantified by PCR (1, 2). This highlights one of the ongoing challenges in the field, namely achieving consensus on standardisation. A higher number of biopsies may be more practical in the large joints focused on in this article. The author notes that there remains a lack of standardisation of techniques for biopsy procedures, and we will later see how this lack of standardisation extends to tissue handling and processing, as well as to reporting in manuscripts. Risks are discussed in general, as well as relevant joint-specific risks, and again these are shown to be acceptable. Lazarou et al. provide a review for US guided biopsy of the small joints, including wrist, MCPs and MTPs, and they also discuss biopsy of a tendon sheath.

Orr et al. describe the technique used for arthroscopically guided synovial biopsies. They provide a comprehensive, step by step explanation of the procedure itself, as well as a discussion regarding the available safety data Orr et al. They describe how arthroscopy represented a pioneering approach in researching inflammatory arthritis, and will continue to complement sonographically guided approaches.

Another major advancement in synovial tissue research has been the increasing accessibility in facilitating the sampling itself. When compared to what has been considered the gold standard procedure for retrieved samples, arthroscopy, US guided procedures have been shown to be less expensive, to yield similar quality tissue, to require less training, and to be more suitable to biopsy small joints (often preferentially involved in rheumatoid arthritis). Humby provides a historical framework to the field, beginning with the first "blind" biopsies by Forestier in the 1930's. The paper discusses in detail the differences between tissue retrieved from arthroscopically guided, as well as the two US guided techniques, outlining the advantages and challenges associated with each. Although there remains robust debate, depending on the quantity of tissue required, whether obtaining lining layer is important, the clinical or research question being addressed, and the joint involved, there is broad consensus that either approach is acceptable in the clinical or research setting. In addition, the attention of the reader is once again drawn to the many studies confirming acceptable safety and tolerability, regardless of technique. Humby also introduces two major international multicentre clinical trials, R4RA and Stratification of Biologic Therapies for RA by Pathobiology, each investigating the role of synovial biopsy in realising precision medicine for rheumatoid arthritis (RA).

Smits et al. discuss the current landscape of synovial sampling to describe to what extent clinical implementation is possible today. They identify that the successful acquisition of synovial tissue is operator dependant, and that although it is obvious that skills need to be retained by regular performance, no minimum requirement in this respect is currently known. They delve into the issues regarding quality assurance and standardisation in all respects of synovial biopsy research, which have been alluded to above. These matters have been the subject of recent intensive and ongoing attempts to harmonise. Synovial biopsy is rarely used in the differential diagnosis of inflammatory arthritis, and it is not entirely clear in which circumstances a synovial biopsy may aid in diagnosis. One definite sub-group that may benefit are those where infective causes are high on the differential, but the synovial fluid has not revealed an organism, or where a non-inflammatory cause for synovial hyperplasia is being considered. The review concludes by articulating the barriers to advancing to more widespread clinical implementation, including in the first instance, making a determination as to what the best quality control to ensure that synovial instead of other joint tissue is acquired.

Manzo et al. also consider the current role of synovial biopsy in the clinics, but they also look towards the objective of being able to stratify individuals with inflammatory arthritis both within and across varying diseases. The authors explain, "One of the most compelling working hypothesis is that the cellular/molecular patho-biology of the inflamed synovial membrane might delineate specific discriminative traits able to improve early diagnosis of

undifferentiated forms and patients' stratification into treatment-specific response groups…If differences in the synovial characteristics can be captured between different clinical entities, a cutting-edge question is whether clinically relevant differences can be reliably distinguished also within the same disease, a fundamental premise to conceive the possible integration of synovial biopsy into a precision medicine algorithm."

While Manzo et al. articulate the hopes for synovial biopsy research to pave a way forward to precision medicine for those with inflammatory arthritis, they also provide an honest account of where we currently are and what challenges remain. There are some "circumstantial" data currently available to support the use of synovial biopsy in the inflammatory arthritis clinical setting, but the discriminative power of this tool to accurately diagnose and prognosticate, as well as to point to a candidate target, remains unproven. Furthermore, they suggest that a stratified approach rather than truly precise approach may hold more promise in the short or medium term.

Arguably, the last number of years have seen the most rapid advances in our understanding of synovial pathobiology, and more precisely, our ability to start considering how to stratify within diseases using these novel insights. Initial attempts were made to achieve this using traditional histopathological observation with important clinical correlates. Later the clinical correlates were related to whole tissue gene expression profiling, with both microarrays and next generation RNA sequencing. Laser capture microdissection and technologies for single cell analysis, are starting to contribute to the effort to stratify disease subtypes. Discrete phenotypes recognisable by varying synovial "signatures," are now well-described.

In relation to these transcriptomic technologies, Carr et al. detail their development and application, with a particular emphasis on studying fibroblast sub-populations in the synovium (3). The authors explain the initial use of PCR to probe for specific gene expression, which requires pre-identified genes. The ability to test for thousands of genes was provided by cDNA microarrays, and this opened the door to more discovery-based, as distinct from hypothesis-based research. Most recently, RNA sequencing technology has been developed, where the entire transcriptome can be analysed, negating the need to examine specific targeted genes. The potential opportunities as well as caveats are discussed. In addition to the high-throughput transcriptomic technology and methods, Carr et al. discuss how advancements in separating cell subtypes within the joint can be combined with RNA sequencing, to give novel insights into pathobiological processes. One obvious benefit to this approach of separating cell subsets from within the joint rather than analysing whole tissue, is the reduction in the potential to miss subtle but important gene expression signatures from important but numerically few cell subtypes, an observation also made by Triaille and Lauwerys.

Undoubtedly one of the most exciting developments in therapeutics for inflammatory arthritis over the last decade has been the advent of agents targeting the IL-17 pathway. Robert and Miossec discuss IL-17 pathobiology in the joint, examining the role of IL-17 in cartilage and joint destruction, neoangiogenesis, and synergistic effects with TNF alpha. The clinical effects of the various targeted IL-17 therapies in clinical trials are discussed, and possible explanations for the conflicting findings are considered. While accepting that there are "mixed" results in the clinic to targeting IL-17 in RA, they suggest that it may be possible to identify a subset of RA patients, possibly through synovial biopsy analysis, for whom IL-17 is a relevant target. Celis et al. review the synovial biopsy observations in psoriatic arthritis, noting the many similarities with RA, but examining what may be learnt from the differences. They also examine the importance of the role of IL-17, and the pathways associated with it.

A significant challenge is the heterogeneity of the RA phenotype, and this too is reflected in studies of the RA synovium. In the contributions of both Ouboussad et al. as well as Triaille and Lauwerys, this heterogeneity, and the relevance of this to RA research and clinical therapeutics is discussed in detail. Ouboussard et al. provide a review of the effects of biologics and targeted therapies on the synovium, as well as examining synovial predictive markers of response to these therapies.

Triaille and Lauwerys discuss the complexity and challenges of synovial biopsy research to date. They identify some of the key limitations of the data thus far available, including the poor stratification of patients enrolled in synovial biopsy studies, the limited numbers of biopsies performed, as well as the use of retrospective material for research. To further complicate interpreting the data so far collected, the authors draw attention to the plasticity of the RA synovium, varying according to disease duration, serological status, treatment, and, most obviously, disease activity. They also call into question the concept of discrete interpretation of the myeloid and lymphoid pathotypes and suggest a degree of interdependence, offering intriguing evidence to support this viewpoint. The authors hypothesise that a poor response to a specific targeted therapy could either represent an absence of that pathway in the inflamed synovium, or simply disease severity, expressed by several active pathways, overwhelming a highly specific, targeted approach to treatment.

An article collection focussing on synovial sampling and its relevance to precision medicine in arthritis has never been more apt. The last number of years have seen significant advances in our knowledge of synovial pathobiology. New technologies and investigative techniques will likely see this advance further. Despite progress to date, significant challenges remain, and the articles assembled here reflect this reality. The community interested in synovial sampling has never been so large, and the efforts to achieve standardisation never so intense. The largest ever international collaborations utilising synovial biopsies are hopefully about to bear fruit. There is much cause for optimism that we really are about to turn the page to precision medicine in arthritis.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REVIEW
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IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels

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Interleukin (IL)-17A has a direct contribution in early induction and late chronic stages of various inflammatory diseases. *In vitro* and *in vivo* experiments have first characterized its local effects on different cell types and then its systemic effects. For instance, IL-17 axis is now identified as a key driver of psoriasis through its effects on keratinocytes. Similar observations apply for rheumatoid arthritis (RA) where IL-17A triggers changes in the synovium that lead to synovitis and maintain local inflammation. These results have prompted the development of biologics to target this cytokine. However, while convincing studies are reported on the efficacy of IL-17 inhibitors in psoriasis, there are conflicting results in RA. Patient heterogeneity but also the involvement of mediators that regulate IL-17 function may explain these results. Therefore, new tools and concepts are required to identify patients that could benefit from these IL-17 targeted therapies in RA and the development of predictive biomarkers of response has started with the emergence of various bioassays. Current strategies are also focusing on synovial biopsies that may be used to stratify patients. From local to systemic levels, new approaches are developing and move the field of RA management into the era of precision medicine.

Keywords: synovitis, rheumatoid arthritis, interleukin-17, interleukin-17 inhibition, precision medicine

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INTRODUCTION

Interleukin (IL)-17A is a pro-inflammatory cytokine that contributes to the pathogenesis of several auto-immune and inflammatory diseases (1). *In vitro* and *in vivo* experiments have identified IL-17 effects on various cell types explaining its involvement in early induction and late chronic stages of many diseases. For instance, IL-17A acts on keratinocytes to induce the expression of several chemokines leading to the recruitment of immune cells that characterized psoriasis (2). Furthermore, in rheumatoid arthritis (RA), the most prevalent chronic inflammatory disease (3), IL-17A acts locally on synoviocytes and osteoblasts contributing to synovitis and joint destruction (4, 5).

Abbreviations: IL, interleukin; RA, rheumatoid arthritis; Th, T-helper; ROR, retinoic acid receptor-related orphan receptor; IL-17R, IL-17 receptor; TNF, tumor necrosis factor; NF-κB, nuclear factor-κ B; OA, osteoarthritis; LIF, leukemia inhibitory factor; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of MMP; RANKL, receptor activator of NF-κB ligand; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN, interferon; sIL-17R, soluble IL-17R; ACR, American College of Rheumatology.

These observations have prompted the development of biologics targeting IL-17A and various strategies are currently being tested (2). In psoriasis, inhibitors of IL-17A axis bring a clear benefit in patient care management. Among diseases affecting joints, IL-17 inhibitors are effective in active ankylosing spondylitis and psoriatic arthritis, whereas conflicting results are reported for RA with a high degree of heterogeneity in response (6–9). To potentiate the use of such therapies in RA, an effort is needed to precisely identify patients that would respond to IL-17A inhibition. Current strategies are focusing on the development of biomarkers (5, 10) but also on synovial biopsies (11) to explain patient heterogeneity and treatment response.

The present review discusses the effects of IL-17A on synovium, its regulation and current strategies to detect bioactive IL-17A. Regarding the role of IL-17 in RA pathogenesis, these observations emphasize that this cytokine and its inhibitors should now be considered in the development of precision medicine in RA.

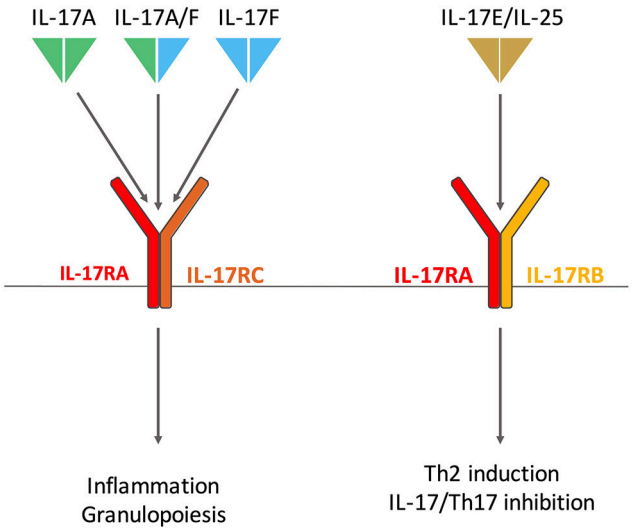

FIGURE 1 | Interleukin (IL)-17 and Receptor family involved in rheumatoid arthritis. IL-17A and IL-17F homodimers and the IL-17A/IL-17F heterodimer bind the same receptor composed of IL-17RA and IL-17RC subunits. IL-17RA is also involved in IL-17E (also known as IL-25) receptor with IL-17RB. IL-17A/F and IL-17E have distinct biological effects, the first triggers inflammation and granulopoiesis; the latter promotes T-helper (Th) 2 responses in host defense against parasites and allergy. IL-17E also regulates Th17 inflammatory response.

IL-17 AND SYNOVITIS

The IL-17 Family

IL-17A, IL-17F, and IL-17E

The IL-17 family is composed of six members: IL-17A to IL-17F. The IL-17A was the first isoform discovered in 1993. Initially described as cytotoxic T lymphocyte-associated antigen 8, a product of T cells in rodents, the effects of human IL-17A were then characterized (12, 13). One of its earliest documented biological activities was its effects on RA synoviocytes (14). Then, it was shown that this cytokine promotes granulopoiesis and protects the host against bacterial and fungal infections (1).

Among the IL-17 family, IL-17A and IL-17F share the greatest homology with a 50% sequence identity and can be secreted as homodimer or heterodimer (15, 16). Many of the effects of IL-17A and IL-17F are found similar even if IL-17F is usually less active at inducing inflammation (1).

Conversely, IL-17E (also known as IL-25) has the lowest homology with IL-17A with only 20% sequence identity (17). IL-17E is a mediator of T-helper (Th) 2 cell responses especially in host defense against parasites (18) and allergy (19). In addition, it also regulates Th17 inflammatory response and IL-17 function (20) (**Figure 1**).

IL-17 Producing Cells

The first cellular source of IL-17 was identified in 1999 as a particular subtype of CD4+ T cells (21). Th17 cells were finally described in 2005 in the mouse being different from the classical Th1 and Th2 cells (22). The differentiation of Th17 cells is a multi-step process involving transforming growth factor ß, IL-21, IL-1ß, IL-6, and IL-23 in humans (23, 24). The lineage-specific transcription factor retinoic acid receptor-related orphan receptor (RORc, RORγt in mice) is required for the differentiation (23). Other subsets of immune cells can produce IL-17 including γδ T cells, natural killer cells, invariant natural killer T cells, innate lymphoid cells and CD8+ T cells (2).

IL-17 Receptor Family and Signaling

The first receptor of IL-17 to be identified was discovered in 1995 (25). The IL-17 receptor (IL-17R) family now includes 5 subunits, from IL-17RA to IL-17RE (26). IL-17A, IL-17F, and IL-17A/F bind the same receptor composed of IL-17RA and IL-17RC subunits (27). IL-25 binds a receptor made of IL-17RA and IL-17RB (28). Despite their opposite biological effects, IL-17A and IL-25 share a common receptor chain, an important point to consider when targeting IL-17RA in clinic (2) (**Figure 1**).

Upon ligand binding, the association of IL-17R with Act1 (also known as connection to iκB kinase and stress-activated protein kinases) induces the recruitment and the ubiquitination of tumor necrosis factor (TNF)-receptor associated factor-6 triggering nuclear factor-κ B (NF-κB), CCAAT/enhancer binding protein-ß, CCAAT/enhancer binding protein-δ and mitogen-activated protein kinase pathways. IL-17R and Act1 also activate extracellular signal-regulated kinase-5. These two signaling pathways mediated by IL-17 induce the transcription of inflammatory genes. IL-17 signaling also increases mRNA stability of IL-17 target genes (29). mRNA stabilization is one of the process by which IL-17 and other cytokines synergize, as described below for TNFα (30). Interestingly, peptide that blocks the interaction between Act1 and IL-17RA decreases both IL-17A and IL-25-induced inflammation (31).

Production by and Effects of IL-17 on Synovitis

Many changes occur in the RA synovium, which is characterized by hyperplasia, neoangiogenesis and local infiltration by immune

cells (32, 33). These modifications trigger the destruction of cartilage and bone. The role of IL-17 in the synovitis pathogenesis was first characterized by observations on RA explants. Then, its effects on synovial and bone biopsies and *in vitro* are described.

IL-17 and Th17 Cells in RA Synovial Tissue Pathobiology

Shortly after the description of IL-17, observations on synovial tissues and fluids of RA patients suggest that this cytokine may be involved in joint destruction. Indeed, immunostaining of the synovial tissues of RA patients demonstrates that a subset of CD4+CD45RO+ memory T cells produces IL-17; these IL-17 positive cells being not detected in synovial tissue from osteoarthritis (OA) patients. Moreover, concentration of IL-17 in synovial fluid is also higher in RA patients than in OA, trauma and gout patients (34). Interestingly, there is a spontaneous secretion of IL-17 by RA synovium compared with OA and normal synovium (35, 36). IL-17 synovial membrane mRNA level predicts damage progression (37). Double-immunofluorescence studies show that RORc co-localized with IL-17A and IL-17F staining suggesting that Th17 cells participate to the local cytokine production. IL-17A and IL-17F producing-cells are detected in the lymphocytic infiltrates and in hyperplasic lining cells of RA synovium (30). The recruitment of Th17 cells to the joint leads to interactions with local cells that perpetuate chronic inflammation (38). Specifically, cell interactions between Th17 cells and synoviocytes are crucial as they lead to a massive production of IL-17. The interaction molecule podoplanin contributes widely to this high IL-17 secretion (39, 40). *In vitro* and *in vivo* experiments show that IL-17A and IL-17F-producing cells have a plasma-cell like morphology (30, 41). This morphology has been associated with increased secretion *in vitro* and probably *in vivo*. Experiments on synovial explants from RA show that the Th2 cytokines IL-4 and IL-13 completely inhibit the production of IL-17 (35).

All together, these findings suggest a local production of IL-17 in RA synovium, mainly mediated by Th17 cells. The interactions between local mesenchymal cells and Th17 cells are crucial for a higher and more sustained production.

Effects of IL-17 in RA Pathogenesis

Having characterized the production of IL-17 in RA synovitis and the cells involved, IL-17 effects on synovial and bone explants are now described.

Structural damage in RA includes cartilage destruction and bone erosion (42). Cartilage damage is partially induced by synovial cytokines such as IL-17. Experiments on RA synovial samples show that IL-17 triggers the production of IL-6, leukemia inhibitory factor (LIF) and macrophage inflammatory protein (MIP)-3α/chemokine (C-C motif) ligand-20 by RA synovium (35, 43, 44). Moreover, the addition of an anti-IL-17 antibody to RA synovium cultures significantly decreases matrix metalloproteinase (MMP)-1 production, collagenase activity but not tissue inhibitor of MMP (TIMP)-1 production suggesting the direct contribution of IL-17 to joint destruction (45). The MMP/TIMP system plays a role in the collagen tissue turnover; a shift toward MMP production suggests degradation of the collagen framework. MMP-1 induces collagen degradation and the release of carboxy-terminal telopeptides. IL-17 increases carboxy-terminal telopeptides production in RA synovium explants, an effect that is reversed when adding an anti-IL-17 antibody (45, 46). Keeping with this, the C-pro-peptide of type I collagen, representing the production of type I collagen as part of repair efforts, is inhibited when adding IL-17 to RA synovium (46). All together, these results suggest that IL-17 promotes cartilage destruction at the expense of cartilage synthesis.

As mentioned above, RA also leads to bone erosion and particularly to early juxta-articular bone loss (42). Keeping with the results on RA synovium, IL-17 alone, and more in combination with IL-1 or TNFα, increases the production of IL-6 by RA bone explants (46, 47). In addition, IL-17 reduces bone formation and increases its destruction (46).

To go further into the comprehension of IL-17-induced destruction, effects of IL-17 on isolated cells are now described (**Figure 2**). IL-17A and IL-17F induce synoviocyte activation with increased cytokine and chemokine production, especially of IL-6 and IL-8 (4, 48–51). Moreover, IL-17 triggers synoviocyte migration and promotes an invasive phenotype that favors tissue destruction (33, 52, 53). Tissue destruction includes cartilage matrix destruction and bone erosion. Matrix destruction is mainly mediated by MMP. Among them, MMP-1,−2,−9, and−13 are induced by IL-17 in RA synoviocytes and chondrocytes (45, 54).

Bone remodeling roughly depends on the balance between the activity of osteoclasts, that favor destruction, and osteoblasts, that promote bone formation. IL-17 promotes the expression of receptor activator of NF-κB ligand (RANKL) on osteoblasts and synoviocytes and then activates RANK signaling in osteoclasts (1, 55, 56). These results suggest that IL-17 plays a role in osteoclastogenesis, thereby promoting bone destruction (34, 57). Moreover, IL-17A could inhibit osteoblast and osteocyte activity *in vitro* but this should be confirmed (58).

Neoangiogenesis is crucial for pannus development in RA synovium. IL-17 is involved in this process inducing the production of vascular endothelial growth factor by synovial fibroblasts (59, 60). The RA synovium is also characterized by hyperplasia of synovial lining cells. IL-17 stimulates synoviocyte proliferation (61). This excessive proliferation combined with apoptosis resistance causes synovial hypertrophy. More specifically, IL-17 up-regulates anti-apoptotic genes and down-regulates pro-apoptotic genes (61, 62). IL-17 alone, and especially when combined with TNFα, increases the expression of the anti-apoptotic adhesion molecule Amigo 2 (63) and that of synoviolin, that prolong the survival of RA synoviocytes (50, 64). IL-17 also impairs apoptosis through activation of autophagy (65).

Therefore, observations on synovial and bone samples from RA patients and *in vitro* experiments confirm the role of IL-17 in synovitis.

REGULATION OF IL-17 FUNCTION

Even if IL-17 effects on RA synovitis are clear, some mediators interfere with this system by regulating positively and negatively IL-17 function (**Figure 3**).

Synergistic Effects

IL-17 and TNFα

Concomitant with the description of human IL-17A (13), experiments showed that TNFα potentiates the effect of IL-17A on IL-6 and IL-8-induced secretion by rheumatoid synoviocytes (14, 56). Similar results were obtained on RA synovium explants (37, 44). Moreover, IL-17F also synergizes with TNFα (4, 48). Mechanisms underlying this synergistic interaction were later described when IL-17A and IL-17F were shown to induce TNF receptor II expression and production. Microarrays analysis reveal that almost 90% of genes modified by the combination of IL-17A and TNFα showed a pattern of additivity and 1% of synergy (4, 30). Interestingly, IL-17 and TNFα mainly synergize through the induction of mRNA stabilization independently of TNF-receptor associated factor-6 (66, 67). Some genes synergistically induced by this combination are of importance in RA (e.g., IL-6, IL-8, chemokine (C-C motif) ligand-20, etc …). For instance, IL-17 and TNFα promote an invasive phenotype in synoviocytes (53) but also neutrophil survival (68).

IL-17 and IL-1

IL-1 is involved in RA pathogenesis through bone and cartilage destruction (69). *In vitro* experiments on RA synoviocytes show a synergistic effect of IL-17 and IL-1ß on the production of IL-6 whereas an additive effect is observed for LIF production (43, 70). Interestingly, IL-17 and IL-1ß induce synergistically chemokine (C-C motif) ligand-20 production, which in turn recruits Th17 cells (44). The synergistic effect of IL-17 and IL-1ß is also demonstrated in RA bone explants where the two cytokines increase bone destruction and reduce its formation (46). Similar results are observed with the collagen-induced arthritis mouse models (71, 72). Blocking of both IL-1ß and IL-17A with a bi-specific antibody appears to reduce joint inflammation, destruction and synovial proliferation notably through the reduction of NF-κB activation (72). While IL-1 inhibitors (anti-IL-1ß antibody or soluble type I IL-1 receptor) have modest effect in RA, it would be of interest to develop biological agents that block both IL-17 and IL-1 (69).

IL-17 and GM-CSF

Granulocyte-macrophage colony stimulating factor (GM-CSF) is produced by many cell types (e.g., myeloid cells, tissue-resident cells) and plays a key role in the differentiation of

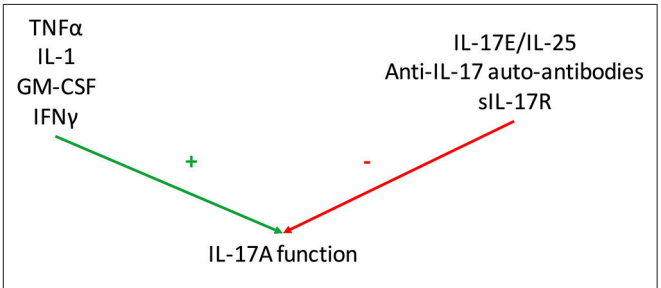

FIGURE 3 | Regulation of Interleukin (IL)-17 function. Various mediators regulate IL-17A function. Some have additive or synergistic effects with IL-17A as tumor necrosis factor-α (TNFα), IL-1, granulocyte-macrophage colony stimulating factor (GM-CSF) and interferon (IFN) γ. Conversely, IL-25 (also known as IL-17E), anti-IL-17 autoantibodies and soluble IL-17 receptor (sIL-17R) inhibit IL-17 function.

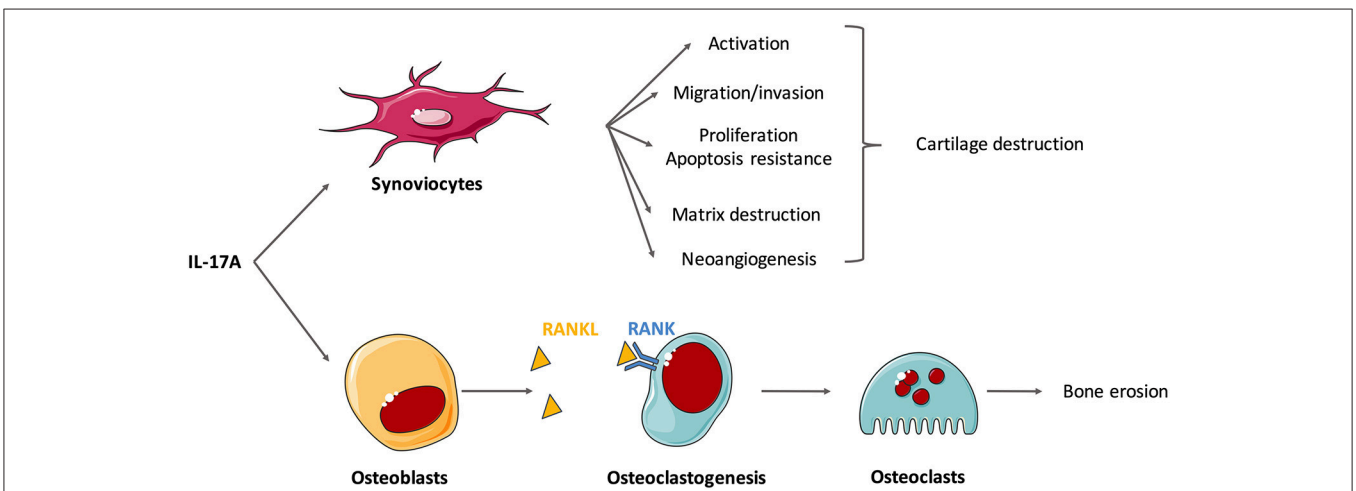

FIGURE 2 | Effects of interleukin (IL)-17A on isolated cells involved in the synovitis. Rheumatoid synovium is characterized by hyperplasia, neoangiogenesis and excessive inflammation. IL-17A mediates cartilage and bone destruction that occurs in rheumatoid arthritis (RA) mainly through its action on synoviocytes and osteoblasts. *In vitro* experiments show that IL-17A induces synoviocyte activation (e.g., production of IL-6 and IL-8), migration and invasion that promote cartilage destruction. Through the induction of matrix metalloproteinases (MMP), IL-17A induces matrix destruction. It also favors proliferation and apoptosis resistance and the neoangiogenesis required for pannus development. In addition, IL-17 promotes receptor activator of NF-κB ligand (RANKL) expression on osteoblasts that binds to RANK, activates osteoclastogenesis and finally triggers bone erosion by osteoclasts.

myeloid cells and in the production of neutrophils, eosinophils and monocytes. GM-CSF is also involved in adaptive immunity. *In vivo* experiments show that GM-CSF is involved in RA pathogenesis (69). GM-CSF level is also increased in synovial fluid and blood from RA patients (73). In experimental arthritis, the combination of IL-17 and GM-CSF shows complementary and local additive effects and induces a more severe phenotype (74).

IL-17 and IFNγ

Interferon (IFN) γ plays a role in anti-infectious host defense, in inflammatory and in auto-immune diseases (75). IL-17A and IFNγ have an additive effect on IL-6 secretion by RA synoviocytes (14). Experiments on other cell types show that the combination of IL-17 and IFNγ increases the production of IL-6, IL-8, intracellular adhesion molecule-1 and nitric oxide (50, 76).

Antagonist Effects

IL-17 and IL-25

As described above, IL-17A and IL-25 bear the lowest homology and their receptors share the common chain IL-17RA (1).

In a mouse model of type I diabetes, IL-25 effect is similar to that of anti-IL-17 to reduce peri-islet CD4 and CD8 T-cell infiltrates while increasing the proportion of the Treg cell population. Interestingly, only IL-25 treatment reduces the amount of autoreactive Th2 and Th17 cells in delayed recurrent autoimmunity. This study highlights the potential shift induced by IL-25 into the Th17/Treg balance (77).

Administration of IL-25 reduces collagen-induced arthritis development in mice and suppresses Th17 cell responses in an IL-13 dependent manner (78). Similar observations are made in experimental autoimmune encephalomyelitis mice where IL-13 is also required to induce Th17 suppression (20).

IL-25 level is higher in serum and synovial fluid from RA patients compared with OA patients and healthy controls (78, 79). Similarly, IL-25 level is correlated with disease activity and with inflammatory cytokines (e.g., TNFα, IL-1ß, IL-17A, IL-6) in RA patients. Moreover, when stimulated peripheral blood mononuclear cells from RA patients are treated with recombinant IL-25, Th17 cells and IL-17A expression are inhibited and that of IL-4 increased (78).

Interestingly, there is a spontaneous secretion of IL-25 by RA synoviocytes that is delayed compared with the production of IL-6. Similar results were obtained in a model known to mimic the inflammatory site of RA synovium (synoviocytes/peripheral blood mononuclear cells coculture), IL-25 production being delayed compared with that of IL-17A. In turn, IL-25 can inhibit IL-17A function acting as a receptor antagonist (79).

Considering the interaction between IL-17A and TNFα, IL-25 reduces the production of IL-6 induced by these two cytokines. Interestingly, IL-17A and TNFα decrease IL-25 production while TNFα alone increases IL-17RB in synoviocytes, being a potential way for TNFα to regulate inflammation (79). Indeed, IL-17RB is required for IL-25 signaling that in turn controls Th1 and Th17 responses and inhibits monocyte-derived inflammatory cytokines (20, 79–81).

All of these results suggest that IL-25 acts as a regulatory pathway in response to inflammation to then down-regulates excessive Th17 and IL-17 immune response.

IL-17 and Autoantibodies Against IL-17

Autoantibodies against pro-inflammatory cytokines were first described for IL-1α and constitute a marker of good prognosis in RA (82, 83). They bind their antigen and form immune complexes with the cytokine. Anti-IL-17 antibodies are absent in healthy controls while there are detected in almost 40% of RA patients. As opposed to bioactive IL-17A, anti-IL-17 antibodies are increased in non-severe RA and so linked to a better prognosis. As expected, higher titers of immune complexes are detected in non-destructive compared with destructive RA (10).

IL-17 and sIL-17R

The expression of cytokines is regulated by various mechanisms. For instance, IL-1 receptor antagonist binds to IL-1 receptors, competitively antagonizes the binding of IL-1 and finally decreases its biological effects (84). Soluble type II IL-1 receptor also acts as an inhibitor of IL-1 function (85).

Although not fully demonstrated, it makes sense to consider the contribution of soluble IL-17R (sIL-17R) in the regulation of IL-17 function. Interestingly, sIL-17RB is increased in alveolar echinococcosis infected patients compared with controls and its level is correlated with disease severity. Conversely, sIL-17RA shows an opposite trend. These variations of soluble receptors may silence the IL-25 mediated response, thereby promoting disease progression (86).

This example, far from the RA, illustrates that sIL-17R is involved in the modulation of IL-17 levels. *In vitro* and *ex vivo* experiments with RA samples have shown that the combined inhibition of IL-17, IL-1 and TNFα with soluble receptors increased the degree of response (44, 87, 88).

Therefore, many mediators regulate positively or negatively IL-17 function; these results are summarized in **Figure 3**. Considering all these interactions, it remains a challenge to detect the specific effect of IL-17 both at local and systemic level.

IL-17 DETECTION

From Local Production to Circulating Levels

It makes sense that patients with high level of IL-17 would be more sensitive to an anti-IL-17 inhibitor. This concept was developed after the emergence of TNFα inhibitors. In a majority of patients, this treatment leads to the reduction of symptoms, inflammation and bone destruction. However, around 30 % of these RA patients do not respond. To better understand this observation, a bioassay was developed to evaluate TNFα bioactivity before treatment (89). It is based on the ability of synoviocytes to produce IL-6 in response to TNFα (90). Indeed, 60% of patients have a good ability of their plasma to induce IL-6 production before infliximab therapy (a TNFα inhibitor), this production being inhibited 4 h after the first infliximab infusion. Another pattern of patients has

moderate or no IL-6 production before infusion, therefore no inhibition by infliximab. The difference of IL-6 production before and 4 h after the first infliximab infusion is correlated with clinical response. This may explain the heterogeneity in treatment response to TNFα inhibitors (89). Interestingly, intra-articular administration of etanercept (a TNFα inhibitor) results in a significant improvement of the composite change index compared to placebo in RA and psoriatic arthritis patients. Serum etanercept levels were comparable between composite change index good and non-responders, thus indicating that local inhibition of TNFα would be effective (91).

Similar experiments would be of interest for IL-17 since IL-17 systemic inhibitors show heterogeneous results in RA. Interestingly, IL-17A synovial fluid levels are higher than serum levels in early RA cases, suggesting that local production may be reflected by circulating levels (92).

More recently, studies from the Pathobiology of Early Arthritis Cohort have been set up with the aim to define from synovial biopsies and blood samples the involvement of cellular/molecular signatures in determining clinical phenotypes (11, 93, 94). For instance, in early RA, synovial transcripts correlating with disease activity (disease activity score-28/C-reactive protein) are significantly enriched in TNFα-induced genes and predict poor response to first-line therapy (95). Considering the important interpatient heterogeneity, such approaches on synovial biopsies may be used to stratify patient for tailored drug delivery strategies (94), especially in the case of IL-17 inhibitors where results showed a high heterogeneity. Indeed, IL-17 and its receptor are up-regulated within synovial ectopic lymphoid structures and further contribute to the chronicity of local inflammation (64, 96). These structures have the ability to function as germinal centers and there is a significant association between their presence and erosive disease. Aggressive treatments are recommended for these patients to prevent the onset of erosions. Considering the key role of IL-17 in the formation of these ectopic lymphoid structures, IL-17 inhibition would be of interest in these patients selected with a synovial biopsy (97, 98).

Methods to Detect IL-17 (ELISA and Bioassay)

Considering the results described above, there is no doubt that IL-17 is involved in RA pathogenesis. However, IL-17A circulating levels measured by ELISA vary a lot across studies, from undetectable to pg/ml or even ng/ml concentration (99, 100). Moreover, these tests do not detect the bioactive form that is crucial since there are circulating inhibitors (IL-25, anti-IL-17 autoantibodies, sIL-17R) and activators of IL-17 (TNFα, IL-1, GM-CSF, IFNγ) (**Figure 3**). To measure the level of bioactive IL-17A, a cell-based bioassay was developed on the ability of RA synoviocytes to produce IL-6. RA synoviocytes are exposed to plasma samples and IL-6 production is measured with or without an anti-IL-17 antibody (35). The test was then extended to human endothelial cells that are able to produce IL-8 in presence of IL-17A (5). By blocking IL-17A, it allows to quantify its specific contribution in the production of pro-inflammatory cytokines.

TOWARD PRECISION MEDICINE IN RA

While many studies are performed to identify predictive biomarkers of RA development (e.g., cigarette smoking, infection), another issue is also to predict which therapy is the best suited for patients that have developed RA (101).

Predictive Biomarkers of Response to IL-17 Inhibition

Using the bioassay described above, bioactive IL-17A is higher in RA patients compared with healthy controls and its level is correlated with destruction (5). As mentioned earlier, anti-IL-17 antibodies and immune complexes are elevated in non-destructive RA (10). Detection of these biomarkers represents an interesting tool to identify patients with an IL-17 driven disease that could respond better to IL-17 inhibitors.

Identification of Patients That Would Benefit From Anti-IL-17

In RA patients, a meta-analysis shows the superiority of secukinumab (anti-IL-17A) and ixekizumab (anti-IL-17A) compared with placebo based on American College of Rheumatology (ACR)-20 and ACR50 clinical response. However, it does not reach statistical significance for ACR70 response and analysis of individual response rate shows a high degree of heterogeneity. Moreover, brodalumab (anti-IL-17RA) is not effective in achieving ACR20 (2, 102). These observations rely on different explanations. First, immunohistochemical analysis reveal a high variability of IL-17A, IL-17F and their receptor expression in RA synovitis (103). IL-17 inhibition would not be sufficient in these patients with low expression of IL-17. Then, different strategies are developed to block the IL-17 pathway with anti-IL-17A, anti-IL-17A/F and anti-IL-17RA (2). These antibodies may encounter some pitfalls; for instance, the inhibition of IL-17RA could inhibit the anti-inflammatory effect mediated by IL-25 (28). The dual inhibition with bi-specific antibodies against TNFα and IL-17A would have been of interest to prevent their synergistic interaction but recent papers show no clear benefit, especially when compared to TNFα inhibition alone (104–106). The structure of the dual inhibitor and the respective location of the two binding sites have to be considered.

Therefore, as for TNF inhibitors, an effort is needed to identify RA patients that would benefit from IL-17 targeted therapies. The development of predictive biomarkers of response to IL-17 inhibitors is beginning; for instance, the cell-based bioassay detecting bioactive IL-17A is of interest but only constitutes the spearhead of more research. IL-17 expression in synovial tissue may be another way to stratify patients to potentiate the beneficial effect of these inhibitors (11, 96, 103). Even if robust evidence is still needed to confirm the use of such biomarkers in clinical routine, these strategies can move the field of RA management into the new era of precision medicine in the future.

CONCLUSION

IL-17A is involved in early induction and late chronic stages of various inflammatory diseases. The inhibition of its signaling

brings a clear improvement in psoriasis, psoriatic arthritis and in ankylosing spondylitis treatment but results are less convincing in RA. However, *ex vivo* and *in vitro* studies clearly show that IL-17A is one of the culprit that perpetuates local inflammation in synovium and especially in RA. Explanations of such unexpected results may come from the many mediators that modulate IL-17 function, with either agonist or antagonist effects. The significant heterogeneity of IL-17 expression between patients also imposes a stratification of them to identify the ones that could benefit from IL-17 inhibitors. The development of predictive biomarkers as bioactive IL-17 or anti-IL-17-autoantibodies or the use of synovial biopsies still requires robust evidence but would be interesting to turn the page to precision medicine in RA.

DISCLOSURE

PM holds a patent on the IL-17 bioassay.

AUTHOR CONTRIBUTIONS

MR: writing and figures. PM: concept and proof reading.

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MINI REVIEW
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Ultrasound-Guided Synovial Biopsies of Wrists, Metacarpophalangeal, Metatarsophalangeal, Interphalangeal Joints, and Tendon Sheaths

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Ultrasound-guided synovial biopsy (UGSB) is a minimally-invasive procedure which allows quality synovial tissue retrieval. In this article, we will discuss overarching principles of the procedure performed in wrists, metacarpophalangeal (MCP), metatarsophalangeal (MTP), interphalangeal joints (IP), and tendon sheaths, including basic sonoanatomy, entry site and biopsy technique, as well as special considerations for each structure whenever relevant.

Keywords: ultrasound-guided synovial biopsy, wrist, metacarpophalangeal, metatarsophalangeal, proximal interphalangeal

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INTRODUCTION

Synovial tissue analysis is fundamental for basic research on inflammatory arthritis (IA) pathobiology, and in the quest of biomarkers of response to treatment (1–5). The advance of musculoskeletal ultrasound (US) over the past decades as a reliable imaging tool has led to development of US-guided synovial biopsy (UGSB) techniques, using a portal-and-forceps or semi-automatic biopsy needle (6, 7). UGSB is increasingly used to harvest synovial tissue in the setting of clinical trials (8) and clinical routine. UGSB is a well-tolerated procedure (6), has a favorable safety profile and can be repeated on a serial basis as it does not alter subsequent clinical or US joint assessments (9). Recommendations for minimal standards for reporting (10) and for sample retrieval for small joint biopsies (11) have already been published and will not be discussed in this review, which focuses on technical aspects of the procedure in wrists and small joints.

Prerequisites are sufficient musculoskeletal US experience and US-guided procedures or specific training in UGSB. A learning curve certainly exists with regards to procedure duration and tissue quality-RNA yield. It is strongly recommended to keep records of these three parameters as well as patient tolerance data as an index of quality control. The percentage of graded samples and RNA yield should be coupled to disease activity measures, US findings of the biopsied joint and personal experience (a highly inflamed large joint will probably provide more tissue and RNA in the hands of an experienced operator).

PRIOR TO THE PROCEDURE

Patient eligibility should be verified and include but not limited to absolute and relative contraindications to UGSB, such as active skin infection and anticoagulant/antiplatelet treatment,

TABLE 1 | Pre-procedure checklist.

Signed consent form	Sample container	Sterile swabs
Biopsy needles	10 mls / 20 mls syringes	Sterile gown and gloves
21G/19G needles	Antiseptic solution	Sterile drapes
Procedure pack	Local anesthetic	Sterile ultrasound sheath
Face mask and hair cover	Non-adhesive dressing	Sterile gel (optional)

respectively. A written informed consent should be obtained before the procedure and documented in patient notes. We usually consent patients for the most common or severe complications such as wound and joint bleeding, swelling, pain, neurovascular, and tendinous-ligament damage, wound and joint infection, thrombophlebitis and vein thrombosis.

The choice of the area to biopsy (joint and specific joint compartment) depends on local (synovial thickening-ST and Power Doppler -PD) and global patient factors (such as patient's preference and comorbidity). According to the previously published algorithm for biopsy site selection, the ideal candidate joint will demonstrate significant ST with high grade PD (6).

An appropriate clean room must be available for the procedure. Any room suitable for patient care as per local policies may be used, as long as adequate space is provided for the patient, the operator and assistant, the US machine, and the procedure tray.

A checklist of the required material and standardized operating protocol can be found on synovialbiopsy.com (see **Table 1**) and should appear on the report form. The choice between a portal-and-forceps and a needle approach depends on the joint (needle biopsy is adequate for most small joints) and personal preference. Their differences and similarities have been discussed elsewhere (10).The most common biopsy needle calibers used for the wrist and small joints are 16-Gauge (5 French) and 18-G. Sterile gel use is optional. Alternatively, a chlorhexidine solution can be used as a contact medium.

After the procedure, compression of the entry site is followed by application of a small adhesive dressing and a quick neuro-vascular assessment of the hand or foot. Contact details of the department, as well as a prescription for painkillers on demand must be handed to the patient before discharge.

WRIST BIOPSY

Together with the knee, the wrist is one of the most commonly biopsied joints (10), due to its relative ease of access and prevalence of involvement in IA especially rheumatoid arthritis (RA).

Normal sonoanatomy of the wrist is shown in **Figure 1A**. It is important to identify the various structures and area of interest before the procedure, bearing in mind that in cases of IA this might be challenging due to bone erosions and osteophytes and loss of normal architecture. A suitable path for the needle should thus be planned beforehand.

The patient must be comfortable, supine or recumbent at 45°, with the hand placed on a table next to her/his bed or seat, palm downwards. An absorbent pad is placed on the table. Significant arm elevation and/or abduction should be avoided and the bed should be moved instead. After wearing a mask and hear cover, the operator proceeds to hand disinfection and then wears a

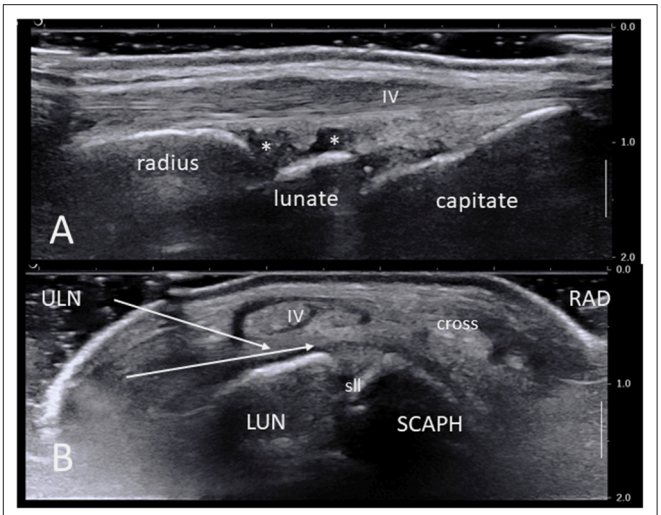

FIGURE 1 | (A) Ultrasound long-axis scan of a normal wrist joint using a high frequency linear transducer. Note the radius, lunate, and capitate bone surfaces, the synovial recesses (asterisks), the IV extensor tendon compartment and its retinaculum. **(B)** Short axis ultrasound scan of a normal right wrist. The area of maximal synovial thickening usually lies posterior and radially to the scapho-lunate junction. White arrows show the two most common needle paths between extensor tendons compartments V and IV and VI and V. IV: fourth extensor tendon compartment; cross: crossing point between compartments III and II; LUN, semi-lunate bone; RAD, radial side; SCAPH, scaphoid bone; sll, scapho-lunate ligament; ULN, ulnar side.

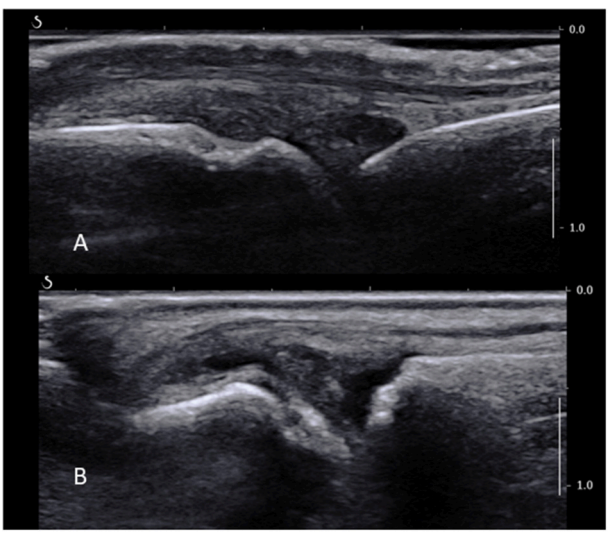

FIGURE 2 | (A) Normal MCP joint on a longitudinal ultrasound view. Note the joint space, metacarpal head, proximal phalanx, and extensor tendon. **(B)** Long axis ultrasound scan of a fifth MTP joint showing synovial thickening proximally to the joint line overlying the metatarsal head, as well as a small amount of fluid.

sterile gown and sterile gloves. Wearing a second pair of gloves that is discarded after disinfection is preferred by many, even though there is –to our best knowledge- no evidence to support fewer infectious complications in the setting of UGSB. Patients are asked to rest their arm on their elbow and then the hand, wrist and forearm are prepped with a disinfectant soap or solution with emphasis on the entry site. Once clean, the patient is asked to lift her/his arm, then sterile drapes are positioned on the table and a sterile drape used as a cuff at the mid forearm. The hand is then placed in pronation and gentle flexion (one may use sterile gauzes under the volar side of the wrist). A sterile sheath is placed around the US probe, and 10 ml of lidocaine are aspirated in a syringe.

In general, the most suitable harvesting site lies dorsally (anatomically) and slightly radially to the scapho-lunate junction (**Figure 1B**). On a short axis US scan this corresponds to the area between the scaphoid, scapholunear ligament and lunate bone at the bottom, and the extensor tendon compartments II-IV overlying the long radio-luno-triquetral ligament on the top. Indeed, this area is often the most significantly thickened in IA, but the ulnar synovial recess (deep to and on the ulnar side of the extensor compartment IV) may also be suitable.

The local anesthetic and biopsy needles are thus most commonly inserted on the ulnar side of the wrist, distally to the ulnar styloid process. The exact entry site will depend on case-specific anatomy, but a suitable path can be found between the extensor carpi ulnaris (ECU) and extensor digiti quinti proprius (EDQ) tendons, or –less often- between the EDQ and extensor digitorum communis (EDC) tendons or even on the volar side of the ECU tendon (although not privileged because of the risk of lesion of the triangular fibrocartilage complex). As with any US-guided procedure, once the needle trajectory has been determined by the operator, the US probe must remain still while the needles are inserted in a longitudinal plane (i.e., following the probe's long axis). The subcutaneous tissue at the entry site is first injected with a small volume of local anesthetic such as lidocaine 1% 1–3 ml. Once the skin is anesthetised (usually within 1–3 min), the tissue layers lying between the skin and joint capsule are injected with a suitable volume of lidocaine, e.g., using a 19G needle. Any synovial fluid should be aspirated before the intra-articular lidocaine injection (to prevent false negative culture results owing to its bactericidal effect and false positive due to preparation and handling) and sent for analysis as indicated. The joint space itself is then injected e.g., with 2–5 ml of 1% lidocaine, which is also helpful in distending the joint and allowing for better identification of the synovial membrane under US once the synovial fluid has been removed.

The biopsy needle should be primed before insertion. Once in the area of interest, the throw is opened and must be visualized on the US scan. It is then positioned with a gentle pressure against the synovium to maximize tissue yield. Likewise, applying mild pressure on the probe might also prove helpful in keeping the throw against the synovial surface. The tip of the biopsy needle should be free to move, i.e., not abut against bone or osteophytes, as the triggering mechanism tends to cause a backwards move of the whole needle due to recoil momentum.

Keeping continuous hand- patient skin contact is preferred by some operators for a better control of the biopsy needle.

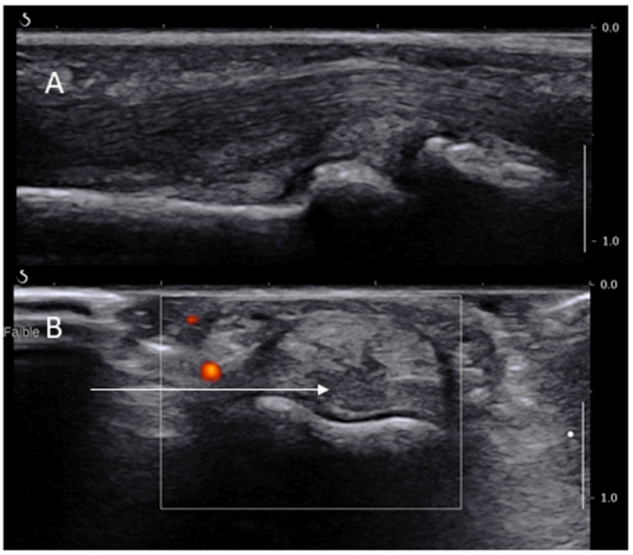

FIGURE 3 | Long **(A)** and short **(B)** axis views of a PIP joint showing the long axis of the needle path (arrow) dorsally to the digital artery.

Some operators opt for use of a portal, while others, including the authors, avoid it, as the path created by the first couple of insertions is considered sufficient. Our experience is that this does not increase the risk of complications and published data on safety using this approach is excellent (6).

Synovial tissue is first harvested from the area overlying the biopsy needle, i.e., in the direction of the probe. The three-dimensional structure of the joint space should be kept in mind: once no more tissue is retrieved from a given area, the synovial membrane distally, proximally, and deeply may be biopsied. This is achieved using the same path, while rotating the needle accordingly once inserted and applying pressure against the membrane as described before.

Bleeding –while rare- is a complication of virtually any invasive procedure. Special care must be given to avoid visible vessels such as the branches of the radial artery lying between the V and IV, and IV and III extensor tendon compartments. These arteries are not inside the synovial membrane, but could in theory be punctured if overzealous pressure is applied on the biopsy needle during triggering.

MCP AND MTP JOINTS

Normal sonoanatomy of the joint is shown in **Figure 2A**. Selection of the joint to be biopsied, operator room setup and equipment, and patient positioning for the MCP joints are as per wrists. For an MTP joint biopsy, the patient is recumbent at 45° with the lower limb in flexion and the foot lying flat on the bed with special care to avoid the limb slipping during the procedure. Skin is prepped as per wrist joint with perhaps a more restricted forearm and calf area to disinfect when targeting MCP and MTP joints, respectively.

The area of interest lies proximally to the joint line and metacarpal or –tarsal head, where most of the ST is found in MCPs and MTPs (**Figure 2B**). A high-frequency US transducer, such as a hockey stick, should be used. The digital vessels and nerves should be identified and a path of entry to the synovium planned accordingly; this is usually dorsal to the neurovascular bundle. The MCP and MTP joints can be accessed from either their medial or lateral sides depending on the room setup and personal preference, bearing in mind that it is much more comfortable to avoid working over the patient's opposite hand or foot. The probe is placed over the area to biopsy on a short axis, and the skin and joint capsule are injected with a small volume of local anesthetic, e.g., lidocaine 1% 1 ml through a subcutaneous needle. A fine biopsy needle (16G or smaller) is used. It is inserted as the local anesthetic needle in the area of interest lying between the bone and the extensor tendon. Care should be taken not to apply too much pressure to penetrate the joint capsule, as this may lead to the needle passing through the capsule and exiting on the opposite site of the joint and finger or toe. Counter-balancing pressure by keeping a continuous contact with the skin with one or two fingers may provide more stability. Another caveat is the width of the hypertrophied synovium compared to the needle throw. This should be assessed before the procedure proceeds, as the throw (usually 10 mm) is sometimes wider than the area of ST itself, which may cause unnecessary soft tissue and/or skin damage. In this case, an alternative biopsy site must be considered. The rest of the procedure and post-procedure care follow the general rules described before.

PIP AND DIP JOINTS

These joints are much smaller and certainly not the favored biopsy site. However, they are preferentially affected in some cases of IA and non-inflammatory conditions such as osteoarthritis. Their approach is identical to the MCP and MTP joints with two major differences with regards to pain control and entry site. The local anesthetic may be administrated through a ring block, procedure in which an anesthetic solution is injected into the base of a finger or toe thus blocking all four digital nerves (which is more time-consuming, therefore should be done after the disinfection and before the rest of the equipment is fully prepared). However, some experts report a good efficacy of a local anesthetic injection of the joint as per MCPs. The ST is more prominent on the volar side of the joint (**Figure 3**), just proximal to the joint line and over the distal part of the phalanx. The US probe is hence placed on the volar side of the joint and the needles are introduced medially or laterally on a short axis view deeply to the flexor tendons while avoiding neurovascular structures (see **Figure 3A**). It is helpful to separate the finger (or toe) from the rest by placing some sterile gauze perpendicular to its axis.

TENDON SHEATHS

Tendon sheaths can also be biopsied in the same way as any other synovial site, provided that they demonstrate at least moderate tenosynovitis and extra care is taken to avoid damaging the tendon itself. Entry site and technique (short or long axis approach) must be individualized to the specific condition and location.

CONCLUSION

UGSB of the small joints is of particular interest in the setting of IA and can be safely performed with basic sonoanatomy knowledge provided that the overarching principles of tissue sampling are respected.

AUTHOR CONTRIBUTIONS

IL, SK, and LM contributed to the conception, drafting, and revision of the work. All authors approve its content for publication and take responsibility for its accuracy and integrity.

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MINI REVIEW
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US-Guided Biopsies: Overarching Principles

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Gathering synovial tissue from any swollen joint especially in early arthritis patients is critical for good quality research and to obtain further insight into the pathophysiology of inflammatory joint diseases. Multiplying biopsy sites is a challenge in terms of the techniques needed for each different joint but also in terms of safety and tolerability. It is important to provide the best care especially in very early arthritis patients who have only had the disease for a few months. This review discusses the minimal requirements applying to antiseptic techniques for the operator's hands, patient preparation, local anesthesia, and post-procedure care.

Keywords: synovial biopsy, anesthesia—local, arthritis (including rheumatoid arthritis), antisepsis, ultrasound guided biopsy techniques, local anesthesia

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INTRODUCTION

Synovial biopsy with ultrasound guided techniques is a safe and well-tolerated procedure however it remains invasive. As such, rheumatologists have to follow some basic aseptic techniques in order to avoid complications.

This review will first discuss the preparation of the patient and the operator, then local anesthetics, the possibility of corticosteroid injections, and post-procedure care.

ASEPSIS FOR THE OPERATOR

Surgical Hand Antisepsis

Preoperative cleansing of hands and forearms with an antiseptic agent has been an accepted practice since the late 1800s (1). Despite a large body of indirect evidence for the need of hand antisepsis prior to surgical interventions this has never been proved by randomized, controlled clinical trials.

United States of America (USA) guidelines recommend the use of agents for surgical hand scrubs which substantially reduce microorganisms on intact skin, contain a non-irritating antimicrobial preparation, have broad-spectrum activity, and are fast-acting and persistent (2, 3).

Reducing resident skin flora on the hands of the surgical team for the duration of a procedure reduces the risk of bacteria being released into the surgical field if gloves are punctured or torn during surgery (4–6).

The World Health Organization (WHO) has provided very precise definitions and has also described operator's hand antisepsis step by step (7–9). Surgical handscrubbing refers to the use of soap and water, while surgical handrubbing is the use of a waterless, alcohol-based solution. The alcohol-based (hand) rub is an alcohol-containing preparation (liquid, gel, or foam) designed to be applied to hands to kill microorganisms and/or temporarily suppress their growth.

Which Products Should be Used for Surgical Hand Preparation?

There are slight differences in terms of requirements between USA and European guidelines. Guidelines in the USA recommend that agents used for surgical hand preparation should significantly reduce microorganisms on intact skin, contain a non-irritating antimicrobial preparation, have broad-spectrum activity, and be fast-acting and persistent (10). In Europe, all products must have at least the same efficacy as a reference surgical rub using n-propanol, as outlined in the European Standard EN 12791. In contrast to the USA guidelines, only the immediate effect after the hand hygiene procedure and the level of regrowth after 3 h under gloved hands are measured. The cumulative effect over 5 days is not an EN 12791 requirement.

Surgical hand antisepsis can be achieved using medicated soap such avec chlorhexidine gluconate (CHG) 4% or povidone-iodine which both result in similar reductions of bacterial counts (70–80%). Despite both *in vitro* and *in vivo* studies demonstrating that povidone-iodine is less efficient than chlorhexidine, it remains one of the widely-used products for surgical hand antisepsis, although it induces more allergic reactions, and does not have similar residual effects (11, 12).

Surgical hand preparation with alcohol-based handrubs seems to be a safer method with a higher reduction of bacterial counts compared to other agents and a greater acceptability and fewer adverse effects on skin. Only alcohol-based hand gels which have passed the EN 12791 test or an equivalent standard for handrub formulations e.g., FDA TFM 1994, should be used (13). Such preparations usually contain 60–95% ethanol or isopropanol.

Both methods are suitable for the prevention of Surgical Site Infections (SSIs) but WHO panel experts have declared a preference for alcohol-based products.

Key Steps Before Entering the Operating Theater

Rings, wristbands and watches must be removed and nails must be short and clean without nail-polish. False nails should also be avoided.

Hands and forearms may be washed with non-medicated soap and water. This part is not necessary unless hands are visibly soiled or dirty but it is highly recommended to eliminate any risk of colonization with bacterial spores (14–16).

Aseptic Procedure

Here we describe the alcohol-based handrub (ABHR) according to WHO recommendations. Apart from a few cases of very large hands and forearms, 15 ml of ABHR are usually enough for the whole procedure.

First, fingertips and forearms are cleaned using 5 milliliters—or 3 doses of ABHR—for each side. This takes ∼1 min with an emphasis on the forearms. Second, hands are then rubbed with 5 ml of ABHR keeping the hands held higher than the elbows.

The whole handrub procedure lasts 1.5 min with the recommended ABHR formulations.

The operator's hands are then considered sterile and the operator can enter the procedure room and put on the sterile gloves (2 pairs) and gown.

MATERIAL NEEDED AND TECHNIQUE

Table Preparation

On a sterile drape, place sterile gauzes (5 × 5 or 7.5 × 5 cm), sterile drapes (adhesive 75 × 75 or 140 × 190 non-adhesive), 10–20 needles for biopsy collection, 1 sterile probe sheath, needles for local anesthetic (1 26-G for the skin, 1 20G 50 mm and 1 18G 50 mm), syringes (20 ml for CHG, 10 ml for lidocaine) (**Figure 1**). The disposable biopsy needle or the instruments portal & forceps (18 G needle, wire, and dilators, optional metallic instruments, flexible, and/or rigid forceps) are also placed on the sterile drape. According to the antimicrobial agents chosen, 150 ml of CHG or PVI-I are usually enough.

The sterile probe sheath may need to have non-sterile ultrasound gel poured inside it to maintain contact with the probe. Some probe sheaths have an adhesive area for the probe so that no gel is needed. As a contact medium between the sheath and skin, we prefer to use chlorhexidine gluconate 4% rather than a sterile gel. A volume of 20 ml is usually sufficient for the whole procedure.

Ultrasound-Guided Synovial Biopsy Techniques

Ultrasound-guided synovial biopsies can be performed using two different techniques. One uses a portal and forceps (P&F) where a modified Seldinger technique is used to position the coaxial sheath and also to provide a portal for irrigation. The biopsies are then performed with a flexible or rigid forceps under ultrasound guidance (17). The other technique uses a dedicated disposable semi-automatic guillotine-type biopsy needle (BN). It can be used with or without an introducer according to the size of the biopsied joint. Several disposable devices are available, e.g., Tru-Cut (UK Medical) or Quick-Core (Cook Medical), Temno Evolution (BD). There may be some differences found in needle

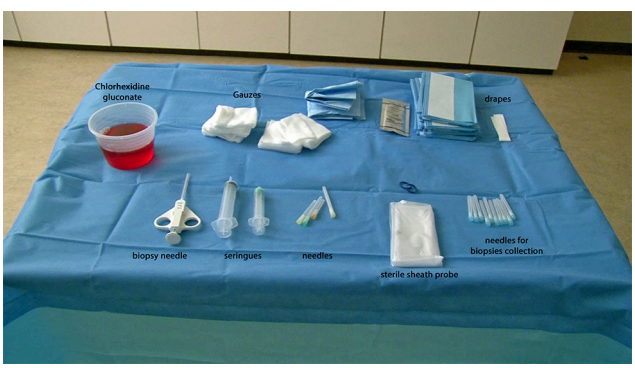

FIGURE 1 | Prepared table with the material needed for ultrasound-guided synovial needle biopsy.

rigidity, the shape of needle bevel, the sensitivity of the semi-automatic mechanism trigger.

The USG biopsies performed either with P&F or a disposable needle offer the same quality of histological analysis of the tissues and the same safety in terms of side effects. Tolerability of both is also good and comparable.

The main differences between these two techniques are that procedure duration is marginally higher for P&F, that BN uses disposable material compared to autoclavable equipment for P&F, and that P&F often requires two operators (18).

PATIENT PREPARATION

Patient Position and Procedure Room

The patient may be sitting or lying comfortably on a bed according to the target joint. Comfort is particularly important for arthritic patients with active disease.

The patient has to remove rings and bracelets, and has to wear a mask. Shaving of the area where the needle is to be introduced is not required.

Enough space must be provided for the ultrasound machine on the side of the patient opposite the operator, and for the sterile gown to be put on with the aid of an assistant. A dedicated room for the procedure is recommended but any space fulfilling local patient safety standards may be used.

Patient Asepsis

The aim of this procedure is to reduce the microbial load on the patient's skin as much as possible before breaking the skin barrier.

In Europe, the antimicrobial agent recommended is chlorhexidine gluconate 4% (CHG) but povidone-iodine is also frequently used. In the USA, despite the fact that chlorhexidine gluconate is superior to povidone-iodine for patient preoperative skin preparation, it is still not eligible for that use because of different standards for efficacy.

CHG is a cationic bisbiguanide developed in England in the early 1950s. It is effective on Gram positive and negative bacteria and also against lipophilic viruses (Human Immunodeficiency Virus, influenza, herpes simplex). It has a persistent antimicrobial action that prevents regrowth of microorganisms for up to 6 h. There is no evidence of CHG being toxic if it is absorbed through the skin. This point is crucial and explains why sterile gels are not essential as a contact medium during the ultrasound procedure, as they can be replaced by CHG. Finally, there is a low incidence of hypersensitivity reactions and skin irritation but one has to keep in mind that some severe allergic reactions have been reported (including anaphylaxis).

Povidone-iodine contains 9–12% available iodine and is eligible for patient antiseptic skin preparation, health care personnel hand washing and surgical hand scrubbing. Bacteria do not develop resistance to PVP-I (19).

The area to wash will obviously depend on the target joint:

- For the wrists, metacarpophalangeal (MCP), and proximal interphalangeal (PIP) joints: hand and forearm up to the elbow (**Figures 2C,D**).

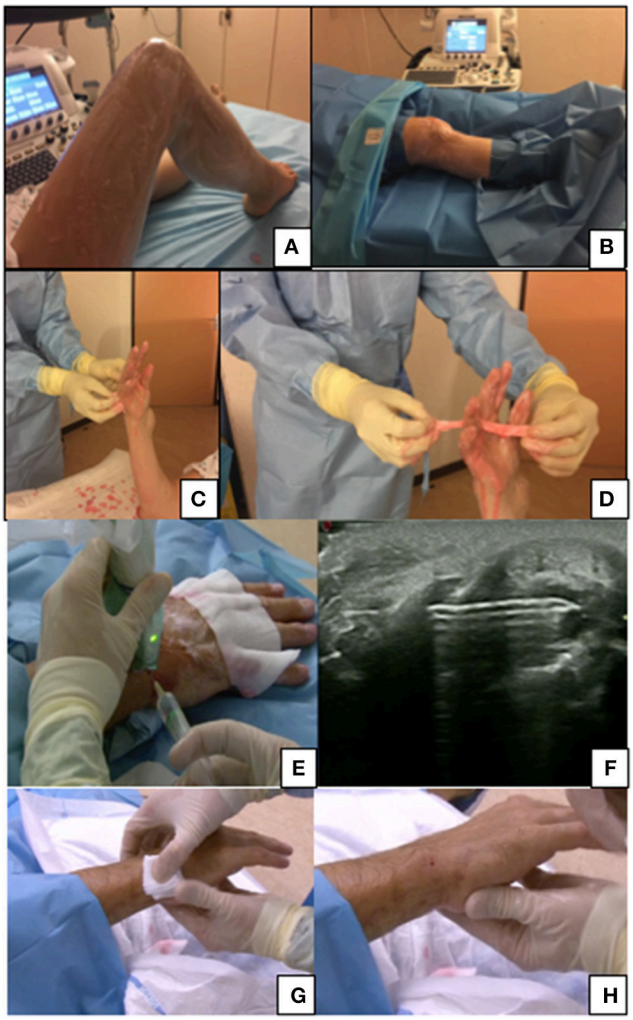

FIGURE 2 | (A,B) Preparation for knee biopsy, patient lying. **(C,D)** Hand washing before a biopsy of wrist/PIP with a focus on the interdigit region washing. **(E,F)** Articular anesthesia after skin anesthesia with 18G 50 mm needle under ultrasound guidance. **(G,H)** Post-procedure care, a 2 min compression of the hole entrance before putting a small dressing.

- For the elbow: most of the arm and forearm excluding the distal third of the forearm and proximal third of the arm
- For the knee; most of the thigh and the leg excluding the distal third of the leg and proximal third of the thigh (**Figures 2A,B**).
- For the ankles, metatarsophalangeal (MTP), and proximal interphalangeal (PIP) joints: the leg up to the knee, the ankle and foot.

The area of interest is washed with sterile compresses dipped in the chosen antimicrobial agent solution (CHG or PVP-I). A couple of rubbings of the area of interest are usually enough. For hands and feet, special care should be taken for the interdigital areas and nails. After disinfecting, sterile drapes should be placed to isolate the target joint.

The first pair of gloves (no longer considered sterile) should be discarded.

The assistant (e.g., nurse) has to wear a mask and gloves. One member of the family may be present according to local policies, and they should also wear a mask.

LOCAL ANESTHESIC

Lidocaine 1% (w/v) is recommended for the local anaesthesic (LA). The volume depends on the size of the target joint from 3 ml for MCP and PIP to 10 ml for big joints such as knees. The maximal dose is 4–5 mg/kg. In adults, the average lidocaine injected dose is far below the maximal dose. For example, in a 60 kg patient, injection should not exceed 300 mg of lidocaine, whereas 10 ml of lidocaine 1% (10 mg/ml) only correspond to 100 mg. Doses must be carefully calculated for children.

The LA is performed under ultrasound guidance with a suitable needle from the skin to the synovial hypertrophy and the anesthetic effect is usually very quick (1–3 min). Alternatively, a subcutaneous needle can be used for the skin followed by the deeper injection with a thicker needle (**Figures 2E,F**).

Adverse Events

Side effects—neurological and cardiovascular—are more common in cases of overdosing or intravascular injection. Patients may experience paraesthesia, a metallic taste, blurred vision, tinnitus, an increase in blood pressure or cardiac arrhythmias.

Chondrotoxicity of Anesthetic Agents

Severe cartilage damage has been reported with the use of local anesthetic but mainly with continuous intra-articular infusion with bupivacaine, the gleno-humeral joint being the most commonly affected. Thus far, there is no clinical evidence of chondrolysis resulting from a single injection of local anesthetic but rheumatologists have to be aware that *ex-vivo* studies have demonstrated that bupivacaine, lidocaine, ropivacaine, and levobupivacaine are toxic for cartilage. The mechanisms are still unknown, but mitochondrial DNA damage or chemical incompatibility have been suggested and there seems to be a dose- and dose-over-time effect on toxicity (20, 21).

In animal models the assessment of *in vitro* chondrotoxicity showed a dose- and time-dependent effect of lidocaine on the viability of articular cells (22, 23).

Antimicrobial Effect of Lidocaine

Lidocaine like the other local anesthetic agents possesses bacteriostatic, bactericidal, fungistatic, and fungicidal properties. This role has been documented with *in vitro* and *in vivo* studies since 1950. The exact mode of action is not known but some believe that local anesthetics cause a disruption of microbial cell membrane permeability, leading to a leakage of cellular components and subsequent cell lysis. Lidocaine demonstrated a dose-dependent inhibition of growth for all strains of bacteria tested, with the most activity against gram-negative organisms, and the least against *Staphylococcus aureus*. The addition of epinephrine to the local anesthetic had no effect on the susceptibility of the bacteria to lidocaine.

Thus, on the one hand lidocaine is beneficial in preventing joint infections after invasive procedures, but on the other hand, it could lead to false-negative results or suboptimal culture yields for biopsies (24, 25).

POST-PROCEDURE CARE

Theoretically ultrasound guided synovial biopsy procedure may cause infection, bleeding, or lesions of tendons or nerves. This is why the ultrasound pre-biopsy assessment is important, with the identification of the vascular structures and the tendons in the joint of interest. The continuous visualization of the needle and its tip throughout the procedure is also important for the same reason. This is important for complex joint such as wrists, elbows, or ankles biopsies. In the event of unexpected bleeding, clinical examination, and surveillance is recommended.

The very good tolerability of the ultrasound guided synovial biopsy has been demonstrated in many studies and no intense pain should be expected at short- or long-term after biopsies (26).

In practice, once the procedure is finished, the entry site is gentle cleansed with sterile water. A small dressing is placed after 1–2 min of compression on the entry site where there should be a tiny red spot (**Figures 2G,H**). A bandage can be put around the biopsied joint but is not essential. The dressing and the bandage can be removed the next morning.

Contact details of the operator or department should be given to the patient in case of significant pain, swelling, bleeding, or neurologic symptoms during the week after the procedure.

INTRA-ARTICULAR GLUCOCORTICOID (GC) INJECTIONS

Methylprednisolone 40 mg/ml, triamcinolone acetonide 40 mg/ml or triamcinolone hexacetonide 20 mg/ml can be injected into the joint under ultrasound guidance of the at the end of the biopsy procedure if needed. A 50 mm long 20G needle is suitable for the injection. With the exception of large joints, some of the GC might leak from the needle entry site since pressure builds up during the procedure and LA injection.

In terms of safety, intra-articular glucocorticoid injections are safe with a low incidence of septic arthritis: 1/27.000 in a Dutch retrospective study from 2008 and 2013 (27). In a retrospective multicentric study in patients undergoing synovial biopsies using different techniques (ultrasound-guided or arthroscopic-guided), Soeren et al. recently reported 38 intra-articular joint injections without any increase in adverse events including infections. They were also associated with a statistically significant reduction in post biopsy swelling (28).

CONCLUSIONS

Aseptic techniques for preoperative preparation of patient's skin may vary slightly according to your country or your hospital but their basis and definitions are precise and based on numerous studies. Every rheumatologist who starts performing synovial biopsies has to refresh or acquire knowledge in this specific domain. To date, precise, validated and easily accessible recommendations are published. USG biopsies either with P&F of with a disposable needle biopsy require

heeding these precautions. Chlorhexidine gluconate and povidone-iodine can be used for patient skin preparation while alcohol-based handrubs are used for surgical hand preparation.

One has to be aware of the maximal dose, side effects and potential chondrotoxicity of local anesthetics. Ultrasound pre-biopsy examination is important so as to choose the joint of interest and to assess biopsy feasibility. Identification of the different structures (tendons, blood vessels, nerves) along the needle path toward the synovial thickening prevents many problems.

Finally, intra-articular glucocorticoid injections can be safely performed at the end of the procedure if clinically necessary.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Synovial Tissue Sampling in Rheumatological Practice—Past Developments and Future Perspectives

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Synovial biopsies are performed in routine clinical care in order to refine diagnosis as well as within a research setting. Progress in the development of minimally invasive synovial sampling methods in the last century has accelerated and facilitated novel insights into disease pathogenesis. This review discusses the development of synovial biopsy techniques as well as examining the three currently most commonly used approaches: arthroscopic, blind needle biopsy and ultrasound guided approaches. It also highlights major research advances driven through synovial research and considers future developments.

Keywords: synovium, biopsy, ultrasound, arthroscopy, rheumatoid arthritis

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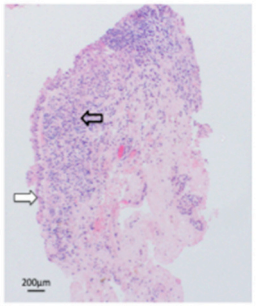
INTRODUCTION: HISTORICAL PERSPECTIVE

Synovial tissue lines the diarthrodial joints, tendon sheathes, and bursae, and functions to supply nutrients to the avascular cartilage and to lubricate the joint. In the clinical setting synovial tissue sampling is infrequently required to exclude either infection, when insufficient information is gained from sampling of synovial fluid or peripheral blood, or to refine the diagnosis of an inflammatory synovitis through identifying conditions such as sarcoid (1), Behcets (2), or pigmented villonodular synovitis (3). However, since the term rheumatoid arthritis (RA) was first proposed by Garrod (4) synovial tissue analysis has been utilized as a research tool to examine disease pathogenesis and/or dissect pathogeneic processes determining prognosis and/or response to therapeutic intervention. However, early research efforts in this regard were hampered by access to synovial samples derived from only post mortem specimens or open arthrotomy and thus end stage disease. Although the development of arthroplastic surgery in the early 1930s began to provide a more consistent source of synovial tissue concerns that these samples might not be truly representative of RA pathogenesis were confirmed by later reports that demonstrated significant differences in synovial cellular infiltrate between established and end stage disease (5). Notwithstanding these limitations an observed diversity in synovial histopathological characteristics between patients was noted early on and fuelled efforts to develop novel less invasive methods to sample synovial tissue and examine whether such diversity translated to significance differences in clinical phenotypes. One of the first attempts to develop a minimally invasive sampling technique was by Forrestier who described the application of a modified dental nerve extractor inserted into joints through a larger needle to sample synovial tissue (6). However, formal reports of the method were never published and therefore the technique not translated to clinical practice. Subsequently an approach applying the insertion

of a percutaneous needle inserted via a trochar to perform punch biopsies of synovial tissue was reported with success rates approaching 86% for sampling synovial tissue (7–9). However, due to the requirement for an incision and the insertion of a relatively large instrument, although significant complications were not reported, considerable soft tissue trauma was inevitable and this approach therefore not widely adopted. Despite this by 1960 joint features such as histological synovitis, proliferating invasive pannus and cartilage erosions were well described (10). The next major advance arrived with the development of the Parker-Pearson needle in 1963 which utilized a small bore 14G needle and did not require a skin incision (11). A case series of 125 patients documented a success rate of >95% in sampling synovial tissue and moreover demonstrated its safety in this context (11). The subsequent application of the Parker-Pearson needle biopsy or a modification of it (12, 13) led to significant progress in the understanding of RA pathogenesis with reports describing synovial lining layer infiltrates (14) as well as histopathological features of early synovitis (15) (**Figure 1A**) and remained the instrument of choice for acquiring synovial tissue for diagnostic or research purposes until the 1980s. However, blind needle biopsy was primarily used for sampling synovial tissue from knee joints and was not a useful technique for joints with limited synovitis (16). Thus the transfer of arthroscopy from a primarily diagnostic tool used by orthopedic surgeons to rheumatology research in the 1980s particularly with utilization of smaller bore needle arthroscopes, which permitted access to joints other than the knee and those with minimal synovitis, offered significant advantages, and was readily adopted by the academic rheumatology community (17). Despite this a number of issues associated with arthroscopy such as requirement for highly specialist training, dedicated space and equipment and relatively high cost limited the adoption of arthroscopy outside of large academic rheumatology centers. However, the development of musculoskeletal ultrasound (US) as a diagnostic and management tool for patients with inflammatory arthritis in the mid 1990s presented the opportunity to overcome these limitations by guiding minimally invasive biopsy instruments to synovial tissue via live ultrasound images. Two US-guided biopsy techniques have been reported firstly applying a semi-automatic needle (18) and latterly using a portal and forceps approach (19) to sample synovial tissue. Efforts to validate US-guided biopsy have demonstrated that it appears to be well tolerated (20), able to access a wide range of synovial joints (20–22) provide good quality and quantity of synovial tissue (23) and when applying a semi-automatic needle able to access joints with minimal synovitis (21). Such data therefore supports the current uptake of the technique into both clinical trial protocols as well as routine clinical care (24). The development of synovial sampling techniques over the last century is summarized in **Figure 1B**.

OVERVIEW OF BIOPSY TECHNIQUES

At present there are broadly three techniques used to sample synovial tissue, which will be discussed briefly below.



B

Year	Procedure
1930	Arthroplasty
1932	Dental Nerve extractor (Forestier)
1952	Needle biopsy
1963	Parker Pearson Needle
1968	Arthroscopy
1995	Needle arthroscopy
1997	US guided synovial biopsy

FIGURE 1 | Synovial sampling techniques. **(A)** Representative image of RA synovial tissue demonstrating hypertrophy of lining layer (white arrow) and sublining infiltration by lymphocytes (open arrow). **(B)** The development of minimally invasive synovial sampling techniques.

Blind Needle Synovial Biopsy

This is performed following administration of local anesthesia to the skin and subcutaneous tissues up to the joint capsule. Following standard aseptic techniques a trochar is inserted into the joint capsule through which a 14G Parker-Pearson needle is positioned to retrieve synovial tissue. Although most frequently performed on the knee joint, biopsy of the shoulder, wrist, ankle, and elbow has been described and with the introduction of a modified short 2.5 cm needle synovial tissue within the metacarpal phalangeal joints (MCP) has been sampled (25). Sampling of synovial tissue from joints with minimal or no inflammation has also been reported with installation of isotonic saline solution into the joint space prior to biopsy (26, 27) although success rates for successful sampling are lower (16). A comparative study of synovial tissue obtained from clinically active joints using either blind needle biopsy or under direct vision with arthroscopy demonstrated good correlation in terms of microscopic measures of inflammation (27). However given the technical difficulties in successful sampling of synovial tissue from joints with little or no synovitis current recommendations suggest its application should be restricted to diagnostic procedures or cross sectional studies of patients with active arthritis (28). The benefits of blind needle biopsy are that it is technically simple, does not require specialist equipment and is safe (**Table 1**).

US Guided Synovial Biopsy

US-guided synovial biopsy can be performed using either a portal and forceps approach or using a semi-automated needle.

TABLE 1 | Considerations for selection of biopsy technique.

	Arthroscopic	U5-NB	US·P&F	Blind needle biopsy
Synovial sampling success rates	+++	+++	+++	+++
Technically simple	+	++	++	+++
Patient acceptability	++	++	++	++
Suitable for serial biopsies	+++	+++	+	+
Cost	+++	++	++	+
Suitable for large or small joints	++	+++	+++	+

Both approaches use standard aseptic protocols and require the installation of local anesthesia to the soft tissues up to the joint capsule and into the joint space. If applying a portal and forceps approach a percutaneous sheath introducer is inserted into the joint under US guidance and either a rigid or flexible forceps introduced to sample synovium (18). Similarly when using a semi-automated needle the closed needle is inserted into the joint and directed to an area of synovium under US guidance (19, 20). The throw of the needle is then opened and synovial tissue sampled. The needle is repeatedly introduced into the joint for multiple biopsy pieces. Although the most recently developed of the available sampling techniques there is an increasing data set to demonstrate its safety, tolerability, and success in reliably sampling synovial tissue both in large and small joints (20, 21, 23) (**Table 1**). In addition serial sampling of joints is feasible although the quantity of tissue for histological and/or molecular analysis decreases dependent on the degree of pre-biopsy US synovitis (20, 21).

Arthroscopic Synovial Biopsy

Under the supervision of rheumatologists arthroscopic synovial biopsy is in general performed using a small bore (1–2.7 mm) arthroscope under general or regional anesthesia as a day case procedure. It is technically the most complex of the synovial sampling procedures available and requires two portals. Arthroscopy also requires a dedicated procedure room or theater space and two operators. It does however have a number of advantages including capacity to be performed in MCP, wrist, ankle and knee joints as well as in joints with minimal or no synovitis with excellent success rates for obtaining synovial tissue (29–31). There is also extensive data evaluating its safety including a study evaluating 15,682 procedures performed by rheumatologists (17) demonstrating equivalence in complication rates to those performed by orthopedic surgeons. Furthermore it has been demonstrated to be well tolerated by patients (32). Thus despite the increased training requirements and cost associated with arthroscopic sampling it remains the gold standard procedure for synovial sampling within clinical trials (28).

SELECTION OF APPROPRIATE SYNOVIAL SAMPLING TECHNIQUE

Historically data examining performance of synovial biopsy techniques was frequently performed in isolation with little opportunity to compare techniques (20, 22, 32) and thus guide selection of ideal method for a specific setting. Such comparative analyses became increasingly important with the advent of US-guided synovial biopsy, which although readily adopted by the rheumatology community at least initially was not validated against the gold standard arthroscopic approach. In order to tackle these issues validation measures for US-guided biopsy were defined at OMERACT 2014 (33) and since then have steadily begun to be addressed. For example a retrospective analysis of evaluation of 159 biopsy procedures suggested that US-guided procedures, though not those performed using blind needle biopsy, were as successful as arthroscopic in retaining sufficient synovial tissue for histological and molecular analyses (23). In addition recent data examining safety and tolerability of synovial biopsy in a cohort of 524 patients under arthroscopic or US-guided biopsy procedures suggested no differences in outcomes (34). Importantly two large scale biopsy based multicentre international clincial trials, the National Institute Health Research funded Response, relapse, resistance to rituximab (R4RA) (Trial)[1] and the Arthritis Research-UK (vs. Arthritis)/Medical Research Council funded STratification of Biologic Therapies for RA by Pathobiology (http://www.matura-mrc.whri.qmul.ac.uk)[2] are due to report outcomes including performance of biopsy techniques in 2019/2020 and will provide the first prospective data sets from randomized controlled clinical trials in which to evaluate performance of both arthroscopic and US-guided biopsy techniques. Considerations for selection of appropriate biopsy technique is summarized in **Table 1.**

MAJOR RESEARCH OUTCOMES

Since synovial tissue was identified as the target tissue in RA it's analysis has led to invaluable insights into disease pathogenesis, in addition to the identification of potential therapeutic targets. Furthermore, with the advent of an era of personalized medicine understanding mechanisms of drug response/resistance as well as defining disease prognosis have been major areas of research focus. There are many examples that have been reviewed extensively elsewhere (35). For example the identification that lymphocytic aggregates capable of functioning as ectopic germinal centers and producing disease specific antibodies within synovial tissue in approximately 30% of patients with RA has identified mechanisms driving local autoimmunity (36) and furthermore such structures have been identified as putative biomarkers of response to TNF inhibition (37). Work evaluating synovial tissue response to therapeutic intervention also identified synovial sublining macrophages as key mediators of RA pathogenesis through demonstrating consistent statistically significant reduction in infiltration following therapeutic response (38) an effect that was

[1] Trial, R. Available online at: http://www.r4ra-nihr.whri.qmul.ac.uk

[2] Available online at: http://www.matura-mrc.whri.qmul.ac.uk STRAP trial.

consistent between centers and across therapies (39). Sublining macrophage number has been translated to a research tool to identify clinical efficacy of novel drugs in early stage clinical development and validated as an outcome marker by OMERACT (39). Potential biomarkers in early arthritis include differential infiltration by CD22+ve B cells and CD38+ plasma cells in patients with early arthritis differentiating RA vs. non RA inflammatory arthritis (40). More recent developments include the identification of joint specific methylation and transcriptomic signatures of synovial fibroblasts (41, 42) providing a potential mechanism to explain both RA joint distribution and differential joint specific therapeutic responses.

FUTURE DEVELOPMENTS

Reliable access to synovial tissue from patients with inflammatory arthritidies is becoming increasingly feasible largely due to the advent of minimally invasive US-guided procedures. Such approaches should facilitate the rapid translation of synovial biomarkers to routine clinical practice once identified. However, further validation of US guided procedures are required including the evaluation of procedures along with arthroscopic within the context of prospective large scale randomized controlled trials with robust reporting measures for defining successful sampling as well as capturing adverse events and patient tolerability in standardized patient cohorts. In addition training requirements for rheumatologists undertaking such procedures, such as have been developed for arthroscopic synovial sampling, need developing.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Psoriatic Synovitis: Singularity and Potential Clinical Implications

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Psoriatic arthritis (PsA) is an immuno-inflammatory disease with a heterogeneous clinical presentation as affects musculoskeletal tissues (arthritis, enthesitis, spondylitis), skin (psoriasis) and, less frequently, eye (uveitis) and bowel (inflammatory bowel disease). It has been suggested that distinct affected tissues could exhibit different immune-inflammatory pathways so complicating the understanding of the physiopathology of psoriatic disease as well as its treatment. Despite of the key pathogenic and clinical relevance that enthesitis has in PsA, peripheral arthritis is more easily perceived. At the macroscopic level, PsA synovitis has predominantly tortuous, bushy vessels, whereas rheumatoid arthritis (RA) is characterized by mainly straight, branching vessels so reflecting prominent neo-angiogenesis in PsA. Synovial biopsies have demonstrated a similar cellular and molecular picture in PsA and RA, although some differences have been reported at the group level, as higher density of vessels, CD163+ macrophages, neutrophils and mast cells in PsA. In fact, synovial IL-17+ mast cells are significantly increased in PsA and produce more IL-17A compared with RA, and a proof of concept study supports its relevant role in the synovitis of SpA, included PsA. As firstly reported in RA, synovial lymphoid neogenesis is found also in the same proportion of PsA as in RA patients, despite the lack of autoantibodies in PsA. These lymphoid structures are associated with activation of the IL-23/Th17 pathway in RA and seemly in PsA, which could be useful to stratify RA patients. Immunohistochemical and transcriptomic methodologies have still not found synovial biomarkers useful to distinguish psoriatic from rheumatoid synovitis at the patient level. However, modern methodologies, as MALDI-Mass Spectrometry Imaging, applied to the study of synovial tissue have revealed metabolic and lipid signatures which could support clinical decision-making in the diagnosis of PsA and RA and to go further toward the personalized medicine.

Keywords: psoriatic arthritis, rheumatoid arthritis, synovitis, immunohistochemistry, macrophage-polarization, microarrays, mass spectrometry image

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INTRODUCTION

Psoriatic arthritis (PsA) is an immune-mediated inflammatory disease with a wide range of clinical manifestations: synovitis, enthesitis, spondylitis, dactylitis, skin, and nail psoriasis. More rarely, it involves the eye (uveitis) and the bowel (Crohn's disease). PsA is included in the spondyloarthritis (SpA) concept, which encompasses a group of diseases sharing immunogenetic, pathophysiological, clinical, and radiological features, which differ from rheumatoid arthritis (RA) (1). McGonagle et al. hypothesized that the primary lesion of SpA is enthesitis, that enthesopathy

may be the common link between all forms of SpA, and that enthesitis in SpA synovial joints is frequent (2, 3). The close anatomical relationship between the enthesis, prone to mechanical stress, and the vascular synovium, in contact with a variety of immune mediators, may provide the pathogenic basis for joint inflammation in SpA, including PsA. The functional unit formed by enthesis and adjacent synovium was termed as synovial enthesis complex (SEC). The SEC represents a conceptual framework, which may explain the tissue specificity and highlights the role of mechanical stress in SpA, while at the same time providing a unifying pathophysiological concept for PsA based on the idea that specific tissues may be particularly sensitive to mechanical triggers (4). Paramarta et al. challenged the hypothesis of enthesitis being the primary lesion in SpA leading to a secondary synovitis over time, although the authors recognized some limitations in their study (5). Also, the study of enthesitis pathophysiology is limited by the difficulty to obtaining biopsies from the enthesis due to potential adverse effects.

Arthritis is more easily perceived that enthesitis, as clinical trials and registries of patients with PsA have showed. Peripheral arthritis is a key target of the pathogenic process which may lead to joint destruction and associated impaired function and quality of life (6). Therefore, psoriatic synovitis has been widely studied, generally as part of other peripheral SpA and has been compared with RA, the most prevalent peripheral arthritis (7).

The synovial membrane (synovium) borders the joint cavity and attach to the bone-cartilage interface. A healthy synovium consists of a thin layer (lining) 1-2 cells thick containing synovial fibroblasts and macrophages. Below this layer is the sublining, which is composed of loose connective tissue with blood vessels, lymphoid vessels, fibroblasts, nerve fibers, and few leucocytes. The inflamed synovium (synovitis) has three histological characteristics: lining hyperplasia (proliferation of synovial fibroblasts and accumulation of macrophages); neoangiogenesis (blood vessel proliferation in the sublining), and huge infiltration of the sublining by inflammatory cells, including lymphocytes, macrophages, dendritic cells and mast cells, which produce proinflammatory cytokines, growth factors and metalloproteases contributing to persistent synovitis and joint destruction (1, 7).

The study of synovitis in PsA, RA and other chronic arthritis is being driven by mini-arthroscopy and ultrasound-guided biopsies, which are safe and well-tolerated techniques and allow synovial tissue samples to be obtained from large and small joints at any stage of activity of disease: early, established, active or remission, as well as before and after therapeutic interventions. Taken together, easier extraction of synovial tissue together with the application of powerful new methodologies (trancriptomics, single-cell RNA, proteomics, metabolomics, new inmmunohistologic markers, mass spectrometry image analysis) will accelerate the study of synovitis to better understand their diagnostic and prognostic implications (8). Most studies on synovitis have focused on RA, while others comparing RA and SpA, included PsA; however few studies have focused specifically on PsA. We review PsA synovitis from the macroscopic (arthroscopy) and microscopic perspective, highlighting the cellular and molecular characteristics of each of the histological alterations of synovitis mentioned above as compared with RA. **Table 1** displays some key clinical and pathogenic differences between PsA and RA.

TABLE 1 | Differences between PsA and RA.

Features	Psoriatic arthritis	Rheumatoid arthritis
Clinical	Asymmetrical arthritis lower limbs DIP joints, enthesitis, dactylitis, Axial arthritis	Symmetrical MCP and wrist joints
Genetics	HLA-B38, -B39, and HLA-B27 HLA-cw6 IL-23/IL-17 pathway-related genes	HLA-DRB1 PTPN22
Pathogenesis		
Autoantibodies	No	ACPA and RF
Synovial immunopathology	Innate immune cells (IL-17A+ Mast cells, Neutrophils)	Adaptive immune cells (B and T-cells)
Synovial neoangiogenesis	Intense	Moderate
Vessels morphology*	Bushy, tortuous vessels	Straight, branching vessels
Radiology	Bone erosion and neoformation	Erosion
Therapeutic targets	TNFi Anti-IL-23/IL-12 Anti-IL-17A Anti-IL-23	TNFi Anti-B cells (anti-CD20) Anti-T cells (CTL4-Ig) Anti-IL-6

*MCP, Metacarpophalangeal; DIP, distal interphalangeal; ACPA, Anti-Citrullinated Peptide Antibody; RF, Rheumatoid Factor; IL, Interleukin; TNFi, Tumor Necrosis Factor inhibitors; *Macroscopy vessels morphology as seen by arthroscopy.*

SYNOVIAL TISSUE FEATURES IN PSA SYNOVITIS

The morphologic and cellular heterogeneity of synovitis requires review of the macroscopic features, which appear to differ between PsA and RA, and subsequent description of the cellular features according to the key changes that occur in inflammation: lining hyperplasia, neo-angiogenesis and leukocyte infiltration.

Macroscopic Features of PsA Synovitis

Using rheumatologic arthroscopy, Reece et al. (9) found significant differences in the pattern of new blood vessel between psoriatic and rheumatoid synovitis. PsA synovitis is characterized by erythematous villae with dilated, bushy and tortuous vessels (**Figure 1A**) whereas RA synovitis predominantly shows straight, branched vessels. This distinct pattern probably reflects a distorted proliferation of neovessels (neo-angiogenesis) due to increased expression of pro-angiogenic mediators, such as VEGF and Angiopoietin-2 (Ang-2), in PsA (10). Other studies confirmed these findings in PsA and peripheral SpA, with some differences in the frequency of the straight and branched

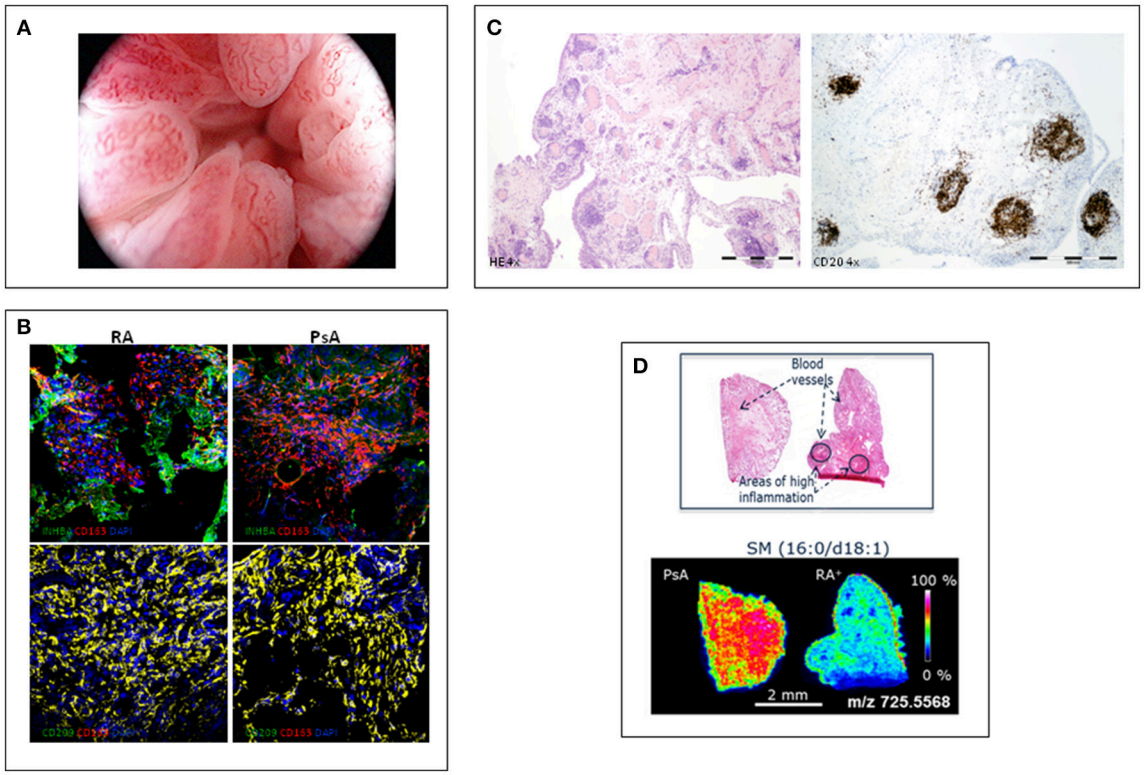

FIGURE 1 | Different features of psoriatic synovitis are represented. **(A)** Arthroscopic view of psoriatic synovitis with erithematous villae plenty of dilated, tortuous vessels. **(B)** Immunofluoresence analysis of the expression of macrophage-polarization markers in synovial tissue CD163+ macrophages from RA and PsA patients, as determined by confocal microscopy using anti-INHBA (Activin A) –a GM-CSF induced gene- and CD209 –a M-CSF induced gene- specific antibodies; nuclei were counterstained with DAPI (Courtesy of A Puig-Kröger, PhD, Madrid, Spain). **(C)** Staining of inflammed synovium from a patient with PsA; left: H-E staining (4x) showing a general view of synovial membrane standing out abundant vessels surrounded by folicular aggregates; right: CD20 staining (4x) highlighting the B-cell folicles in PsA synovitis. **(D)** Mass Spectometry Image analysis showing spatial mapping positive-lipid ion in synovium sections of PsA and RA. Scale bar shows normalized intensities (Courtesy of Prof. F Blanco, A Coruña, Spain).

pattern of RA (11–13). Despite its high sensitivity and specificity, the bushy and tortuous pattern is not diagnostic of PsA, although it may be a useful guide in the diagnostic work-up of undifferentiated arthritis (12).

Cellular Features of PsA Synovitis

Hyperplasia of the Synovial Lining

Fibroblast-like synoviocytes (FLS) and macrophages are the cellular components of the lining. Inflammation induces activation, proliferation and/or diminished FLS apoptosis, whereas macrophages increase due to infiltration from the peripheral blood. Studies of FLS in PsA are scarce compared with RA, where it has been shown that FLS undergo epigenetic changes, becoming persistently activated and forming the invasive front of synovial tissue in the joint cartilage (pannus) (14). A study of the effects of Janus kinase inhibitor tofacitinib on synovial fibroblast function suggested that PsA fibroblasts are activated similarly to RA fibroblasts (15). In RA, FLS change phenotypically and functionally at different anatomical sites and contribute to the identity of individual tissue, and they are capable of actively participating and orchestrating inflammation and immunity (14). A single-cell RNA sequencing and immunohistochemistry based study has described three functionally distinct subsets of FLS in RA: lining, immunoregulatory, and pathogenic fibroblasts populations. Pathogenic fibroblasts are located in the sublining around the vessels (CD34-CD90+) and they are the only FLS subset significantly increased in RA compared with osteoarthritis (16). Although several studies have reported increased lining hyperplasia in RA compared with PsA, others have found no differences (7). Using the Hsp47 antibody, a new specific marker of lining and sublining FLS (17), we have found a significant increase of sublining FLS, but no lining FLS, in RA compared with PsA, without between-group differences in systemic inflammation markers (CRP) (18). Lining CD68+ macrophages are functionally heterogeneous and include proinflammatory and tissue resident macrophages, a population not well-defined by lack of markers, but there are no differences in their cellular density between PsA and RA synovitis (19).

Neoangiogenesis

In line with the macroscopic hypervascularization that characterizes PsA synovitis, several studies have found an increase of vessels density in PsA compared with RA.

Furthermore, different pro-angiogenic factors are expressed in the two diseases, with increased Ang-2 in PsA and Ang-1 in RA (20–22). Successful treatment with anti-TNF therapy in PsA synovitis reduces expression of VEGF and its receptors VGFR1 and VGFR2, but not Ang-2 expression, leading to regression of neovessels, probably by inducing endothelial cell apoptosis (23).

A recent study comparing CD31+ synovial vessels between PsA ($n = 38$) and RA ($n = 40$) patients found no significant differences between the two diseases (18).

Synovial Leukocyte Infiltrate

A vast influx of inflammatory cells of the innate and adaptive immune system populates the inflamed synovial membrane, with the most being macrophages, neutrophils, mast cells, and T and B-lymphocytes. All these cells are activated and produce multiple pro-inflammatory and pro-angiogenic cytokines, chemokines, growth factors, metalloproteases, and other mediators, which contribute to the persistence of synovitis and joint destruction. Global cell infiltration in PsA and RA synovitis in the histologic analysis is similar, although characterization by immunohistochemistry of the infiltrating cells could encounter differences, as synovial infiltration by mast cells, CD15+ neutrophils and CD163+ macrophages is increased in SpA, included PsA, compared with in RA (7).

Synovial Macrophages

CD68+ macrophages accumulate in the synovium of RA and PsA joints, where they exhibit destructive and remodeling potential and contribute considerably to joint inflammation and joint destruction (24, 25). In RA and in SpA, including PsA, macrophage density correlates with disease activity (19). Sublining CD68+ macrophages density has been shown to be similar in PsA and RA synovitis (18, 26). A small study comparing RA and PsA synovitis found that synovial p53 expression and CD68+ macrophages density was associated with erosive disease only in RA suggesting that CD68+ macrophages differ in the destructive potential between RA and PsA (27).

Few studies have analyzed macrophage subsets in chronic arthritis, but have shown differences, probably due to the markers used. CD163-positivity has been proposed as a biomarker of anti-inflammatory macrophages and CD163+ macrophages were found overexpressed in SpA synovitis, whereas RA was characterized by overexpression of pro-inflammatory macrophage markers (19). A study using surface markers (CD14, CD163, CD68, CD32, CD64, CD200R, CD80) on synovial tissue macrophages from RA and SpA patients found that macrophages had a mixed M1-proinflammatory/M2-anti-inflammatory phenotype, with M1 predominance in RA and IL-10-expressing macrophages in SpA (28).

The characterization of *ex-vivo* CD14+ macrophages isolated from the synovial fluid of patients with active RA indicates that they exhibit a transcriptomic and protein profile that is compatible with a GM-CSF-skewed macrophage polarization (29). The proteins encoded by several of the GM-CSF-associated gene markers have also been detected in macrophages from active RA synovial tissue, including activin A, MMP12 and CCR2 (29). We analyzed the expression of markers of GM-CSF derived macrophages (INHBA, MMP12, and TNFα) and M-CSF derived macrophages (CD209) on CD163+ macrophages, and found a similar expression of GM-CSF- and M-CSF-associated markers in synovial tissue of RA and PsA patients (30) (**Figure 1B**). These results support the presence of similar GM-CSF and M-CSF skewed macrophages in RA and PsA synovitis.

Synovial Mast Cells

Mast cells have been reported to have a potential sentinel function as innate protective cells which is supported by their strategic location in skin, gut, and airways, and their expression of specific danger signal receptors such as TLR2 and TLR4. Mast cells also have the ability to synthesize and, in addition, release preformed mediators including cytokines, proteases, and anti-microbial defensins (31). Mast cells play a previously- unappreciated role in synovial inflammation in SpA, included PsA, as it has been shown that they are significantly more abundant in PsA than in RA synovitis and, importantly, they are also the main cellular source of IL-17 A in PsA synovial tissue. These findings are independent of the disease stage and anti-TNF therapy (32). However, the absence of IL-17A mRNA in mast cells has also been demonstrated and a novel mechanism whereby mast cells capture and store exogenous IL-17A in specialized intra-cellular vesicles through receptor-mediated endocytosis, releasing bioactive IL-17 A after mast cell stimulation, has been discovered (33).

New findings reporting IL-17A-loaded mast cells in the normal skin and gut, in SpA synovial tissue before and after anti-IL-17 A antibody secukinumab, and in the inflamed gut, support the concept of mast cells as sentinel cells, as IL-17A-positive mast cells are readily available in non-inflamed tissues, and the IL-17A content decreased during inflammation in the gut lamina propria and increased upon anti-inflammatory treatment of SpA synovitis (31). Therefore, the presence of IL-17A-positive mast cells across different SpA target tissues and the inverse correlation between their IL-17A-content and inflammation indicate that the IL-17A content in mast cells can be regulated (31). Understanding how IL-17A can be controlled locally during tissue inflammation may result in novel therapeutic strategies to target IL-17A, a key cytokine in PsA (31).

In RA synovitis, high synovial mast cell counts are associated with local and systemic inflammation, autoantibody positivity and high disease activity. They are located at the outer border of lymphoid aggregates. Furthermore, mast cells promote the activation and differentiation of naïve B cells and induce ACPA production, mainly via contact-dependent interactions (34). Although synovial mast cells are also the main IL-17A positive cells in RA synovitis, its role remains to be studied (35).

Synovial Neutrophils

Polymorphonuclear cells have been reported to be increased in synovial tissue of axial and peripheral SpA, including PsA synovitis, compared with RA, and correlated with disease activity. Their reduction after treatment was associated with a good therapeutic response, leading to them being

defined as a biomarker of response for SpA (36, 37). In fact, after mast cells, neutrophils (CD15+ cells) are the most frequent IL-17 A+ cells in SpA and PsA (32). Neutrophils are scarce in RA synovitis, but a recent study comparing sinovial CD15+ cells (neutrophils) in PsA and RA synovitis found no significant differences between the two diseases (18).

Lymphocytes and Ectopic Lymphoid Neogenesis

Although PsA seems to have a partial autoinflammatory pathophysiology whereas RA has a strong autoimmunity component (38), in general sinovial T and B-lymphocytes, and plasma cells have been found to be similar in PsA and RA synovitis (7). However, beyond the number and type of infiltrating leukocytes, their spatial organization in the sinovial microarchitecture may be of pathophysiological relevance (7). Ectopic lymphoid neogenesis (ELN) is characterized by lymphocyte aggregates (**Figure 1C**) with prototypical features that recapitulate those of germinal centers, such as the presence of high endotelial venules and folicular dendritic cells (39). As ELN resembles secondary lymphoid tissues, it has been proposed that sinovial ELN may play a role in mounting immune responses, and specifically the autoimmune response observed in RA (40). However, sinovial ELN is similarly found in PsA and in RA, and there is no association with the presence of RA-specific autoantibodies (41–43). However, synovial ELN in PsA and RA have been associated with a different cytokine profile characterized by specific expression of the IL-23/Th17 cytokines axis (44, 45). These findings suggest that an important subgroup of RA patients express high IL-23/IL-17 cytokines, introducing the potential of stratification of patients by ELN in exploratory clinical trial for anti-IL23 or anti-IL-17 antibodies.

Microarray Analysis of Synovial Tissue in PsA

Comparison of synovial biopsies of patients with RA and SpA, including PsA, to analyse synovial molecular and cellular processes by pan-genomic microarray, has revealed a myogene signature specific for SpA, which was independent of disease duration, treatment and SpA subtype (non-psoriatic vs. psoriatic). These findings were confirmed by qPCR and immunohistochemistry analysis, and the synovial cells expressing myogenes were identified as vimentin-positive, prolil4-hydroxilase-positive, CD90+,CD146+ mesenchimal cells in the lining and sublining layers. This specific myogene signature did not change after anti-TNF therapy (46).

A study of gene array in paired skin and synovial biopsy samples from 12 patients with both PsA and psoriasis, confirmed by PCR and immunohistochemistry, showed that gene expression patterns in psoriatic skin and synovium differed, with a stronger IL-17 signature in skin than synovium, while TNF was higher in synovium (47). These transcriptomic analysis reveal new molecular pathways that open new avenues in the knowledge of the differential pathogenesis of synovitis in PsA and RA as well as between different tissues involved in PsA.

Mass Spectrometry Imaging Analysis

A pionner study used Mass Spectrometry Imaging (MSI) to identify lipid and metabolic profiles in the synovial tissue of 25 patients with PsA, 21 with RA (16 seropositive and 5 seronegative) and 10 with undifferentiated arthritis. Tissue sections were deposited on conductive slides and coated with different matrices for lipid and metabolite extraction. MALDI images were acquired on a rapifleX MALDI Tissuetyper time-of-flight instrument. Multivariate data analysis was used to search for the lipids and metabolites with the highest between-group differences.

MALDI-MSI revealed differentiated lipid and metabolic profiles in all the groups studied. Discriminant analysis of the lipid data acquired in positive ion mode displayed a good separation of patients with PsA and RA, especially seropositive RA (**Figure 1D**). PsA synovium was characterized by a higher content of phospholipids compared to seronegative and seropositive RA. However, sugar metabolites displayed a stronger intensity in RA than in PsA synovium. Metabolic and lipid signatures reported with this new methodology could support clinical decision-making in the diagnosis of RA and PsA (48).

CONCLUSIONS

Globally, PsA synovitis has more similarities than differences when compared with RA at the histologic and immunohistochemical level. However, there is some singularities in PsA that merit more in-depth research: the role of IL-17-positive mast cells in PsA inflammation and in IL-17 A regulation; the role of ectopic lymphoid neogenesis in PsA, and to know if there is distinct functional subsets of synovial FLS in PsA as in RA. New research tools as pan-genomic microarrays and metabolomics/proteomics associated to mass spectrometry image analysis are full of promise to reveal new cellular and molecular features specific to PsA synovitis which improve our diagnostic and prognostic potential.

AUTHOR CONTRIBUTIONS

JC revised the references and wrote the first draft of the manuscript. RC, AC, and JR revised the manuscript and collaborate in the discussion and writing to the last version. All the co-author revised and approved the manuscript.

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MINI REVIEW
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Synovial Tissue Heterogeneity in Rheumatoid Arthritis and Changes With Biologic and Targeted Synthetic Therapies to Inform Stratified Therapy

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The treatment of rheumatoid arthritis (RA) has been transformed with the introduction of biologic disease modifying anti-rheumatic drugs (bDMARD) and more recently, targeted synthetic DMARD (tsDMARD) therapies in the form of janus-kinase inhibitors. Nevertheless, response to these agents varies such that a trial and error approach is adopted; leading to poor patient quality of life, and long-term outcomes. There is thus an urgent need to identify effective biomarkers to guide treatment selection. A wealth of research has been invested in this field but with minimal progress. Increasingly recognized is the importance of evaluating synovial tissue, the primary site of RA, as opposed to peripheral blood-based investigation. In this mini-review, we summarize the literature supporting synovial tissue heterogeneity, the conceptual basis for stratified therapy. This includes recognition of distinct synovial pathobiological subtypes and associated molecular pathways. We also review synovial tissue studies that have been conducted to evaluate the effect of individual bDMARD and tsDMARD on the cellular and molecular characteristics, with a view to identifying tissue predictors of response. Initial observations are being brought into the clinical trial landscape with stratified biopsy trials to validate toward implementation. Furthermore, development of tissue based omics technology holds still more promise in advancing our understanding of disease processes and guiding future drug selection.

Keywords: rheumatoid arthritis, biologics, JAK inhibitors, synovial tissue, histology, cytokine, gene expression, pathotypes

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INTRODUCTION

Rheumatoid arthritis (RA) is a complex, genetically and biologically heterogeneous autoimmune disease. It is characterized by a systemic inflammatory arthritis. The treatment of patients with RA has evolved considerably in recent years owing to the successful development and widespread use of biologic disease modifying anti-rheumatic drug (bDMARD) therapy, with more recent introduction of targeted synthetic DMARDs (tsDMARD) in the form of small molecules inhibitors.

However, up to 40% patients in clinical trials fail to respond, also reflected in real-world practice; and a sizeable proportion fail to achieve the target of therapy, mainly clinical remission where appropriate or low disease activity (1, 2). Personalized medicine, i.e., tailoring therapy to individual patient (or, put simply, "choosing the right drug for the right patient"), has the potential to improve response rates, but has proven challenging to implement. If it is to be successful, the identification of reliable biomarkers will be of prime importance.

In this mini-review, we summarize the evidence for synovial tissue heterogeneity, and tissue studies that have evaluated change in cellular and molecular markers following currently available bDMARD and tsDMARD specifically that could aid treatment selection.

THE SYNOVIUM, PRINCIPAL TARGET OF INFLAMMATION

The synovium is the principal target of inflammation in RA, undergoing marked pathological changes compared to healthy tissue. The study of RA synovial tissue has offered insights at a cellular level into multiple aspects of the disease, from identifying pathogenic processes and pathways (3, 4); to explaining clinical manifestations. Furthermore, changes in synovial tissue following successful treatment allow better understanding of mechanism of drug action (5–7).

Synovial tissue samples can be obtained via arthroscopic or ultrasound (US)-guided biopsies. The US-guided approach has been shown to be safe, with reproducible tissue quality/RNA yield (8), and has the advantage of enabling joint assessment for synovial thickness (gray-scale score) and vascularity (Power Doppler-PD), associated with active synovial inflammation (9).

HEALTHY SYNOVIUM

In health, the synovial membrane contains relatively few cells, consisting of an intimal lining layer of 1–2 cell thickness and a distinct synovial sublining layer (10). The intima comprises fibroblast-like synoviocytes (FLS, also known as synovial fibroblasts or type B synoviocytes) intercalated with macrophage-like synoviocytes (MLS, also called type A synoviocytes) (11). The sub-lining layer is a well-vascularized connective tissue, containing collagen fibers and evenly dispersed FLS and MLS (11).

The synovial membrane is key to the structure and function of the healthy synovial joint. The synovial membrane controls transport to and from the synovial cavity, thus maintaining the composition of synovial fluid as well as overall joint homeostasis and integrity. The intimal lining is particularly important, as its lack of tight junctions or a true basement membrane allows the ingress and egress of various cells and proteins (12). Intimal FLS orchestrate proceedings, controlling the synovial fluid volume, secreting hyaluronan for lubrication, clearing intra-articular debris, regulating various immunological processes, and maintaining the extracellular matrix (ECM) of the sublining (13).

RA SYNOVIUM

In RA, the synovial tissue becomes markedly expanded, with a striking increase in cellular infiltration. This leads to hallmark "pannus" formation at cartilage-bone interfaces; pannus can be composed of macrophages, FLS, leucocytes, plasma cells, and mast cells (14), and behaves like a locally invasive tumor, mediating damage and erosion formation in later disease (15). The intimal lining can expand to 10–20 cells in thickness, partly due to an increase in FLS, but mostly due to infiltration by bone marrow-derived MLS recruited from the circulation (15). Highly activated macrophages send pro-inflammatory signals to intimal FLS, inducing invasiveness, and to B cells, which in turn produce various pro-inflammatory mediators. Paracrine and autocrine signaling networks develop in this way, further propagating synovitis (16). Sub-lining MLS have been associated with disease activity (17) and synovial inflammation measured on magnetic resonance imaging (MRI) (18), and therefore appear of paramount importance to the inflammatory joint reaction (19). Proliferation of FLS are a prime cause of synovial hyperplasia, and major mediators of damage to cartilage and bone, via both direct and indirect interactions, including production of inflammatory mediators, adhesion molecules, proteolytic enzymes and pro-osteoclastogenic factors (13). T cells are able to establish important crosstalk with antibody-producing plasma cells (15, 20, 21). When present, CD3+ T cells in the RA synovium are mostly found in deeper sub-lining layers, where they may be homogeneously or randomly distributed, or clustered in follicle-like structures (19). Similarly, B cells, when present, are mostly organized in follicular structures, which can act as pro-inflammatory, immunological niches (19).

HETEROGENEITY OF RA SYNOVITIS

RA synovitis is highly heterogeneous, with diverse cellular and molecular signatures (22, 23). In recent years distinct patterns have been recognized, primarily according to the composition, organization and localization of cellular infiltrates. Studies have revealed RA synovial 'pathotypes' (7, 24), namely, lymphoid, myeloid, pauci-immune, and fibroid variants (other patterns, such as granulomatous synovitis, have also been described). The lymphoid pathotype is characterized by lymphoid infiltrates, which may be diffuse (small, loosely arranged lymphocyte clusters) or follicular (large aggregates of lymphocytes organized in ectopic lymphoid structures). The latter may develop germinal centers containing T follicular helper (Tfh) cells highly expressing of programmed cell-death (PD-1), C-X-C chemokine receptor 5 (CXCR5), B-cell lymphoma (Bcl6), and Inducible T cell costimulator (ICOS) (7, 24, 25). Cellular composition of tissue defined as myeloid pathotype shows a less abundant B and T cells aggregates compared to the lymphoid subgroup, and presence of sublining macrophages. By contrast, the 'pauci-immune' (7) (or 'low inflammatory') pathotype shows minimal infiltrating immune cells (24). The fibroid pathotype has complete absence of aggregates and little immune infiltration comprising hyperplastic tissues.

FLS are also not a uniform population but segregate into different phenotypes based, in part, on their cytokine profiles (26). Additionally, functionally distinct disease-associated subsets of fibroblasts are recognized in RA synovium (27) including a study based on surface expression of CD34, THY1, and CDH11 (28). T and B cells infiltrating the inflamed synovium in RA show the highest degree of qualitative and quantitative heterogeneity. Whilst the relation of fibroblast subsets to clinical outcomes remains to be elucidated, these may prove to be instructive biomarkers.

SYNOVIAL TISSUE GENE EXPRESSION PROFILES

Early gene expression of RA synovial tissue studies identified distinct profiles and revealed the presence of multiple activated signaling pathways (29–31). Perhaps unsurprisingly given its clinical heterogeneity, expression of molecular signatures in RA is likewise heterogeneous. Gene expression profiles can be modulated by disease activity and the burden of inflammation in synovial tissue (32). Gene expression in RA synovial (intimal) lining cells specifically has been analyzed using a laser mediated micro-dissection (LIMM) approach (33). Data analysis using clustering revealed two distinct RA subgroups associated with increased expression levels of inflammation-related genes [compared with osteoarthritis (OA) control tissue] involved in the tumor necrosis factor TNF-activated interferon regulatory factor (IRF1)- interferon (IFN)- signal transducer and activator of transcription 1 (STAT1)- pathway (34). Three molecularly distinct forms of RA tissues have also been identified by the same group; the first characterized by genes involved in inflammation and the adaptive immune response [matrix metalloproteinase (MMP) 1 and 3 genes, STAT-encoding and -induced genes and antigen-presenting-cell–related genes], the second characterized by genes involved in extracellular matrix remodeling (genes involved in degradation of cartilage and subchondral bone), and the third with a low-inflammation gene signature similar to that of osteoarthritis (30, 31). Increased receptor activator of nuclear factor kappa-B ligand (RANKL) (35) and decreased osteoprotegerin expression (36) have also been detected in actively inflamed RA synovial tissue. These findings, along with the lack of tissue repair signatures, support the hypothesis of inflammation-driven joint remodeling in RA, characterized by uncoupling of destructive and reparative processes (37). A number of transcription factor families, such as nuclear factor kB (NF-kB) and the activator protein 1 (AP-1), were established early on as chief regulators of gene expression in the inflamed synovium (38). Gene expression analysis of FLS indicates the presence of 2 subtypes, with high-inflammatory FLS expressing transforming growth factor (TGF)-β/activin A–inducible genes and FLS from low inflammatory synovial tissue predominantly expressing growth factor genes (39). Distinct molecular signatures indicating pathways relating to T cell-mediated immunity and major histocompatibility complex (MHC) class II mediated immunity (amongst others) upregulated in early RA, and pathways relating to the cell cycle upregulated in later disease (40) have been reported. Similarly differential gene expression between high and low inflammatory subsets of RA patients in relation to disease duration has been observed (29).

GENE EXPRESSION ANALYSIS ACROSS SYNOVIAL PATHOTYPES

Differential gene expression has also been confirmed across the RA synovial pathotypes described earlier, providing further evidence for different molecular mechanisms underlying these variants. The lymphoid type is characterized by increased expression of genes associated with B cell and plasmablast activation and differentiation [including CD19, CD20, X-box binding protein XBP1, immunoglobulin heavy and light chains, CD38 and C-X-C motif chemokine ligand 13 (CXCL13)], as well as the Janus kinase JAK/STAT pathway and interleukin 17 (IL-17) signaling (24). In another study, patients with lymphoid aggregates again displayed activation of the JAK/STAT pathway, but also the IL-7 pathway, as well as genes associated with lymphoid neogenesis [such as CXCL13, C-C chemokine ligand 21 (CCL21), and receptor CCR7 and Lymphotoxin alpha (LTα)] and B-cell receptor activation, supporting the existence of a link between tertiary lymphoid structures and the local humoral response (41). In the myeloid pathotype, activation of NF-κB pathway genes (including TNFα, IL-1β, IL-1RA, intracellular adhesion molecule ICAM1, and MyD88), the inflammatory chemokines CCL2 and IL-8, and granulocyte and inflammatory macrophage lineage genes (such as S100A12, CD14, and OSCAR) were identified. In the fibroid pathotype, genes associated with fibroblast and osteoclast/osteoblast regulation were found to be involved, including fibroblast growth factor FGF2, FGF9, BMP6, and osteoprotegerin. Higher expression of Wnt and TGFβ signaling pathway components, as well as "angiogenesis module" genes, were also identified (24). The pauci-immune variant shares characteristics with the aforementioned pathotypes in terms of inflammatory response gene expression, with "M2 monocyte module" genes particularly activated (24, 42). Expression of IL-6, IL-6 receptor components (IL-6R and IL-6ST/gp130), and its associated signaling component STAT3 was broadly observed across all phenotypes, consistent with the multiple roles of the IL-6 pathway in both lymphocyte and fibroblast biology (24, 43). The existence of different gene expression profiles according to RA histological pathotype was also confirmed by Klimiuk et al. who demonstrated increased transcriptional activity of TNFα, IL-1, IFNγ, IL-10, and TGFβ in follicular synovitis, compared with diffuse synovitis (44).

Recently, a machine learning algorithm was able to predict RA synovial gene expression subtype according to 20 histological features. Three subtypes were pre-identified based on RNA-seq clustering: high inflammatory, low inflammatory, and mixed. The high inflammatory subtype showed enrichment of pathways of immunity, immune cell signaling (including SH2, SH3, JAK/STAT, and TNF-mediated signaling), immunoglobulins, chemokines, and cytokines. The low inflammatory subtype was

defined by enrichment of transforming growth factor β pathways, glycoprotein synthesis, and cell adhesion genes (45). Distinct myeloid and lymphoid synovial histological subtypes were not identified, in contrast to previous studies (24), but the high inflammatory subtype displayed elevated expression of genes previously attributed to these in the literature.

SYNOVIAL TISSUE STUDIES TO PREDICT RESPONSE TO BIOLOGIC AND TARGETED THERAPIES

General Synovial Tissue Biomarkers of Response To Therapy

CD68 Macrophage

Effective treatment can modify synovial histology, cytokine and gene expression, with ineffective treatment having little impact, thus providing a means to assess for pathological response (46). Synovial sublining (CD68) macrophage numbers and macrophage expressed cytokines have been shown to correlate with disease activity, and change in sublining macrophage to be the optimal indicator of effective therapy, thus providing a potential early predictive biomarker of drug response (6, 47, 48). A recent study demonstrated that the transcriptional profile of isolated RA synovial macrophages highlighted different subpopulations of patients and identified 6 novel transcriptional modules that were associated with disease activity and therapy (49). The authors suggest that transcriptional signatures in macrophages regardless of location (sublining vs. synovial lining) predict responsiveness to specific non-biologic and/or biologic therapies.

Synovial Pathotypes and Response

A study by Dennis et al. suggested myeloid and lymphoid pathoypes may predict therapeutic sucess with TNF inhibitors (TNFi) and IL-6-targeted tocilizumab, respectively (24). Analysis of serum chemokines further suggested these two pathotypes correlate with raised serum suloble intercellular adhesion molecule 1 (sICAM) and CXCL13 (sICAM/CXCL13) compared to high CXCL13/sICAM, respectively. These initial observations however have not been validated in other cohorts using the serum correlates (50) indicating the need for additional such synovial tissue studies. Nevertheless, stratifying patients by synovial pathotype may inform choice of targeted therapy.

Multiple types of therapies will be discussed in detail below, these are summarized in **Table 1** together with key findings which indicate response to biologic and synthetic targeted DMARDs.

Anti-cytokine Therapies

Tumor-Necrosis Factor-Inhibitors

Synovial studies have offered useful insights into the mechanism of action of TNFi. TNFi have been shown to regulate chemokine and leukocyte trafficking (69) likely explaining the reduction in the synovial cellular infiltrate observed; with reductions in synovial tissue expression of IL-6, IL-8, granulocyte macrophage colony stimulating factor (GM-CSF), macrophage chemoattractant protein-1 (MCP-1), IL-1β, TNF, and vascular endothelial growth factor (VEGF) (70).

Several studies have sought to identify predictors of response to TNF blockade through examination of synovial tissue cytokine expression. Baseline synovial TNF levels (intimal and sub-lining) predicted response to infliximab in one study (54), although another similar study did not reproduce this finding (53). Decreased sub-lining TNF expression was, however, seen in responders. A prospective study of 86 patients found higher proportions of synovial lymphoid aggregates in poor responders to treatment, despite higher rates of TNFi use. Baseline lymphoid aggregates were an independent predictor of poor response in multivariate analysis, and reversal of these histological changes was seen in over half of treatment responders (57). Addition of lymphocyte aggregates to sub-lining TNF expression (54) improved infliximab response prediction, but still only accounting for 29% variance (71); thus insufficient for clinical application. An early RA synovial gene expression study found that mRNA levels pertaining to several inflammatory pathways were associated with response to TNFi therapy, suggesting a role for synovial gene expression profiles as response predictors (72). Another study identified a number of negative predictors of response to adalimumab, another TNFi biologic, including baseline synovial expression of IL-7 receptor alpha chain (IL-7R), CXCL11, IL-18, IL-18 receptor accessory (IL-18rap), and MKI67 (63). However, a larger gene expression study using whole synovial tissue samples pre- and post-infliximab did not identify any predictors, perhaps because of the confounding presence of lymphoid aggregates (55).

Tocilizumab

Tocilizumab is a clinically effective humanized anti–IL-6R monoclonal antibody that inhibits membrane IL-6R– and soluble IL-6R (sIL-6R)–mediated signaling. The aforementioned study by Dennis et al. (24), suggested lymphoid pathotype as predictive of response. In another study, paired synovial tissue biopsies taken at baseline and post-treatment with tocilizumab showed a significant decrease in the expression of various chemokines and T-cell activation genes (51). When compared with gene expression data following other treatments, results showed strong correlation with methotrexate and B-cell depleting agent rituximab, but notable differences with adalimumab (51). A further study of synovial histology post-tocilizumab demonstrated a complete block of synovial IL-6 and a significant reduction of B-cells, CD29 and phospho-JNK. ERK was increased in the tocilizumab group compared to a methotrexate-treated control group, whilst TNF, MMP-3, and CD68 were similarly expressed in both groups. Therefore, inhibition of IL-6/CD20/CD29 may be differentially involved in tocilizumab efficacy compared with methotrexate (52). A more recent study in 33 early RA patients suggested higher expression of TNF-induced transcripts in early RA synovitis was associated with higher disease activity, and predicted poor response to first-line therapy (that comprised either methotrexate, tocilizumab or rituximab therapy) (65). Finally, an exploratory study by Das et al. suggested persistent synovial IL-6 mRNA expression

TABLE 1 | Rheumatoid synovial tissue studies of biologic and targeted synthetic DMARDs.

	Drug	RA Population	Analysis type	Key findings	References
ANTI CYTOKINE THERAPY					
IL-6 blockade	TCZ	30 early RA (disease duration <1 year); treatment-naïve	Gene expression microarrays, IHC	Significant decrease in the expression of various chemokines and T-cell activation genes.	(51)
	TCZ	10 bDMARD treated RA patients 10 controls: RA patients on no bDMARDs	IHC	Complete blockade of IL-6. Inhibition of CD20, CD29, and JNK in MAPK implicates TCZ efficacy compared with MTX.	(52)
TNF inhibitors	IFX	32 RA patients	IHC	Reduction in synovial TNF expression in IFX responders and non-responders. Unchanged TNF in extreme non-responders	(53)
	IFX	143 active RA patients	IHC	Higher intimal and sub-lining TNF expression in IFX responders vs. non-responders.	(54)
	IFX	62 RA patients	IHC and gene expression arrays	Baseline whole synovial biopsy microarray unable to identify TNFi non-responders.	(55)
	ADA	25 RA patients	Global gene expression profiles arrays at T0 and T16, IHC	Poor response to ADA associated with: - Upregulation of genes from cell division and immune responses pathways in poor responders. - High baseline synovial expression of IL-7R, CXCL11, IL-18, IL-18ra), and MKI67.	(56)
	Several TNFi	86 RA patients	IHC	High synovial lymphoid neogenesis, with B and T cell aggregates, correlated with poorer clinical outcomes. Reversal of these aggregates associated with good response.	(57)
CELL-MEDIATED THERAPY					
B-Cell depletion	RTX	13 RA patients	IHC, digital image analysis, gene expression	Significant decrease synovial B cells post-RTX but not completely depleted compared to peripheral B cells. No strong correlation with clinical response.	(58)
	RTX	20 RA patients	qPCR	Responders have higher expression of macrophage and T cell genes. Non-responders showed higher expression of interferon-α and signaling genes.	(59)
	RTX	24 RA patients	IHC, flow cytometry	Significant lower infiltration of $CD79^{+}CD20^{-}$ plasma cells in the synovium associated with the reduction in peripheral blood B-cell repopulation.	(60)
	RTX	24 RA patients	IHC	Clinical response predicted by changes in cell types other than B cells, mainly number of synovial plasma cells.	(61)
	RTX	17 RA patients	IHC	RTX treatment associated with rapid decrease in synovial B cell numbers.	(62)
T-CELL CO-STIMULATION BLOCKADE					
	ABT	16 RA patients	IHC	Significant downregulation of pro inflammatory genes, notably IFNγ. Only specific reduction in synovial $CD20^{+}$ B cells, in responders.	(63)
	ABT	20 RA patients (10 ABA and 10 MTX)	IHC	Increase in CD29 and ERK in MAP kinases.	(64)
MIXED BDMARD COHORT					
	NSAIDs and DMARDs with/without bDMARD (ADA, ETN, IFX, ANK, RTX)	49 RA patients and 29 RA	GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix, Inc.) ELISA, IHC	A myeloid phenotype (high serum sICAM1/low CXCL13) prevalent in responders to TNFI therapy A lymphoid pathotype (high serum CXCL13/low sICAM1) prevalent in responders to TCZ.	(24)

(Continued)

TABLE 1 | Continued

Drug	RA Population	Analysis type	Key findings	References
TCZ, MTX, RTX	Early RA (mainly <1 year disease duration), pre- and post-3 months TCZ (n =13 and 12 respectively) or MTX ($n = 2 \times 8$ samples) TNFi-failure RA pre- and post 3 months RTX ($n = 2 \times 12$ samples)	GeneChip Human Genome U133 Plus 2.0., Affymetrix, IHC	Over-expressed baseline tissue GADD45B and PDE4D in first-line MTX and bDMARD non- responders	(65)
SMALL INHIBITORS (JAKi)				
TOFA	14 RA patients	ELISA, IHC, qPCR.	Reduced synovial mRNA expression of MMP1 and MMP3 and IFN-regulated genes. Clinical improvement correlated with reductions in STAT1 and STAT3 phosphorylation.	(66)
TOFA	Varied/unclear	Synovial explants and tissue culture of primary RASFs, qPCR, WB, and ELISA	Decrease in metabolic functions (mitochondrial pathways, ROS production and glycolysis), indicating that the JAK-STAT signaling is a mediator between inflammation and cellular metabolism.	(67)
Baricitinib	27 RA samples	Tissue culture experiments on FLS	Abrogation of IFNγ-stimulated FLS invasion by targeted inhibition of JAK.	(68)

ABT, abatacept; ADA, adalimumab; ANK, anakinra; bDMARD, biologic disease modifying anti-rheumatic drug; ELISA, enzyme-linked immunosorbent assay; ERK, Extracellular signal-Regulated Kinase; ETN, etanercept; FLS, fibroblast-like synoviocytes; GADD45B, Growth Arrest And DNA Damage Inducible Beta; IHC, immunohistochemistry; IFN, interferon; IFX, infliximab; JAKi, janus kinase inhibitor; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; MTX, methotrexate; PDE4D, Phosphodiesterase 4D; qPCR, quantitative polymerase chain reaction; RA, rheumatoid arthritis; RTX, rituximab; SF, synovial fibroblast; STAT, signal transducers and activators of transcription; TCZ, tocilizumab; TNF, tumor necrosis factor.

(following rituximab inefficacy) associated with subsequent tocilizumab response (73).

Cell Mediated Therapies

B-Cell Depletion: Rituximab

Treatment with the anti-CD20 monoclonal antibody rituximab significantly decreases synovial B cells, but, unlike in the periphery, does not completely eradicate them. In addition, synovial B cell depletion does not correlate strongly with clinical response in RA, suggesting the effects of rituximab on synovial B cells may be necessary but not sufficient for inducing clinical efficacy (58). A separate study of RA synovial histology pre- and post-rituximab confirmed these findings, but also examined changes in other cell populations at 4 and 16 weeks. A reduction in short-lived $CD138^+$ plasma cells, possibly generated locally within the synovial membrane, was found to predict clinical response, whilst delayed reductions in T cell, intimal macrophages and lymphoid aggregates were also seen, highlighting the role of B cells in sustaining inflammation and cell recruitment (74). Another study suggested that clinical response to rituximab is associated with higher residual levels of $CD79^+CD20^-$ plasma cells in the synovium (together with persistence of circultaing ACPA+ IgM plasmablasts) (60). In addition, there is evidence that baseline synovial gene expression may be able to predict response to rituximab (and lack of response), as composite "gene scores" were found to correlate with changes in disease activity (DAS-28 score) in one study (59). Genes relating to macrophage and T cell function were activated in responders.

At a more fundamental level, B cells have shown to be central to T-cell mediated synovial inflammation. This was elegantly demonstrated by a study showing that synovial T-cell clones adoptively transferred into human leukocyte antigen (HLA)-DR-matched synovial tissues xenotransplanted into severe combine immunodeficient (SCID) mice are able to enhance local production of IFNγ, TNF, and IL-1β, but only when transplanted tissues contain B-cell follicles (75). Furthermore, treatment of synovial grafts with anti-CD20 depleting agents induces not only a decrease in B-cell density but also a disruption of the overall lymphoid architecture and reduction of cytokine expression, as well as a dramatic depletion of T cells and macrophages, in keeping with the existence of an active cell network supported by B cells.

T-Cell Co-stimulation Blockade (Abatacept (CTLA4-Fc))

Abatacept, a recombinant fusion protein approved for the treatment of RA, blocks T cell co-stimulation by competing with CD28 for CD80/86 on antigen presenting cells. Synovial studies of the effect of and mechanism of abatacept are relatively lacking. A study of 16 RA patients compared synovial tissue pre- and 16 weeks post-abatacept in terms of gene expression and immunohistochemistry. Amongst responders, there was notable downregulation of several pro-inflammatory mediators, particularly the T-cell-related cytokine IFNγ. However, only

a specific reduction in synovial $CD20^+$ B cells without significant disruption in other cell populations was observed (contrasting with the observations following anti-cytokine therapies, perhaps in keeping with the more immunomodulatory role of CTLA4) (63). Whilst effects on tertiary lymphoid structures were not analyzed, these observations suggest that disruption of T-/B-cell interactions may be critical to abatacept's mode of action. In contrast to this study, a smaller study on 5 patients treated with abatacept indicated inhibition of cell proliferation, with decreases in the expression of MMP-3, CD68, CD4, CD8, CD20, CD80, and CD86 in the synovium (64).

Small Molecule Janus-Kinase (JAK) Inhibitors

Multiple inflammatory cytokines signal via JAK-STAT pathway. Thus, JAK/STAT signaling plays a key role in several immune mediated inflammatory diseases, including RA (76). As small molecules with intracellular targets (i.e., JAK family members), JAK inhibitors represent a novel targeted therapeutic approach in RA (77).

Tofacitinib is an oral JAK inhibitor effective for the treatment of RA (78). It is a pan-selective JAKi, blocking signaling mediated via JAK1, JAK3 and, to a lesser extent, JAK2 (79). A comparison of RA synovial tissue at pre- and 4 weeks post-treatment with tofacitinib showed no change in an overall inflammation score or levels of T cells, B cells or macrophages, but reduced expression of MMPs (MMP1 and MMP3) and interferon-regulated genes, notably CXCL10. Furthermore, clinical improvement at 4 months was found to correlate with reductions in STAT1 and STAT3 phosphorylation, indicating the importance of IFNγ and IL-6 inhibition, respectively (66). In addition, a recent metabolomics study showed that adding tofacitinib to RA synovial explants and synovial fibroblasts *in vitro* led to decreased mitochondrial pathway activity, reactive oxygen species (ROS) production and glycolysis, suggesting modulation of cellular metabolism may contribute to its therapeutic effect (67).

Baricitinib, a JAK inhibitor targeting JAK1/JAK2, is another licensed treatment for RA (80). A study specifically examining FLS activity in RA showed that baricitinib abrogates IFNγ-induced invasiveness of FLS (68), which is of importance given their key contribution to pannus formation (aggressive cell masses that destroy articular cartilage and bone), one of the hallmarks of RA synovial pathobiology (81).

CONCLUSION

It is well-accepted that the considerable advances in the treatment of RA need to be accompanied by a stratified approach that mitigates against the current trial and error approach of treatment decision-making, and the associated individual patient and health-economic consequences. Significant investment in biomarker studies has failed to deliver clinically meaningful tools, with the vast majority focusing on peripheral blood-based evaluation. The emphasis on synovial tissue, the primary site of RA is intuitive, from which tissue and thus disease subtypes are emerging.

The need to pull through benchside investigation of tissue biomarkers to the bedside demands more refined and innovative stratified trial design (82). We will soon see the outcomes of such initiatives [including STRAP—Stratification of Biologic Therapies for RA by Pathobiology (ISRCTN10618686) and R4-RA—A Randomized, open labeled study in anti-TNFa inadequate responders to investigate the mechanisms for Response—Resistance to Rituximab vs. Tocilizumab in RA (ISRCTN97443826)] that will inform future tissue driven trial design. These trials and other tissue-based programmes such as the recently established NIH Accelerating Medicines Partnership (AMP) RA/SLE network will also exploit high-dimensional analyses including mass cytometry, RNA-seq of selected cell populations, and single cell RNA-seq (83). Whilst the sheer volume of data in itself presents massive challenges in the clinically meaningful interpretation, the richness of data matched with improved sophisticated analytical techniques holds the promise of being able to join the field of personalized RA targeted therapy use.

AUTHOR CONTRIBUTIONS

LO, AB, and AM: literature Search, write up, final approval of the manuscript; MB: conception and design of the work, write up, final approval of the manuscript.

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REVIEW
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Synovial Tissue: Turning the Page to Precision Medicine in Arthritis

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Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease targeting the joints. Current treatment strategies are based on clinical, biological and radiological features, yet still fail to reach the goal of early low disease activity in a significant number of cases. Hence, there is a need for refining current treatment algorithms, using accurate markers of response to therapy. Because RA induces histological and molecular alterations in the synovium even before apparition of clinical symptoms, synovial biopsies are a promising tool in the search of such new biomarkers. Histological and molecular characteristics of RA synovitis are heterogeneous. Variations in synovial lining layer hyperplasia, in cellular infiltration of the sublining by immune cells of myeloid and lymphoid lineages, and in molecular triggers of these features are currently categorized using well-defined pathotypes: myeloid, lymphoid, fibroid and pauci-immune. Here, we first bring the plasticity of RA synovitis under scrutiny, i.e., how variations in synovial characteristics are associated with relevant clinical features (disease duration, disease activity, effects of therapies, disease severity). Primary response to a specific drug could be, at least theoretically, related to the representation of the molecular pathway targeted by the drug in the synovium. Alternatively, absence of primary response to a specific agent could be due to disease severity, i.e., overrepresentation of all synovial molecular pathways driving disease activity overwhelming the capacity of any drug to block them. Using this theoretical frame, we will highlight how the findings of previous studies trying to link response to therapy with synovial changes provide promising perspectives on bridging the gap to personalized medicine in RA.

Keywords: synovial biopsies, rheumatoid arthritis, precision medicine, response to therapy, biologics

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting mostly joints. RA diagnosis using the ACR/EULAR 2010 criteria relies on clinical and biological criteria (1, 2), resulting in early diagnosis and differentiation from other conditions. Yet, making a diagnosis of RA is not informative about the strong clinical heterogeneity that prevails regarding many aspects of the disease such as disease severity, development of erosions, functional impact, and last but not least, response to therapy. Several features at diagnosis are classically associated with more severe disease: elevated serum CRP, presence of anti-citrullinated peptides antibodies, x-ray erosions at baseline (3). Yet, these features perform poorly at the individual level and do not allow any accurate prediction regarding outcomes and response to treatment.

Treatment strategies in RA changed dramatically over the last decades. First, the development of biological or targeted synthetic Disease Modifying Anti-Rheumatic Drugs (b or tsDMARDS) provided physicians with new powerful targeted drugs. Second, the growing body of evidence underlining the importance of early disease control led to the current consensus on treat-to-target therapy. Yet, the current recommendations are built on a trial and error approach despite the inclusion of clinical, biological and radiological prognostic factors (4). Therefore, failure to achieve early low disease activity is not uncommon using the current treatment strategies (5, 6).

There is a strong rationale in refining treatment strategies in RA in order to tackle the heterogeneity in treatment responses and reach the goal of early disease control in a majority of patients. In a single patient perspective, the question is simple: what drug from our large arsenal should this particular patient receive to reach early disease control? Within a broader perspective, can rheumatology enter a new era of precision medicine?

Besides the clinical benefit urging us to choose the drug with the highest probability of resulting in low disease activity or remission, some other factors make this choice critical. First, preventing patient from exposition to inefficient, yet potentially toxic, medication is a must. In addition, one cannot overlook the economic considerations raised by these new drugs.

Analysis of synovial tissue in RA seems a promising approach to search for markers of disease severity and response to therapy. However, as opposed to other medical specialists, rheumatologists did not systematically harvest synovial biopsies in clinical practice, and their use long remained limited to research areas, despite the development of safe, non-invasive procedures (7–10).

As a consequence, the biology of RA synovitis did not unveil all its secrets, to say the least. In fact, out of the evidence available until now, it appears that heterogeneity is probably the most appropriate attribute to characterize RA synovitis, both from a histological and from a molecular point of view. Although the observation of such heterogeneous patterns holds promises in the search for correlations with heterogeneous clinical outcomes, our understanding of the in- or extrinsic factors driving the observed variations in synovial features is still limited, partly because most studies were performed on retrospective material, collected in small numbers of patients, resulting in significant methodological issues regarding patients' stratification. Despite these limitations, several intelligible patterns have emerged, which we will describe in the paragraphs below, with a particular focus on the use of gene expression profiling in RA synovitis in order to predict response to therapy.

SYNOVIAL PATHOTYPES IN RA

RA is characterized by distinct changes in synovial architecture: proliferation of lining cells (macrophage and fibroblasts), proliferation of blood vessels in the sub-lining and infiltration by mononuclear cells (macrophages, T and B lymphocytes) (11). These changes are not specific to RA, but are also found in other rheumatic conditions, albeit with different amplitudes [e.g., higher grades of synovial hyperplasia or mononuclear cell infiltration in RA (12), increased hypervascularity in spondyloarthropathies (13, 14)]. Conversely, histological markers of synovitis vary significantly within the same condition. Ulfgren et al. reported back in 2000 that the degree of immune cell infiltration in RA synovitis can range from highly infiltrated to a low inflammatory pattern (15).

In 2014, Dennis et al. (16) introduced the concept of synovial pathotypes in RA according to the cellular and molecular composition of the synovium, and proposed a subdivision in 4 categories: lymphoid, myeloid, fibroid and pauci-immune. Thus, hierarchical clustering of microarray gene expression data led to the identification of these 4 subgroups of RA synovitis in a cohort of 49 patients based on gene expression profiles, and this corresponded to immunohistochemical evidence of T and B cell enrichment in the lymphoid subgroup, proportional enrichment of macrophages in the myeloid subgroup and a relative higher proportion of fibroblasts in the fibroid subgroup. Of interest was the increase in synovial myeloid scores (i.e., a quantitated evaluation of the overall expression of myeloid-associated transcripts in the synovium) in good-responders to TNF blockade, while lymphoid scores were equally distributed in non-, moderate-, or good-responders to these drugs.

Of note, the samples used in this study were obtained from RA patients with established disease undergoing arthroplasty or synoviectomy, treated with conventional synthetic or bDMARDs, and we will see below how these factors impact synovial features in RA. However, the concept that intrinsically distinct pathotypes underpin the organization of RA synovitis is a potential breakthrough, and deserves further discussion (17).

Identification of a lymphoid pattern characterized by a strong synovial enrichment in T and B cells is reminiscent of previous work related to the presence of lymphoid aggregates in RA synovitis. Ectopic lymphoid neogenesis occurs in 25% of RA synovial samples, and results in some cases in the formation of follicular dendritic cell-positive germinal centers (18–20). In previous studies, the presence of lymphoid aggregates was associated with disease severity, i.e., the risk of developing x-ray erosions (21). However, these results were not confirmed in later studies, performed on larger numbers of patients, in which no association was found between synovial lymphoid aggregates and clinical outcomes such as the development of erosions or increased disease activity (19, 22). In addition, these studies showed that lymphoid aggregates are also present in the synovium of other inflammatory diseases and correlated with the degree of overall synovial infiltration by inflammatory cells.

Positive correlations between the presence of synovial lymphoid cells and overall synovial inflammation suggest that synovial lymphoid and myeloid scores might be inter-dependent, rather than mutually exclusive. Using the scores developed by Dennis et al. (16), we mined high-throughput transcriptomic data generated in two series of 20 RA biopsies, and found a strong correlation between both scores, with very few outliers displaying a preferential myeloid or lymphoid signature (**Figure 1**), indicating that activated myeloid and lymphoid cells in RA synovitis are part of a coordinated inflammatory response.

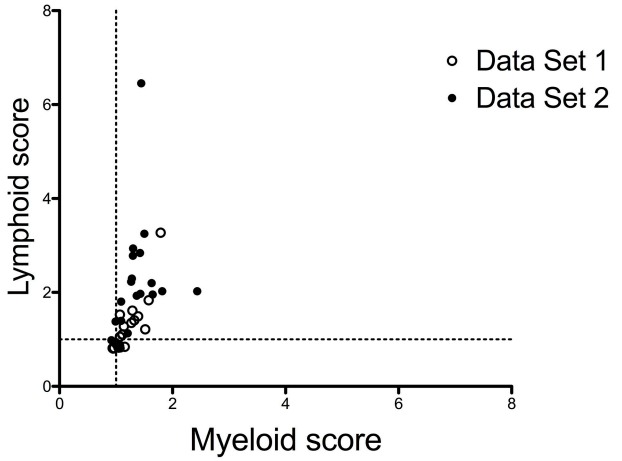

FIGURE 1 | Correlations between myeloid and lymphoid scores in RA synovitis. Lymphoid and myeloid gene scores were calculated in 2 sets of 20 biopsies from patients with active RA, based on gene lists used by Dennis et al. (16), downloaded from https://arthritisresearch.biomedcentral.com/articles/10.1186/ar4555#MOESM3. Gene scores are the median values of the log2- transformed fold changes of each transcript belonging to the score compared to a reference group of 4 OA samples. Data set 1: synovial samples from RA patients with early disease, published in Ducreux et al. (23). Data Set 2: unpublished set of synovial samples, from RA patients with established disease. The characteristics of the patients in both data sets are displayed in **Supplementary Table 1**. Spearman correlation coefficient $r = 0.7801$.

Yet, it remains plausible that differential activation of specific lymphoid cell subsets in RA synovitis is associated with relevant clinical outcomes. In this perspective, it should be stressed that transcriptomic studies performed on whole synovial biopsies might easily miss signals generated by rare cell populations, and do not always allow to differentiate between differential cell activation vs. representation across samples (24). The results of single synovial cells RNA sequencing studies (25) will obviously increase our ability to understand associations between specific synovial cell subsets and clinical phenotypes. For example, previous descriptive and functional approaches suggested an association between synovial B cell enrichment and early development of erosions in RA (26). Synovial B cells undergo affinity maturation and clonal selection in ectopic lymphoid structures (27, 28), especially in early disease. They locally produce ACPA (29) that have the known ability to activate osteoclasts (30). In addition, synovial B cells activate T cells (31), display an antigen-presenting cell phenotype (32), directly activate osteoclasts through production of RANKL (33), and are involved in the production of various cytokines (34).

Regarding high and low inflammatory synovitis, it is unclear how both patterns relate to each other. Two main hypotheses are currently proposed to explain this variability. First, intensity of synovial inflammation and disease activity could be linked. Second, high and low inflammatory synovitis could represent distinct entities driven by different physiopathological mechanisms. To address this issue, one main question arises: do high and low inflammatory synovitis differ in terms of clinical phenotype (disease activity) or biological mechanisms? In a recent study (35), Orr et al. used a semi-quantitative score of inflammation to evaluate synovial biopsies obtained from 189 RA patients. They showed a significant, albeit weak, correlation ($r = 0.23$) between inflammatory scores and DAS28-CRP. The correlation with serum CRP was significant as well, and stronger ($r = 0.43$), suggesting that synovial tissue infiltration by immune cells could be related to global disease activity.

By contrast, in a study performed on 39 synovial samples from patients with longstanding RA (36), global RNA sequencing results divided patients in 3 subgroups according to their gene expression profiles: high, medium and low inflammatory subtypes. Deconvolution algorithms indicated that the 3 subtypes displayed small but significant variation in terms of inferred immune cell subsets. Of note, the 3 subtypes differed in markers of systemic inflammation (CRP, ESR) but not clinical markers (swollen joint count, tender joint count) and treatment, and it is therefore unclear whether the level of synovial inflammation was or not an independent variable in this group of samples.

Finally, in another study (37), Kasperkovitz et al. studied gene expression profiles in both whole synovial biopsies and cultured fibroblast-like synovial cells (FLS) from 10 RA patients. Intriguingly, they found that cultured FLS from high and low inflammatory synovitis kept distinct gene expression profiles *in vitro*, thereby suggesting that the differences between these conditions could be driven internally by a stable phenotypical trait in non-autoimmune synovial cells.

Additional work is needed in order to assess whether synovial pathotypes, in particular low- vs. high-inflammatory synovitis, are associated with different underlying pathogenic mechanisms, hence require differential diagnostic and therapeutic approaches. From the evidence accumulated thus far, synovial phenotypes display a high level of plasticity. As expected, extrinsic factors, in particular disease activity, display a significant influence on synovial phenotype, and this is further illustrated in the following paragraphs describing variations in synovial gene expression profiles in different clinical situations. Yet, evidence suggests that a pauci-immune, by opposition to a high-inflammatory, pathotype is found in RA synovial biopsies as an intrinsic presentation of the disease, independently of disease activity. How this observation translates in clinically relevant decisions further needs to be evaluated.

FACTORS CONTRIBUTING TO SYNOVIAL HETEROGENEITY IN RA

Diagnosis and Stage

Not surprisingly, gene expression profiles in the synovium are dependent on the underlying disorder. Thus, Nzeusseu Toukap et al. compared gene expression patterns in synovial biopsies from patients with systemic lupus erythematosus (SLE), RA, osteoarthritis (OA), psoriatic arthritis and gout (12). SLE biopsies were characterized by the spontaneous overexpression of interferon-induced genes. RA biopsies had a typical lymphoid signature (overexpression of T- and B cell activation-associated transcripts) and OA samples were characterized by the overexpression of transcripts associated with

extracellular matrix turnover. Because it is a hallmark of synovial inflammation, a dominant myeloid signature was not found in any of these conditions, thereby also demonstrating how the choice of the comparator impacts the results of *ex vivo* studies.

Patients with longstanding RA display joint modifications associated with secondary or primary OA, which probably impact the results of synovial gene expression profiling experiments, although the evidence is scarce, and not always concordant. Thus, comparison of 10 patients with end-stage destructive disease undergoing joint replacement to 13 RA patients also with established disease, but active synovitis showed higher numbers of macrophages in the lining and sublining of patients with active synovitis, whereas differences in B and T cells were not significant (38). By contrast, Baeten et al. did not evidence any difference in histological features and immune cell proportions between early and longstanding RA synovial samples (13). Using 10,000 probes cDNA microarrays, Lequerré et al. compared gene expression profiles between 4 early and 4 longstanding RA synovial samples (39). Early RA synovitis was enriched in transcripts involved in the following processes: immunity and host defenses, stress responses, T cell-mediated immunity, and tumor suppressor and major histocompatibility complex (MHC) class II-mediated immunity whereas longstanding RA was enriched in cell cycle, cell surface receptor-mediated signal transduction, cell cycle control, ligand-mediated signaling, apoptosis inhibition, and granulocyte-mediated immunity. By contrast, Tsubaki et al., using 23,040-probes cDNA microarrays, studied synovial biopsies from early ($n = 12$) vs. longstanding ($n = 4$) RA undergoing arthroplasty, and did not evidence significant transcriptomic and histological differences between both groups (40). Overall, these results are compatible with the presence of a lower inflammatory load in longstanding RA synovitis. However, they also demonstrate how complex the interpretation of synovial biopsy studies might be when performed on low numbers of samples or retrospective material, in which interfering variables such as disease activity, ACPA status, or therapies potentially play a confounding role.

Whether patients with undifferentiated arthritis (UA) display RA-like synovial gene expression patterns before they progress to full-blown RA is also a question requiring large prospective studies to be addressed properly. Using a set of 100 transcripts, based on their ability to discriminate RA from other inflammatory disorders in synovial tissue, we found that an accurate diagnosis of RA could be predicted in UA patients only when a combination of synovial transcriptomic and clinical data were combined, in line with the hypothesis that synovial samples rather display an undifferentiated synovial gene expression pattern when they originate from UA patients (41).

ACPA Status

van Oosterhout et al. compared synovial histological and immunohistological features in 34 ACPA+ vs. 23 ACPA– RA patients with established disease (average disease duration: 9.2 years). Expression of CD3 and CD8 was significantly higher in ACPA+ compared to ACPA– patients, while there was no difference in expression of CD4, CD19, or CD68. Semi-quantitative evaluation of synovial lining layer thickness and synovial fibrosis were higher in ACPA– patients (42). Similarly, Orr et al. compared synovial biopsies from 78 ACPA+ vs. 45 ACPA– patients, and found increased expression of CD3, CD8, and CD19 and more B cell aggregates in ACPA+ compared to ACPA patients (but not CD4 nor CD68) (26). In both cases however, disease activity scores were significantly higher in ACPA+ patients, which introduced a potential bias in the analyses, underscoring again the need for extensive patients' stratification in synovial biopsy studies.

Clinical Disease Activity

We discussed previously the link between disease activity and histological signs of inflammation in synovial tissue. Not surprisingly, variations in disease activity also translate in variations in transcriptomic signatures observed in synovial biopsies from RA patients. van Baarsen et al. studied 17 RA synovial biopsy samples (43). Unsupervised hierarchical clustering divided them in 2 groups characterized by high vs. low inflammatory molecular signatures. The high inflammatory group was enriched in transcripts involved in the following biological processes: T-cell mediated immunity, cytokine- and chemokine-mediated signaling pathway and B-cell- and antibody-mediated immunity. Conversely, the low inflammatory group overexpressed genes associated with developmental processes, ectoderm development, and signal transduction. Disease activity (DAS28, TJC, ESR, CRP) was higher and disease duration was shorter in the high inflammatory group.

We looked at the link between synovial transcriptomic profiles and disease activity (DAS28CRP, CDAI, SDAI) in a series of 65 RA synovial biopsies (44) and found a strong correlation between all 3 measures and transcripts associated with an overwhelming lymphoid, but also, to a lesser extent, myeloid (TNFα-dependent) signature. Of note, the samples used in this study were obtained from untreated patients, but also from patients treated with methotrexate, tocilizumab and rituximab, drugs that preferentially down-regulate lymphoid transcripts in RA synovitis (see below). Because these drugs also decrease disease activity, correlations between disease activity and gene expression patterns using such samples necessarily increase the weight of lymphoid transcripts. Restricting the analyses to the 21 samples obtained from untreated patients restored the balance between lymphoid- and myeloid-associated transcripts in the correlation study with disease activity. These results point to an important link between clinical disease activity and synovial molecular signatures, thereby opening stimulating questions about the mechanisms driving disease activity in RA. Clinical disease activity measures the global burden of disease, and is based on the integration of systemic variables: number of tender/swollen joints, acute phase reactants and patient's, sometimes physician's, assessment of global disease activity. The meaning of the link between such global measures and gene expression profiles in a single joint remains to be elucidated. Is synovial gene expression the reflection of a disseminated systemic inflammation or is systemic disease activity driven by locally-initiated inflammatory processes? Finally, these results also demonstrate how clinical parameters (in this case therapies)

affect the results of synovial gene expression studies, hence need to be tightly controlled.

Effect of Therapies

RA drugs display significant effects on synovial cell populations and transcriptomic profiles, as evidenced by several longitudinal studies in which synovial biopsies were collected prospectively before and after administration of therapy. These results were determining in the identification of synovial molecular pathways correlating with response to therapy, and contributed to a better understanding of the mechanisms driving synovial inflammation. They also opened new perspectives in terms of personalized medicine and prediction of response to therapy, as discussed below.

Immunohistochemistry studies showed differences in cell populations before and after administration of effective drugs. As expected, proportions of all infiltrating inflammatory cells decreased in response to therapy, although the amplitude of the changes observed in specific cell populations were different according to the modes of action of the drugs. Thus, 3 months after initiation of tocilizumab (an anti-IL6R antibody) therapy in early RA patients (23), a relatively stronger decrease in infiltrating CD3 positive T cells was observed compared to other cell types, in line with the known T cell growth factor properties of IL6. By contrast, adalimumab (a TNFα-blocking antibody) displayed relatively stronger effects on proportions of CD68 positive cells compared to other synovial cell populations, 3 months after administration of the drug to methotrexate-resistant RA patients (45). We also investigated the effects of rituximab (anti-CD20 antibody) therapy on synovial cell populations before and 3 months after therapy (46). We found that B cells were depleted in the majority (18/20) of the samples, but the drug also displayed a significant effect on IL17 producing T cells (47), thereby supporting the hypothesis that B cells also play a role as antigen-presenting cells in RA synovitis. Differential responses of synovial cell populations to rituximab vs. TNF-blocking agents is also apparent from observations reported by other groups (48–51).

Interestingly, Bresnihan et al. (52, 53) found a correlation between the overall effects of several drugs (prednisolone, methotrexate, gold salts, leflunomide, infliximab, and rituximab) on disease activity in groups of patients and the decrease in CD68 positive macrophages in the sublining: the stronger the overall decrease in disease activity, the stronger the decrease in sublining CD68 positive cells in response to a given drug. It is not clear whether the association holds at the individual level, but these observations are of interest from a pathogenic point of view, as they support the role of synovial macrophages as a common mediator of disease activity in RA.

In several studies, the global molecular effects of therapies in RA synovitis were also investigated. Most of these studies were performed on low numbers of patients, yet delivered interesting clues on the modes of actions of these drugs. Lindberg et al. (54) performed transcriptomic studies on synovial biopsies from 10 RA patients before and 9 weeks after administration of infliximab therapy. A positive TNFα stain was detected at baseline in 4 of them, in whom the infliximab-induced molecular changes were the most striking, i.e., differential expression of 1,058 transcripts involved in immune responses, cell communication, signal transduction and chemotaxis. Similar patterns of differential gene expression were found in the subgroup of patients who were good-responders.

Our group carried out gene expression profiling of synovial biopsies from 12 patients before and 3 months after adalimumab therapy (45). In good responders ($n = 6$), adalimumab induced the down-regulation of 632 transcripts that were mainly involved in regulation of inflammatory responses (production of chemokines and cytokines) and cell division.

Both studies showed a good overlap of the biological themes affected by TNF inhibitors in RA synovitis. By contrast, we observed very different results when the molecular effects of other drugs were evaluated. Thus, we performed pathway analyses of differentially expressed genes in the synovium of TNF inhibitor-resistant patients prior to and 3 months administration of rituximab therapy (46) ($n = 12$ patients). In these samples, rituximab induced the downregulation of transcripts involved in immune responses, chemotaxis, T cell activation and immunoglobulins. In addition, rituximab led to upregulation of genes involved in wound healing. A similar study (23) was performed on synovial biopsies obtained from 12 early RA patients before and 3 months after administration of tocilizumab therapy. Pathway analyses of differentially expressed indicated a downregulation of transcripts involved in T cell activation and chemokines and an induction of transcripts involved in healing processes. A very similar pattern of molecular changes was observed in the synovium of early RA patients in response to methotrexate therapy, although the amplitude of the effects was lower.

When comparing the transcriptomic effects of the drugs reported in the previous paragraphs, we found a striking overlap between the effects of tocilizumab, rituximab and methotrexate (23). A transcript down- or upregulated by one of these drugs had a high probability to be similarly up- or downregulated by another drug. By contrast, there was no common ground between the molecular effects of adalimumab and the three other drugs. These observations lend further support to the concept that molecular pathways in RA synovitis are built along two axes: a lymphoid axis (T cell activation, chemokines) targeted by drugs such as tocilizumab, rituximab or methotrexate, and a myeloid axis (inflammation, cell division) targeted by TNF blocking agents.

It is important to keep in mind that changes in transcriptomic profiles in RA synovitis in these studies are influenced by changes in cell populations before and after administration of the drug. The results of single cell RNA sequencing studies will make it easier to understand how specific drugs interfere with dysregulated cellular pathways in the disease. This is for example the case regarding the induction of transcripts involved in wound healing pathways in response to several drugs (23, 46), an effect in which increased representation of resident cells (following a decrease in the presence of inflammatory cells) could play a role. Nevertheless, such description of the global effects of these drugs in the synovium provided clinicians and researchers with unique tools, leading to new research hypotheses on e.g., potential drug interactions in the treatment of RA (drugs that do not share the

same molecular effects might have additive or synergistic effects) and response to therapy.

Gene Expression Profiling in RA Synovitis and Prediction of Response to Therapy

Response to therapy in RA follows a prototypical pattern, common to all drugs used in the disease: 30–40% good response, 30–40% moderate response, and 30–40% poor response (6). Response to therapy is usually assessed after 3 months (55) (although some drugs, such as methotrexate, may still improve their therapeutic effect after a longer period of time). However, in the context of a treat-to-target strategy aiming at achieving early remission, clinical care of RA patients would be improved if response to therapy could be predicted prior to initiation of therapy, using accurate markers.

Response to therapy as such is a complex phenotype. Whether ACR or EULAR response criteria (56, 57) are used, response to therapy is a composite score that integrates changes in objective (acute phase reactants, swollen and tender joint counts) and more subjective (patient's or physician's global assessment of disease activity) variables that do not necessarily overlap. For example, searching for clinical measures correlating with the expression of TNFα-induced transcripts in RA synovitis (44), we found a very poor correlation with patient's global assessment of disease activity, while the best correlation was found with physician's global assessment, indicating that both variables do not reflect similar features. It is therefore important to keep in mind that response to therapy is not a homogeneous variable, which strongly affects the ability to predict it accurately.

Theoretically, absence of adequate clinical response to a specific drug could be due to synovial underrepresentation of the pathway targeted by the drug. Alternatively, disease severity could drive poor response to therapy, through synovial overexpression of several (if not all) molecular pathways driving disease activity in the synovium, overwhelming the capacity of the drug to inhibit any of them (a situation that could characterize patients failing one drug after the other). Finally, secondary loss of response to therapy due to the development of anti-drug antibodies (58, 59) is a situation very different from primary lack of response, which is discussed in the following paragraphs.

Several studies were performed in order to find associations between synovial molecular patterns at baseline and response to several drugs, in particular TNF-blocking agents (**Table 1**). In 2008, Van der Pouw Kraan et al. (61) compared baseline synovial gene expression profiles in 12 responders (defined based on a DAS28 reduction>or = 1.2 at 4 months) vs. 6 non-responders to infliximab therapy. Molecular pathways upregulated in responders included: T-cell mediated immunity, cell surface receptor mediated transduction, MHCII mediated immunity, cell adhesion, cytokine and chemokine mediated signaling pathway, cell adhesion mediated pathway, signal transduction, macrophage mediated immunity. In 2010, Lindberg et al. (54) obtained synovial biopsies from 62 methotrexate-resistant patients prior to initiation of infliximab therapy, and compared gene expression profiles in 18 good- vs. 14 poor-responders according to EULAR response criteria. At first, they also found a slight enrichment in transcripts involved in chemotaxis, inflammatory responses and leukocyte activation in good-responders. However, they observed that these molecular patterns were rather associated with the presence of lymphoid aggregates in synovial tissue, found more often in good-responders in this group of patients. It was however unclear whether the overrepresentation of lymphoid aggregates positive patients in good responders was a confounding factor or reflected a real biological difference. After stratification of the samples for the presence and size of lymphoid aggregates, they could not observe any robust gene expression differences between good- and poor-responders to the drug. In this context, it is noteworthy that presence of synovial lymphoid aggregates was associated with a better response to infliximab in histological study on 97 methotrexate-resistant RA patients (63).

As discussed previously, Dennis et al. (16) mined a set of transcriptomic data generated from 49 synovial biopsies before administration of infliximab therapy. They found a higher myeloid score in good- compared to moderate- or non-responders to infliximab therapy, while lymphoid scores were not different across the three groups. Interestingly, they further investigated the concept by measuring serum biomarkers correlating with synovial myeloid (sICAM1) or lymphoid (CXCL13) scores in an additional cohort of 198 patients prior to administration of tocilizumab vs. adalimumab [ADACTA trial (64)]. In line with their synovial transcriptomic data, they found a positive association between the relative levels of both serum markers and response to infliximab or tocilizumab. Thus, high ICAM1/low CXCL13 patients had a 42% probability of reaching a ACR50 response to adalimumab vs. 13% in low ICAM1/high CXCL13 patients. Conversely, 69% low ICAM1/high CXCL13 patients reached a ACR50 response after administration of tocilizumab vs. 20% of the high ICAM1/low CXCL13 patients. Overall, the accuracy of these serum markers in predicting response to therapy was rather low. However, the experimental approach adopted by the authors was highly rational, and highlighted how synovial biopsy studies could lead to the development of useful biomarkers in daily clinical practice.

Using synovial biopsies (45) obtained at baseline in 25 methotrexate-resistant RA patients prior to administration of adalimumab therapy, we reported contrasting results in comparison to the previous observations. Thus, we found that transcripts overexpressed in poor-responders to adalimumab therapy were induced by TNFα itself or by IL1β (or by a combination of both cytokines) in cultured FLS, and confirmed these results by immunohistochemistry on the same samples. IL7R was one of the transcripts most overexpressed in poor-responders, and this observation led us to identify how the soluble form of the receptor (sIL7R) is produced by stromal cells in response to inflammatory cytokines and secreted in the serum. Hence, serum sIL7R measurements are a marker of tissue (instead of systemic) inflammation in RA, high serum concentrations being indicative of high concentrations of inflammatory cytokines (TNFα, IL1β) in the synovium. We measured sIL7R serum concentrations in sera from DMARD-resistant RA patients prior to initiation of infliximab therapy (65), and found significantly higher concentrations in 18 poor-

TABLE 1 | Studies using high-throughput gene expression profiling of the synovium to identify predictive markers of response to treatment.

Reference	Treatment	Disease duration	Number of patients and response	Pretreatment disease activity score across groups	Response to treatment criteria	Ongoing DMARD or steroid treatment	Histology	Main findings	Platform	Transcriptomic data available	Sampling technique
Lindberg et al. (60)	IFX	0.6–26 years	GR = 3 MR = 5 NR = 2	N/A	EULAR response criteria	MTX: 10/10 Pred (<7.5 mg/j): 5/10	N/A	Up regulated pathways in GR: Immune response, cell communication, chemotaxis, signal transduction	KTH microarray	Array express	Arthroscopy
van der Pouw Kraan et al. (61)	IFX	0.6–26 years	R = 12 NR = 6	DAS R: 6.0 (4.7-7.7) NR : 5.3 (3.7-7.2) CRP(mg/dl): R: 28(3–110) NR: 13 (5–49)	1.2 decrease in DAS28 score is response	MTX: 18/18 Pred: 5/18	CD3 and CD163 higher in R	Upregulated pathways in R: Immunity and defense, T-cell mediated immunity, Signal transduction, MHCII-mediated immunity	43 k human cDNA micro array (Stanford University)	Stanford micro array database	Arthroscopy
Badot et al. (45)	ADA	1–36 years (mean 10 years)	R = 20 NR =5	DAS28-CRP (±SEM) R: 5.289 (±0.213) NR:4.774 (±0.186)	EULAR response criteria	MTX (23/25), Lefl (2/25) Pred (18/25)	CD3, CD15, CD20, CD68 staining showed no difference between R and NR	Genes overexpressed in NR vs. R are implicated in cell division and regulation of immune response and are induced by TNF-α	GeneChip HG u133Plus 2.0 array, Affymetrix	GSE15602	Needle arthroscopy
Lindberg et al. (54)	IFX	Mean(±SD) : 11 years (±9.5)	R= 48 NR = 14	DAS28 (±SD) R: 6 (± 0.9) NR 5.9 (±0.9) CRP (mg/dl) (±SD) R: 17 (±16) NR 14 (±18)	EULAR response criteria	MTX (62/62) Pred (16/62)	Over representation of lymphoid aggregates in GR	Presence of aggregates dominates the gene expression profile. GR group is enriched with aggregates positive patients	In-house array 17972 genes (KTH micro array)	GSE21537	Needle arthroscopy
Hogan et al. (62)	RTX	>1 year	R = 10 NR = 10	N/A	EULAR response criteria	MTX (18/20), Prednisone (11/20)	N/A	Higher expression of macrophages and T-cell related genes in R Higher expression of Interferon-α in NR	Home made qPCR panel (125 genes)	N/A	Arthroscopy

(Continued)

TABLE 1 | Continued

Reference	Treatment	Disease duration	Number of patients and response	Pretreatment disease activity score across groups	Response to treatment criteria	Ongoing DMARD or steroid treatment	Histology	Main findings	Platform	Transcriptomic data available	Sampling technique
Dennis et al. (16)	TNFi/TCZ	See Lindberg et al. (54)	Pathotypes and corresponding gene scores were computed on 69 treated patients with >3 years disease duration. Patients from Lindberg et al. (54) were used for prediction of treatment response R = 48 NR = 14	See Lindberg et al. (54)	EULAR response criteria	See Lindberg et al. (54)	Immune cells subsets correlate with transcriptomic-defined clusters in patients used for pathotype determination	Myeloid Score at baseline is higher in GR vs. NR to IFX Identification of circulating biomarker associated with better response to TNFi or TCZ in ADACTA trial patients	GeneChip HG u133Plus 2.0 array, Affymetrix	GSE48780	Joint replacement or surgical synovectomy
De Groof et al. (44)	MTX or TCZ	Mean (±SD) 0.5 year (± 1.1)	GR = 9 MR = 7 NR = 5	DAS28CRP MTX group: 4.51 ± 1.49 TCZ group: 4.5 ± 1.1	EULAR response criteria	Untreated	N/A	Genes over expressed in NR are TNF-α and IL6-dependent	GeneChip HG u133Plus 2.0 array, Affymetrix	GSE45867	Needle arthroscopy

GR, Good responders; MR, moderate responders; NR, non-responders; R, responders; MTX, Methotrexate; Lefl, Leflunomid; Pred, Prednisolone; RTX, Rituximab; TCZ, tocilizumab; TNFi, TNF-inhibitor; IFX, Infliximab; ADA, Adalimumab; ANA, anakinra.

compared to 57 good- and moderate-responders (predictive positive and predictive negative values in predicting response to therapy were 87 and 71%, respectively). These observations support the hypothesis that non-response to TNF blockade in these two groups of RA patients was driven by higher disease severity, resulting in higher synovial expression of pro-inflammatory cytokines, not adequately blocked by the administered antibodies. These results are not necessarily in contradiction with the ones reported in the previous paragraphs, but they do not intuitively point at the same concepts. In one case, response to TNF blocking agents requires the presence of a TNF signature to be blocked. In the other case, too much synovial impregnation in TNFα opposes the effect of TNF blocking agents. If anything, these results demonstrate the need for large scale prospective trials on large numbers of patients, in order to reconcile all observations and identify accurate markers of response to TNF blocking agents in RA.

We also performed transcriptomic and immunohistochemistry studies in synovial biopsies obtained in early RA patients (44), in order to identify markers of poor response to first line therapies. GADD45B stains (GADD45B is induced in macrophages upon stimulation with TNFα) were carried out in synovial samples obtained in two different groups ($n = 46$ and $n = 35$) of patients before initiation of first-line therapies, in particular methotrexate ($n = 17$ and $n = 35$). We observed that GADD45B expression in synovial tissue was significantly higher in non-responders to methotrexate or to any first line therapy in both groups, thereby supporting the hypothesis that higher levels of synovial inflammatory cytokines drive poor response to any therapy in RA, in line with the concept linking higher disease severity and treatment responses.

In 2012, Hogan et al. (62) performed qPCR studies on synovial biopsies from 20 RA patients resistant to TNF blockade prior to administration of rituximab therapy in order to study correlations between gene expression at baseline and short- (3 months) or long-term (21 months) response to therapy. When computed in a gene score, 16 out of the 125 genes tested were found to correlate with decrease in DAS scores over time. Genes associated with response to therapy were mostly involved in macrophage- and T-cell biology whereas genes associated with absence of response were involved in bone remodeling and IFNα response. The link between IFN signature and poor response to rituximab in RA is still elusive. It was however confirmed by studies performed on PBMC from patients with RA, in which a similar association between type 1 IFN signature and poor-response to the drug was found (66, 67).

CONCLUSION

Synovial tissue in RA is heterogeneous from a cellular and molecular point of view. However, it seems that at least some part of this heterogeneity is explained by clinically relevant variables such as disease duration, disease activity and effects of therapies. Although numerous therapies are available to treat RA, it appears from their molecular effects in synovial tissue that they mainly target two major pathways in the synovium, associated with activation of myeloid vs. lymphoid cells. Whether these pathways define distinct RA subgroups or pathotypes, in addition to a low inflammatory pathotype, or whether RA synovitis is a molecular and cellular continuum, is a pending issue. It is also unclear at this stage whether enrichment of selected pathways in the synovium is associated with a preferential response to selected drugs. In some studies, evidence also links absence of response to therapy to RA drugs with the presence of markers of disease severity in the synovium rather than markers of response to a specific agent.

Most of the studies addressing these questions were performed on small number of samples. Comparing studies is a difficult endeavor not only because of the heterogeneity of the analytical techniques used by different groups, but even more because of the heterogeneity of the patients' populations included in these trials, in terms of disease duration, exposure to previous therapeutic agents, disease activity, and disease severity, all variables known to influence molecular patterns in synovitis.

Large-scale, multi-centric trials are ongoing, and will undoubtedly cast new lights on many uncertain matters raised in this review article. Homogenization of the pre-processing and analytical steps, in addition to international agreements on minimal reporting requirements in synovial biopsy studies are also highly expected outcomes of ongoing multi-centric initiatives in the field (68, 69). Finally, technological [single cell RNA sequencing (70)] and analytical [machine learning (36)] developments will undoubtedly enable us to capture meaningful patterns in RA synovial tissue, in order to help clinicians tailor their clinical decisions according to the individual characteristics of their patients.

DATA AVAILABILITY

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45867.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00046/full#supplementary-material

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frontiers in Medicine

MINI REVIEW
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Synovial Tissue Biopsy Research

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Synovial tissue is a key structure in diarthrodial joints and is the primary target of inflammation in autoimmune arthritis. The study of synovial tissue has developed significantly in the last two decades as arthroscopic and ultrasonographic techniques have allowed visualization and access to synovial biopsy. Further progress in synovial tissue processing and analysis has improved studies of disease pathogenesis, biomarker discovery, and molecular therapeutic targeting with increasingly specialized analytical and technological approaches. In September 2018 the first course on Synovial Tissue Biopsies was convened in Brussels, in this Mini Review these approaches will be described and I will summarize how synovial tissue research advanced.

Keywords: synovial tissue biopsy, rheumatoid arthritis, single cell analysis, biomarkers, synovial tissue biomarkers

KEY POINTS

- Synovial tissue is the target tissue of rheumatoid arthritis (RA).
- Synovial biopsy, under local anesthetic, is safe and well-tolerated by patients
- Cellular and molecular analysis of the synovial tissue of RA patients might identify novel targets for therapy and specific biomarkers.
- Technological advances in single cell and molecular analysis provides new opportunities for discovery.

INTRODUCTION AND HISTORY

The main focus of synovial tissue research has been rheumatoid arthritis (RA), as the most prevalent cause of inflammatory synovitis. In the last two decades, considerable advances have been made in the diagnosis and therapy of RA (1). However, early diagnosis and precision medicine remain a challenge. In the 1970's Ralph Schumacher and Barry Bresnihan pioneered synovial biopsy research using the Parker-Pearson needle to obtain biopsies and study the cellular composition of the tissue.

SYNOVIAL JOINT

Normal synovial tissue contains specialized fibroblast-like synoviocytes (FLS) interspersed with macrophages (2). In RA the synovial tissue becomes hypervascular and hyperplastic (**Figure 1**) while microscopic analysis reveals hyperplasia of the intimal lining layer, primarily due to increased accumulation of FLS and macrophage cells in the synovial lining (3). Angiogenesis, the development of new blood vessels is probably an early event enabling infiltration of immune cells such as T cells, B cells and monocytes, and is aberrant resulting in an abnormal blood vessel pattern (4). The new blood vessels appear immature and permit increased leukocyte migration, transforming the synovial tissue into an aggressive "pannus" characterized by release of proinflammatory cytokines from macrophages, T and B cells that stimulate FLS activation and subsequent cartilage and bone destruction (5–7). Although angiogenesis leads to increased blood vessels the tissue is markedly hypoxic *in vivo* (8).

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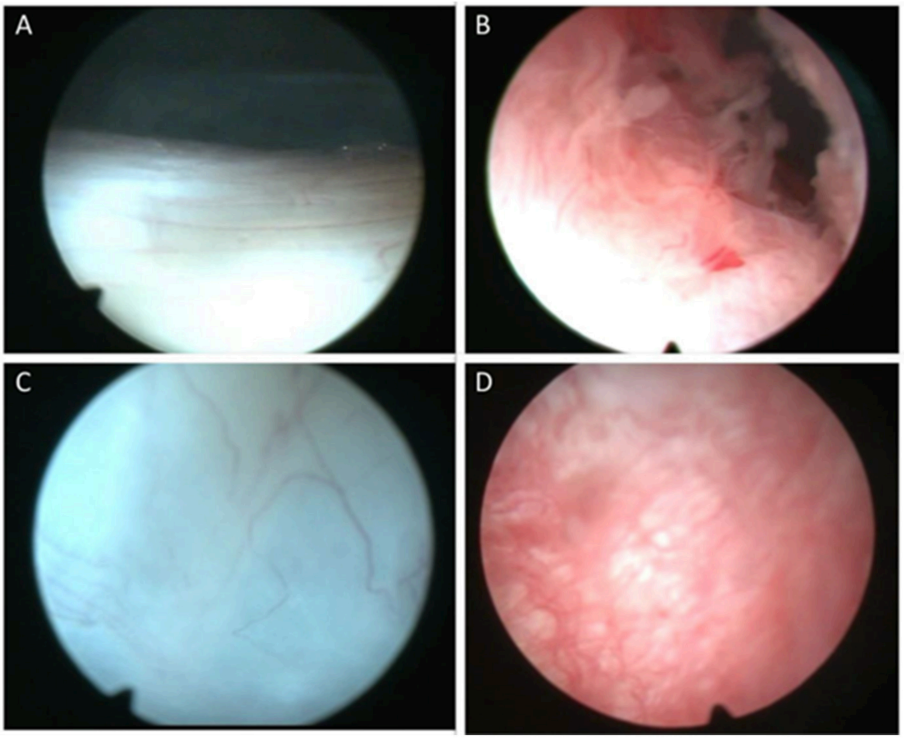

FIGURE 1 | Representative macroscopic appearance of synovial tissue. Macroscopic images of the synovial tissue demonstrating normal synovial tissue **(A,C)** compared to inflamed and hyperplastic synovial villi in rheumatoid arthritis **(B,D)**.

SYNOVIAL BIOPSY

The analysis of synovial tissue biopsies has advanced our understanding of RA pathogenesis, yielded potential therapeutic targets, and allows detailed evaluation of new therapies (9, 10).

Synovial tissue biopsies have been obtained by blind needle biopsy, arthroplasty, arthroscopy, and more recently using ultrasound- (11). Arthroscopic and ultrasound-guided (USG) biopsy procedures are safe and well-tolerate, both provide good biopsy material. The main benefit of USG appears to be access to small joints, however the yield of synovial tissue is often lower (∼80%) (12, 13), while in the authors experience arthroscopy provides 100% synovial tissue yields. There is a low adverse event rates of 0.9% for haemarthrosis, 0.2% for deep vein thrombosis, and 0.1% for both wound infection and joint infection (14). Similarly, a systematic review reported an overall major complication rate of 0.4% for ultrasound-guided biopsy procedures (15).

SYNOVIAL TISSUE ANALYSIS

Immunohistochemical analysis of synovial tissue has a clinical role in the differential diagnosis of arthritis (e.g., infectious, granulomatous, infiltrative, or crystal arthropathies), the benefit in studies of personalized medicine have yet to provide a substantial advance (16). Interestingly though, studies of the synovium beyond immunohistochemistry involving whole-tissue culture, tissue digestion, homogenization, and single cell analysis with detailed molecular profiling including -omic technologies are now possible (**Figure 2**). Direct analysis of synovial tissue—the target of inflammation in RA—is critical to the investigation of pathogenesis in RA. Monocytes, T and B cells are expanded in the blood as well as in the synovial tissue of RA patients and this has provided the rationale for development of novel biological therapies including anti-cytokine antibodies, abatacept, and rituximab.

PREDICTORS OF ARTHRITIS AND RESPONSE TO THERAPY

In the last 15 years, synovial tissue analysis has impacted the treatment of early RA using clinical, pathological, and -omic data analysis. A link between circulating ACPAs and the development of RA in subjects with arthralgia, and bone damage has been described in patients with early arthritis (17, 18). The predictive value of a positive ACPA status in RA patients has been reported, however, in those with arthralgia it is highly variable with 30–70% subsequently developing RA on follow-up (19).One study has identified a highly expanded, T cell clone in RA synovial tissue early in the disease underlining the importance of T cells at this stage (20). Epigenetic changes in synovial tissue FLS might also define the different stages of RA after clinical onset (21).

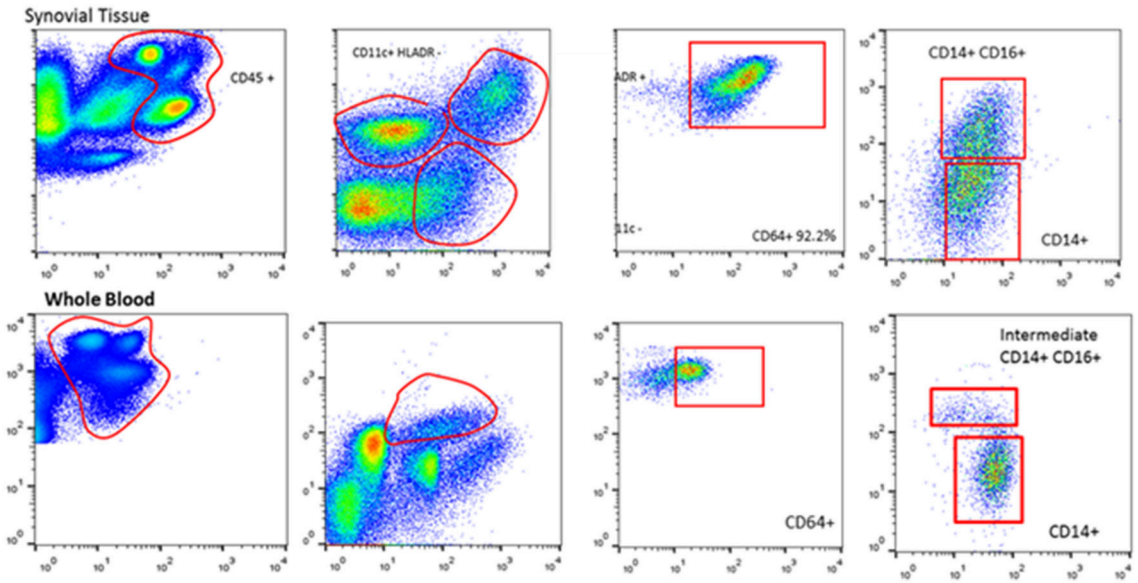

FIGURE 2 | Synovial tissue and whole blood cell isolations with flow cytometry. Flow cytometric analysis of paired peripheral blood and synovial tissue derived cells illustrating the ability to isolate and identify single cell populations of key immune cells.

Inflammatory genes overexpressed in pre-treatment biopsies might predict those RA patients most likely to responded to TNF inhibitor therapy. Another study of synovial tissue RNA suggested that transcripts associated with lymphocyte aggregates predicted response to infliximab therapy (22). The role of macrophages and T cells as biomarkers of response is also supported by gene-expression analyses of paired RA synovial biopsies before and after rituximab treatment, that showed clinical response was greater in those with high expression of macrophage and T cell associated synovial genes (23).

CHALLENGES IN BIOMARKER DISCOVERY

Recent advances in -omic techniques are allowing deeper molecular analysis of synovial tissue, however several challenges remain. The new technologies have become faster, better value and provide a more detailed analyses of genes, proteins, and epigenetic modifications. However, a number of commonly used microarray platforms have yielded poor reproducibility causing some problems with interpretation of data. In addition, the results of initial whole tissue transcriptional profiling await more detailed analysis. Therefore, in the last 2 years, we have developed laboratory techniques to dissociate the synovial biopsies into viable single cell subsets allowing specific analysis of the genes, proteins, and functions of the cell subsets that comprise the population in the actively inflamed synovial joint tissue (24).

CONCLUSIONS

RA is characterized by inflammation of the synovial tissue, which therefore represents the target tissue of autoimmune arthritis. Various methods of sampling synovial tissue have now been validated as safe, well-tolerated by patients and minimally invasive thus they have become more widely practiced. In this Mini review I have focused on the development of synovial tissue biopsy studies including the current technological advances in analysis that allow detailed cellular and molecular experiments that define the functions of immune cells in the RA synovial tissue. These studies might allow greater understanding of the pathogenesis of RA and development of a "precision medicine" approach with improved therapy, patient stratification, development of new therapeutic targets, and development of specific biomarkers of response.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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MINI REVIEW
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Clinical Applications of Synovial Biopsy

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The synovial tissue is a primary target of multiple diseases characterized by different pathogenic mechanisms, including infective, deposition, neoplastic, and chronic immune-inflammatory pathologies. Synovial biopsy can have a relevant role in differential diagnosis of specific conditions in clinical practice, although its exploitation remains relatively limited. In particular, no validated synovial-tissue-derived biomarkers are currently available in the clinic to aid in the diagnosis and management in most frequent forms of chronic inflammatory arthropathies, namely rheumatoid arthritis (RA) and the spondyloarthritides (SpA). In this brief review, we will discuss the current spectrum of clinical applications of synovial biopsy in routine rheumatologic care and will provide an analysis of the perspectives for its potential exploitation in patients with chronic inflammatory arthritides.

Keywords: synovial biopsy, arthritis, synovitis, biomarkers, precision medicine

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The assessment of the pathologic process at peripheral sites has been proved as a source of clinically relevant information in different human pathologies including cancers and systemic autoimmune diseases. Examples of the latter group are the assessment of the salivary glands in sialo adenitis, the muscle in idiopathic inflammatory myopathies or the kidney in systemic lupus erythematosus, in which the qualitative and/or quantitative analysis of local inflammatory processes can be exploited to corroborate diagnosis/classification, facilitate discrimination among disease entities, evaluate prognosis and guide the choice of appropriate treatments (1–3). Similarly, the synovial membrane, being the target of different rheumatologic conditions, holds an intrinsic potential for wide clinical applications, although its exploitation remains, at present, relatively limited. If, on the one hand, the synovial biopsy may offer unique information aiding the diagnosis of infectious and other rare diseases, on the other, no validated synovial tissue-derived biomarkers are currently available in the clinic to support early diagnosis/classification or to guide individual patients' management in most frequent forms of chronic inflammatory arthropathies.

Based on the existence of major unmet needs and on data derived from previous proof-of-concept studies (see next paragraphs), there is now growing attention in expanding the translational applicability of synovial tissue analysis also in this direction (4). One of the most compelling working hypothesis is that the cellular/molecular patho-biology of the inflamed synovial membrane might delineate specific discriminative traits able to improve early diagnosis of undifferentiated forms and patients' stratification into treatment-specific response groups. The introduction of mini-invasive approaches allowing targeted tissue sampling of large and small joints under direct vision of a standard ultrasound (US) machine (US-guided biopsies) is now contributing to make this perspective more realistic, favoring synovial biopsy widespread applicability and allowing the development of multi-center research and clinical trials (5–10). For a detailed update on currently available synovial biopsy techniques, including their advantages, limitations and validation requirements the reader can refer to a recently published review (11).

In the following paragraphs, we will provide a summary of current applications of synovial biopsy in clinical practice and of the background data that are allowing to conceive their extension into the field of stratified medicine.

CURRENT CLINICAL APPLICATIONS OF SYNOVIAL BIOPSY: DIFFERENTIAL DIAGNOSIS IN ROUTINE CARE

For the majority of rheumatologic diseases, patients' interview, clinical examination, imaging and serological tests are usually sufficient to establish a diagnosis and monitor treatment response. The analysis of the synovial tissue can be, however, of assistance for diagnostic purposes in course of arthritis of undetermined origin, allowing the identification of specific traits of a restricted, though defined, spectrum of pathologies, including infective, neoplastic and some deposition diseases (**Table 1**). Whilst specific markers (cellular and/or molecular) related to several of these conditions can be readily identified also through less-invasive approaches, such as the analysis of synovial fluid, the biopsy can be a relevant implementation tool in different situations. Firstly, the collection of synovial tissue can be essential to ensure sampling of joint environments characterized by lack of or limited effusion, as a primary approach or as a "failsafe" mechanism (12, 13). Under certain circumstances, the synovial biopsy can be important also as a complementary or second level approach in the case of fluid availability. Indeed, despite comparative data on fluid vs. tissue diagnostic accuracy for most conventional approaches (microbiological cultures, PCR for infective agents, detection of crystals) remain limited (14–17), results derived from the two compartments, even if focused on the same downstream procedure, have been shown to lack systematic redundancy and either may contemplate false negative results (13–21).

Beyond expanding the analytical substrate, the availability of biopsy specimens may also offer specific information by allowing the integration of microbiological and molecular screenings with the analysis of characteristic histopathologic traits of some infections and rare diseases (see next paragraphs for details).

Deposition Diseases

US-guided dry needle synovial tissue aspiration (22) or synovial biopsy (23) can be considered as diagnostic options when crystal-associated arthropathies are suspected, in particular in patients without synovial effusion or in the case of negative results from synovial fluid. Both monosodium urate (after tissue fixation in absolute alcohol) and calcium pyrophosphate crystals (24) can be detected within tissue specimens as focal deposits of amorphous material or as birefringent structures by polarized light microscopy. As a general notion, inferred from a recent retrospective study of biopsy reports involving synovial tissue between 1998 and 2015, a confirmatory diagnosis of crystal associated arthritis can be established in ∼40% of the cases in which the procedure is performed for a primary clinical suspicion (23).

Both synovial fluid or synovial tissue analyses can also contribute to the differential diagnosis of other rarer deposition diseases including amyloid arthropathy, through Congo-red stain and the identification of typical apple-green birefringent deposits of immunoglobulin free light chains, as well as ochronotic arthritis, typically associated to local accumulation of homogentisic acid polymers and characteristic yellow-brown cartilage debris (25–28).

Infectious Arthritis

In keeping with deposition diseases, the diagnosis of suspected infectious arthropathies can be approached either through the analysis of synovial fluid or synovial tissue. Both type of samples have been successfully exploited for the identification of pathogenic organisms through microbiological cultures and molecular analyses. Unfortunately, due to the scarcity of systematic studies, no definite guidelines are currently available to assist clinicians in the selection of the most appropriate strategy when both sites are accessible. Notwithstanding this gap, the availability of synovial specimens can represent, however, a benefit in certain circumstances by offering a wider spectrum of analytical perspectives. These perspectives, which may be of particular relevance in the case of negative results from cultural examinations, include the direct analysis of bacterial and fungal localization *in situ* through conventional stainings (Gram, Ziehl, Dieterle, periodic acid-Schiff), as well as the evaluation of indirect signs, such as the presence/pathologic aspect of local granulomatous reactions or the degree of perivascular neutrophilic infiltration (29, 30). The latter parameter, though not specific *per se,* has been reproducibly shown to be a valid discriminative marker of septic arthritis if quantitatively addressed, either through conventional haematoxylin and eosin (H&E) stain or CD15 immunohistochemistry (31–34).

Broad-range 16S rRNA bacterial PCR has not proved to offer major advantages over bacterial culture in the standard diagnostic setting (35) and is considered prone to non-specific results (36). It has been however proposed as a candidate method to monitor the presence of bacterial DNA in synovial samples from patients with septic arthritis during antibiotic treatment (19). Targeted-PCR testing for mycobacteria and difficult-to-culture atypical germs (Borrelia, Tropheryma whipplei) has been similarly applied to both synovial fluid and synovial membrane and can be considered in the case of suspicion of mycobacterial- (37, 38), Lyme- (20, 39) and Whipple's arthritis (20, 40) when a sensitive approach is required.

Synovial Tumors and Histiocytic Disorders

Tissue-directed analyses give also the unique opportunity to broaden the diagnostic spectrum in patients with unclassified arthritis, allowing the identification of specific (non-infective) conditions characterized by typical synovial histopathologic features and less traceable changes in the synovial fluid. Examples of these conditions, in which the synovial biopsy may have a primary diagnostic role, include primary synovial malignancies (lymphomas, sarcomas), metastatic tumors and some benign proliferative lesions like pigmented villonodular synovitis and synovial chondromatosis (the latter characterized by a minor

TABLE 1 | Clinical utility of synovial biopsy in differential diagnosis.

Deposition diseases	Crystal arthropathies Amyloidosis Ochronosis Hemochromatosis
Infectious arthritis	Low-grade infections by common bacteria Mycobacterial arthritis Spirochetal arthritis (Lyme disease, syphilis) Whipple's disease Fungal arthritis
Synovial tumors	Synovial cell sarcoma/synovial chondrosarcoma Lymphoma and metastatic carcinoma Pigmented villonodular synovitis Synovial chondromatosis
Histiocytic disorders and others	Multicentric reticulohistiocytosis Erdheim-Chester disease Chronic sarcoidosis Foreign-body arthritis

risk of malignant transformation). In all these conditions, the *in situ* evaluation by conventional histopathologic analyses can be required to integrate and corroborate imaging findings for a defined differential diagnosis (20, 41–43).

The standard histologic analysis of the synovium can be instrumental also in the diagnosis of arthritis in patients affected by some non-Langerhans cell histiocytic disorders, uncommon conditions characterized by multi-system involvement due to dysregulated accumulation of mononuclear phagocytes. In some forms with adult-onset (multicentric reticulohistiocytosis, Erdheim-Chester disease), patients can display severe joint involvement, typically associated to abnormal sub-lining infiltration of CD68-positive (CD1a- and S100-negative) histiocytes and multinucleated giant cells with a lipid-laden or PAS-positive ground-glass cytoplasm (44–46).

Diagnostic Value of Synovial Biopsy in Real-Life Clinical Practice

Altogether, these data, generated over the last decades, demonstrated the utility of synovial tissue collection for differential diagnosis, but left partially unclear the actual output of the procedure in the real-life setting of a rheumatology clinic, in particular for what concerns most recent approaches, such as US-guided biopsy. This issue has now been addressed by independent groups demonstrating, quite consistently, a success rate of around 82–96% in obtaining samples suitable for analysis with the potential to achieve a diagnosis in between 16 and 20% of the cases (13, 20, 47), depending on the inclusion criteria and study design (13, 20, 47).

TRANSLATIONAL APPLICABILITY OF SYNOVIAL BIOPSY IN CLINICAL TRIALS: SURROGATE BIOMARKERS OF CLINICAL RESPONSE TO TREATMENT

Beyond its possible application for differential diagnosis of unclassified arthritis, synovial tissue examination has been also proved to be a valuable source of surrogate biomarkers of response-to-treatment. Evidence supporting this concept derives from pioneering studies performed in the last two decades demonstrating, through the evaluation of serial arthroscopic biopsies, the sensitivity-to-change and external responsiveness of sub-lining CD68+ macrophages in relationship to variations of clinical composite indices in rheumatoid arthritis (RA). The reduction of CD68+ sub-lining macrophages has been shown to be associated to effective treatment, less influenced by placebo compared to clinical parameters, and to be a valid measure of response to treatments characterized by different mechanisms of action (48–51). Collectively, these observations have corroborated the value of synovial tissue analysis for the development of markers of early patho-biologic effect, thus potentially exploitable to accelerate decisions (including dose selection) in early phase I/II clinical trials. Strengthening the general applicability of these data, the correlation between modulation of the number of CD68+ sublining macrophages with clinical response to treatment has been recently confirmed also through the assessment of US-guided biopsies restricted to tissue collection from small joints (8).

CLINICAL PERSPECTIVES: EARLY DIAGNOSIS OF CHRONIC INFLAMMATORY ARTHRITIDES

If, on the one hand, the studies presented in the previous sections delineated the conditions that can be diagnosed through a synovial biopsy, on the other, they also shed further emphasis on what synovial tissue sampling cannot currently offer in routine clinical practice. The possibility to identify specific traits for most common forms of systemic chronic inflammatory arthropathies, namely RA and spondyloarthritis (SpA) remains, indeed, impracticable. This issue is relevant both in patho-biologic and clinical terms and has been the object of intense investigation in the past. Indeed, since early diagnosis and treatment in these conditions are linked to improved long-term outcomes (52, 53), the identification of disease-specific pathologic changes would contribute not only to improve comprehension of disease pathogenesis but, potentially, also to improve current models for early outcome prediction in undifferentiated forms (54–56).

RA and SpA synovitis (evaluated at a group level) do display measurable differences compared with post-traumatic and degenerative conditions, in terms of gene expression (57), histopathologic score (Krenn's, IMSYC) (58, 59), and cell proliferation rate (60). None of the analyzed parameters, however, has so far proved a sufficient degree of diagnostic accuracy due to intra-disease variability and overlapping features. The same concept applies for what concerns the overall level of micro-anatomic organization of inflammatory infiltrate that has been shown, as expected from studies in different pathologic contexts (61), to present similar qualitative characteristics (62, 63).

Despite these data and the observed gross analogies, there is now growing evidence from independent studies that a detailed

comparative analysis of specific components of the inflammatory process may actually allow to detect multiple and congruent biological differences among diseases, in particular if patients' characteristics and the overall degree of joint inflammation are appropriately matched. One of the most compelling aspects that has been reproducibly confirmed relates to the characteristics of the vascular system. Synovial vascularity has been shown to display macroscopic and microscopic differences between RA and SpA, with the latter associated to an increased distribution of tortuous blood vessels in the sub-lining both in early and established disease (64–67). Accordingly, the level of synovial production of angiogenic factors (VEGF and Ang2 mRNA and protein) is significantly increased in psoriatic arthritis (PsA) compared to RA, with a prominent differential expression in perivascular regions. Since Ang2 expression in the presence of VEGF is functionally implicated in angiogenesis and vessel destabilization, it has been proposed that the observed high levels of Ang2/VEGF in PsA joint could inhibit stabilization of the new vessels, resulting in the formation of more "plastic" vessels (68).

The existence of peculiar biological traits characteristic of SpA synovial stroma has been confirmed by gene expression analyses. In this context, of particular interest is the work performed by Yeremenko et al. (69) who, by pan-genomic microarrays of synovial samples from patients with SpA and RA matched for the local degree of histological inflammation, demonstrated a robust disease-specific, inflammation-independent myogene expression signature in SpA synovitis. Synovial tissue staining identified the myogene expressing cells as α-SMA positive, vimentin-positive, prolyl 4-hydroxylase-positive, CD90+ and CD146+ mesenchymal cells, confirming their significant over-representation in the lining and sub-lining of the inflamed SpA synovium.

No differential characteristics, instead, have been reproducibly recognized in the distribution of major lymphocyte populations (conventional CD3+ T cells, CD8+ T cells, B cells, plasma cells) and of lining/sub-lining CD68+ macrophages (65–67, 70), although an increased prevalence of alternatively activated CD163+ macrophages (67, 71) and IL17 producing mast cells (72) has been reported in SpA.

In conclusion, data derived from several independent studies demonstrate that, despite a shared inflammatory background, the inflamed synovium of different forms of chronic inflammatory arthritides can associate to differential cellular and molecular traits. Further research and novel multi-center observational studies (56, 73) are needed to improve our mechanistic comprehension of these traits and delineate their predictive value in real-life clinical practice.

CLINICAL PERSPECTIVES: PATIENTS' STRATIFICATION WITHIN AND ACROSS CHRONIC INFLAMMATORY ARTHRITIDES

If differences in the synovial characteristics can be captured between different clinical entities, a cutting-edge question is whether clinically relevant differences can be reliably distinguished also within the same disease, a fundamental premise to conceive the possible integration of synovial biopsy into a precision medicine algorithm.

Precision medicine is an approach to disease treatment and prevention that takes into account individual patho-biologic variability, thus allowing to predict more accurately which treatment or prevention strategy for a particular disease will be more suitable in specific groups of patients. This perspective, which differs substantially from conventional approaches based on the "average person," represents a major objective of modern healthcare systems due to both clinical and socio-economic needs. Chronic inflammatory arthritides have several characteristics that make them ideally suitable for stratification. These include the high degree of clinical heterogeneity that characterizes both RA and SpA, the degree of variability of response to specific treatments within each disease, and the similar degree of efficacy of specific treatments across individuals affected by different diseases (74). Whilst a rudimental level of stratification is already applied to RA, through the distinction of autoantibody-positive and -negative sub-groups (75), it is quite clear that these categories, *per se*, are not sufficient to entirely explain the heterogeneity of the disease and that a finer profiling is required (76). Since the synovial membrane represents one of the primary targets of these conditions, it is expected that dissecting its pathologic traits could be a privileged window on disease pathogenic spectrum (4).

Several studies performed in recent years have set the technical, pathological and clinical bases to support the scientific rationale of exploiting synovial biopsy for a precision medicine approach to arthritis.

In technical terms, the collection of a limited amount of tissue from a single procedure has been proved sufficient to obtain a reliable assessment of different histopathologic markers (6, 8, 77–79) and gene expression (80) in one joint. Despite differences among studies, depending on the adopted technique and measurement unit (number of specimens, mm^2), all reported data were falling within the feasibility range of a routinely applicable procedure. As a complementary observation, the assessment of different characteristics (selected histopathologic markers and of T cell clonal expansions) in one joint has been shown to be representative of the same parameters in other joints in RA (81, 82). Notwithstanding the possible existence of variability in transcriptome signatures and epigenetic traits among different sites (83), current results collectively suggest that the analysis of few synovial specimens from a single accessible site can be informative on the systemic process.

In pathological terms, there is now extensive evidence indicating that the cross-sectional evaluation of the synovium from a single joint does actually allow the identification of defined inter-individual differences in RA. This concept has been supported by independent studies focused on different analytical perspectives: immuno-histology (84, 85), gene expression profiling of whole tissue (86–92), and RNA-seq data from isolated synovial cells (93).

A critical issue remains the interpretation of the observed heterogeneity and two main models are currently emerging. In particular, whilst some studies have described the variability of synovial characteristics primarily as a function of the overall

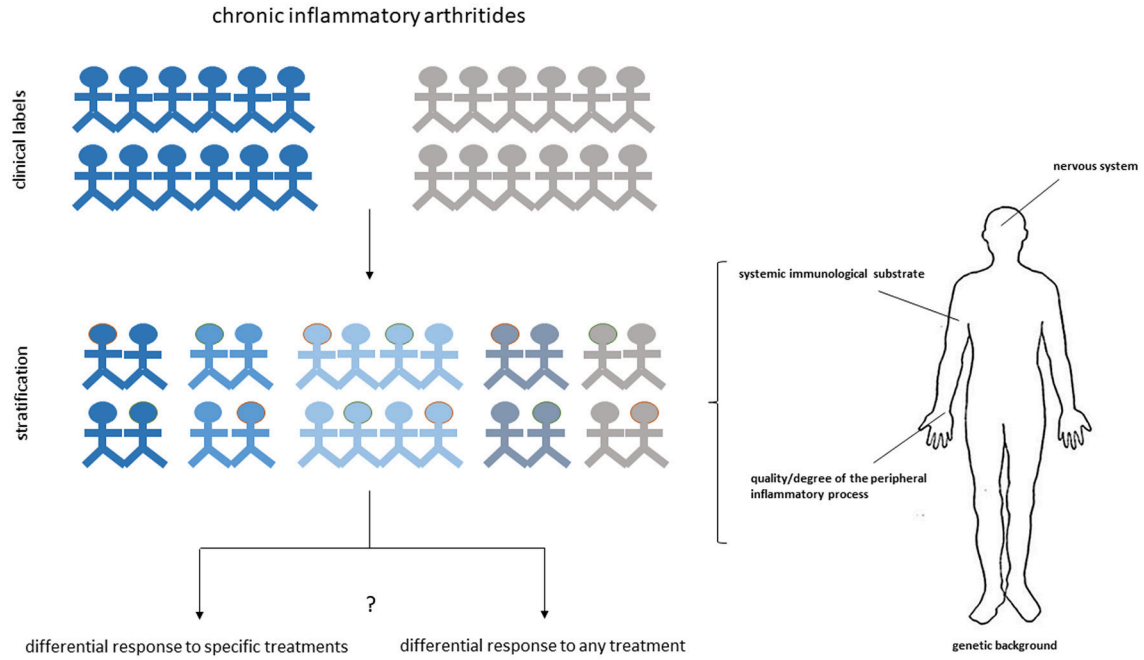

FIGURE 1 | Multidimensional approach for personalized medicine in arthritis. Different anatomic and functional dimensions can cooperate to delineate the heterogeneous phenotype of chronic inflammatory arthritides and the predisposition of subgroup of patients to respond to specific treatments or to respond to any treatment. Dissecting the synovial histological and molecular characteristics of each individual patient may provide a fundamental contribution to the stratification process by offering a privileged window on the differential expression of the disease at target sites.

degree of inflammation intensity (92, 94), other analyses have proposed the existence of a more qualitative spectrum, with the identification of distinct synovitis categories, each characterized by congruent histological, molecular and cytological correlates (95, 96). Based on the relative enrichment of specific gene sets, these categories have been defined by Dennis et al. (95) as: (i) *the lymphoid phenotype*, enriched in genes related to B-T lymphocyte activation-differentiation, immunoglobulin production and antigen presentation; (ii) *the myeloid phenotype*, also characterized by processes associated with TNFα and IL-1β production, TLR and NOD-like receptor signaling, Fcγ-receptor-meditated phagocytosis; (iii) *the fibroid phenotype*, enriched for genes associated with TGFβ and BMP signaling, together with SMAD binding, but lacking enrichment of any immune system processes; (iv) *the low inflammatory phenotype*, showing only enrichment for inflammatory and wound response processes. These phenotypes, or similar patterns according to a recently revised classification (97), have been shown to present measurable associations with specific biomarkers in peripheral blood (CXCL13 and soluble ICAM-1 for the lymphoid and myeloid phenotype, respectively) and to be detectable in early-untreated RA, strengthening their differential biologic impact also at systemic level and in the absence of treatment biases (95, 97).

In clinical terms, despite it remains unclear whether the heterogeneity of synovial features does reflect fixed characteristics of specific disease subsets or dynamic phases conditioned by fluctuations of the inflammatory process, we have now proof-of-concept evidence that the assessment of synovial inter-individual differences does actually have the potential to predict clinically-relevant outcomes. Data supporting this idea derive from independent observational studies based on patients' stratification through either histological parameters or molecular signatures. Associations between synovial pathologic traits and clinical response to specific treatments has been obtained in studies focusing on agent targeting different molecular pathways, including anti-TNF (95, 98–104), IL-6 inhibitors (105), or B cell depleting agents (106–108), pointing at a wide spectrum of applicability. The assessment of synovial patho-biology in single joints has been shown also to hold an intrinsic potential for the development of prognostic biomarkers, as it can be inferred, for example, by the association between B cell-rich/lymphoid synovitis (109) and radiographic progression, recently confirmed in independent RA cohorts (85, 97, 104).

Is a Multidimensional Approach Required for Stratification of Systemic Inflammatory Arthritides?

Despite current advancements, the development of a valid personalized approach to RA or SpA, based on synovial biopsy and applicable at community level still remains a very ambitious target. It should be indeed emphasized that data derived from available prediction studies, though promising, did not always led to univocal conclusions. Although differences might be obviously related to the limited sample size, differences in

the definition of exposure variables and pre-set confounders, we might also consider that both RA and SpA are likely to be determined, as the majority of immune-inflammatory diseases, by a complex series of events controlled by polygenic, environmental and endocrine factors (110, 111). Some of these events might express themselves also at systemic level and in different anatomic compartments (112–115), providing a source of variability that may be missed by restricting the analysis to downstream inflammatory reactions. Response to treatment can be also influenced by patient-related subjective factors not directly reconcilable to measurable peripheral events (116). Thus, unlike oncology, in which the conception of a precision approach can be primarily based on genetic drivers, the approach to systemic immune-inflammatory diseases might require considering additional levels of complexity through the integration of different systems and clinical parameters (117) (**Figure 1**). A direct example supporting this hypothesis derives from the work performed by Lauwerys et al. demonstrating that the diagnostic accuracy of synovial analyses based on gene expression data increases from 56.8 to 98.6% by the addition of specific clinical symptoms in the prediction algorithm (57). Large size prospective multi-center clinical trials testing the relevance of biopsy-based patient stratification are currently in progress and are expected to offer direct insights into the actual predictive weight of synovial biopsy.

CONCLUSIONS

Taken together, the studies discussed in this review highlight the important, though circumstantial role that synovial biopsy can have in current clinical practice. Depending on the clinical context, it may complement and in some cases substitute less invasive procedures, offering the possibility to integrate microbiologic and histopathologic data. The combination of these approaches in certain circumstances can be essential to achieve a definite diagnosis in patients with arthritis of undetermined origin. The spectrum of applicability of synovial biopsy remains, however, relatively limited mostly due to the lack of validated markers for the diagnosis and management of major forms of chronic inflammatory arthritis.

The next challenge is thus to define the exploitability of the heterogeneous molecular and cellular patterns that characterize the synovial tissue in RA and SpA for the development of novel diagnostic markers and multi-dimensional precision medicine algorithms. Based on recent data from observational studies and the technological advancements in synovial tissue sampling and analysis this perspective seems now more realistic. Of considerable relevance in this direction is the recent introduction of novel cutting-edge tools allowing transcriptional profiling and single-cell RNA sequencing of infiltrating cells isolated from synovial samples (118, 119). This technology, which has already contributed to the achievement of important goals in the characterization of novel cell subsets in RA (93, 120, 121), is expected in the near future to play a key role also in the field of biomarker discovery and in clinical translation. It is indeed likely that, compared to whole-tissue gene expression analyses, the assessment of synovial characteristics at single cell level might dramatically expand our possibilities to screen specific aspects of the pathogenic process and to unravel intrinsic characteristics of the disease. The fine deconstruction of the histopathological, molecular and cellular heterogeneity of the synovial inflammatory process by means of integrated high-throughput approaches might also lead in the near future to a novel taxonomic classification of chronic inflammatory arthritides, firmly rooted in basic pathogenic processes and, possibly, spanning across the boundaries of conventional clinical labels.

AUTHOR CONTRIBUTIONS

AM, SB, and SR contributed to literature review and preparation of the manuscript.

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Ultrasound-Guided Biopsies: Medium and Large Joints

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Ultrasound-guided needle synovial biopsies are useful for clinical practice and research in rheumatology. With the emergence of personalized medicine for the treatment of inflammatory rheumatic diseases, it is predicted that this technique will be increasingly used in the near future. Standardized characterization of the technical aspects of ultrasound-guided needle synovial biopsies is needed in order to produce solid evidence on the safety and effectiveness of the technique.

Keywords: synovium, ultrasound, ultrasound guided needle biopsy, ultrasound guided-procedures, synovial membrane

INTRODUCTION

Synovial biopsies have been used for several decades to study synovium. In clinical practice, they have been mostly used to enhance the differential diagnosis in cases of monoarthritis, mostly chronic, being particularly useful for the diagnosis of fastidious infectious agents, infiltrative diseases, and for some selected cases of crystal induced arthropathies. In research, synovial biopsies have been mostly used to clarify the pathogenesis of rheumatic inflammatory diseases, namely rheumatoid arthritis and spondyloarthritis. More recently synovial biopsies are being used to aid in the personalized treatment of rheumatic diseases. Despite all the advances in the treatment of rheumatoid arthritis in recent years, with several biologic agents available, there is still a lack of markers of response to treatment. Synovial membrane studies may aid in this objective (1–4).

There are several ways of collecting synovial tissue, and the four most commonly used nowadays include blind needle, arthroscopic, ultrasound guided with portal and forceps and ultrasound guided needle biopsy (2).

The late 1990s blind needle biopsy was a natural evolution of the older Parker and Pearson's blind biopsy allowing easier collection of synovium (5, 6). Blind needle biopsies are relatively safe, easy to perform with appropriate training but don't allow accurate sampling of the joint (7). Arthroscopic guided biopsies allow direct visualization of the synovium, but, although feasible in medium and large joints (not so in small ones), require operating theater or similar room (8).

With the increasing use of ultrasound in rheumatology two minimally invasive techniques of synovial biopsy were developed: portal and forceps and needle, allowing the study of smaller joints (1, 3). Both techniques seem safe and well-tolerated with appropriate training. Tissue quality/RNA yield is preserved in subsequent biopsies following therapeutic intervention for the ultrasound guided needle biopsies (3). For ultrasound-guided needle biopsies, there seems to be a trend to have a greater yield for large joints, but this aspect lacks confirmation in larger groups of patients (3). The grade of gray scale synovitis seems to be the best predictor of biopsy yield (3).

In a recent multicentre study comparing four different techniques: blind needle, ultrasound guided portal and forceps, ultrasound guided needle biopsy, and arthroscopy biopsy, including biopsies of knees, wrists, ankles, metacarpophalangeal, and proximal interphalangeal joints, it

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seemed that blind needle biopsy is less reliable than either arthroscopy or ultrasound-guided biopsy, namely regarding the lower amount of gradable synovial tissue. Arthroscopy seems to yield higher amount of tissue but seems only feasible for bigger joints (2).

There is a long time history of performance of blind synovial biopsies, namely through the use of a Parker Person trocar (5). Apart from that there is a significant experience of performing blind, fluoroscopy-guided and, in the past 10–15 years, ultrasound guided joint injections using previously described methods (9).

With this paper we aim to describe our procedures on how to perform an ultrasound guided needle biopsy of the shoulder, elbow, hip, knee, and tibiotalar joints.

GENERAL TECHNIQUE

The general technique to perform the biopsy of the medium and large joints is an extrapolation of Kelly et al. (3). Video material is available in the website of the author[1].

Patient positioning is a key for an uneventful procedure. Most of the medium and large biopsies are best performed with the patient supine and the physician seated, but the shoulder joint, by posterior access can be performed with the patient prone and the physician seated or the patient in lateral decubitus and the physician standing. Both patient and physician shall be comfortable in order to safely access the target joint. As in ultrasound guided injections, before the procedure a scan is used to plan the most adequate needle trajectory in order to avoid neurovascular structures (9).

The procedure shall be performed in sterile conditions, in a clean procedures room or in an operating theater. Anesthetic injection (1–3 mL) is performed in the skin and subcutaneous tissue, up to the joint capsule. Afterwards, anesthetic injection of nearly 5 mL in medium joints and 10–15 mL in large joints. The biopsy needle is the Quick Core 16G 10 mm biopsy needle or equivalent. Longer needles are needed to reach deeper joints in obese patients (such as hips or shoulders). A coaxial sheath is not obligatory but aids when the trajectory is long or when the trajectory is close to neurovascular structures. A maximum number of biopsies shall be obtained, without patient discomfort aiming at a total number of 12. At least six to eight shall be used for paraffine embedding and/or frozen (according to local procedures) and the remainder six immersed in RNA-Later for RNA extraction. Six samples per technique shall guarantee good joint sampling, but standardization is required (3). As in ultrasound guided injections an in-plane approach, trying to keep than the needle as parallel as possible to the probe, is the best approach to perform a synovial guided biopsy. If the angle between the probe and needle is superior to 40° the needle is difficult to see, and two possible strategies to enhance needle visualization include either toeing in the probe or doing the puncture site farther away from the probe (9, 10).

[1]http://www.synovialbiopsy.com

Specific Joints Technique

All procedures described and specific risks are summarized in **Table 1**.

Elbow Joint

For ultrasound guided joint injection, the usual approach is laterally through the radiocapitellar joint or posteriorly at the medial or lateral side of the triceps tendon. The medial approach shall be avoided due to the presence of the ulnar nerve that goes through the medial aspect of the triceps tendon (10). However, none of these approaches allows good visualization of the needle throw in ultrasound-guided needle biopsies. One good technique to surpass these difficulties is to approach the elbow joint anterolaterally and proximally, through the long extensor carpi radialis and brachioradialis muscles, posterior to the radial nerve in the radial fossa of the humerus (**Figure 1**). For this approach, the elbow must be extended and the hand supinated. The proximity of the radial nerve is a caveat and the use of a coaxial sheath may diminish the risk of nerve injury, despite the lack of evidence. After reaching the joint recess the needle throw shall be directed in multiple ways for better sampling (**Figure 2**).

Patients with limited elbow extension may need to perform the biopsy through the posterior approach with the elbow flexed, laterally to the triceps tendon. The medial approach shall be avoided due to the proximity of the ulnar nerve, as previously referred for the injection.

TABLE 1 | Summary of preferential approach for ultrasound guided biopsy of medium and large joints.

Joint	Patient positioning	Biopsy approach	Specific risks
Elbow	Patient supine, shoulder slightly abducted and elbow extended and supinated	Anterolateral and proximal approach, through the long extensor carpi radialis and brachioradialis muscles	Muscle rupture or hematoma; Radial nerve lesion
Shoulder	Patient prone or in lateral decubitus with shoulder adducted and with neutral or slight internal rotation	Lateral to medial and posterior approach, through the infraspinatus muscle	Suprascapular nerve or circumflex artery lesion
Tibiotalar	Patient supine with knee with 90° flexion and tibiotalar joint with slight plantar flexion	Anterolateral approach posterior and inferior to the extensor digitorum longus	Tibialis anterior artery, deep peroneal, and superficial peroneal nerve lesion
Knee	Patient supine and knee slightly flexed and supported	Superolateral approach of the suprapatellar pouch	Same as knee joint aspiration
Hip	Patient supine with hip with neutral or slight external rotation and knee extended	Lateral to medial approach, puncture posterior to the sartorius muscle, aiming at the femoral head to neck transition	Lateral femoral cutaneous nerve; femoral neurovascular bundle

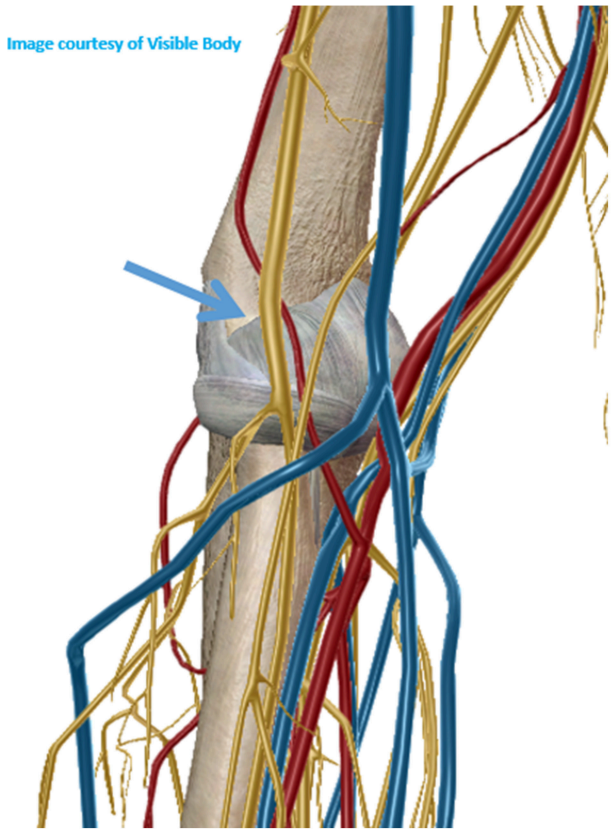

FIGURE 1 | Anterolateral approach for elbow biopsy.

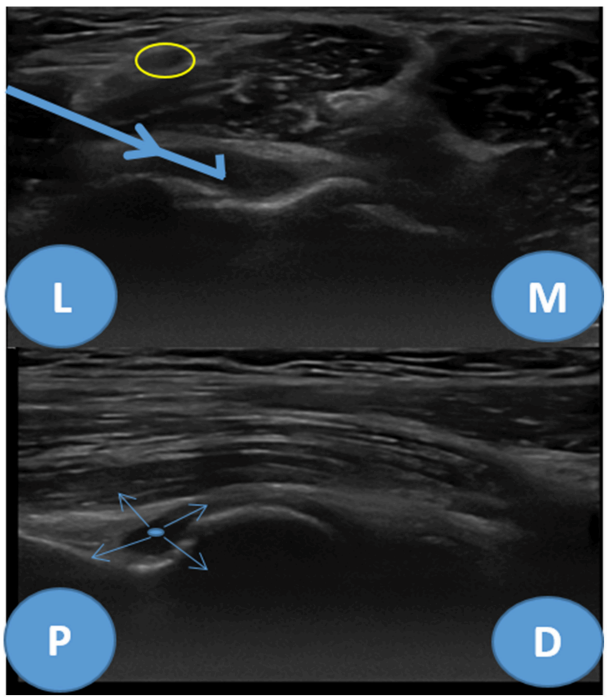

FIGURE 2 | Anterolateral approach for elbow biopsy, ultrasound images. Upper image—transverse view, L, lateral; M, medial. Yellow circle—radial nerve, Bold blue arrow—biopsy needle trajectory. Lower image—longitudinal view, P, proximal; D, distal. Thin blue arrows—needle throw directions.

Shoulder Joint

Usually the posterior approach is the preferred for the ultrasound guided shoulder injection, and the same applies for the ultrasound guided needle biopsy (9, 11). To perform this approach, the patient shall be either in lateral decubitus or prone, with the shoulder neutral or with slight internal rotation. The transducer shall be aligned with the long axis of the infraspinatus muscle and the lateral to medial approach is usually the better to execute the biopsy due to better placement of the needle throw (more parallel to the probe) (9, 11) (**Figures 3**, **4**).

Tibiotalar Joint

The best position to perform either the injection or the biopsy of the tibiotalar joint is with the patient lying supine, with the knee flexed around 90 degrees and the tibiotalar joint in slight plantar flexion.

For the injection, usually the preferred approach is between the tibialis anterior and extensor hallucis longus, in order to avoid damage to the anterior tibial artery or to the deep peroneal nerve, which are more lateral (10). However, this approach, being done ultrasound guided, provides poor needle visualization on its long axis, since it is placed almost perpendicular to the probe. One good alternative for the injection, that can be used for the ultrasound guided biopsy, is to perform it with the probe in coronal plane, either medially, just below the tibialis anterior tendon or laterally, just below the extensor digitorum longus (**Figures 5**, **6**).

Knee Joint

To perform a knee biopsy the patient is placed supine with the joint slightly flexed and supported, as for the knee joint aspiration (12).

The knee is a large, but quite superficial joint, hence can be easily injected or aspirated, even without ultrasound guidance (13). For blind injections the medial parapatellar approach, 1 cm deep to the patella is easy to perform, but doesn't allow ultrasound guidance because the needle is hidden by the patella. The best approach for the knee ultrasound guided biopsy is therefore the superolateral approach through the suprapatellar pouch, deeply to the quadriceps tendon (**Figures 7**, **8**) (13). Caution shall be taken when doing the puncture in order to avoid the quadriceps tendon, which is quite painful, if punctured (12).

Although easily accessible blindly there is evidence that either injection or biopsy are more precise and better tolerated when ultrasound-guided (2, 12, 14, 15).

Hip Joint

Although there are descriptions of approaching the hip to inject or aspirate blindly it is a very deep seated joint that shall be injected through imaging guidance (16). Fluoroscopy guided techniques can be performed to target the femoral head, through the sartorius and rectus femoris muscles in a vertical trajectory,

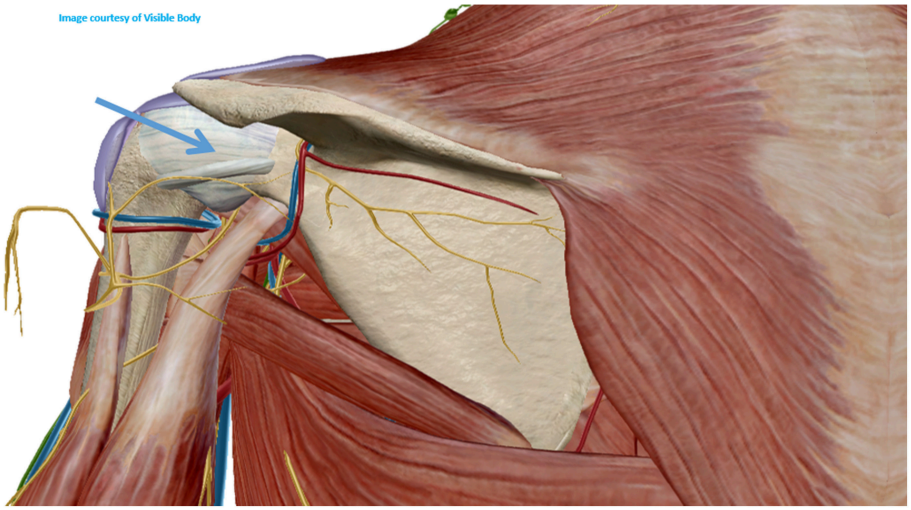

FIGURE 3 | Posterior approach of the shoulder joint.

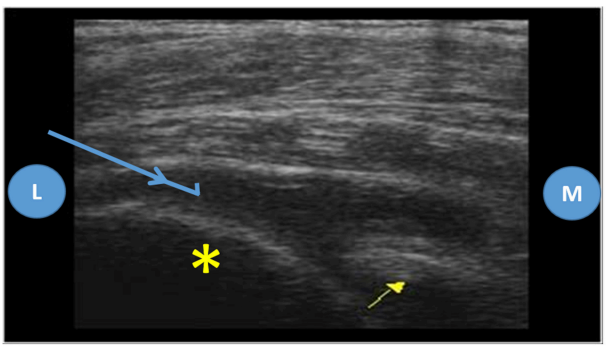

FIGURE 4 | Posterior approach of the shoulder joint, ultrasound images. Probe placed on the long axis of the infraspinatus muscle, L, lateral; M, medial; Yellow asterisk—humeral head; Yellow arrow—glenoid; Blue arrow—biopsy needle trajectory.

or more obliquely, deeply to the sartorius, targeting the femoral neck. These are performed with the patient supine and the hip 10 to 15 degrees internally rotated (17). These techniques are, however, unable to be applied ultrasound-guided. The most commonly used technique to perform an ultrasound guided injection is performed with the patient supine and the hip 20 degrees externally rotated (but according to some authors in neutral position), with the probe longitudinally placed in relation with the femoral neck. The puncture site is usually the more lateral, and the needle shall be placed in perfect alignment with the probe in order to guarantee that the femoral nerve and vessels, which are placed medially, are not harmed. The puncture is done through the rectus femoris and iliopsoas muscles (9, 10, 18). These techniques, albeit suitable for joint injection, due to the fact that the needle trajectory is too steep, don't allow proper needle positioning that permit appropriate ultrasound visualization for the biopsy, nor allow proper biopsy needle throw pressure against the synovium to effectively harvest synovial membrane. One alternative to surpass these limitations is a lateral to medial approach, directed to the femoral head and neck transition, placing the needle more horizontally than the fluoroscopy guided injection technique aiming at the femoral neck described previously by Duc et al. (17) (**Figures 9**, **10**). With this technique, more technically challenging, there is always good needle and femoral neurovascular bundle visualization in relation to the needle (9, 17).

GENERAL RISKS

When compared to other biopsies, such as renal biopsy, synovial biopsies (irrespective of the technique used) are very safe when performed by orthopedic surgeons or rheumatologists, and according to some authors lead to <1% of adverse events (19). Up to 25% of patients undergoing ultrasound guided needle biopsy report, at least, mild discomfort or pain and few patients develop a vagal crisis. Joint and skin infections, bleeding and hemarthrosis, post biopsy pain, and neurovascular lesion are rare (3, 20).

Specific Risks

Elbow Joint

Apart from the general risks previously referred the specific risks of the anterolateral approach of the elbow joint are related with the structures that may be injured by the needle trajectory. Long extensor carpi radialis and brachioradialis muscle hematoma or muscle rupture are possible risks as well as lesions of the radial nerve (which can be minimized using a coaxial sheath). There is no evidence on the prevalence of these complications but are expected to be low with practitioners experienced on the use of ultrasound guided procedures.

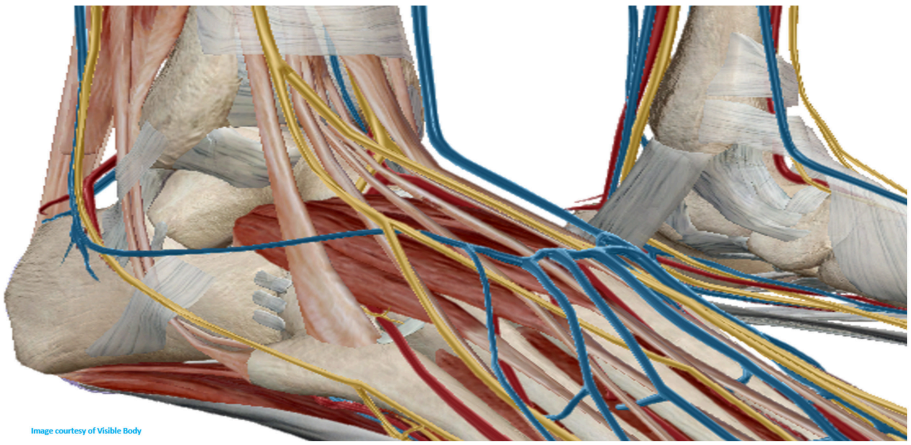

FIGURE 5 | Anterolateral biopsy approach for the tibiotalar joint.

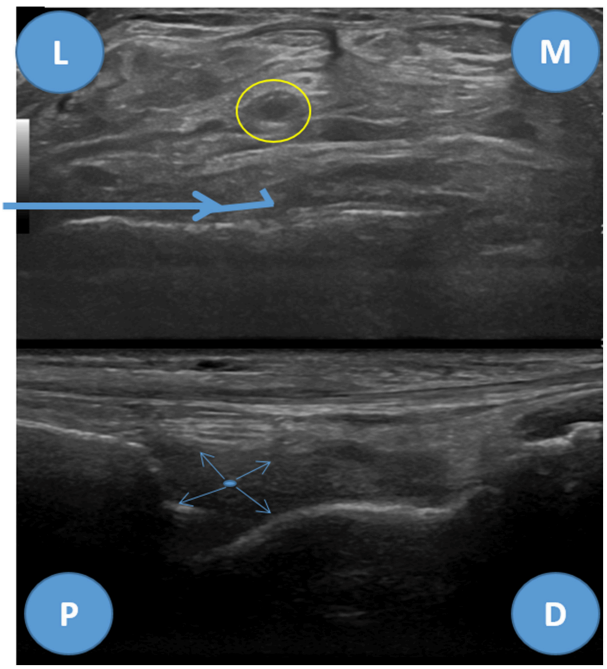

FIGURE 6 | Anterolateral biopsy approach for the tibiotalar joint, ultrasound images. Upper image—transverse view of the tibitalar joint, L, lateral; M, medial. Yellow circle—tibialis posterior artery and deep peroneal nerve, Bold blue arrow—biopsy needle trajectory. Lower image—longitudinal view, P, proximal; D, distal. Thin blue arrows—needle throw directions.

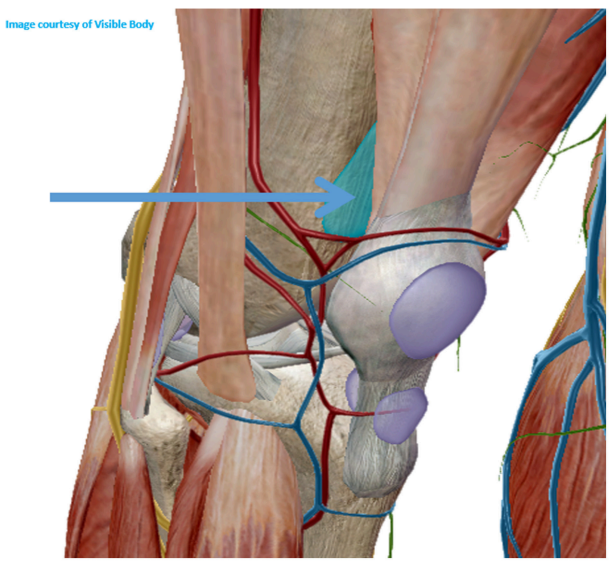

FIGURE 7 | Superolateral approach for knee biopsy.

Shoulder Joint

A theoretical risk of this biopsy is the lesion of the suprascapular nerve and circumflex scapular vessels in the spinoglenoid fossa, but that only happens if the needle is placed too medially (9, 11).

Tibiotalar Joint

The main structures to avoid regarding the anteromedial or anterolateral pathways for the ultrasound guided biopsy are the anterior tibial artery and the deep peroneal nerve, which are easily seen in the midline (yellow circle in **Figure 6**) (9). One nerve that can be accidentally punctured in the anterolateral approach is the superficial peroneal nerve, however, in most individuals it is placed superficial to the extensor digitorum longus and, therefore, the biopsy can be safely performed when the needle is placed deeply in relation to this tendon. This nerve, purely sensitive, is a branch of the common peroneal nerve and can be located either in the anterior or the lateral compartment in up to one third of the patients (21–23). In the anteromedial approach, just posterior to the tibialis anterior tendon, care shall be taken in order to avoid the saphenous nerve and the great saphenous vein (21).

Knee Joint

Neurovascular structures are far from the superolateral approach hereby described. However, care shall be taken not to puncture

the periosteum of the femur or the quadriceps tendon which are significantly painful (12).

Hip Joint

One structure than can be harmed in the lateral to medial approach of the hip biopsy is the lateral femoral cutaneous nerve, which is usually located posteriorly to the sartorius and superficially to the rectus femoris and tensor fasciae latae muscles. However, despite in most of the individuals the nerve is located medially to anterior superior iliac spine, there are a lot

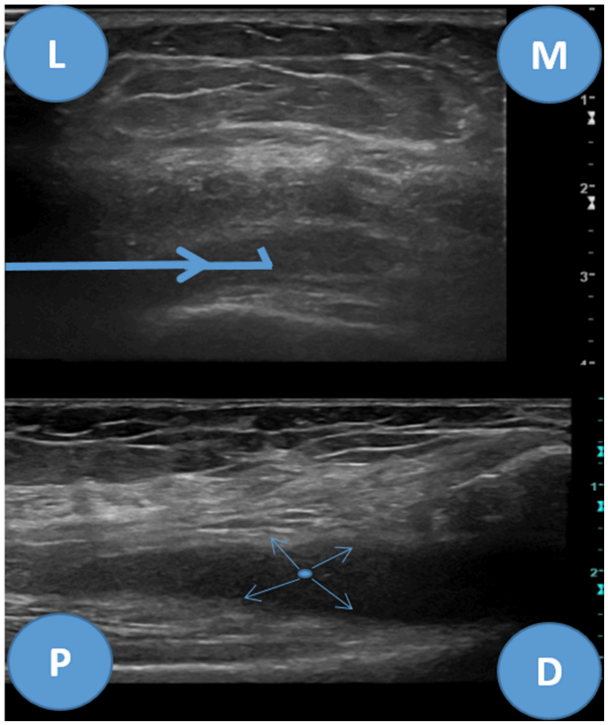

FIGURE 8 | Superolateral approach for knee biopsy, ultrasound images. Upper image—transverse view of the suprapatellar pouch of the knee joint, L, lateral; M, medial. Bold blue arrow—biopsy needle trajectory. Lower image—longitudinal view, P, proximal; D, distal. Thin blue arrows—needle throw directions.

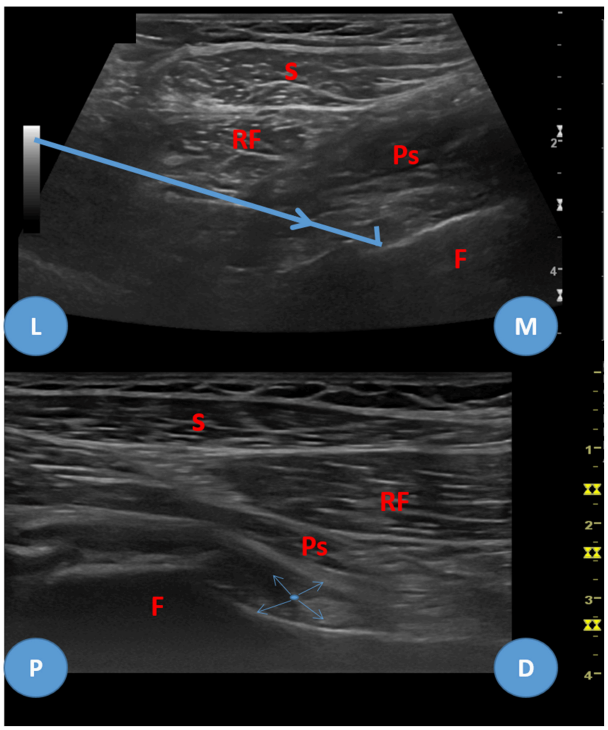

FIGURE 10 | Lateral to medial approach for hip biopsy, ultrasound images. Upper image—transverse-oblique view of the hip joint, L, lateral; M, medial. Bold blue arrow—biopsy needle trajectory. Lower image—longitudinal view, P, proximal; D, distal. Thin blue arrows—needle throw directions. S, sartorius muscle; RF, rectus femoris muscle; Ps, Psoas iliacus muscle and tendon; F, femur.

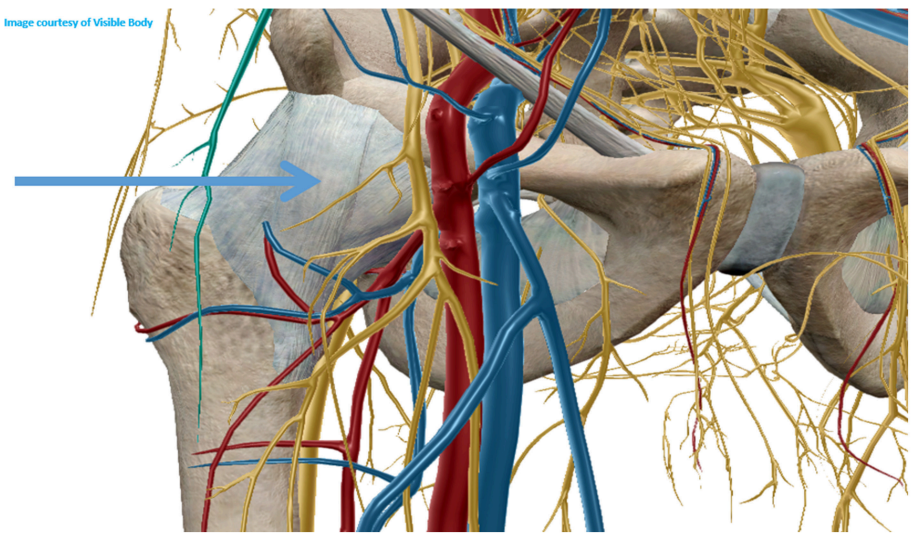

FIGURE 9 | Lateral to medial approach for hip biopsy.

of variations (it can be located from 6.5 cm medial to 6 cm lateral) (24). For most individuals the nerve courses distally through the flat-filled flat tunnel that lies between the sartorius and tensor fasciae latae muscles, therefore, if the puncture site is through the tensor fasciae latae muscle, there is a low likelihood of harming the nerve (25). The use of a coaxial sheath may diminish this risk.

The most important risk is to harm the femoral neurovascular bundle, if the needle is placed too medially, but this risk is expected to be minor in an experienced practitioner (9, 16, 17).

CONCLUSION

Synovial biopsies are of great value even in rheumatology clinical practice nowadays. Ultrasound-guided biopsies are safe, well-tolerated and effective but evidence is lacking. In knee joints the quality of tissue harvested seems superior when compared with wrists, ankles, metacarpophalangeal and proximal interphalangeal joints. There is no evidence of the safety and effectiveness of the procedure for some joints, such as the shoulder or hip. In this paper there is a description of some ways these biopsies can be performed, but technical agreement on how to perform them is needed in order to standardize procedures and to allow the production of solid evidence (2, 3).

AUTHOR CONTRIBUTIONS

JP-P is responsible for the conception of the manuscript, including the review of literature, and the individual experience that conducted to the idea.

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REVIEW
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Synovial Tissue Biopsy Collection by Rheumatologists: Ready for Clinical Implementation?

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Synovial tissue from arthritis patients is increasingly used for both basic pathophysiological and clinical translational research. This development has been spurred by the development of biotechnological techniques for analysis of complex tissues and the validation of ultrasound guided biopsies for easier tissue sampling. This increasing use of synovial tissue raises questions on standardization of methodologies for tissue processing and cellular & molecular analyses. Furthermore, it raises the question if synovial tissue biopsy analysis may be more widely implemented in clinical practice, what are the methodological hurdles for implementation and what are the lessons that can be learned from previous experience. This will be the focus of this review.

Keywords: arthritis, synovial tissue, biopsy, clinical practice, implementation

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ACQUIREMENT OF SYNOVIAL BIOPSIES

There are several possible approaches to the acquisition of synovial tissue (1, 2). In most clinical practices tissue acquisition is performed by orthopedic surgeons at the operating theater, with the patient under sufficient anesthesia. For large joints arthroscopic biopsy is generally accepted as the gold standard, which gives a good quality and size of biopsy specimens in most cases (3). To acquire sufficient tissue from small joints an arthrotomy could be performed. During the past 25 years arthoscopic biopsy procedures have been increasingly used by academic rheumatological expert groups for basic pathophysiologic and clinical translational research. A number of their studies have addressed the minimal requirements for arthroscopic or ultrasound guided synovial tissue biopsies for scientific research.

In these studies the minimum number of biopsies to be retrieved was addressed. A minimum of 6 biopsies per procedure was shown to be sufficient to reduce sample variability in T cell numbers as analyzed by immunohistochemistry (2, 4–6). Other papers addressed the locations in the joint from which synovial biopsies should be acquired. It was found that macrophages and associated cytokines were unevenly distributed within the joint, while T cells and expanded T cell clones were more evenly distributed (7–9). The amount of synovial tissue needed depends on the clinical or translational questions and further research is needed for validation.

ULTRASOUND-GUIDED SYNOVIAL BIOPSIES

A relatively new method to obtain synovial tissue is ultrasound (US) guided synovial biopsy, which is performed by trained rheumatologists. It can be performed by portal and forceps or Quick core needle. US biopsies are less invasive than arthroscopic biopsies and can be performed in both small and large joints (3, 6, 10–12). An advantage of US biopsy is that it is relatively easy

to learn and it has a relatively small chance on side effects. A caveat is that synovial tissue yield is operator and index joint dependent and the operator needs to perform biopsies at regular intervals to retain skills to maintain a high success rate in obtaining good quality synovial tissue samples (6). The minimal requirements to retain skills is the subject of ongoing investigations. Furthermore, synovial tissue yield depends on the level of synovial inflammation as visualized by ultrasound. This seems to limits the application into research for conditions with low level of gray scale synovitis. Good quality synovial tissue was obtained from the knee in a cohort of RA patients in disease remission, but the success rate and tissue quality was not precisely reported (13). It has been shown that US guided synovial biopsies of joints selected on ultrasound parameters yield synovial tissue in 80–90% of cases of sufficient quality for histological evaluation and RNA extraction in both small and large joints (6). One study showed that the histological analysis of 2.5 mm^2 from 4 biopsies of synovial tissue acquired by US biopsies is representative of the joint status in small joints of RA patients (14). In a recent multicenter retrospective study comparing arthroscopic biopsies with ultra-sound guided and blind needle biopsies on 159 procedures from 5 different academic rheumatology centers, there was no significant difference in the proportion of graded synovial tissue or total graded synovial tissue area and containing enough RNA of significant quality and quantity for transcriptomic analysis (15). These studies on tissue quality have only been investigated for a number of general assays, such as immunohistochemical staining of T cells and general retrieval of RNA. These diagnostic tests are not used in the clinical setting. Studies for these diagnostic tests have not been performed. It is therefore not precisely known what the density is of pathophysiological aberrations measured with various techniques or if there is an uneven distribution of biomarkers for different clinical conditions. Furthermore, if a number of diagnostic tests are combined within one patient, it is not known if the synovial tissue yield is similar between the first vs. later biopsies.

Clinical Value of Synovial Tissue Sampling

Most clinical translational research focuses on prognostication and prediction of treatment response in patients with rheumatoid arthritis or psoriatic arthritis. To better understand the hurdles toward clinical implementation of potential biomarkers it is informative to critically appraise the use of synovial tissue diagnostic tests in current clinical practice. At this moment, synovial tissue analysis is infrequently used for differential diagnosis in patients with arthritis. There are many different causes of arthritis. For the rheumatologist it is frequently problematic to discriminate between these different causes. In a patient with arthritis the rheumatologist first analyzes the development in time and the number and pattern of involved joints. A major distinguishing factor for differential diagnosis is the presence of a mono- vs. oligo- or polyarthritis. Second, investigations such as imaging studies and blood tests may give additional clues for the cause. Also, examination of synovial fluid, when it is possible to aspirate this, can be of aid. Despite this, the rheumatologist can often not make a certain diagnosis (16). In most clinical practices synovial biopsies are performed by surgeons. Unfortunately, this can result in considerable delay. Sometimes, a biopsy is even omitted and patients are first treated with a trial of immunosuppressants and a biopsy is only performed if they do not respond. This can result in a prolonged period before an effective treatment is found with a long period of illness, invalidity and risk on permanent joint damage. Implementation of synovial biopsy sampling in these patients is also hampered by the relative limited amount of scientific reporting on this issue. In various case reports and series synovial biopsy analysis has shown an added value in addition to other diagnostic tests (17). From these reports it is however not entirely clear in which circumstances a synovial biopsy may precisely aid in diagnosis and what are the chances on sampling error. This is relevant because ultrasound-guided synovial biopsies are smaller compared to arthroscopic or arthrotomic tissue specimens. A careful reading of reported literature may give clues to the opportunities and hurdles for implementation in clinical practice for synovial biopsy analysis with existing diagnostic tests and this may also give insight into the hurdles for implementation of potential future diagnostic tests.

Infectious Arthritis

There are many different pathogens that can infect synovial tissue. Below we discuss different causes of infectious arthritis and the value of synovial tissue analysis.

Acute Infectious Arthritis

Synovial tissue analyses can assist in the detection of joint infections (18). Most infections present as an acute onset mono-arthritis accompanied by fever. Less frequently, infectious arthritis presents as an indolent mono- or oligoarthritis. Causative organisms range from common gram-positive and gram-negative bacteria to *gonococci, Borrelia Burgdorpheri, mycobacteria, fungi,* or *Tropheryma Whippleii* infection. Synovial fluid culture yields growth of pathogenic bacteria in only a proportion of cases depending on the causative organism. Synovial fluid with a nucleated cell count $\geq$2,000 white blood cells/mm^3 is considered inflammatory, the higher the leukocyte count (>10,000/mm3) and the greater the percentage of polymorphonuclear neutrophils (PMNs) (>90%), the higher the likelyhood of septic arthritis (19). Bacterial joint infections often have more than 75% of PMNs (20). In a recent study Coiffier et al. performed ultrasound guided synovial biopsies in patients with an acute monoarthritis (defined as <6 weeks duration). A total of 51 synovial biopsies were obtained from these patients from which 11 were positive on culture and defined as septic arthritis. Three of these biopsies had a positive synovial tissue culture and no bacterial growth on synovial fluid. This suggests it is useful to obtain synovial tissue in patients with an acute mono-arthritis and negative synovial fluid culture. Also the presence of perivascular infiltration of neutrophils in synovial tissue had a sensitivity and specificity of, respectively, 81.8 and 842% which leads to a likelyhood of 5,2 for the diagnosis septic arthritis (21).

Neisseria gonorrhoeae septic arthritis is often difficult to diagnose, for which mostly PCR or culturing on synovial fluid is performed. *N. Gonorrhoeae* is fragile and difficult to grow (22)

The Gram stain reveals intra- and extracellular Gram-negative diplococci in <50% of cuture-positive fluids. Polymerase chain reaction (PCR) for *N. Gonorrhoeae* has a high specificity, which is estimated at 96–98% and a sensitivity of 78–80% (22). Broad-range bacterial primers to analyze genes coding for ribosomal RNA (16S rRNA) by polymerase chain reaction (PCR) may also show bacterial species (23–25). The available literature on the performance of these diagnostic tests mostly consists of case reports and series. It is therefore unknown in which cases and to what extent synovial tissue analysis is of added value compared to synovial fluid analysis. The use of 16S rRNA in diagnosis is for example under discussion since this test has also been reported positive in cases of rheumatoid arthritis and spondyloarthritis (25, 26). 16S rRNA analysis has also yielded positive results in uninfected liver and lymph node specimens. It is thought that this may be caused by amplification of RNA from bacterial fragments in endosomes of macrophages. In these cases 16S rRNA mostly yielded multiple organisms. Infectious arthritis might be characterized by the presence of rRNA from a single organism in multiple tissue specimens (27–29). A single study suggested that serial sampling could help in the decision to discontinue antibiotic treatment (29). However, the minimum amount of tissue that is required for immunohistochemical staining, culture and RNA analysis has not yet been systematically investigated.

Lyme Arthritis

A number of studies have focused on synovial tissue analysis in Lyme arthritis (23, 30–37). Lyme disease is a tick-borne infectious disease caused by different subspecies, most often *Borrelia Burgdorferi, B. Garinii, and B. Afzelii*. Lyme arthritis most common present as an intermittent or chronic mono-arthritis of the knee joint and less common an asymmetrical oligoarthritis (23). The causative agents and disease course and manifestations vary between continents. In the USA *Borrelia Burgdorferi* is the primary cause of Lyme disease (38). In Europe Lyme arthritis is most commonly caused by *B. afzelii, B. garini,* and *B. burgdorferi* occurs less often (39). About 60% of the untreated patients with Lyme disease develop Lyme arthritis as a manifestation of Lyme disease and about 10% of these do not respond to antibiotics (23, 36). Hypothetical explanations for this problem include the persistent presence of the organism or development of post-infectious inflammatory arthritis. *Borrelia Burgdorpheri* grows in blood and skin biopsies, but synovial fluid is a toxic environment for Borrelia species and successful cultivation is rarely seen (31, 32, 37). In spiked cultures adding small amounts of joint fluid results in rapid killing of spirochetes. For the diagnosis of Lyme disease it is recommended to use a two test approach for active disease and for previous infection using a sensitive enzyme immunoassay (EIA) or immunofluorescent assay (IFA) followed by a Western immunoblot. Negative EIA or IFA make a diagnosis of Lyme arthritis highly unlikely and remove the need for further testing (40). Lyme arthritis is a late stage of Lyme borreliosis and occurs several months after initial infection. Persons tested for Lyme disease almost always have a strong IgG positive response to *Borrelia Burgdorpheri* or blot antigens (41). However, positive serology may also reflect past (asymptomatic) Lyme infection.

PCR testing of synovial fluid for *Borrelia Burgdorpheri* DNA may be helpful for establishing a diagnosis of Lyme arthritis. There are different ways of PCR testing, qualitative PCR and quantitative PCR testing which detect different DNA sites encoding for *Borrelia Burgdorpheri* genes. Sensitivity of PCR testing on synovial fluid varies between 76 and 88% depending on which test is used in patients with clinical suspected Lyme arthritis and positive serology (30). Lyme arthritis can respond to antibiotic treatment despite a negative baseline Borrelia-PCR (23, 31, 32, 36). PCR-results vary, because technical execution is variable and different primer sets against different genes and subtypes of *Borrelia Burgdorpheri* are used. It is uncertain to what extent the sensitivity of Borrelia-PCR testing is diminished by cytotoxic effects of the synovial fluid on live Borrelia bacteria shed from the synovial tissue. Borrelia-PCR positivity often decreases after successful antibiotic treatment but may also persist. It persists more often in those with antibiotic refractory arthritis, but it may also disappear without further antibiotic treatment and does not correlate with time to remission in patients treated with DMARDs (33). This suggests that a persistent positive Borrelia-PCR test may result from either persisting living bacteria or prolonged but temporary presence of bacterial components in the absence of living bacteria in the synovial tissue.

Data on synovial tissue are limited. In two European studies Borrelia-PCR remained positive in the synovial tissue but negative in the synovial fluid in a small number of patients with Lyme arthritis persisting 2 months after antibiotic therapy. In one of these studies arthritis resolved post or propter additional antibiotic treatment (30, 34). In two USA studies Borrelia PCR was negative in all patients with antibiotic refractory arthritis 7–12 months after multiple antibiotic treatments (32, 33). In another study it was shown that susceptibility to antibiotic treatment differs between Borrelia subtypes so data between Europe and the USA may not be well comparable (31). Furthermore, it is uncertain if a positive Borrelia-PCR that persists in the synovial tissue despite antibiotic treatment reflects persisting live or dead/moribund bacteria. Other tests that may better reflect Borrelia viability, such as detection of Borrelia-mRNA, have been developed but not tested in this context (32). At the same time there is a lack of data on Borrelia species in the synovial tissue vs. fluid of patients with a persistent arthritis despite first-line antibiotic treatment. Overall, it can be clinically difficult to diagnose Lyme arthritis and to determine if the persisting arthritis is caused by persistent infection, post-infectious reactive arthritis or another rheumatological disease and challenging to manage the optimal duration of antibiotic vs. immunosuppressive treatment. Performance of current or new diagnostic tests in synovial tissue biopsies might be of added value, but this is uncertain.

Mycobacterial Arthritis

Tuberculous and non-tuberculous mycobacteria are an infrequent cause of arthritis and diagnosis is typically delayed from 5 to 50 months because of low initial clinical suspicion because of the very indolent onset, accounting 7% of all

extrapulmonary tuberculosis (42) These patients most often present with a slowly progressive and destructive monoarthritis, mostly affecting knee and hip, while systemic symptoms can be absent. Chest radiography shows pulmonary involvement in around 50% of patients with osteoarticular tuberculosis. Tuberculin skin and quantiferon assay maybe falsely negative as a result from immunosuppression or natural waning of protective immunity. Ziehl-Nielsen is only positive in 10–20% of cases and cultures of synovial fluid in 80% and synovial tissue in 94% (42, 43). Histology showed caseating granulomatous inflammation in 90% of specimens, which can be hard to discriminate from granulomatous inflammation in other conditions including fungal joint disease, sarcoidosis, erythema nodosum, Brucellosis, Crohn's disease, and foreign body giant cell reaction (42). Diagnosis is made with PCR and/or culture in synovial fluid or tissue (24, 44). Synovial biopsy culture may be positive while culture of synovial fluid and blood is negative. In one series in 20% of all cases synovial biopsies were needed to detect *M. tuberculosis* (43). Mycobacterial infection may also result in a type of reactive oligo- or polyarthritis called Poncet's disease. In these cases it may be particularly challenging to discriminate infectious from reactive arthritis. Data lacks on the minimum amount of tissue to be acquired for the performance of relevant diagnostic tests.

Mycobacterium leprae can occur without cutaneous manifestations and present with articular features, mostly combined with neurologic involvement. Acute and chronic symmetric polyarthritis of hands, wrists, elbows and knees, and tenosynovitis are described. It may result from direct infiltration of the synovial membrane with *M. Lepra* bacilli or because of reactive arthritis. Occasionally, Lepra bacilli have been reported in synovial biopsies, but it has not been investigated how much synovium should be acquired to differentiate infectious from reactive arthritis (45).

Non-tuberculous mycobacteria (NTM) are very slowly growing bacteria and need special medium and prolonged incubation. PCR techniques are less sensitive but faster to diagnose NTM and can distinguish mycobacterium tuberculosis from non-tuberculous mycobacteria. Chronic granulomatous infection of tendon sheats, bursa, joints, and bone are most commonly caused by *Mycobacterium marinum*, *Mycobacterium avium intracellulare M kansasii*, *M terrae* complex, *M. Abscessus*, *M. Fortuitum*, and *M chelonae* most commonly seen in immune compromised patients. Surgical excision and antibiotic therapy is needed in these patients to prevent musculoskeletal damage (46, 47).

M. Whipple

Whipple's disease is caused by Tropheryma Whipplei, 65–90% presents with arthralgias. It typically presents as a chronic, often migratory and intermittent polyarthritis (48). It is most often accompanied by gastrointestinal complaints, signs of malabsorption, and in a proportion of patients, neurological, and cardiac complaints. A diagnosis is made by PAS staining and PCR from duodenal or jejunum biopsies, but has also been reported from blood, synovial fluid, or synovial tissue (48).

Local Proliferative Conditions

Local proliferative and neoplastic conditions often result in abnormalities in conventional, ultrasound, or MRI images (49). However, these are absent in some cases, while specific pathological changes can be detected in the synovial tissue (50, 51). Synovial chondromatosis is a rare, benign condition that can occur as a primary condition but also secondary to joint damage. It involves metaplasia of synovial tissue into cartilaginous nodules. These gradually enlarge en eventually break loose to form intra- and periarticular loose bodies. These may ossify, continue to grow and induce tissue destruction. Especially at this later stage it may be hard to distinguish from intracapsular chondroma, chondrosarcoma, and there is a small risk on malignant transformation. Synovial tissue analysis may assist diagnosis both in very early stage and in late stage patients (52).

Pigmented villonodular synovitis (PVNS) is a benign disorder that involves hypertrophy of villonodular synovial tissue that gradually fills up the joint space. MRI typically shows a low signal on T1 and T2 eighted images because of hemosiderin content, but this may be masked by secondary synovitis, hemorrhage, or fat deposition. Based on imaging it may be difficult to differentiate from synovial sarcoma, recurrent hemarthrosis, or hemangioma. Synovial fluid may be bloody, xanthochromic, or clear. Synovial biopsy is considered the gold standard for diagnosis. It shows nodular fragments of hemosiderin and fat (53).

Synovial lipoma arborescens is a rare proliferative fatty process of the synovium. It may develop as a primary process or secondary to inflammatory or traumatic synovitis (54, 55). Synovial proliferation may also occur in response to a foreign body, such as surgery material, wood splinters, plant thorns, or sea urchin spine (56). Synovial biopsy may assist in diagnosis of these conditions in cases without clear etiology.

Local Degenerative Conditions: Recurrent Hemarthrosis

Spontaneous recurrent hemarthrosis is a condition that can occur secondary to a number of conditions, such as osteoarthritis, torn lateral menisci, synovial proliferative lesions, or after arthroplasty. Cases caused by torn lateral menisci may be treated with meniscectomy and those with a synovial bleeding source by synovectomy or arterial embolization (57). Synovial tissue analysis shows hemosiderin depositions and may have assisted in diagnosis in isolated cases (58–60).

Deposition Diseases

Gout, Pseudogout, Basic Calcium Phosphate Deposition Disease

Gout, pseudo-gout, and basic calcium phosphate deposition disease cannot always be diagnosed by synovial fluid analysis but can involve deposits of crystalline material in the synovial tissue (61–63). In case of suspected gout the tissue should be preserved with alcohol because the monosodium urate crystals can dissolve in other fixatives. Sections can be examined using a polarization microscope or using the DeGolanthal staining method. In a recent case series a group from Copenhagen University Hospital, Denmark, introduced the use of synovial

biopsies to diagnose gout in patients without clinical arthritis or tophi. Biopsies were performed from MTP or ankle joints of 9 patients suspected of gout. Joints were selected that showed signs of gout on ultrasound, being intrasynovial hyperechogenicity, or articular double contours. Biopsy was performed with a sterile no-touch technique, as used for joint punctures, with an intramuscular needle (21 gauge/0.8 mm). It showed synovial urate crystal deposition in 8 out of 9 patients (64). The authors argue that the 1 case in which no crystals were found might have been caused by sampling error. Synovial biopsies were also shown to assist in diagnosis of pseudogout patients with a seronegative polyarthritis (65). Basic calcium phosphate induced arthritis is hard to formally prove since the crystals are too small to be identified by (polarizing) light microscopy. They can be visualized using the calcium stain alizarin red S. A definite diagnosis can be made using transmission or scanning electron microscopy coupled with energy dispersive analysis, but this is mainly limited to the research setting.

Amyloidosis

Amyloid arthropathy results from deposition of immunoglobulin free light chains in patients with monoclonal gammopathies, multiple myeloma, or Waldenström's macroglobulinemia (66). It can manifest as joint and peri-articular soft tissue swellings or as arthritis. Most often it presents as a symmetric polyarthritis of small and large joints, but sometimes fewer or one joint may be involved (67). It may be the presenting symptom of multiple myeloma (68). Patients often have an increased erythrocyte sedimentation rate, Bence jones proteins in urine, anemia, hypercalcemia, and/or renal insufficiency. There can be clinical doubt whether the arthritis is caused by amyloid deposition in the presence of these clinical parameters. Amyloid deposits can be detected in the synovial tissue with Congo red staining with polarization microscopy and most sensitively fluorescent microscopy or immunohistochemical staining of light chains (69). Of 70 reported cases synovial biopsy was positive in 69 (99%) cases. In one case synovial biopsy was negative for amyloid and a subsequent renal biopsy was positive. In another an initial synovial biopsy was negative, but a subsequent synovial biopsy was positive. This indicates there sampling error may occur in this condition.

Hemochromatosis, Wilson's Disease, Ochronosis

Hemochromatosis involves arthralgia in a proportion of patients, which frequently involves a metocarpophalangeal osteoarthritis-like arthropathy. Sometimes a patient may present with episodes of acute arthritis of various joints that may be caused by pseudogout. Also case reports have been published of acute arthritis (70), apparently without signs of pseudogout, where synovial biopsies showed extensive cellular iron accumulation (71, 72).

Arthritis has been reported as a manifestation of Wilson's disease in isolated case reports. Synovial tissue X-ray energy spectroscopy of a synovial biopsy yielded the diagnosis in one case (73, 74).

Ochronosis is a rare genetically inherited metabolic condition that manifests as dark discoloration of the urine, dark pigmentation of the skin, and eyes and a progressive axial and peripheral degenerative arthropathy due to loss of cartilage integrity. The clinical manifestation and pathology results from joint replacement surgery sufficed for diagnosis in most reported cases, but synovial tissue biopsy might have assisted diagnosis in some cases. It shows necrotic, brown cartilage debris, and sometimes foreign body type reactions including histiocytes and giant cells containing ochronotic material (75).

Systemic Proliferative Conditions

Rare systemic proliferative non-infectious conditions and neoplastic conditions such as histiocytositic conditions, sarcoidosis, melanoma, leukemia/lymphoma, and metastasis often can be diagnosed based on pathological changes in other tissues or organs, but these sometimes lack and typical synovial tissue pathological changes may yield a diagnosis (76–78). Histiocytic conditions, such as multicentric reticulohistiocytosis, Langerhans cell histiocytosis, and Erdheim–Chester disease, typically involve tissue infiltration of bones, the reticuloendothelial system and various organs (79–83). They have been associated with mono-, oligo-, and polyarthritis and synovial biopsy has assisted in differential diagnosis in multiple reported cases. It typically shows infiltration by disease associated histiocyte subtypes and various subset of giant cells (17, 84–86).

OPPORTUNITIES AND HURDLES FOR CLINICAL IMPLEMENTATION OF SYNOVIAL TISSUE ANALYSIS BY RHEUMATOLOGISTS

Opportunities

Taken together, the validation of ultrasound guided synovial biopsies and development of novel potential diagnostic tests offers an opportunity for synovial biopsy analysis by rheumatologists. This is relevant for patients with arthritis in whom synovial tissue analysis is considered, since tissue acquisition is currently generally performed by surgeons. This may lead to a considerable delay. There especially seems to be an indication for a synovial biopsy in patients with a monoarthritis where blood, synovial fluid, X-ray and MRI investigations yield insufficient clues. Still, the jury is out whether a rheumatology center can best invest in an efficient referral system to their surgical or radiological colleagues or start performing these biopsies themselves.

Hurdles

There seem to be some hurdles for implementation of ultrasound guided biopsies. Case studies concern relatively rare etiologies and these vary between countries. Furthermore, the technical approach and analytic yields vary. Besides, the reports often lack full description of other diagnostic clues. Most importantly, there is a lack of systematic prospective investigations in at risk populations. Therefore, it is controversial how often a synovial biopsy is of added value. It is also not known if ultrasound guided biopsy can reliably substitute arthroscopic or arthrotomic procedures, especially when multiple tests need to be performed.

In a recent case series of 74 patients with undifferentiated arthritis by Najm et al. (16) synovial biopsy analysis was performed with ultrasound guided biopsies of large and small joints in a number of rheumatological expert centers in France. 58 patients had an acute or chronic monoarthritis, 7 an oligoarthritis, and 6 a polyarthritis. Biopsy size was assessed sufficient if larger than 0.5 mm^2 based on previous literature assessing heterogeneity of histology in RA (16). The biopsies were of sufficient quality in 82% of patients, the yield depended on learning curve and joint accessibility. These allowed a definite diagnosis in 16% of the patients. Five patients underwent a secondary arthroscopy/-tomy because of suspicion of a septic arthritis which yielded a diagnosis of pseudogout in one patient. A case of Lyme and Whipple were diagnosed based on PCR in 2 patients (16). These data are promising but a number of questions have not yet been systematically addressed:

What is the number of procedures that should be performed yearly to retain skills in routine clinical practice? What is the minimum of synovial biopsies that should be taken for each diagnostic test, especially in patients in whom multiple tests need to be performed (6)? Should different joint sites be biopsied to exclude specific conditions, such as Borrelia, which might have a predilection for initial infection of the hamstring tendons (45)? What is the best quality control to ensure that synovial instead of other joint tissue is acquired for culture or RNA analysis?

CONCLUSION

Analysis of synovial biopsies has been extensively validated for experimental research and increasingly for clinical translational research and clinical practice. Further concerted international collaboration is needed to understand the utility of synovial biopsies in clinical decision making in patients with mono- oligo-, or polyarthritis in the context of other clinical clues. Furthermore, the technical constraints of ultrasound guided biopsies need to be studied in comparison with the gold standard: surgical biopsies. Participation in research networks or quality registries is essential for successful clinical implementation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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New Developments in Transcriptomic Analysis of Synovial Tissue

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Transcriptomic technologies are constantly changing and improving, resulting in an ever increasing understanding of gene expression in health and disease. These technologies have been used to investigate the pathological changes occurring in the joints of rheumatoid arthritis patients, leading to discoveries of disease mechanisms, and novel potential therapeutic targets. Microarrays were initially used on both whole tissue and cell subsets to investigate research questions, with bulk RNA sequencing allowing for further elaboration of these findings. A key example is the classification of pathotypes in rheumatoid arthritis using RNA sequencing that had previously been discovered using microarray and histology. Single-cell sequencing has now delivered a step change in understanding of the diversity and function of subpopulations of cells, in particular synovial fibroblasts. Future technologies, such as high resolution spatial transcriptomics, will enable step changes integrating single cell transcriptomic and geographic data to provide an integrated understanding of synovial pathology.

Keywords: transcriptomics, synovium, sequencing, single-cell, microarray, stratification, fibroblast, pathotype

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INTRODUCTION

Research into transcriptomic changes in the diseased synovium in rheumatoid arthritis (RA) has significantly enhanced our understanding of disease pathogenesis. This review details the research pathway taken to study gene expression in the synovium, including methods for synovial cell isolation, and analysis of tissue and cells by microarray and RNA-sequencing technologies. The progression of gene expression technologies which have increased our understanding of disease processes will be described, with a focus on understanding fibroblast populations, on which new transcriptomic technologies have had their most profound impact.

The synovium is a thin tissue lining the interior of the fibrous capsule, enclosing diarthroidal joints, and facilitating the normal function of the joint (1). In healthy, uninflamed joints the synovium produces compounds such as hyaluronate and lubricin. These are vital components of synovial fluid which fills the joint space and provides lubrication to aid load-bearing and flexion without damage to the cartilage and underlying bone (1, 2). By contrast, the inflamed synovium in RA is characterized by tissue hyperplasia and angiogenesis, accompanied by infiltration of multiple leukocyte populations, most significantly including monocytic cells, T and B lymphocytes. The latter frequently form organized aggregates that persist over time, releasing inflammatory cytokines and chemokines, and contributing to autoantibody production while remaining resistant to the normal processes of apoptosis and resolution that characterize acute inflammation (3, 4).

Fibroblasts play key roles within the RA synovium both in the regulation of leukocyte influx and efflux, and in damaging cartilage and driving indirect damage to bone (5–11). In RA, fibroblasts within the synovium become persistently activated, resulting in reduced responses to apoptotic signals, increased proliferation, production of proinflammatory molecules, such as IL-6 and CCL5, and the release of matrix remodeling enzymes, such as matrix metalloproteinase-3 (MMP-3) and MMP-9 (12–19). Consequently, the synovium develops an aggressive phenotype, leading to inflammation and hyperplasia, causing pain, and loss of function (17, 20). Whilst the role of fibroblasts in supporting these processes has been described *in vitro*, our knowledge of fibroblast heterogeneity and functions *in vivo* has been limited to decades-old histological observations of synovial microanatomy. This was dominated by the differing appearances of lining layer cells, which undergo hyperplasia in RA and appear to be continuous with destructive pannus tissue that damages cartilage, and sublining cells that are located alongside leukocyte infiltrates and synovial blood vessels. This heterogeneity led to synovial fibroblasts being termed fibroblast-like synoviocytes (FLS) historically, however recently developed transcriptomic technologies, specifically single-cell sequencing, have allowed for the recognition of subpopulations of fibroblasts. Understanding these subpopulations and their differing functions has the potential to open new doors for therapeutic intervention.

ISOLATING CELLS FROM SYNOVIAL TISSUE

Studies are generally completed at two levels of resolution, from either whole-tissue or individual cellular populations. Whilst the methods for obtaining whole-tissue samples of synovium are fairly standardized (arthroplasty or biopsy), the methods of obtaining cellular populations are varied, which confounds the results of downstream analyses.

Two main methods, the enzymatic digest method and the explant-outgrowth method, are widely used to isolate and study synovial cells, in particular synovial fibroblasts. The enzymatic method of isolation focuses on disrupting the extracellular matrix and adhesion of cells to this matrix to create a suspension of synovial cells. However, a range of enzymes, concentrations, and lengths of digestion have been used to achieve this, with limited investigation of the efficacy of the isolation or the effect of the digestion on marker expression and cellular viability. This complicates interpretation when examining surface protein expression or gene expression *ex vivo*, making it challenging to compare between studies.

The explant outgrowth method is more consistent between publications, possibly due to the relative simplicity of the protocol. Small segments of synovial tissue are placed into tissue-culture, allowing adherent cells to migrate out of the tissue and begin proliferating (21, 22). After 7 days the remaining tissue is removed and the cells are cultured. Whilst easier to implement than the enzymatic digestion protocol, the explant-outgrowth technique is not without its caveats. There is a significant risk of selecting for synovial populations that proliferate rapidly and are able to migrate out of the tissue, meaning that other populations may not be accounted for.

To address the heterogeneity in isolation methods the National Institutes of Health (NIH) Accelerating Medicines Partnership (AMP, https://www.nih.gov/research-training/accelerating-medicines-partnership-amp), a consortium of research groups funded by government, industry, and non-profit organizations, developed optimized protocols for the isolation of cells from multiple tissues, including the synovium, alongside a protocol for the cryopreservation of synovial tissue allowing for later digestion, which aids in reducing batch effects (23). This approach brings consistency to the field of synovial dissociation, allowing for more robust comparisons between results from different research groups.

WHOLE TISSUE APPROACHES TO SYNOVIAL TISSUE ANALYSIS

Studies investigating gene expression in the synovium initially took whole tissue approaches to assess broad, organ level changes. Whilst this work serves a purpose with regards to biomarker screening and understanding links between synovial and systemic inflammation, it is difficult to determine which specific cells are responsible for the changes in gene expression. Furthermore, subtle yet relevant alterations in gene expression amongst small cell populations are likely to be masked by changes in more dominant ones, meaning important mechanisms may be missed.

INTERROGATING THE SYNOVIUM BY GENE EXPRESSION ARRAY

Whilst the investigation of specific genes using PCR can be a powerful tool for testing pre-existing hypotheses, the ability to screen thousands of genes can extend gene expression analyses into discovery-focussed approaches. The development of cDNA microarray technology filled this niche. cDNA microarrays are inert supports, such as glass slides, upon which probes are printed or "grown" using masking techniques in spots, with each spot containing probes for a single gene (24, 25). In the most common platforms RNA is converted to cDNA before being labeled with a fluorescent dye. The cDNA is then hybridized to the probes in the microarray. After removing unbound RNA, the fluorescence measured from each spot can be used to calculate relative gene expression.

The strength of cDNA arrays is in the number of genes that can be simultaneously screened. Lindberg et al. (26) used an in-house generated microarray to investigate the expression of 16,164 genes in RA synovial tissue. The authors investigated differences in gene expression between synovial biopsy material obtained during arthroscopy and arthroplasty from several different sites within each joint to quantify the variation in gene expression dependent on the sampling procedure. Interestingly, even when non-inflamed biopsy samples were excluded from the analysis, a large number of differentially expressed genes were found between biopsy samples from the same joint. Despite

this finding, the authors were able to identify patient-specific gene expression signatures, indicating that the variation imparted by the biopsy site does not completely obscure the variation between patients.

Several publications have investigated the contribution of anatomical origin to the variance seen in fibroblast gene expression. Chang et al. (27) and Rinn et al. (28) used microarrays to investigate the differences in gene expression between skin fibroblasts isolated from different locations of the body. The major determinant of differential expression between sites was shown to be amongst genes encoding proteins involved in cell development during embryogenesis. Fibroblasts isolated from the same anatomical location in different donors showed closer gene expression profiles than those from a different anatomical location in the same donor (29). This indicates that developmental pathways may impact more upon fibroblast gene expression than inter-individual variation, as might be expected for cells involved in tissue specific structural patterning.

Microarray platforms can be applied not only to whole tissue but to any sample from which sufficient RNA can be isolated. Microarrays have been used with laser capture microdissection, a technique for isolating specific regions of tissue from histological sections, to compare gene expression in the synovial lining layer between RA and osteoarthritis (OA) samples (30). One hundred ninety-seven genes were detected as differentially expressed between the two diseases. Samples clustered according to disease, indicating that the lining layer is significantly different in phenotype between RA and OA. Additionally, the RA samples could be sub-clustered into high- and low-inflammation samples, a feature that was supported by similar high and low levels of serum C-reactive protein or histological inflammation scores.

The concept of subclassifying RA samples on the basis of gene expression has been explored by other investigators. van der Pouw Kraan et al. (31) used hierarchical cluster analysis of microarray data generated from the synovial tissue of RA patients undergoing arthroplasty to find subgroups based on differences in synovial gene expression. In concordance with later work by Yoshida et al. (30), the samples clustered into high and low inflammatory groups. The high inflammatory group was characterized by high *CD3D*, *IL2RG*, and *CD8* expression, suggesting that these differences were driven by differential immune-cell infiltration into the synovium. However, the investigators subsequently showed that synovial groupings could be mapped onto the transcriptomes of synovial fibroblasts cultured from the same tissues, demonstrating a link between fibroblast, and leukocyte populations (32).

Microarrays can also be used to correlate gene expression with response to therapy. This holds particular importance as, without stratification, RA patient therapeutic responses are characteristically <70%, even to targeted biologic agents. If gene expression could inform which therapeutic regime is likely to have the most effect, it would not only benefit patients but also reduce the cost of care (33). One study used an in-house developed microarray targeting 17,972 genes to measure gene expression in the synovial tissue of 48 RA patients who responded to therapy to varying degrees, and 14 non-responders. The main source of variance in gene expression was the presence or absence of inflammatory aggregates within the synovial tissue, however no clear differences between responders or non-responders were found (34). In a seminal paper, Dennis et al. used microarrays to assess response to first anti-TNF therapy, discovering modules of response corresponding to dominance of different cell types (myeloid, fibroid, or lymphoid) that were correlated to immunohistochemistry (35).

Whilst the data provided by microarrays has provided insight into the pathogenesis of inflammatory synovial disease, this technology is limited in the number of genes that can be simultaneously assessed. With the development of RNA-sequencing that allows measurement of the entire transcriptome, attention has turned toward the use of these techniques and the unique opportunities, and problems, they present.

RNA-SEQUENCING

RNA-sequencing (RNA-seq) offers significant advantages over microarrays with regards to assessing gene expression, namely not targeting a specific selection of genes. RNA-seq can measure protein coding and non-coding genes, and even micro-RNAs, increasing the utility of this tool. In addition, RNA-seq provides data over a larger dynamic range with lower background noise than microarray technology (36). Untargeted RNA-seq consists of the isolation of RNA and conversion to a cDNA library, which is then sequenced. Computational alignment to the genome or transcriptome can then be performed, avoiding the need for pre-selected targets.

Orange et al. (37) used bulk RNA-seq to investigate total synovial gene expression in tandem with histology, with an aim to subclassify RA. The samples could be clustered as high- or low-inflammatory tissues along with an additional mixed cluster. Six thousand five hundred eighty-two genes were detected as differentially expressed between the clusters, with the high inflammatory cluster expressing increased levels of genes associated with immune related pathways. Investigation of key histological variables in the tissue samples showed concordance with the sequencing results, with the high-inflammatory samples possessing high levels of immune cell infiltrate. Additionally, in a longitudinal consortium study taking synovial biopsies at first presentation of new RA, significant correlations were observed between histological type, whole tissue bulk RNAseq-derived gene clusters, and clinical response to first therapy (38, 39). This powerful approach elegantly demonstrates the predictive value of cellular gene clusters derived from whole tissue signatures.

SINGLE CELL APPROACHES TO SYNOVIAL TISSUE ANALYSIS

Although bulk RNA-seq provides in-depth information on gene expression in whole tissues or pre-defined populations of cells, assumptions made regarding the number and type of cells present may bias the results. Single cell RNA-sequencing (scRNA-seq) can reduce this bias by allowing independent sequencing of every cell within a tissue, or within a subset of cells (**Figure 1**). The subsequent use of unsupervised clustering techniques to

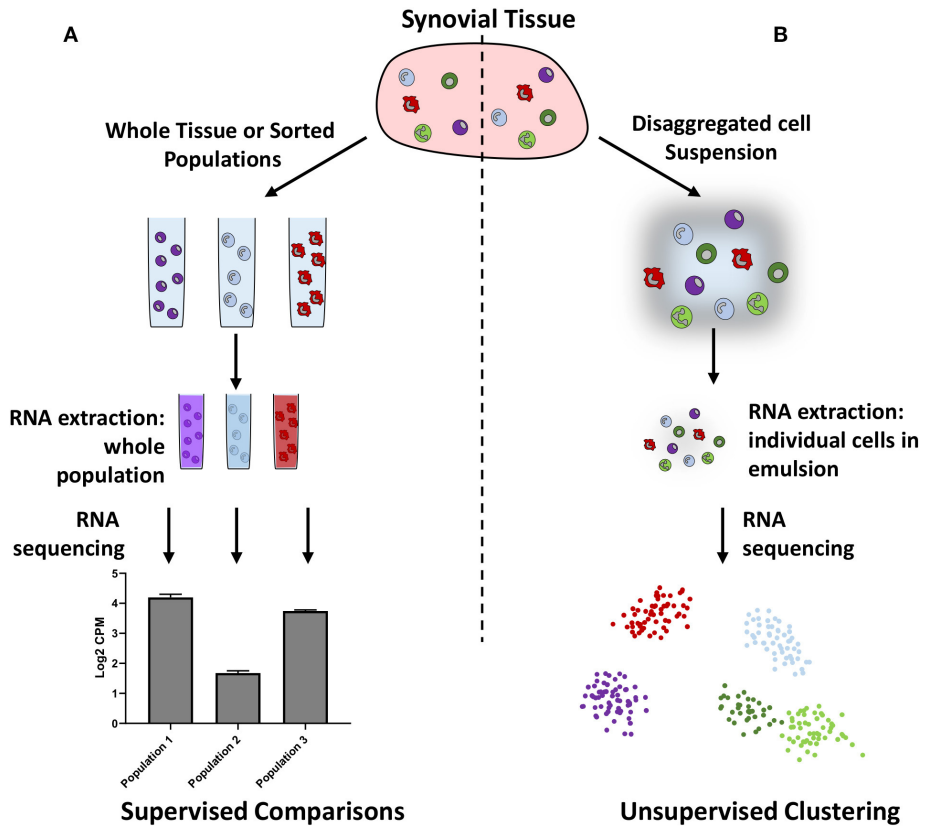

FIGURE 1 | Schematic illustrating bulk **(A)** vs. single cell **(B)** RNA sequencing. Bulk RNA sequencing requires researchers to preselect cellular populations or sequence whole samples, whereas single cell RNA sequencing does not require pre-selection and can be used to identify cellular populations in an unbiased manner.

find related cells, as defined by gene expression, can confirm the presence of previously defined populations or help in the identification of unknown populations of cells. However, caveats exist for readers of such papers: firstly, it is possible to over-cluster the data, leading researchers to believe there are more discrete populations present than actually exist. Secondly, multiple dimensionality reduction techniques and clustering methods exist, which can lead to different interpretations of the data, and which should therefore be clearly stated by authors and noted by readers. Thirdly, the depth of information obtained by scRNA-seq can be limited by low amounts of starting RNA, meaning that the absolute number of genes that can be recognized and quantified is lower than that of bulk RNA-seq. To leverage the strengths of this technique, both approaches are commonly used in tandem to identify key subpopulations and then further investigate gene expression.

Mizoguchi et al. (40) used a combined approach of microarrays, bulk, and single-cell RNA-sequencing to identify subsets of fibroblasts in synovial tissue from RA and OA patients. $CD45^{-}CD31^{-}CD146^{-}$ cells were found to split into seven populations by flow cytometric analysis of CD34, CD90, PDPN, and CDH11 expression. However, following microarray and bulk RNA-seq of these populations, clustering of the data identified three populations in both datasets, $CD34^{-}CD90^{-}$, $CD34^{-}CD90^{+}$, and $CD34^{+}$. scRNA-seq was then used on a small number of samples (2 RA and 2 OA) to confirm this finding. The $CD34^{-}CD90^{+}$ population was expanded in RA compared to OA samples, as measured by flow cytometry, possessed high *RANKL* and low *OPG* expression, and was capable of differentiating monocytes to osteoclasts *in vitro* suggesting a role in bone regulation and damage. The $CD34^{+}$ population expressed high levels of *IL-6*, *CXCL12*, and *CCL2,* whereas the $CD34^{-}CD90^{-}$ population expressed high levels of *MMP1, MMP3, PRG4, HAS1*, and *CD55*. These findings indicate that the traditional split of lining vs. sublining does not capture the full heterogeneity of synovial fibroblast populations.

Unbiased single cell sequencing platforms have helped our understanding of the true heterogeneity of fibroblast subpopulations and therefore synovial pathology. Within the NIH AMP consortium, the exploration of synovial fibroblast subsets was extended through a combined investigation of larger numbers of RA and OA samples using bulk and scRNA-seq, and flow and mass-cytometry. Zhang et al. (41) used canonical correlation analysis to integrate this multimodal data from 51 RA and OA samples, facilitating a linked transcriptomic and proteomic analysis of single cells. In addition to defining the myeloid, T and B cell subpopulations present in the RA synovium, this study

identified four synovial fibroblast populations: a $CD55^+$ lining layer population and three sublining populations identified as $CD34^+$, HLA^{hi}, or $DKK3^+$. With regards to gene expression, the sublining populations showed greater similarities with each other than with the lining population, a feature reflected in the shared enrichment for extracellular matrix related pathways within the sublining populations. HLA^{hi} synovial fibroblasts were the predominant source of IL-6. When classifying RA samples on the basis of immune infiltrate identified by histology, the HLA^{hi} population was expanded in leukocyte-rich synovium, hinting that this population may be driving classical inflammation within the synovium, whereas others, such as the $CD55^+$ lining layer population, may be responsible for aspects of cartilage destruction.

The function of individual synovial fibroblast populations has been further explored using murine models of arthritis. Croft et al. (42) used the serum transfer induced arthritis model in combination with the deletion of fibroblast activation protein-α (FAPα) expressing cells to interrogate the role of synovial fibroblast subsets. FAPα is expressed on both lining and sublining fibroblasts in RA, therefore offering a mechanism for the global deletion of synovial fibroblasts (42–45). The deletion of $FAP\alpha^+$ cells during arthritis led to both a significant reduction in inflammation and accelerated resolution. This was accompanied by reductions in synovial cellularity, joint damage, and leukocyte infiltration, highlighting that the changes observed were not solely due to a reduction in the number of cells within the synovium. Bulk-sequencing of sorted populations mirrored the findings of Zhang et al. (41), with the largest differences being observed between the lining layer and sublining layer (45). Further similarities existed in the gene expression profile of these populations with the $FAP\alpha^+CD90^+$ cells expressing higher levels of chemokines and cytokines, including IL-6, whereas the $FAP\alpha^+CD90^-$ cells showed higher levels of RANKL and MMPs. The functional behavior of these populations was confirmed by adoptive transfer of the sorted populations into the joints of arthritic mice. $FAP\alpha^+CD90^+$ cells increased inflammation but not joint damage, whereas the reverse was observed with the $FAP\alpha^+CD90^-$ cells. Finally, scRNA-seq of murine synovial fibroblasts revealed that five independent populations could be found, one lining layer, three sublining, and an additional cycling population.

FUTURE PREDICTIONS

The investigation of synovial fibroblasts provides an excellent illustration of the power of single cell analyses. Our previous knowledge was restricted to speculation based on anatomical sub regions in the synovium and limited fibroblast surface markers. For the first time investigators have been able to identify and assign putative functions to individual fibroblast subpopulations that were previously unknown using the power of single cell sequencing. This provides exciting new opportunities to understand the pathobiology of inflammatory arthritis, and to target novel therapeutic approaches to fibroblast cells. Even the field of lymphocyte biology, in which multiple cellular subsets have already been identified, has been changed by single cell analyses of synovial tissue, as in the recent identification of a novel pro-inflammatory synovial T cell subpopulation in RA assisted by mass cytometry (46). Techniques such as cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) and RNA expression and protein sequencing assay (REAP-seq) are enabling the simultaneous resolution of transcriptomic and proteomic data at an individual cell level (47, 48).

The challenge now is to recast previously published findings into this new framework of individual subpopulations, and to integrate isolated cell transcriptomic and proteomic data with multiparameter platforms providing spatial proteomic and transcriptomic data alongside established techniques such as laser capture microdissection.

AUTHOR CONTRIBUTIONS

HC, JT, TM, DS-T and AF all contributed to the design of the review. All authors wrote, revised, and edited the final submission.

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Arthroscopic Guided Synovial Biopsies

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Synovial tissue can be safely and reliably collected for research and clinical purposes using arthroscopy. This technique offers the obvious advantage of allowing direct visualization, and targeted biopsy of specific areas of interest within the joint, as well as for the collection of tissue which will include a lining layer. Much has been learnt by studying the synovium retrieved using this technique concerning the pathobiology of inflammatory arthritis. Furthermore, recent evidence suggests that the tissue retrieved may enable the identification of unique pathotypes that will allow for a precise approach to treatment selection in individual patients. Although ultrasound guided techniques for sampling synovial tissue have gained in popularity over the last decade, both methodologies are expected to compliment each other, each having unique benefits and drawbacks. We present here a detailed description of the arthroscopy technique reporting on our collective experience at two centers in Europe.

Keywords: arthroscopy, synovial tissue, synovitis, inflammatory arthritis, synovial biopsy

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INTRODUCTION

The synovium is the primary target tissue in inflammatory arthritis (IA), and it therefore follows that analysis of this tissue must yield important clues to advance our understanding of the underlying pathobiology of these heterogenous diseases. The field has rapidly expanded over the last three decades, and this has led to some very significant developments in unraveling the cellular and molecular networks underlying the development and perpetuation of IA (1–4). Putative targets have been identified by synovial tissue (ST) analysis (5). ST has been used in the evaluation of current and potential treatments, in both *in vivo* and *ex vivo* settings (6, 7). Recent evidence suggests that it may be possible to stratify patients with rheumatoid arthritis (RA) on the basis of histopathology and transcriptomic analysis, into groups with differing underlying pathobiology, and with differential responses to therapies (8–10). These developments have depended on the ability to reliably retrieve ST, in a safe manner, and in such a way as to be well-tolerated by patients. Although much data has been published on tissue retrieved at arthroplasty, the suitability and applicability of these findings to IA at much earlier timepoints in the disease course remains unclear. Therefore, arthroscopy was adopted by Rheumatologists to allow ST sampling at varying points in the disease course and has long been the favored technique historically. This technique has the advantage of providing direct intra-articular visualization of synovium as well as a therapeutic joint lavage. Both synovial membrane proliferation and vascularization patterns have been described. Certain vascular patterns, although not diagnostic, can be suggestive

of some subtypes of inflammatory arthritis (straight versus tortuous pattern) which can be of particular interest in early and undifferentiated arthritis. Furthermore, crystal deposits, cartilage damage, chondromas and other intra-articular pathologies can be identified contributing for the differential diagnosis of synovitis (11, 12). Sonographically guided techniques have been developed and refined more recently, and it is likely that these techniques will complement ST retrieved under direct arthroscopic guidance. In this review, we describe the general aspects of the technique of arthroscopic guided synovial biopsies (AGSB) of the knee joint under local anesthetic, as performed in two European Rheumatology Centers: St. Vincent's University Hospital, Dublin and Hospital de Santa Maria, Lisbon.

PATIENT RECRUITMENT AND INDICATION FOR AGSB

The authors of this review (CO, EV-S, DV) have experience performing AGSB for patients recruited at the Inflammatory Arthritis Clinics at St. Vincent's University Hospital in Dublin, and from the dedicated Mini-arthroscopy Clinic at Hospital de Santa Maria in Lisbon. Patients are referred either with undifferentiated arthritis or an arthritis flare of an established IA for potential sampling, for diagnostic and/or therapeutic purposes. The specific organization of these clinics allows for enrichment for recruitment, and all medical staff at each center are made aware of the clinical benefits and research programs, and they contribute to patient recruitment. These arthroscopy clinics are focused solely on knee arthritis, and patients with swollen and painful knees are referred.

Knee arthroscopies are performed on patients with a wide range of diagnoses or potential diagnoses: most commonly this is rheumatoid arthritis (RA), undifferentiated arthritis, or spondylarthritis. Furthermore, we have found that most patients are willing to consent to a second arthroscopy and are often less apprehensive about this, having already experienced the procedure (13). This commonly occurs when a treatment change is indicated following arthroscopy, in which circumstance patients are invited to return for follow up ST sampling 12 weeks later.

ETHICS AND CONSENT

The patient is given written information in the form of a Patient Information Leaflet (PIL) that contains relevant information regarding the procedure, the potential risks and instructions post-procedure. For example, patients are instructed not to drive immediately after the arthroscopy, and to rest for the first 48 h. The consent form and the PIL are provided to the patient at the time of recruitment. Local Ethics Committees approve all studies, including the procedure itself where this is conducted exclusively for research purposed. Patient confidentiality is a priority and no identifiable data is retained. If agreeable, patients are scheduled to attend for arthroscopy at the next available slot. We endeavor to ensure that this is within 2 weeks, in order to provide timely access to diagnosis, therapeutic benefit and treatment initiation or change.

On the morning of arthroscopy, patients are requested to bring their PIL/informed consent and there is further discussion with the physician performing the procedure, and an opportunity for any questions to be answered. The patients are invited to consent to the procedure itself, the collection and retention of synovial tissue and blood for research purposes, as well as the collection and anonymous storage of particular demographic data and disease features.

PATIENT DATA

Relevant demographic and disease specific data including ongoing medication, disease activity (e.g., tender and swollen joint counts, patient global health evaluation) as well as validated patient reported outcomes such as the SF-36 and HAQ, as well as the indication for the procedure, are captured. On the morning of the procedure, patients are told to have a light breakfast, and on arrival, the completed forms are checked, and a comprehensive clinical examination is performed and recorded, including various disease activity measurements.

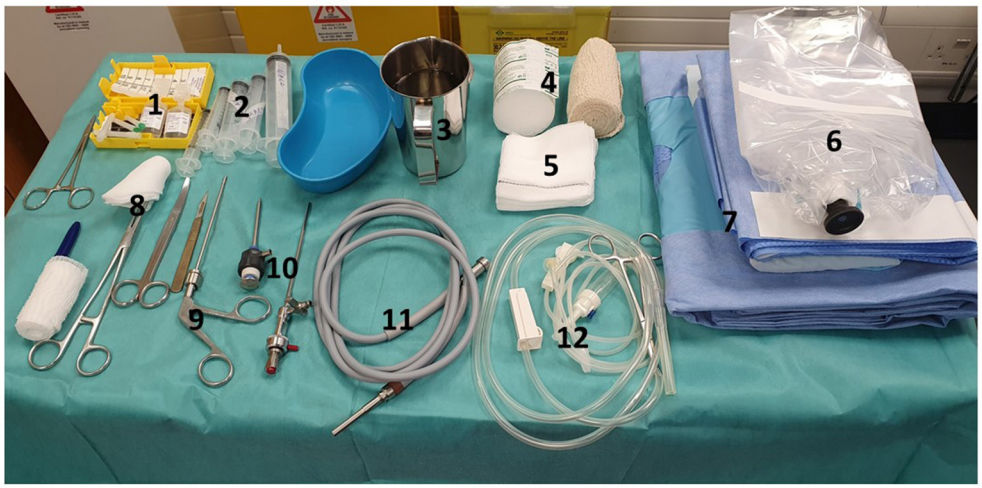
PRE-PROCEDURE ASSESSMENT

A detailed patient's medical history is collected and a plain film radiograph of the knee is requested (if not available) as well as laboratorial evaluation including coagulation parameters. For example, antiaggregant such as clopidogrel and direct oral anti-coagulants or warfarin, are contraindicated unless these can safely be discontinued for an appropriate time before the procedure or occasionally replaced by low molecular weight heparin.

PROCEDURE

Arthroscopies are performed in a dedicated, facility, within the hospital's Clinical Research Center (Dublin) and within the Rheumatolgy Technical Procedures Unit (Lisbon). The unit comprises an anteroom and a procedure room. Patient's change into hospital gowns in the anteroom and usually an intravenous cannula is sited. Cannulation facilitates phlebotomy, for both research bloods and the hospital laboratory to process relevant hematological and biochemical indices. The knee that is to be the site of sampling is marked with a skin marker.

We start by switching the arthroscopy tower on, and by ensuring that both the camera and the light source are working. Patient's hospital identification and knee laterality is inserted for image capture. At both centers, Karl Storz, Germany, equipment are used. In Dublin a 2.7 mm needle arthroscope is used and in Lisbon a 2.7 mm Hopkins II, 30° telescope. The operator scrubs and gowns using standard techniques described elsewhere (14). A theater nurse assists the operator before and throughout the procedure but does not scrub, respecting well-defined aseptic areas.



1. Needle Case and Bupivacaine
2. Labelled Syringes
3. Saline Jug
4. Bandages
5. Gauze
6. Lens (and sheath)
7. Sterile Drapes
8. Gauze in forceps to sterilize
9. Grasping Forceps
10. Trocars and Ports
11 Fibreoptic Cable
12. Inlet and Outlet Drains

FIGURE 1 | Arthroscopy equipment.

All surgical instruments and the Karl Storz hardware kit which contain arthroscopy telescope, trocars, forceps, are assembled in sterile packs and opened on a sterile draped table. In addition, sterile gauzes, syringes, needles, infusion system, sterile bowls and sterile drapes are opened by the theater nurse, received by the operator, and placed on the table. Chlorhexidine and sterile water are poured by the nurse into the sterile bowls on the table. A 20 ml syringe is filled with lignocaine 2%, and another 20 ml syringe filled with bupivacaine 0.5% (Dublin). The local anesthetic products and expiry dates are checked by both the nurse and the operator. **Figure 1** depicts the prepared equipment assembled on a sterile trolley.

Before proceeding any further, a deliberate pause is undertaken to complete a reviewed "WHO Surgical Safety Checklist" for this procedure. Amongst the most important items on the list are a check that we have the correct patient, that the correct knee has been labeled, and that equipment has been arranged for this knee to be biopsied, as well as a final check to confirm the consent form has been completed, and that the patient is not on anti-coagulants/anti-aggregants, and an allergy check. Throughout the procedure we pay careful attention to the patient's comfort and explain each step as we proceed.

A disposable sterile sheet is placed under the lower limb and the leg is raised to 45°, with the assistance of the nurse who holds the patient's heel up. Sterile gauze is held in a pair of forceps and soaked in Chlorexidine. The knee is sterilized by swabbing in ever widening circles from the lateral aspect of the knee, where the two small incisions will be made. The area from mid-thigh to mid-calf is disinfected circumferentially.

In Dublin, a bespoke sterile sleeve closed at one end is placed over the foot and extended the length of the leg with care taken not to contaminate the cleaned field. The leg is next inserted through the fenestration in a sterile drape and the drape advanced to the hip. The two corners of the drape closest to the cephalic end are risen and attached to drip stands either side of the bed. This occludes the patient from inadvertently touching the sterile field. A sterile gauze bandage is applied circumferentially around ankle (which is covered by the sleeve). A window large enough to access the antero-lateral aspect of the knee is cut out of the sterile sleeve using a pair of scissors, taking care not to injure the skin. A similar setup with minor differences is performed in Lisbon.

The anatomical landmarks must now be identified. The knee is placed in 30–45 degrees flexion. Following palpation, a tissue marker can be used to delineate the lateral border of the infrapatellar tendon, the infero-lateral aspect of the patella, the anterolateral border of the tibial plateau, and the infero-lateral surface of the lateral epicondyle. The center of these markings is the site of entry for infero-lateral port. The landmark for the supero-lateral port is 1 cm above and 1 cm lateral to the supero-lateral aspect of the patella, the site used commonly for intra-articular injections. The joint capsule, soft tissues and skin is infiltrated with 10 ml of lignocaine 2%, for what will become the superior-lateral port site and the inferior-lateral port site. About 2–5 min is given to allow for this to take effect. Arthrocentesis is performed using a 21G needle, attached to an empty 10/20ml syringe through the site of the prospective supero-lateral port and synovial fluid is drained until no further fluid can be removed. Obtaining synovial fluid at this point gives high assurance that the tip of the needle is indeed in the joint cavity. Any synovial

fluid removed is carefully placed on the sterile drape covered table. If no fluid can be removed, special care must be taken in the next steps.

The needle is left *in situ* while the syringe is detached. The 20 ml bupivacaine containing syringe is attached to the needle. There should be little or no resistance to the plunger advancing. If the operator encounters resistance, especially where no synovial fluid was obtained, the needle may be misplaced and may require adjustment. After placement of the bupivacaine into the joint cavity, the needle is left *in situ* and a further 2–5 min is given to allow the bupivacaine to take effect.

A sterile sleeve covers the camera wire and camera head, and the sterilized wire light source is attached to the camera. An infusion system attached to a saline solution is also connected to the telescope trocar. The white balance is ensured by placing a gauze in front of the lens.

In Dublin in the next step, the empty syringe is detached from the needle and the 50 ml syringe containing sterile water is attached to the needle. The contents of the syringe are placed into the joint until resistance is felt. Typically, a joint will accommodate up to 60 ml more fluid.

With the knee in a 30–45 degree flexed position, a scalpel is used to make a 1 cm incision in the infra-lateral patella space previously delineated, extending to the joint capsule. The trocar is introduced through the incision using blunt dissection into the joint cavity. It is imperative not to force the trocar if resistance is met. Once *in situ*, the trocar is removed, leaving the port in position, and the rigid camera is inserted through the infero-lateral port.

The cavity of the knee joint can now be inspected. It is important to make certain that the arthroscopy is being video recorded and that photographs are taken. We inspect all visually accessible areas and arbitrarily divide the joint into discrete compartments, as occasionally synovitis can be quite focal. We record synovitis and vascularity scores as appraised by the operator on visual analog scales ranging from 0 to 100 mm (Dublin) or a 0 to 3 severity scale (Lisbon). There have been attempts to develop a reliable scoring system for synovitis observed at arthroscopy, but until recently the numbers studied were small and no system has been validated (15). Our data suggests that there is a correlation between macroscopic synovitis scores and C reactive protein, histological inflammation and the development of erosions (16). More recently, a comprehensive scoring system has been proposed called the "Macro-score," and has been shown to exhibit excellent inter- and intra-rater variation (17). We also record the pattern of vascularity which has been shown to differ between, for example RA and psoriatic arthritis (11, 18). **Figure 2** depicts exemplar images for synovitis and vascularity. Other notable findings are also recorded, such as synovial crystal deposits, tophi or any other intra-articular pathologies. The under-surface of the patella is inspected, and any chondropathy observed is recorded.

To establish the second port required to allow AGSB, the camera is placed such that it points toward to area where the superior-lateral port will be positioned. This can be found by using a finger to exert pressure over the site, and the operator can see the depression made by this action within the joint on screen. Once again, a scalpel is used to make a 1 cm incision extending to the joint capsule and then blunt dissection using a trocar allows the second port be sited.

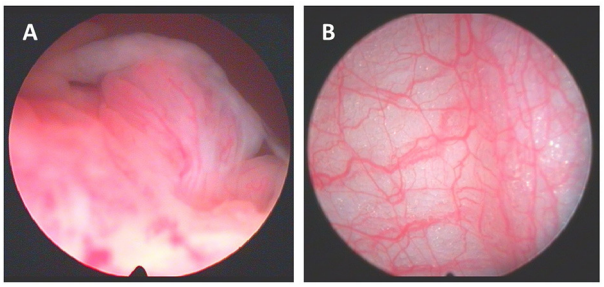


FIGURE 2 | (A) Macroscopic aspects of synovitis with villi formation and **(B)** synovium vascularization.

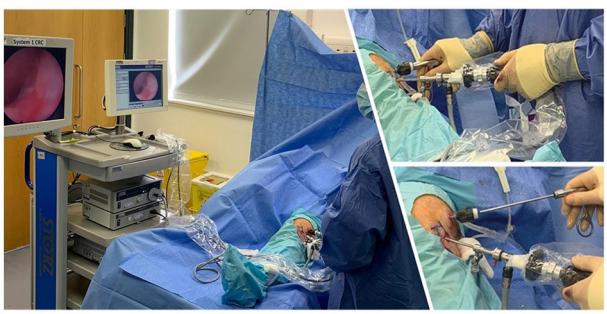


FIGURE 3 | Illustrative images of arthroscopy procedure.

Sterile saline *via* a drip is connected to the camera port, and a drain connected to the grasper port with tubing to a basin or collection bag. A rigid grasper is inserted through the supero-lateral port and synovial biopsies can be collected under direct visualization. **Figure 3** illustrates the positioning of the ports and their relationship to the other equipment discussed above. For time optimization, when diffuse synovitis is present biopsies can be easily performed without direct visualization. The biopsies are placed on saline soaked gauze. When all biopsies have been taken, they are immediately collected to minimize delay in processing.

We check for any bleeding after biopsy collection and complete an effective joint lavage-usually approximately 1,500 ml thought the procedure. The superolateral trocar is removed after draining all the fluid from the joint.

Whenever indicated, intra-articular corticosteroids, are administrated. This is followed by the administration of 8 ml of bupivacaine is intra-articular to allow a sustained anesthetic benefit (Lisbon). The infrapatellar port (and camera) is removed. The two port sites are wiped clean, and either paper stitches or a single stich at each portal are applied to each incision. We next apply a small square dressing to each

incision and apply a waterproof patch. Now the sterile drapes and sleeve are removed, and the knee is wrapped firstly with a cotton wool bandage (Dublin) and then with a crepe bandage (Dublin and Lisbon).

POST-PROCEDURE ASSESSMENT

A procedure note is recorded which includes synovitis and vascularity scores, blood vessel pattern, chondropathy, crystal deposits if present, and whether intra-articular steroids were administered as well as the amount of saline used during the arthroscopy. We have developed a proforma to make this faster and to standardize the procedure notes.

The patient is given written information concerning post procedural care. They are told to remove the crepe and cotton wool bandage after 12–24 h, a waterproof cover is useful to keep the knee dry for 3 days, and to remove the dressing and paper (Dublin) or surgical stitches (Lisbon) in 7 days. They are told to rest, and not to drive for 48 h. If they have persistent swelling we ask them to apply ice. Patients are given emergency contact details for the Rheumatology department, and are given an outpatient appointment for 1 week or 2 weeks' time, when the wounds are inspected, the results from synovial fluid/synovial membrane verified and any indicated change to treatment is implemented.

SAFETY AND TOLERABILITY

Overall safety and tolerability of AGSB in the hands of rheumatologists is reflected in several publications including those from our groups in Dublin and from integrated data from Lisbon in an international study recently published by Just et al. (13, 19). We quote our patients for adverse events related to the procedure such as persistent swelling, haemarthrosis (0.2%), DVT (0.9%), and septic arthritis (<0.1%).

DIAGNOSTIC AND THERAPEUTIC BENEFITS

AGSB performed by Rheumatologists is associated with diagnostic and therapeutic benefits. Collecting the tissue allows for appropriate histologic and microbiologic evaluation of the synovial membrane, but additionally AGSB allows for the direct visualization of intra-articular space as well as facilitating an efficacious joint lavage. Additionally, intra-articular corticosteroids and/or anesthetics can be administered with symptomatic relief. Remarkably, 66.9% (91/136) of our patients felt improvement in their knee symptoms within 2 weeks of arthroscopy (20). Some factors which may explain this include the removal of inflammatory synovial fluid, the knee lavage itself, and the intraarticular injection of corticosteroids in a minority of the patients surveyed. Although few comparative studies have been published, arthroscopic joint lavage plus intra-articular corticosteroids injection is superior to intra-articular injection of corticosteroid alone following joint aspiration in a randomized clinical trial (21).

CHALLENGES

It is widely accepted that AGSB requires technical skills and dedicated training for the operator, which is currently restricted to a small number of academic centers. In addition to this challenge, international guidelines standardizing AGSB as performed by rheumatologists, are scarce (22). As noted by Smits et al., it is certain that ongoing performance of the procedure is important in maintaining skills, which can be appraised by examining biopsy yield and quality, as well as safety record, but it is not known how many supervised procedures are required to attain competency, and how frequently procedures should be performed to maintain this competency, and these factors might be operator dependent (23).

Humby et al. has reviewed the pros and cons of the various methodologies for biopsying synovium (24). The preferred method will likely depend on the clinical or research question being addressed, and in particular, whether lining layer is essential, and how much tissue is required. The obvious benefit of arthroscopy for ST collection is the reliable, relatively large quantity of material retrieved, and the ability to target of areas of most significant synovitis within the joint under direct visualization, as well as reliably collecting lining layer. One limitation of AGSB is its general restriction to large joints. However, there is some evidence to suggest that tissue retrieved from an inflamed knee joint is similar to that obtained contemporaneously from an inflamed wrist or metacarpophalangeal joint (25). Furthermore, in subjects with clinically evident disease manifest in small joints, similar histological abnormalities have been recorded in apparently clinically uninvolved knee joints (26, 27). Taken together, these findings would suggest that AGSB of a large joint, should be representative tissue for studying IA from a histologic perspective. Joint specific synovial fibroblast phenotypes have also been described owing to anatomical transcriptional diversity, and this may have implications for the wider applicability of sampling from any given single joint (28). Of interest, recent evidence suggests that DNA methylation and transcriptome signatures in RA fibroblast-like synoviocytes can vary between knees and hips for example, but the clinical implications for diagnostic or therapeutic decisions in clinical practice from this data is still limited (29).

Additionally, non-swollen joints can also be biopsied with success such as those from patients with osteoarthritis or after successful treatment inflammatory arthritis.

CONCLUSION

AGSB as performed by rheumatologists is a safe and reliable technique for sampling synovial tissue that is most suited

to large joints. It has been the preferred "gold-standard" method, and for the last decade the cornerstone for the development of newer synovial tissue biopsy techniques, namely sonographically guided. It is hoped that the addition of these tools may broaden the accessibility of using synovial biopsies in research and clinical settings in the Rheumatology field. Arthroscopy will however undoubtedly remain an important tool in investigating IA with complementary therapeutic benefits, and specifically identifying synovial biomarkers that will allow the page to be turned toward precision medicine for our patients with heterogenous IA.

AUTHOR CONTRIBUTIONS

CO, EV-S, and DV have extensive experience in performing this procedure, and have contributed to the collection of data relevant to this review. JEF has significantly contributed to arthroscopy implementation in Lisbon. All authors have contributed to writing and editing the manuscript.

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REVIEW
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Ultrasound-Guided Synovial Biopsy: A Review

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Ultrasound-guided synovial biopsy is a safe, well-tolerated, and effective method to collect good-quality synovial tissue from all types of joints for clinical and research purposes. Although synovial biopsy cannot be used to distinguish between types of inflammatory rheumatic disease, analysis of synovial tissue has led to remarkable advances in the understanding of the pathobiology of rheumatoid arthritis and other inflammatory rheumatic diseases. Synovitis is the hallmark of these diseases; hence, accessing the core of the pathological process, synovial tissue, provides an opportunity to gather information with potential diagnostic and prognostic utility.

Keywords: ultrasound-guided synovial biopsy, synovial membrane, synovium, synovial tissue analysis, musculoskeletal ultrasound

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INTRODUCTION

This review aims to gather, in a single source, the most comprehensive information on ultrasound-guided synovial biopsy (USGSB), including the unmet needs of the method.

USGSB is a method for retrieving synovial membrane samples using ultrasonography for guidance. The main advantages of USGSB are that it is well-tolerated by patients; is accessible; can feasibly be performed in large and small joints, as well as bursae and tendon sheaths; and has a low incidence of adverse events. USGSB uses ultrasound to visualize the location of synovial hypertrophy and is simple to perform after adequate training, suitable for serial procedures, and comparable to arthroscopy (which is considered the gold standard for obtaining synovial samples, based on clinical trial data) in terms of sample quality, but is less invasive, is cheaper, and does not require ionizing radiation, unlike fluoroscopy-guided biopsies (1–5). The main disadvantages of USGSB, relative to arthroscopic biopsy, are the limited tissue quantity obtained and lack of direct vision. Moreover, when using USGSB, the synovial tissue retrieved depends on the degree of synovitis and requires musculoskeletal and ultrasound guidance skills. Major contraindications to USGSB are systemic or skin infection, coagulation disorders, or anticoagulant therapy, as well as a non-collaborating patient.

In this review, we address the following questions: Why are synovial biopsies conducted? How are synovial biopsies conducted? What data do synovial biopsies provide? and What are the unmet needs related to synovial biopsy?

WHY ARE SYNOVIAL BIOPSIES CONDUCTED?

Synovial biopsies are conducted for clinical reasons or research purposes (6, 7). In the clinical setting, formal indication for synovial biopsy occurs in cases of monoarthritis for which all other auxiliary diagnostic tests, including synovial fluid examination, are insufficient for diagnosis. Clinical biopsies are performed to exclude infection or because diagnostic clarification is required;

for example, to identify whether synovitis has a non-inflammatory or inflammatory cause. Tumors cause non-inflammatory synovitis, while crystal-related arthropathies and granulomatous and non-granulomatous diseases are responsible for inflammatory synovitis. Crystal-related arthropathies are due to sodium monourate, calcium pyrophosphate, or basic calcium phosphate deposition diseases. Granulomatous synovitis can be related to infection (tuberculosis, brucellosis, fungal infections), immunological (Crohn's disease, erythema nodosum, sarcoidosis), metabolic, and storage diseases, or to a reaction to a foreign body. Non-granulomatous synovitis may be due to septic arthritis, low-grade synovitis (post-traumatic, mechanical, osteoarthritis, or haemochromatosis-related synovitis), or high-grade synovitis (rheumatic inflammatory diseases, such as diffuse connective tissue diseases or spondyloarthritis) (**Figure 1**). Bacterial and fungal diseases can be identified by detection of broad-range 16S and 18S ribosomal RNA in synovial tissue by polymerase chain reaction, which is valuable for the diagnosis of infection by these agents (4).

Synovial biopsies are conducted for research purposes in the context of the study of inflammatory rheumatic disease pathogenesis, early diagnosis, new drugs (through recognition of novel therapeutic targets), identification of biomarkers of disease progression, or advance precision medicine (8, 9). Nevertheless, synovial biopsy is not yet sufficiently discriminative to allow distinction between types of arthritis (10).

HOW ARE SYNOVIAL BIOPSIES CONDUCTED?

Comparison of Synovial Biopsy Approaches

Synovial biopsies can be achieved in the context of surgery (arthrotomies, arthroplasties) fluoroscopic or arthroscopic guided, or conducted as a blind needle- or ultrasound-guided procedure. USGSB is conducted as either a portal + forceps biopsy (PFB) or a guillotine-type semiautomatic needle biopsy (NB). PFB can be rigid or flexible, while NB can be conducted with or without a coaxial; coaxial is helpful when there is a long pathway to deep-seated joints, or when the needle passes near neurovascular structures (**Figure 2**). USGSB clearly has the most well-balanced profile among synovial biopsy approaches, when cost, technical simplicity, patient acceptability, synovial sampling success rate, suitability for large and small joints, and suitability for serial biopsies are considered (2). Of interest, synovial tissue quality is maintained and clinical and ultrasound evaluations do not change when repeat USGSB is necessary for the same joint (2, 7, 11, 12). Blind needle- and fluoroscopy-guided biopsies are more difficult to conduct in small joints and joints without active synovitis and have a lower success rate for obtaining synovial samples than other methods (2, 5). Cost and technical simplicity favor blind needle biopsy, while suitability for serial biopsies and biopsies in small joints favor USGSB, particularly NB (2, 13–15). The major differences between the two types of USGSB are that PFB requires an autoclave for equipment sterilization, one guide, larger ports, and dual operators and is more time consuming (10), while NB uses disposable material, can be easily achieved by a single operator, and is most appropriate for finger, toe, and wrist joints (the joints most frequently affected in rheumatoid arthritis); however, the choice between PFB and NB depends primarily on operator preference and experience.

Compared with arthroscopic biopsy, USGSB is less invasive and can be conducted in both large and small joints (6). Arthroscopic biopsy allows direct visualization of synovial membranes but is more costly, is performed in only a few specialized centers, requires two operators and a dedicated environment (an operating theater or equivalent), and is not viable for small joints (1, 2, 13, 14).

There are no differences between arthroscopic biopsy, PFB, and NB in terms of synovial sample quality, adverse events, or reported patient outcomes. Also, synovial pathotype and degree of inflammatory infiltrate are not influenced by the method used to retrieve synovial samples (2, 3).

Synovitis grade (synovial thickness) on pre-biopsy grayscale ultrasound scan is the best predictor of successful USGSB, in terms of synovial sampling and grading. Kelly et al. proposed a joint selection hierarchy for biopsy, based on joint size and synovitis grade on grayscale ultrasound (16). According to this hierarchy, the first choice should be a medium or large joint, with grade 3 synovial thickening, followed by a joint of any size with synovitis grade ≥ 2, a medium or large joint with synovitis grade ≥ 1, and finally, a small joint with any degree of synovitis. Although not a contraindication, performing a synovial biopsy in a grade 1 synovitis is technically harder to accomplish, especially in the hands of a non-experienced operator.

The USGSB Procedure

In any rheumatological center, several steps should be implemented to adequately perform USGSB. In the author's department, the first step is a checklist, which includes patient reception; exclusion of procedure contraindications; written informed consent; clinical data collection, including biometric items, tender joint count, swollen joint count, erythrocyte sedimentation rate, and C-reactive protein levels; patient- and physician-scored global activity (visual analog scale; VAS); ongoing treatment; and factors related to the joint to be biopsied, including VAS scores for pain, stiffness, and swollenness in the previous week.

The biopsy procedure should be carried out in a theater or clean procedure room, providing sufficient expanse for a sterile trolley, a bed, and an ultrasound machine. Most USGSBs are best performed with the patient in the supine position and the physician seated. Before the procedure, a brief ultrasound examination of the joint to be biopsied is performed to plan the most adequate needle path, in order to avoid tendinous and neurovascular structures, and synovitis grade is determined, according to grayscale and power Doppler.

A support table, with all required equipment and materials, is prepared and a sterile technique implemented. Sterile gel or chlorhexidine solution must be used as a contact medium. An in-plane approach, trying to keep the needle as parallel as possible to the probe, is the best option. Then, each USGSB procedure

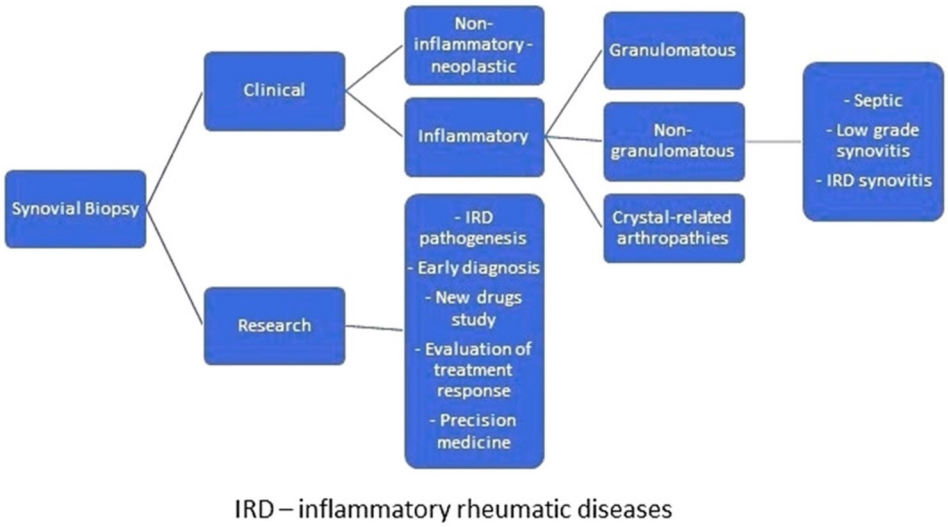

FIGURE 1 | Why are synovial biopsies conducted?

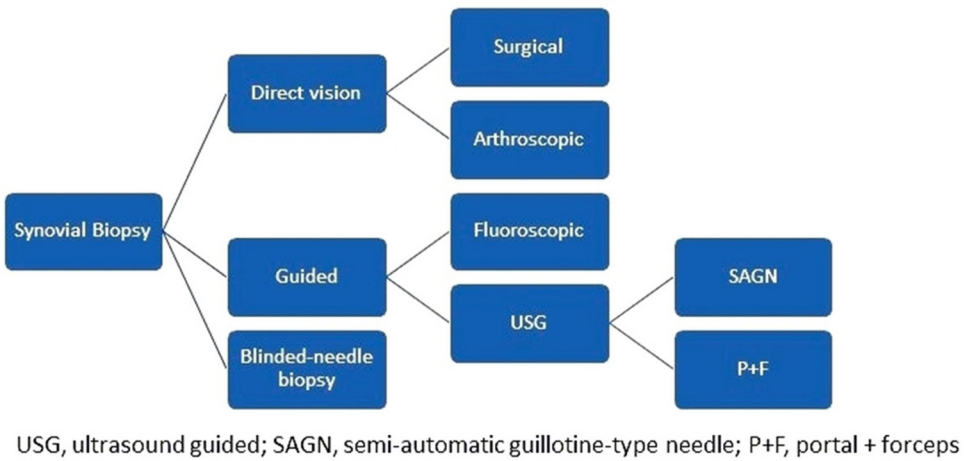

FIGURE 2 | How are synovial biopsies conducted?

follows a similar routine with fluid aspirated (if present) and 1–5 ml of local anesthetic injected into the soft tissues up to the joint capsule, under US guidance. A further 2–5 ml of 1% lidocaine is instilled into small joints, 10–15 ml for large joints. For large joints such the knee, hip, or shoulder, a suitable coaxial outer needle may be used in addition to the 14- or 16-G biopsy needle, to facilitate repeated needle entry over a long path. For small and intermediate joints, a 16-G, throw-length 10-mm biopsy needle is used, without a coaxial sheath. US imaging is used to guide the needle to an appropriate predetermined biopsy site. After the procedure, compression of the entry site is followed by the application of a small adhesive dressing. Some of the steps involved are illustrated in **Figures 3–8**.

When the procedure is completed, the patient is asked to grade his/her tolerance for the intervention. In our experience with NB, the time spent for the whole procedure is <70 min, of which more than half is spent in the pre-procedure steps, with the biopsy itself usually lasting < 35 min. Pre-procedure steps include the checklist (around 10 min); brief ultrasound examination (5 min); and operator, material, and patient preparation (20 min = 5 + 10 + 5 min, respectively). Five to 14 days post-biopsy, patients are asked to grade their discomfort during the procedure and whether they took over the counter analgesics. Besides, they are requested to grade the pain, stiffness, and swollenness in the biopsied joint over the previous 3–4 days and their willingness to repeat the procedure, if needed. Any adverse events that may

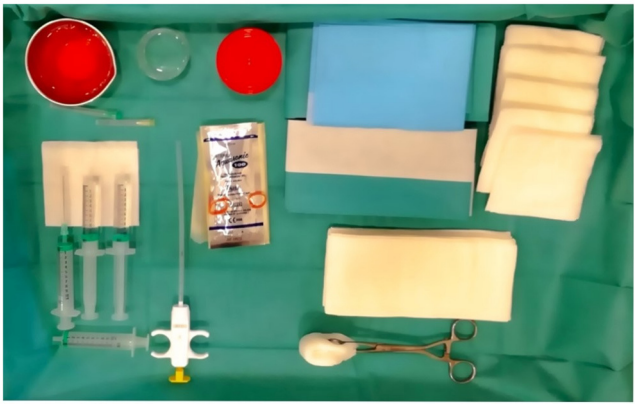

FIGURE 3 | Materials required for USGSB.

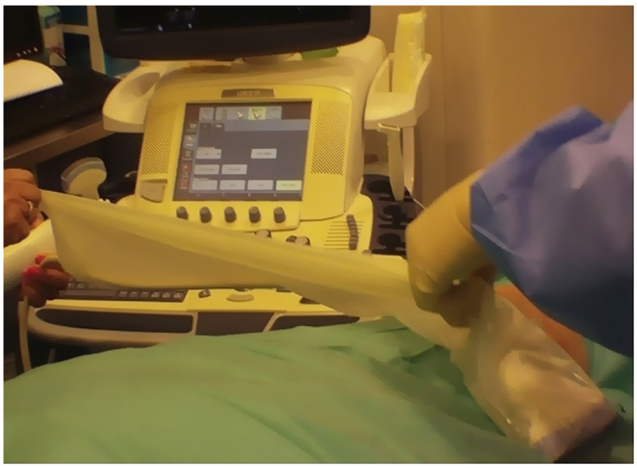

FIGURE 4 | Field and probe cable protection.

have occurred are recorded and ultrasound examination of the biopsied joint performed.

Synovial Sample Collection and Processing

Synovial sample collection is driven by the procedure goal—clinical or research. For clinical purposes, ≥6 fragments are sent for paraffin embedding and ≥5 for microbiology. Fragments sent for paraffin embedding are then sectioned into thin slices using a microtome and sent for histopathological analysis, following staining with hematoxylin–eosin. For research purposes, ≥6 fragments are sent for paraffin or optimal cutting temperature cryoprotective compound embedding. After thin sections are cut, samples are analyzed by immunohistochemistry or immunofluorescence for cell-type identification, according to the research goal. We also store ≥ 6 fragments in RNALater, which is a substance that counteracts RNA degradation by RNAases, for subsequent transcriptome analysis (7). The final number of samples collected inevitably depends on and reflects the research goal, patient tolerability, and time available (10).

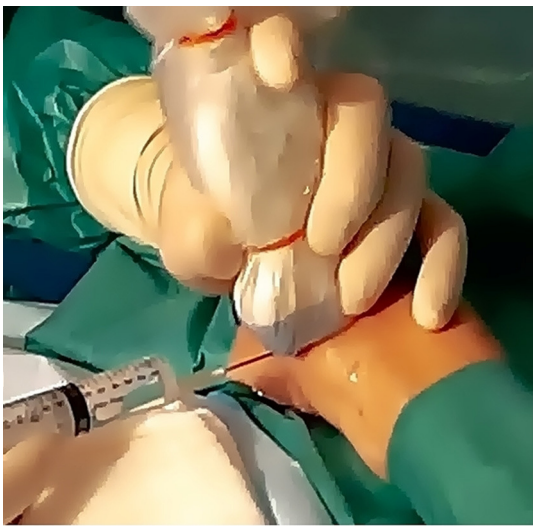

FIGURE 5 | Local anesthesia.

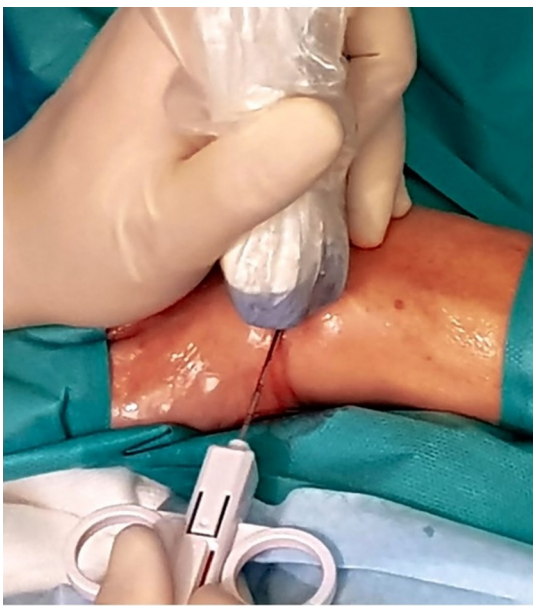

FIGURE 6 | Insertion of biopsy needle.

The European Synovitis Study Group (ESSG) recommends as quality criteria that biopsy size should be >2.5 mm^2 and that the synovial lining layer, as well as the overall morphology of the tissue, must be preserved (17). The same group recommends that synovitis should be quantified; one of the most popular scoring systems for that purpose is Krenn's score, which assesses three features of synovitis: hyperplasia of the lining cell layer, stromal cell density, and intensity of inflammatory infiltrate. Krenn's score can range from 0 to 9, as follows: 0–1, no; 2–4, low-grade; and 5–9, high-grade synovitis (18). The ESSG also recommends that the synovial pathotype, as well as the presence of ectopic

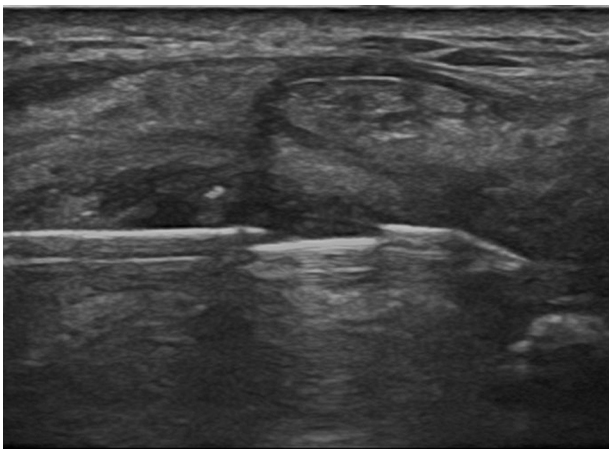

FIGURE 7 | Biopsy needle inside the joint.

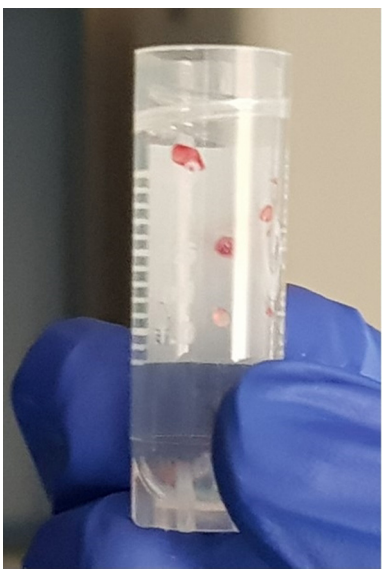

FIGURE 8 | Synovial samples.

lymphoid follicles, should be described (17). Other authors have recommended six as the minimum number of samples to collect for immunohistochemistry, to reduce T cell variability; however, the minimum number of samples for other cell types is less well-established. For macrophages, it depends on the goals pursued by the research (6). Other measures of synovial tissue quality include the number or percentage of synovial fragments and gradable synovial fragments, the total area of gradable synovial tissue, the area of gradable tissue by biopsy sample, and the RNA integrity number (RIN) (2); RIN ranges from 1 to 10, where samples from synovial tissue with values > 3 are considered adequate for subsequent transcriptomic analysis (2), molecular analysis, gene expression profiling, or gene sequencing methods (e.g., next-generation sequencing) (5). The capacity of USGSB to retrieve sufficient RNA is remarkable, even from small joints with minimal synovitis.

WHAT DATA DO SYNOVIAL BIOPSIES PROVIDE?

Healthy Synovial Membrane

Synovial tissue analysis is a fundamental tool for investigating arthritis pathobiology and searching for biomarkers of treatment response in basic and translational research, and is a useful method in routine clinical practice (11). To better understand the value of USGSB, it is crucial to have adequate knowledge of the "normal" synovial membrane or synovium, which is an ectoderm-derived structure that coats the inner surface of diarthrodial joints. The synovium has folds or villi, which provide non-restrictive motion and an augmented absorptive area; may be discontinuous, resulting in occasional bare areas of cartilage or bone; and contains two parts, the intimal lining layer, which is in contact with the joint cavity, and the stroma or sublining layer, with no basal lamina or membrane between them. The microanatomy of the normal synovial membrane can be divided into three types, fibrous, areolar, and adipose, according to the structure and contents of the sublining layer, with the areolar subtype the most typical and ubiquitous, the adipose subtype primarily found in fat pads, and the fibrous subtype found in finger and toe joints (19).

The normal synovial membrane has several functions: (1) it allows the movement of adjacent relatively non-deformable structures; (2) it maintains an intact non-adherent tissue surface; (3) it controls synovial fluid volume and composition; (4) it lubricates cartilage; (5) it nourishes chondrocytes; and (6) it absorbs debris and metabolic waste products (19, 20).

Normal intima is formed by one or two layers of synoviocytes. These can be (1) type A synoviocytes, which are macrophage-like and derived from blood monocytes via subintimal venules, or (2) type B synoviocytes, which are the most abundant, locally derived fibroblast-like synoviocytes of mesenchymal origin. The usual pattern found in the lining layer is a first row of type A synoviocytes, in close contact with the joint cavity, below which there is a row of type B synoviocytes (19). Type A synoviocytes are capable of phagocytosis and pinocytosis and have numerous micro-filopodia and prominent Golgi apparatus. Type B synoviocytes have prominent rough endoplasmic reticulum organelles and synthesize hyaluronan, fibronectin, laminin, collagens, catabolin, lubricin, "superficial zone protein," neutral proteinases, collagenase, and gelatinase. Type B synoviocytes also express several adhesion molecules, including vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), β1 integrins, and the surface markers, CD44 and CD55. The extracellular connective tissue matrix of the lining layer includes a fine fibrillary net of type I, III, IV, V, and VI collagens, as well as variable amounts of hyaluronan, laminin, fibronectin, chondroitin-6-sulfate-rich proteoglycan, and fibrillin-1 microfibrils (10, 19, 20). Large amounts of hyaluronan are present in the lining layer, as well as in the part of the sublining layer that is closer to

the lining layer, while there is no hyaluronan deeper in the subintimal layer.

The normal sublining layer is relatively acellular and contains some blood and lymphatic vessels. Fibroblasts and adipocytes are the dominant cell types in the sublining layer, while a few macrophages, B lymphocytes, plasma cells, $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes, granzyme B-positive cells, interdigitating antigen-presenting dendritic cells, and mast cells may also be found, but in small numbers (19). The part of the sublining layer closer to the lining layer also has a row, two to three cells thick, beneath which there are capillaries, with a deeper plexus of small arterioles and venules, with lymphatic vessels found deepest (furthest from the lining layer) in the sublining layer (19). A rich network of sympathetic and sensory nerves is present in the synovium, usually associated with blood vessels, and extending into the intimal layer. These nerves are myelinated and terminate close to blood vessels, whose vascular tone they regulate. Sensory nerves respond to proprioception and pain via large myelinated nerve fibers or myelinated fibers with unmyelinated free endings (nociceptors), or via small unmyelinated fibers. Synovium nociceptors are reactive to neuropeptides, including vasoactive intestinal peptide, calcitonin gene-related peptide, and substance P (20). The sublining layer contains many elastic fibers, providing tautness to this part of the synovium, which is mainly composed of loose connective tissue, but contains other constituents, including laminin, fibronectin, chondroitin-6-sulfate-rich proteoglycans, and type I, III, IV, V, and VI collagens (19, 21).

Synovial macrophages are $CD163^+$, $CD68^+$, $CD11b^+$, and $CD14^+$; however, subintimal macrophages are strongly positive for CD14, while intimal macrophages are only weakly positive. There are other differences between markers expressed by intimal and subintimal macrophages in healthy synovium: intimal macrophages express strong non-specific esterase (NSE) activity and the immunoglobulin receptor, FcgRIIIa, while subintimal macrophages are weakly positive for NSE activity and express the immunoglobulin receptor, FcgRI, with low or absent levels of FcgRIIIa (19, 21).

Regarding cytokine production in normal synovium, although very small amounts of pro-inflammatory cytokines, such as interleukin-1β (IL1β), interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF) are generated, levels are much lower than those present in any type of synovitis. In contrast, levels of IL1β receptor antagonist and osteoprotegerin, which inhibits receptor activator of NF kappa B ligand (RANKL), in the normal synovium far exceed those of the molecules they inhibit (19).

Synovial Pathology

In the context of clinical arthritis, analysis of synovial tissue allows the identification of lymphocytic aggregates that can produce local autoantibodies, potential biomarkers differentiating rheumatoid arthritis (RA) from other forms of early arthritis, distinct fibroblast cell types in involved or non-involved arthritic joints, and decreased macrophage numbers as a surrogate marker of treatment response (1, 13). Indeed, decreased number of macrophages ($CD45^+$ or $CD68^+$) in the synovial sublining is the most reproducible and validated biomarker of treatment efficacy in RA, and more reliable than disease activity score 28 (DAS28) (12, 13, 22). In contrast, macrophage infiltrate density correlates with progressive structural damage. In addition to macrophages, B cell aggregates and mast cells are also associated with severe disease in RA (23–28). Other potential biomarkers in synovial tissue include different populations of lymphocytes and lymphocyte aggregates, cytokines, chemokines, S100 proteins, adhesion molecules, mediators, and degradation products from bone, cartilage, and synovial membrane, various antigens and antibodies, and genes involved in the regulation of cell division and immune responses (9). The degree of infiltration and aggregation of lymphocytes in synovial tissue, the number of $CD68^+$ macrophages in the sublining, and global synovial inflammation scores, as well as levels of pro-inflammatory cytokines, chemokines, and catabolic enzymes in the synovium, all correlate with disease activity, erosive burden, and radiographic progression. Flow cytometry, bulk-RNA sequencing, and single-cell technologies (mass cytometry and single-cell RNA sequencing), used in judicious sequence, have facilitated high-resolution identification of disease-associated cell subsets in human tissues. These technologies have enabled the identification of four distinct fibroblast populations in the synovium (three in the sublining layer and one in the lining layer); four populations of monocytes; six of T cells (three $CD4^+$ and three $CD8^+$); and four of B cells (naïve B cells, memory B cells, autoimmune-associated B cells, and plasmablasts) (29). Taken together, these data demonstrate that analysis of synovial tissue is an important tool with prognostic value, in terms of phenotypic variability, disease activity, and disease severity (21).

The Doppler signal in RA correlates with hyperplasia of the synovial lining, lymphoid and macrophage infiltration of the sublining, angiogenesis, and lympho-myeloid pathotype (9, 30–32). Both the lining and sublining layers of the synovium exhibit typical features during rheumatoid synovitis. In the lining layer, rheumatoid synovitis manifests as hyperplasia, due to synoviocyte proliferation, with a dramatic increase of type A synoviocytes, which may account for up to 80% of the intimal layer, and mononuclear cell infiltration, caused by recruitment of bone marrow-derived monocytes, reaching up to 12 cells in thickness; these two types of cells are important sources of cytokines, chemokines, matrix-degrading enzymes, adhesion molecules, and osteoclastic and angiogenic factors. Further, increased fibronectin content, with resultant fibrin deposition in the superficial layer of the inflamed synovium and expansion of the lining layer and increased metabolic demands, is observed, but with few blood vessels in the vicinity, suggesting relative hypoxia, a potent stimulus to produce VEGF and other angiogenic mediators.

In the sublining layer, rheumatoid synovitis causes stromal proliferation, with pronounced infiltration by monocytes, macrophages (mainly pro-inflammatory M1 macrophages, monocyte-derived), T cells (mainly, but not limited to, $CD4^+$ Th1 and Th17, and CD45 RO^+ T cells), B cells and plasma cells (mainly antibody-producing, but also chemokine and cytokine producers), dendritic cells (myeloid and plasmacytoid dendritic cells, which are antigen-presenting cells, producers of inflammatory mediators and cytokines, and possibly also

involved in local autoantibody production), NK cells (a source of IL-22, and mediator of fibroblast proliferation), mast cells (antigen-presenting cells, osteoclast promoters, cytokine, and histamine producers), and neutrophils (source of citrullinated peptides, proteases, cytokines, and reactive oxygen species). Metalloproteinases are also increased, alongside stimulators of osteoclast activity, enhanced angiogenesis (with immature blood vessels, expression of adhesion molecules in the vascular endothelium, and formation of numerous endothelial venules), lymphatic congestion, formation of ectopic lymphoid structures (lymphoid aggregates with germinal centers), granulation tissue, fibrin deposition, and fibrinoid necrosis in the sublining layer (8, 10, 19, 21, 30, 33, 34).

Classification of Synovitis Based on Immune Cell Analysis

Immunohistochemistry and immunofluorescence analyses in early arthritis, to identify the dominant cells and the inflammatory infiltrate intensity, allow classification of different pathotypes, namely, the three synovial phenotypes: lympho-myeloid, diffuse-myeloid, and pauci-immune/fibroid, each one affecting about a third of patients (32, 35, 36). Cell types analyzed include $CD3^+$ (T cells), $CD15^+$ (neutrophils), $CD20^+$ $CD22^+$ (B cells), $CD21^+$ (dendritic follicular cells), $CD31^+$ and F VIII (endothelial vascular cells), $CD38^+$ $CD138^+$ (plasma cells), $CD45^+$ $CD68^+$ $CD163^+$ $CD90^-$ (macrophages), $CD90^+$ $CD45^-$ $CD55^+$ (fibroblasts), and $CD117^+$ (mast cells). Biopsies are stratified for each of these groups by semiquantitative grading (0–4) of immunohistochemistry results revealing the degree of immune cell infiltration, which is classified as follows: lympho-myeloid, $CD20^+$ cells $\geq$ 2 and/or $CD138^+$ cells $\geq$ 2; diffuse myeloid, sublining layer $CD68^+$ cells $\geq$ 2, $CD20^+$ cells $\leq$ 1, and/or $CD3^+$ cells $\geq$ 1, and $CD138^+$ cells $\leq$ 2; and pauci-immune/fibroid, sublining layer $CD68^+$ cells $<$ 2 and $CD3^+$, $CD20^+$, and CD 138^+ cells $<$ 1 (18, 32).

The lympho-myeloid pathotype is characterized by the dominance of B cells and plasma cells, together with myeloid cells, and can be associated with autoantibodies, osteoclast-related genes, disease activity, structural damage, poor response to csDMARDs, and good response to anti-IL 6 drugs. The hallmark of the pauci-immune/fibroid pathotype is stroma-resident cells, in association with scarce immune cells, low levels of autoantibodies, lower activity, and less damage/disease progression, but poor response to treatment. The myeloid-diffuse pathotype is characterized by the presence of myeloid cells and scarce B cells, has intermediate features, relative to the two other phenotypes, and may respond well to anti-TNF drugs (32, 35). It has been postulated that, as the pauci-immune pathotype occurs in a considerable proportion of cases of early arthritis, this is a defined pathotype, rather than representing a burned-out end-stage disease (35). Moreover, Lliso-Ribera et al. (36) showed that synovial pathotypes can distinguish between clinical phenotypes, independently of disease duration. Together, these data question the dogma of the "opportunity window" in RA treatment and demonstrate that patients with RA that fulfill the 1987 ACR criteria have an increased probability of needing biologic disease-modifying anti-rheumatic drugs (bDMARDs) at some point during the disease course, relative to those with undifferentiated arthritis or those that fulfill the 2010 ACR/EULAR criteria, but not the 1987 ACR criteria (36). Patients needing bDMARDs exhibit upregulation of genes encoding factors mediating the proliferation, differentiation, and activation of B and T cells, and of matrix metallopeptidase production and cytokine-mediated cell activation. Patients who do not require bDMARDs mainly express genes regulating fibroblast proliferation and cartilage turnover. Finally, the integration of histologic and molecular signatures improves the sensitivity and specificity of a model for predicting which patients would need bDMARDs at some point during the disease course (36).

Regarding established RA, immunohistochemistry and immunofluorescence can also identify several pathotypes, whose various designations include diffuse, aggregate, lymphoid, granulomatous, follicular, myeloid, fibroblastic, and pauci-immune/fibroid synovitis (8, 37–39). Diffuse or myeloid synovitis affects 50–70% of patients, is associated with good responses to anti-TNF drugs, and has one of the most benign clinical phenotypes, where rheumatoid factors tend to be absent, $CD68^+$ cells predominate, and there are few lymphocytes and no ectopic lymphoid structures (39–41). Aggregate, follicular, or lymphoid synovitis is associated with more active disease and the presence of rheumatoid factor and tends to respond to anti-IL6 drugs. It affects 22–50% of patients and may comprise two subtypes, one associated with follicular dendritic cell networks or ectopic/tertiary lymphoid-like structures, and allegedly with worse outcome, and another which lacks those networks/features (37–40). In follicular or lymphoid synovitis, B cell infiltrates and B cell markers predominate, while pauci-immune or fibroid synovitis is characterized by a fibroblast-rich landscape, overexpression of cellular and molecular markers of macrophages and fibroblasts, almost no immune cell infiltration, no associated rheumatoid factor, and a poor response to anti-TNF or anti-IL6 drugs (8, 39); this subtype affects 20–30% of patients. Another much rarer pathotype, granulomatous synovitis, affects $<$ 1% of patients and is associated with extra-articular features (38). Fibroblast-like synoviocytes with distinct genetic signatures are also associated with different disease phenotypes and outcomes (40); however, conflicting results have been reported regarding the association of synovial lymphoid aggregates and disease severity (41–44). Nevertheless, the overall positive correlation between lymphoid aggregates and synovial inflammation may simply be the result of interdependency, rather than mutual exclusivity, between lymphoid and myeloid infiltrates (41). It should also be stressed that synovitis scores and pathotypes may vary among samples from the same biopsy, because of minor differences between sections of the same sample. Hence, final results should be defined only after consideration of several samples and sections (37).

The existence of such diversified pathotypes indicates the presence of distinct pathogenic pathways in the synovial membrane and the need for therapeutic strategies directed toward every scenario (17); however, some authors have proposed that these data provide evidence of two main pathogenic pathways, one through a lymphoid axis, targeted by

drugs like tocilizumab, rituximab, and methotrexate, and another through a myeloid axis, targeted by anti-TNF drugs (41).

Clinical Utility of Synovial Biopsies

Synovial Biopsy to Distinguish Synovitis Pathotypes

In a study conducted in the author's department, the median synovitis score was significantly superior in follicular pathotype RA (5.8 ± 0.0), relative to the diffuse (4.0 ± 1.3) and fibroid (2.5 ± 0.5) pathotypes. In the same study, the median synovitis score was comparable between different inflammatory rheumatic diseases but considerably superior to that in primarily non-inflammatory conditions, such as osteoarthritis, synovial chondromatosis, or foreign body synovitis (37). Comparison of pathotypes among different rheumatic inflammatory conditions did not reveal significant differences between various diagnoses, except for a clear predominance of the diffuse pathotype in spondylarthritis (SpA), the absence of the fibroid pathotype in crystal-related arthropathies, and absence of the follicular pathotype in primarily non-inflammatory arthritis (37).

Synovial membrane activation of JUN N-terminal kinase is increased in early RA, but not in undifferentiated forms of arthritis, and $CD22^+$ and $CD38^+$ cells distinguish RA from other forms of arthritis (9). Compared with psoriatic arthritis (PsA), RA synovial membranes contain fewer vessels, lower lipid content, and fewer neutrophils, mast cells, $CD163^+$, and $CD117^+$ cells, with equal levels of lining and sublining $CD68^+$ cells, sublining $CD3^+$, $CD20^+$, and $CD21^+$ cells, but higher levels of $CD138^+$ cells, and these differences appear to be independent of therapy (26, 45, 46). DMARD-naïve PsA patients that had reached minimum disease activity (MDA) at 6 months had lower levels of $CD3^+$ cells than those that did not reach MDA at 6 months. Similarly, naïve patients with RA that reached DAS28 remission at 6 months had lower levels of $CD68^+$ cells than those that did not reach remission at 6 months (45).

Patients with undifferentiated peripheral inflammatory arthritis that evolved to a definite diagnosis had higher levels of lining and sublining $CD68^+$ and $CD3^+$ cells than those who did not evolve to a definite diagnosis. Similarly, patients with higher grayscale ultrasound and power Doppler ultrasound scores were more likely to evolve to a definite diagnosis than those who did not (47).

Other features that may help to differentiate RA from SpA and other diagnoses are the higher ratios of $CD3^+/CD4^+$ T cells, RANKL/OPG, and $CD20^+/CD22^+$ B cells in the former. RA patients also have more cell infiltration (B and T cells), more lymphoid aggregates, and higher numbers of $CD68^+$ sublining macrophages and $CD38^+$ plasma cells than those with SpA, but lower M2 macrophage populations and, eventually, fewer blood vessels in the sublining layer (21, 27, 34, 48, 49).

Quality and Quantity of Synovial Samples Collected by USGSB

Humby et al. conducted a study in small joints of 35 patients with RA, to determine whether USGSB at baseline and at second biopsy could generate sufficient high-quality synovial tissue for pathotype identification, RNA extraction, and detection of sublining macrophage changes after treatment. They showed that good-quality synovial tissue, adequately reflecting synovial phenotype, was obtained in 81% of biopsies when synovial hypertrophy > 2 was detected by grayscale ultrasound pre-biopsy, opposed to 20% of those with a minimal degree of synovial hypertrophy. In all biopsies, it was possible to retrieve enough RNA for molecular analysis, regardless of pre-biopsy synovial hypertrophy grade, as determined by ultrasound, and a significant correlation was detected between the change in the number of sublining $CD68^+$ macrophages and treatment response evaluated by DAS28. Moreover, they showed that it was necessary to examine multiple biopsy samples, and not only multiple sections of the same sample, to obtain a representative image of the cell infiltrate; use of at least four samples produced a good result, while examination of ≥10 samples (where possible) increased the percentage of evaluable tissue substantially, in terms of $CD3^+$, $CD20^+$, and $CD68^+$ cellular infiltrates. Further, they demonstrated that, in patients with minimal synovial hypertrophy, increasing the number of samples did not improve the quantity of gradable synovial tissue, nor did the presence of Doppler signal predict the success of the procedure (22).

Kelly et al. performed 93 synovial biopsies in large, as well as small, joints, in patients with early RA, of which 36 had a second biopsy 6 months later. A median of 14 samples was retrieved per joint, and 93% of biopsies yielded good-quality synovial tissue, which was maintained in even the second biopsy. RNA samples extracted from all joints and all biopsies were adequate, even those from repeat biopsies, although gradable tissue was only obtained from 40% of small joints. The quantity and quality of synovial tissue retrieved correlated with elevated synovitis score determined by pre-biopsy ultrasound grayscale examination, but not with power-Doppler grade. Self-limited joint discomfort, solved in <24 h, was the most frequent adverse event, occurring in 19% of patients (16).

Published data show that USGSB facilitates the collection of synovial samples of sufficient quality in 82–96% of biopsies, compared with 48–85% of blind needle biopsies (4–6, 32, 37, 50–52). Results are also influenced by operator expertise, joint size and type, and synovitis grade, as determined by grayscale ultrasound (6). In our department, of a series of 64 NB performed in all kinds of joints, synovial bursae, and tendon sheaths, 81% were done within clinical practice to investigate a possible infection, or to help to clarify a diagnosis, and 19% in the context of research activities (37). Synovial biopsy had diagnostic and treatment impact in 37% of cases and, in the research setting, 92% of cases could be used for the proposed objectives. Of all biopsies, 88% yielded synovial tissue, consistent with literature reports (4, 37). Notably, the median sample number was significantly lower in biopsies from which synovium was not successfully retrieved and, of eight unsuccessful biopsies, six were from large joints. Operator experience also had a clear impact on the quality of synovial tissue obtained (37). Remarkably, synovial tissue was less likely to be successfully obtained from samples collected later in the procedure and had fewer concordant pathotypes than those collected early. Consequently, the authors recommend that samples collected for different purposes should be assigned in parallel, rather than sequentially (37).

Synovial Biopsy to Evaluate Disease Progression and Treatment Response

Alivernini et al. used ultrasound and synovial immunohistochemistry to study 42 patients with undifferentiated peripheral arthritis with rheumatoid factor, who were anti-citrullinated protein antibodies (ACPA) negative and naïve to DMARDs. They found a correlation between $CD68^+$ lining and sublining layer cells and CD 31^+ (intravascular) cells and ultrasound scores. The few patients that evolved to a definite diagnosis had significantly higher levels of the aforementioned cells and higher ultrasound scores and $CD3^+$ cell numbers in the sublining layer. These patients also exhibited downregulation of miRNAs 346 and 214 (47).

Just et al. conducted baseline and 6-month ultrasound, magnetic resonance imaging (MRI), and synovial biopsy assessments in 20 patients with early RA and 20 with established RA. They found that EULAR-OMERACT ultrasound score and RAMRIS MRI score were strongly correlated with Krenn's synovitis score at baseline, but not 6-month assessment, in the early RA group. In the established RA group, a moderate to strong correlation was present between the three scores at baseline assessment, except for the RAMRIS 6-month assessment, which was not performed (53).

Rivellese et al. detected $CD20^+$ cell infiltrate $\geq$ 2 (0–4) and B-cell-rich synovitis in 35% of DMARDs-naïve early RA and 47.7% of established RA, with inadequate responses to anti-TNF, which were significant differences. They also found that patients with B-cell-rich synovitis had higher levels of disease activity, rheumatoid factor, and ACPA positivity, but only in early RA, not established RA. Nevertheless, patients with both early and established RA with B-cell synovitis also had higher total histologic synovitis scores. According to the authors, this lack of correlation between B-cell-rich synovitis and clinical disease activity scores (DAS28) could be a sign of the insensitivity of clinical scores for capturing synovial inflammation and may explain the progressive structural damage in patients with low clinical disease activity (54).

Two independent groups compared ACPA-positive and -negative patients with established RA and found that synovial numbers of $CD3^+$, $CD8^+$, $CD19^+$, and B cells were significantly higher in ACPA-positive patients; however, they also had more active disease, introducing bias in the analysis (55, 56).

After 3 months of treatment with tocilizumab, a marked decrease in $CD3^+$ cells was detected in patients with early RA (57). After 3 months of treatment with adalimumab, patients with RA and an inadequate response to methotrexate had a marked decrease in CD 68^+ cells (58). Finally, 3 months of treatment with rituximab resulted in a significant reduction in both B cells and IL-17-producing T cells in patients with RA (59).

The R4RA study investigated the best therapeutic option between rituximab vs. tocilizumab for patients with RA and previous inadequate response to anti-TNF, by assessing whether molecular and cellular signatures of B cells predicted a better response to rituximab. Tocilizumab showed better results in the B cell-poor group and was not inferior to rituximab in the B cell-rich group, although there was a higher incidence of adverse events (60).

Diagnostic Value of USGSB

In RA, there is a tendency to find similar numbers of cells in the sublining layers of different joints in the same patient, even between clinically involved and non-involved joints, although less pronounced in clinically uninvolved joints; however, this does not occur in the synovial lining layer, in which there are no similarities in the number or characteristics of macrophages and fibroblast-like synoviocytes between clinically involved and non-involved joints, with distinctive DNA fingerprints and methylation patterns, according to their positional memory (9).

In septic arthritis, compared with synovial fluid analysis, USGSB increased the percentage of causative agent isolation in non-specific infections, as well as mycobacterial infections (6). Perivascular infiltrate of neutrophils, which typically comprise $>$ 20% of all cells in synovial tissue of septic arthritis, as well as polymerase chain reaction for identification of bacteria and fungi, have remarkably high sensitivity and specificity for detecting the presence of infection. In addition to infectious arthritis, there are several other situations in which synovial biopsy may be diagnostic, including Whipple and Wilson diseases, synovial chondromatosis, pigmented villonodular synovitis, ochronosis, synovial lipoma arborescens, foreign body synovitis, crystal-related arthropathies, amyloidosis, hemochromatosis, histiocytic and neoplastic diseases, and sarcoidosis (6).

Adverse Events Related to USGSB

Adverse events are uncommon in USGSB and usually mild and transient; however, adverse event rates $<$ 0.5%, as described in some series, seem unrealistic and likely reflect a low-sensitivity data collection strategy (3, 5, 7). Reported adverse events include vasovagal reaction; sensory disturbance; nerve, vessel, tendon, ligament, or muscle lesion; ecchymosis, hemarthrosis, skin, or joint infection; sinus tract from joint to skin; fracture of biopsy needle; and thrombophlebitis or deep venous thrombosis (11, 16, 22, 61). Transient post-procedure sensory disturbance, vasovagal reaction (1–2% of cases) and joint discomfort post-biopsy (usually $<$ 24 h; 7–19% of cases) are the most reported adverse events (5, 10, 16, 37). In a series of 524 synovial biopsies (402 NB, 65 PFB, and 57 arthroscopic), adverse events were detected in 1.5% of procedures, with no difference among methods. All patients reported clear improvement 2 weeks post-procedure. Repeated biopsies did not increase the number of adverse events or patient-reported outcomes (3). In our series, adverse events were considered discreet and transient, except for a single case of a slight limitation of fifth finger extension, which persisted after a wrist biopsy (37).

DISCUSSION

What Are the Unmet Needs Related to Synovial Biopsy?

Our capacity to induce sustained remission or cure of RA and other inflammatory arthritis at the individual level remains

BOX 1 | Unmet needs related to USGSB and synovial tissue analysis.

General Framework:
More robust validation in multiple centers
Compare the procedure, performance, and safety of PFB and NB appropriately, to produce a consensual definition of a successful procedure
Define training goals for practitioners learning how to perform USGSB
Specific Technical Issues:
Standardize sample acquisition (number of samples according to goals, define sites within the joint), processing, analysis, and reporting of results
Pathotype:

- How to manage dynamic changes in pathotype that occur over the disease course and after treatment
- How many observations are required to define the dominant pathotype, since it may change according to the harvesting sequence, across samples from the same joint, and across joints from the same patient

Define the best cutoff to differentiate pathotypes that share overlapping features.
Main Technical Goals:
Identification of synovial biomarkers that distinguish between different types of arthritis and other diseases
Determine if synovial histopathological heterogeneity translates into diverse clinical phenotypes
Determine if it is an adequate method for patient stratification and treatment monitoring
Search for molecular signatures modulated by specific therapeutic approaches
Determine whether synovial biopsies early in the disease course can predict outcomes and identify patients that will respond to bDMARDs/csDMARDs and those who will not

limited, due to insufficient information to drive treatment. As synovitis is the hallmark of these diseases, accessing the core site of the pathological process (synovial tissue) provides opportunities to gather information with potential diagnostic and prognostic utility. Synovial tissue biomarkers appear an attractive target for that purpose, due to the inadequacy of peripheral blood biomarkers. An equivalent path has been followed in oncology, where histopathology has demonstrated prognostic value and is now integrated into the standard of care (15).

Several unmet needs related to USGSB and synovial tissue analysis remain a challenge for those working in the field of rheumatology, as outlined in **Box 1**. The ultimate goal is to find the ideal treatment, in the right time frame for each patient, using a precision medicine approach, as applied in cancer therapy. We believe that analysis of synovial tissue will play a decisive role in this strategy, hopefully in the near future (5, 15, 40).

The absence of robust, predictive biomarkers of treatment outcomes is a major unmet need in the management of RA and other types of inflammatory arthritis. A precise understanding of the key events occurring during synovitis will be critical in advancing the era of precision medicine in RA and other inflammatory rheumatic diseases, placing synovial tissue analysis at the core of this journey. However, before proposing routine use of standardized synovial tissue biopsy to guide therapy, several factors must be satisfied, according to the OMERACT Synovial Tissue Biopsy Special Interest Group, including uniformity of biopsy handling and analysis, validation of quality scores, and relationship between immunopathology and therapeutic response and between disease pathotypes and outcomes (62).

Conclusion

In conclusion, available data demonstrate that USGSB is an effective, safe, and well-tolerated method of retrieving quality synovial tissue from any type of joint, with impacts on diagnosis and treatment. In the clinical setting, formal indication for synovial biopsy occurs mainly in monoarthritis cases, to exclude infection, and although synovial biopsy still cannot be used to distinguish between types of inflammatory rheumatic diseases, it has led to remarkable advances in the understanding of the pathobiology of RA and other inflammatory rheumatic diseases. Histopathological analysis, immunohistochemistry, and omic and molecular analyses of synovial tissue have brought us to the cusp of an era of personalized medicine in rheumatology.

AUTHOR CONTRIBUTIONS

FS is responsible for the conception of the manuscript, including the review of the literature and the individual experience that conducted to the idea.

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REVIEW
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The Crucial Questions on Synovial Biopsy: When, Why, Who, What, Where, and How?

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In the majority of joint diseases, changes in the organization of the synovial architecture appear early. Synovial tissue analysis might provide useful information for the diagnosis, especially in atypical and rare joint disorders, and might have a value in case of undifferentiated inflammatory arthritis, by improving disease classification. After patient selection, it is crucial to address the dialogue between the clinician and the pathologist for adequately handling the sample, allowing identifying histological patterns depending on the clinical suspicion. Moreover, synovial tissue analysis gives insight into disease progression helping patient stratification, by working as an actionable and mechanistic biomarker. Finally, it contributes to an understanding of joint disease pathogenesis holding promise for identifying new synovial biomarkers and developing new therapeutic strategies. All of the indications mentioned above are not so far from being investigated in everyday clinical practice in tertiary referral hospitals, thanks to the great feasibility and safety of old and more recent techniques such as ultrasound-guided needle biopsy and needle arthroscopy. Thus, even in rheumatology clinical practice, pathobiology might be a key component in the management and treatment decision-making process. This review aims to examine some essential and crucial points regarding why, when, where, and how to perform a synovial biopsy in clinical practice and research settings and what information you might expect after a proper patient selection.

Keywords: synovial biopsy, synovial membrane, rheumatoid arthritis, inflammatory arthritis, synovial analysis

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INTRODUCTION

Changes in the organization of the synovial architecture are evident in the majority of joint diseases. Thus, the synovium has been studied at the macroscopic, microscopic, and molecular levels as it is an important determinant for the understanding of the biology of the joint and the etiopathogenesis of several joint diseases (1). In rheumatology, synovial tissue analysis provides insight into disease status and disease mechanisms by working as an actionable and mechanistic biomarker.

The synovium is a complex tissue composed of different cell types including tissue-resident macrophages, fibroblasts, and endothelial cells, as well as blood vessels, lymphatic vessels, and nerves (2). The histological analysis shows subcellular compartmentalization in two distinct zones: the lining layer and the sublining layer. The synovial lining has a crucial role in controlling the cellular and molecular exchange with the joint cavity and in maintaining joint integrity by regulating the composition of synovial fluid. In a healthy joint, it is made up of one to three cells thick and it is composed of tissue-resident macrophages and fibroblasts supported by a porous basement-like membrane, while the sublining, aside from fibroblasts and tissue-resident macrophages, includes nerves and blood and lymphatic vessels (2).

When pathology comes in, the normal architecture of the synovial membrane may be disrupted leading to alterations of the lining thickness, stromal cell density, and inflammatory infiltrate.

As in many joint diseases, the changes mentioned above occur early, and synovial tissue analysis might provide useful information for the diagnosis, especially in the case of atypical and rare joint disorders, and might have a supportive value in case of undifferentiated inflammatory arthritis, by improving disease classification. Moreover, it gives insight into disease progression helping patient stratification, a process in constant evolution. Finally, it contributes to an understanding of joint disease pathogenesis holding promise for the identification of new synovial biomarkers and the development of new therapeutic strategies (3).

This review aims to examine some essential and crucial points regarding why, when, where, and how to perform a synovial biopsy in clinical practice and research settings and what information you might expect after a proper patient selection. Given the breadth of the matter, we focus only on those aspects that are of the most interest to the rheumatologist.

WHEN SHOULD THE SYNOVIAL BIOPSY BE DONE?

The synovium is involved in all chronic inflammatory arthropathies. Although in routine clinical practice synovial biopsy is not mandatory for most diagnoses of inflammatory arthritis (e.g., rheumatoid arthritis—RA); in some circumstances it becomes irreplaceable (**Figure 1**). Indeed, when patient history, examination, and diagnostic investigations do not allow to delineate a clear picture and where there is a clinical suspicion of systemic forms, histological examination of synovial tissue with adequate sample processing can allow differential diagnosis between infective, neoplastic, deposition, and histiocytic diseases.

Moreover, a synovial biopsy can be complementary to the synovial fluid analysis. Comparative studies concerning the accuracy of the same diagnostic procedures (microbiological cultures, PCR for infective agents, crystals detection) on synovial fluid and synovial biopsy are not abundant but underline the utility of the two analysis, also in consideration of the possibility of false-negative results of both procedures (4–6).

A synovial biopsy is often performed for research purposes for example in RA patients; synovium histological and molecular alterations are considered a target for identifying new biomarkers to help rheumatologists tailor their clinical and therapeutic decision according to patient characteristics (7). Recently, a multicenter randomized control trial highlighted the possibility to integrate molecular pathology into clinical practice to improve treatment allocation of specific targeted therapies (8).

Finally, in the case of refractory synovitis (to local and systemic treatments), arthroscopic synovectomy could be a viable strategy to reduce local and persistent inflammation (9).

WHY CAN SYNOVIAL BIOPSY BE HELPFUL?

In rheumatology clinical practice, thanks to the great feasibility and safety of ultrasound-guided needle biopsy and needle arthroscopy, pathobiology may become a key component in the management and treatment decision-making process (10). For clinical and research purposes, histopathology and modern applications of molecular biology on synovial tissue are focused on the following major areas:

- ***Classification of early undifferentiated arthritis.*** Since an early diagnosis and treatment of chronic inflammatory arthritis are linked to better long-term outcomes in terms of prevention of irreversible structural damage, nowadays the number of undifferentiated arthritis defined as inflammatory arthritis not satisfying classification criteria for RA (10, 11) is increasing. For this reason, an unmet need is the identification of biomarkers able to detect the patient who will develop RA or peripheral SpAs and differentiate them from those who will develop self-limiting or degenerative diseases. This would allow the use of the so-called "window of opportunity" for the more aggressive forms and, on the other hand, not to overtreat patients who will not develop chronic inflammatory arthritis. As far as concern the histological analysis, the cellular infiltrate and vascularity are informative. In a study of 95 patients with early (<1 year) unclassified arthritis, massive infiltration by CD38+ plasma cells and CD22+ B cells in the synovial sublining was able to predict the diagnosis of RA in the following 2 years of follow-up solely based on histological data with an accuracy of 85%. A diagnosis other than RA can be predicted in 97% of the cases when minimal infiltration by these cells was found (12). Previous research has identified as a possible distinctive marker to differentiate RA from spondyloarthropathies (SpA) and osteoarthritis (OA), the intensity of B and T cells infiltration (13). Several studies have found different characteristics in synovial vascularity among undifferentiated arthritis forms more prone to turn into RA or SpA. In the synovium of those who will develop SpA, blood vessels were increased in the sublining layer and more tortuous compared to the synovium of those who will develop RA (13–15). These findings are complementary to transcriptomic analysis. For example, r synovial markers suggested as specific for RA are the presence of intracellular citrullinated proteins and the differential expression of alpha-V integrin (13, 16).

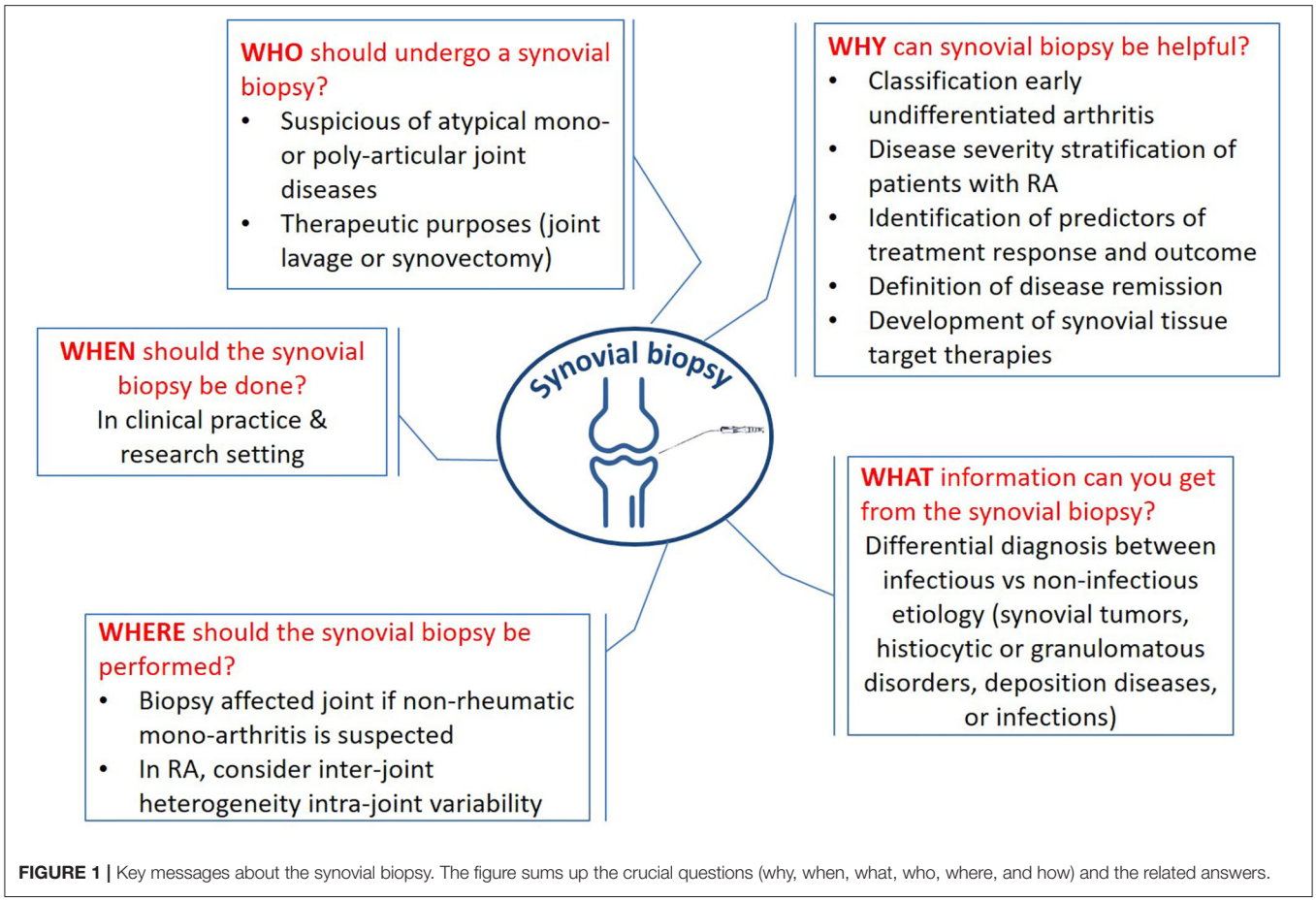

FIGURE 1 | Key messages about the synovial biopsy. The figure sums up the crucial questions (why, when, what, who, where, and how) and the related answers.

Moreover, angiogenic factors such as VEGF and Ang2 (mRNA and protein) were significantly more expressed in the synovial membrane of PsA than RA (17). Yeremenko et al. used pan-genomic microarrays of synovial samples and were able to recognize a myogene expression signature in SpA synovitis distinct from RA (18). Using a set of 100 transcripts on synovial tissue, based on their ability to discriminate RA from other inflammatory arthritic forms, Lauwerys et al. concluded that a diagnosis of RA can be predicted only by combining histological and clinical data (19). The study by Baeten et al. supports the validity of a multivariable prediction model by conjugating histological data (microscopic vascularity, lining layer thickness, assessment of synovial crystal deposition, staining for MHC-human cartilage gp39) with clinical and laboratory data to predict the evolution from undifferentiated arthritis into RA (20).

In particular, in the early disease stage, the presence of specific synovial histopathotypes defines distinct RA subtypes linked to diverse clinical phenotypes, disease activity/severity, and treatment response (21). These findings are further strengthened by the recent identification of different macrophage and fibroblast subsets in RA synovial tissue that are linked with different disease course and treatment responses (22).

- ***Disease severity stratification of patients with RA***. To date, the available risk stratification in RA patients is mainly represented by the presence of RF and ACPA autoantibodies together with CRP, the number of swollen joints at diagnosis, and the presence of erosive disease. Although valid, it certainly does not allow us to explain the great heterogeneity of the disease, prognosis, and response to treatments. Previous works have focused on RA prognosis in terms of disease severity/erosiveness. In a longitudinal study, the number of synovial lining layer macrophages at baseline correlates with the 1-year development of bone erosions in the hands and feet in patients with early (<18 months) inflammatory arthritis (mostly RA) (23). This finding was also confirmed in patients with established RA (24, 25). Furthermore, higher levels of MMP2 in synovial tissue samples from patients with early synovitis were correlated with the development of joint erosions (26). Previous studies have associated the presence of synovial lymphoid aggregates with the development of bone erosions (27), but subsequent studies on a larger number of patients did not confirm this result.
- ***Identification of predictors of treatment response and outcome***. Thanks to advances in molecular biology on synovial tissue, more recent studies have focused their attention on potential predictive synovial biomarkers of response to

therapy. To date, unfortunately, we do not have tools to identify patients who are likely to benefit from a specific therapy. Dennis et al. identified four histological pathotypes confirmed by molecular analysis of gene-expression profile on synovial tissue in patients with RA: *lymphoid phenotype* characterized by enrichment of genes related to B cells and plasmablasts, and T lymphocyte activation and differentiation and antigen presentation; *myeloid phenotype* characterized by M1 monocyte signature with abundance of NFKB-dependent cytokines such as TNF-alpha and IL1-beta; *low-inflammatory phenotype*; and *fibroid phenotype* characterized by genes related to fibroblast and osteoclast/osteoblast regulation, and angiogenesis. In this study, the myeloid phenotype (associated with the circulating marker ICAM1) was more represented in the group of anti-TNF responder patients compared to the lymphoid pathotype (associated with the circulating marker CXCL13), which was more represented in IL6 inhibitor responders (28). In 2019, Humby et al. carried out histopathology and molecular analysis of synovial biopsies in a treatment-naive early RA patient cohort and demonstrated that the "myeloid" synovial pathological groups were most strongly correlated with a greater response to DMARD treatment as opposed to the "pauci-immune/fibroid" group, less responsive to treatment (29). In another treatment-naive early RA patient cohort, a baseline synovial "lymphoid-myeloid pathotype" was significantly associated with the requirement of bDMARD in the subsequent 12 months of follow-up (21). In a recently published study, the "pauci-immune phenotype" achieved a lower clinical response to certolizumab pegol in comparison with lymphoid-myeloid and diffuse-myeloid pathotypes (30). The results of Humby et al. showed that when anti-TNF inadequate responder patients with RA were classified as B cell-poor and B cell-rich by RNA sequencing on synovial biopsies, different responses to successive treatments were observed. While in patients defined as B cell-rich the efficacy of rituximab overlapped with tocilizumab, in the B cell-poor group tocilizumab was more efficacious than rituximab (8). However, studies did not always lead to univocal conclusions: it remains unclear if the response to treatment in RA is dictated by the presence of a marker of response to a specific agent or rather by the presence of a marker of disease severity, including disease duration and the number of previous DMARDs, and consequently a poor probability of response. In this regard, GADD45B expression (macrophage marker of disease severity) in synovial tissue in an early RA patient cohort was significantly higher in non-responders to methotrexate (MTX) or any first-line therapy (31).

- ***Definition of disease remission***. Finally, a synovial biopsy could represent an additional tool to define "real remission" in patients with RA or PsA. Despite apparent clinical remission, about 60% of patients have evidence of a residual power Doppler ultrasound (PDUS) positive synovitis at ultrasound evaluation (32–34). This could explain the joint damage progression in these patients. For this reason, the concept of multidimensional remission has recently been introduced. It includes clinical parameters, PDUS or MRI, and normalization of synovium infiltrates. In particular, synovial mast cell density was independently associated with the clinical flare (35). Alivernini et al. showed that synovial histological features were comparable in patients with RA and PsA in clinical remission or low disease activity, despite being PDUS-negative. Residual synovitis persisted in PsA in clinical remission PDUS-negative patients (in terms of CD68+, CD3+, and CD31+). In this last scenario, treatment reduction or discontinuation would not appear safe in consideration of possible disease relapses (36). The analysis of possible prognostic biomarkers of disease relapse in patients with RA and PSA in remission is needed.
- ***Development* of *new targeted therapies.*** Few studies focused on a possible synovial marker reflecting an early therapeutic effect in the target tissue after a short duration of therapy in RA. By studying serial biopsies (at least two for each patient), a significant result was achieved considering the reduction in the number of sublining CD68+ macrophages as a marker of the effectiveness of treatment independent of the primary mechanism of action (37, 38). This synovial marker could therefore allow an early-stage screening of new therapeutics development on a smaller number of subjects and accelerated decisions in phase I–II clinical trials. In this context, the use of standardized and validated techniques to detect and quantify CD68 macrophages and to obtain reliable results remains critical. Finally, thanks to investigation on synovial tissue biomarkers new targeted therapies have been identified as recently described in detail. These results have the role of improving a more innovative stratified trial design that improves the treatment decision-making (39).

WHO SHOULD UNDERGO A SYNOVIAL BIOPSY?

Patients requiring synovial biopsy represent a selected group in whom specific insights for the differential diagnosis workup of the joint disorder are needed to differentiate the numerous and various entities of atypical and rare mono- or poly-articular joint diseases, or those agreeable to biopsy for research purposes (**Table 1**). Synovium analysis is crucial in the diagnosis of monoarthritis and undifferentiated polyarthritis when synovial fluid cannot be aspirated. Moreover, when synovial tumors, histiocytic or granulomatous disorders, deposition diseases, or infections are suspected, synovial biopsy is often required. What can be seen in the biopsy specimen is directly dependent upon the sample processing and analysis performed, hence on the clinical suspicion selecting who is the patient deserving the procedure. Intending to address the dialogue between the clinician and the pathologist, below listed are few specific clinical findings peculiar to rare diseases with the corresponding histological pattern (**Tables 2**, **3**).

Finally, in some cases, arthroscopy might have also therapeutic purposes; for example, during the surgical procedure, a joint lavage might be useful to treat septic arthritis to remove crystal deposits and sometimes to benefit patients with active RA/PsA (51).

TABLE 1 | Differential diagnosis: WHO deserves synovial biopsy?

Infectious diseases	Presenting mainly with monoarthritis Common bacteria Mycobacterium tuberculosis Fungal arthritis Parasitic arthritis Lyme disease Presenting mainly with polyarthritis Whipple disease Mycobacterium leprae
Deposition diseases	Crystal arthropaties Ochronosis Hemochromatosis Amyloidosis
Systemic diseases	Sarcoidosis Multicentric reticulohistiocytosis
Synovial tumors	Synovial cell sarcoma/synovial chondrosarcoma Pigmented villonodular synovitis Synovial chondromatosis Lymphoma Metastatic carcinoma
Others	Foreign-body arthritis

WHAT INFORMATION CAN YOU GET FROM THE SYNOVIAL BIOPSY?

In the context of refractory monoarticular arthritis, among the infectious etiologies (**Table 2**), typical bacterial agents are more easily suspected, and in the event of unsuccessful isolation of the microorganism, broad-spectrum antibiotics are available. On the other hand, atypical microorganisms deserve special attention due to their less evocative clinical presentation, belated diagnosis, and the need for specific treatments based on the identification of the agent.

To start with, mycobacterium tuberculosis is a typical example of indolent and sometimes destructive arthritis (seldom of a prosthetic joint), where synovial biopsy and culture are required for the diagnosis and for selecting the right treatment regimen given the spread existence of multidrug-resistance bacteria. A detailed history should consider the following: previous TB exposure, living/traveling in endemic areas, concomitant HIV infection, previous trauma causing direct tissue inoculation, and concomitant TNF inhibitor therapy. These forms of arthritis follow a chronic course preferentially involving large joints (hip, knee) and rarely associate with constitutional symptoms or pulmonary findings (41, 52). Fungal arthritis caused by a hematogenous or contiguous spread in the setting of candidiasis, coccidiosis, blastomycosis, scedosporiosis, cryptococcosis, and sporotrichosis are not easily recognized. The patient is usually immunocompromised or of extremes ages, with a background of farm working, traveling in endemic zones, previous surgery, and comorbidities such as diabetes, alcoholism, or intravenous drug abuse. Arthritis again often involves the knee, ankle, elbow, or wrist, and clinical hints are the evidence of coexisting osteomyelitis and extra-articular manifestations of pulmonary and cutaneous relevance. In the case of sporotrichosis, tenosynovitis and bursitis may be present (53).

Parasites (giardiasis, cryptosporidiosis) are usually mentioned as causative agents of rheumatologic disorders mainly due to immune-mediated mechanisms like reactive arthritis. However, sometimes symptoms are directly related to their infiltration of musculoskeletal structures such as in dracunculiasis, strongyloidiasis (54), filariasis (55), and bilharziasis (56) with a predilection for the ankle and knee. Red flags are endemic areas for parasitosis, poor hygiene conditions, hyper-eosinophilia, immunodeficiencies, and concomitant gastrointestinal or pulmonary involvement. It is important to bear in mind the hurdle of isolating and culture parasites, which require a rare medium, such as Harada-Mori moisture for strongyloidiasis or monkey kidney–mosquito cell lines for filariasis (57).

Whipple disease is another challenge that deserves to be mentioned. In 75% of cases, gastroenteric symptoms are

TABLE 2 | Main infectious etiologies for refractory monoarthritis: What do you find?

Microorganism	Medium	Stains	Histology
Mycobacterium tuberculosis (40)	Agar-based and egg-based media incorporating green malachite and Middlebrook broths or solid media	Ziehl-Neelsen	Caseating or non-caseating granulomas
Fungi (41)	Sabouraud's dextrose agar	Gomori methenamine silver, periodic acid Schiff	Candidiasis: thickened synovial membrane with non-specific mononuclear infiltration. Sporotricosis: mixed granulomatous and pyogenic processes. Rarely, asteroid bodies consisting of a central basophilic yeast surrounded by eosinophilic material radiating outward. Coccidioidosis: villonodular synovitis or typical pannus formation with non-caseating granulomas and sphreules containing coccidioidal endospores. Criptococcosis: both acute and chronic synovitis.
Mycobacterium leprae (42)	Almost impossible to culture in a laboratory; PCR techniques for detecting DNA exist, but are currently not used in clinical practice.	Fite-Faraco staining	Non-specific granulomatous synovitis, epithelioid cells

TABLE 3 | Main non-infectious etiologies for refractory mono- or poly-arthritis: WHAT do you find?

Disease	Histology
Ochronosis (43)	Paraffin-embedded sections show yellow-brown shards (able to provoke foreign body reactions with histiocytes and giants cells), scattered over the synovium and brittle pigmented articular cartilage. Haemosiderin and ochronotic pigment in macrophages, and focal inflammatory infiltrate of lymphocytes and plasma cells with some lining layer hyperplasia and hypervascularity may also be seen.
Hemochromatosis (44)	Low degree of synovial hyperplasia with mild infiltration of neutrophils, mononuclear cells -comprising macrophages- and lymphocytes; formation of synovial microvessels; haemosiderin deposition in the synovial lining cells; CPPD crystals may be seen.
Amyloidosis (45)	Diagnosis of amyloidosis requires Congo red staining to show amyloid deposits in the synovium. The immunohistochemical study allows typing of amyloidosis: antibodies directed against light chains of immunoglobulins (AL-amyloidosis), antibodies against the other major amyloid proteins (AA and ATTR).
Multicentric reticulohistiocytosis (46, 47)	Lipid-laden multinucleated giant cells and histiocytes with ground glass PAS-positive cytoplasm, which contains membrane-bound lysosomal granules, with a single large stellate Golgi apparatus.
Sarcoidosis (48, 49)	Histopathological examination of synovium reveals various patterns: diffuse infiltration with histiocytes and lymphocytes, mild lining cell proliferation, seldom vascular congestion. A granulomatous reaction is often absent.
Crystal arthropathies (50)	Gout and pseudogout: deposits of monosodium urate crystals or calcium pyrophosphate dihydrate crystals in the synovium after fixation with absolute alcohol and analysis with a polarization microscope, which shows to evaluate birefringence properties (negative MSU, positive CPPD), or DeGolanthal staining method. Basic calcium phosphate induced arthritis: crystals can be seen using alizarin red staining or transmission or scanning electron microscopy.

preceded by a seronegative oligo- or polyarthritis with a relapsing course (58), and when the diagnosis remains unclear, biopsy specimen clarify the suspicion showing PAS-positive macrophages beneath the synovial lining cells (59).

Arthritis represents one of the well-known late-stage complications of Lyme disease, especially in the United States. After having investigated prior tick exposure, hazard occupation or hobbies (forestry workers, hunters, and hikers), or previous cutaneous manifestation, the clinician will combine serology and PCR-based testing for B. burgdorferi DNA in the synovial fluid to confirm the diagnosis (60). However, some patients, even after being treated, will develop postinfectious antibiotic-refractory arthritis, where synovial biopsy, which usually shows synovial cell hypertrophy, mononuclear infiltration, vascular proliferation, and sometimes obliterative microvascular lesions, could have a role in the understanding of the chronicity of the process which resembles inflammatory arthritis (61).

Keeping in mind the plethora of the aforementioned microorganisms and their relative hints, once the clinical suspicion arises, the dialogue opens up with the infectiologists, microbiologists, and pathologists to manage properly the analysis of the synovial tissue with their respective culture medium and expected histologic findings (**Table 2**).

Amidst deposition diseases, there are few which may manifest as monoarthritis, occasionally resembling aggressive osteoarthritis. Ochronosis is an autosomal recessive disorder where the homogentisic acid oxidation products are in excess and therefore deposit in the connective tissue, making it stiffened and brittle, ultimately leading to ochronotic arthropathy. Suspicion should arise if a patient in its fourth decade of life starts having back pain and subsequently knee pain (or hip, shoulder) with radiological findings showing knee osteoarthritis and wafer-like disc calcification with a reduction of intervertebral spaces in the spine. The clinical examination may reveal deposits of bluish or brownish pigment in the ear cartilage and sclerae (62).

Hemochromatosis arthropathy, where iron in excess deposits in the synovial tissue, may virtually involve any joint. The most reported symptoms resemble osteoarthritis and less often recurrent synovitis. Clinical clues comprise the involvement of the second and third metacarpophalangeal joints with their corresponding radiographic findings (hook-like osteophytes), chondrocalcinosis, abnormal liver enzymes, and hyperferritinemia (63).

In the context of monoarthritis, histology remains the gold standard to characterize the nature of proliferative lesions (**Table 1**). However, most of the time imaging is sufficient to show abnormalities that raise the suspicion and frequently differentiate a local proliferative lesion (50). The topic will be not covered by this review due to its only partial rheumatologic relevance.

After evaluating challenging disease, it is worth mentioning crystal arthropathies, which are supposedly a straight diagnosis. When clinical, instrumental, and synovial fluid analyses are not conclusive, and some uncertainty remains, it must be kept in mind that to indentify crystals under polarizing microscopy, the synovial tissue needs to be processed with absolute alcohol, which is not usually done in the routine analysis, because other fixatives dissolve monosodium urate crystals (45).

Synovial biopsy may be also useful in rheumatic polyarticular disorders (64–68). Osteoarticular manifestations of amyloidosis depend upon the mispleated protein (46). Amyloid light chain (AL) amyloidosis usually presents with an RA-like pattern half of the time associated with cutaneous nodules periarticular or on the extensor surfaces. Bilateral carpal tunnel syndrome is also a frequent finding. Male predominance, the pseudotumoral aspect of the swollen joints, poor response to steroids, radiological evidence of well-circumscribed lytic

lesions together with monoclonal gammopathy, macroglossia, hepatomegaly, and peripheral neuropathy should raise the index of suspicion toward amyloidosis. Transthyretin amyloidosis, whether hereditary or senile, mainly manifests as carpal tunnel syndrome due to peripheral neuropathy often starting with sensitive and autonomic symptoms. Other red flags are concomitant arrhythmias and heart failure symptoms.

Likewise, multicentric reticulohistiocytosis mimics RA, progressing up to a mutilans phenotype. It mainly affects women of Caucasian origin in their fourth decade. While laboratory findings are hardly helpful or specific, except by excluding other etiologies, few clinical hints are the involvement of the distal interphalangeal joints, the appearance of papulonodular skin lesions especially affecting the face and hands, and in 25% of cases the association with neoplasia (69).

Finally yet importantly, among the infectious agents, leprosy is one of the diseases where biopsy remains of key importance to demonstrate the presence of the bacilli in the joint. Arthritis in leprosy may be polymorphic, including acute or chronic polyarthritis, septic arthritis, and Charcot's arthropathy. Clinical clues comprise skin lesions and symptoms suggestive of motor-sensory neuropathy in the context of an endemic area (70).

Sarcoidosis is well-known for being polymorphic, with myriads of different musculoskeletal manifestation: acute arthritis (Lofgren syndrome with symmetric hilar adenopathy and erythema nodosum), chronic symmetric, medium to large joint oligoarthritis (especially manifesting with tenosynovitis and skin involvement), up to Jaccoud arthropathy and dactylitis. During the diagnostic management, X-rays could show bone involvement, equally various with different patterns of bone lesions ("moth-eaten," lytic, and sclerotic lesions). The diagnosis is always challenging, and synovia is one of the precious target tissues that can contribute to it (71).

WHERE SHOULD SYNOVIAL BIOPSY BE PERFORMED?

Synovial biopsy is an invasive procedure; thus, the results expected have to be relevant and informative. All joints are not the same and vary greatly in their vulnerability to different rheumatic diseases. Thus, the choice of the joint to biopsy is crucial and should be guided based on the rheumatologist's purposes.

For example, synovial tissue analysis might be instrumental for the differential diagnosis between rheumatic and non-rheumatic conditions (see paragraph above) of mono-arthritis. In this context, the affected joint should be chosen.

By contrast, in patients with RA, the choice of the joint where synovial biopsy is performed might be based on the published literature (72).

Concerning the inter-joint heterogeneity, in the same patient, it has been demonstrated that synovial samples, taken at the same time, from an active joint are generally representative of other inflamed joints (72, 73). In particular, they provide evidence that cell infiltration of the synovial sublining (i.e., macrophages, T cells, B cells, plasma cells, and IL-6 expression) is similar in large and small joints (74).

In the same patient with RA, a comparison between synovial biopsies in clinically involved and non-involved knee showed that a considerable degree of histological changes, mainly hyperplasia of the synovial lining layer, was present in the uninvolved joint, although changes were less severe than those observed in active joints (72, 73).

Of note, intra-joint variability has also been documented as inflammatory mediators might be differently expressed in different locations of the same joint. In particular, tissue samples from sites close to the cartilage-pannus junction showed a higher level of inflammatory biomarkers that might be underestimated by analyzing specimens from other joint sites (75–77). Although the numbers of T and plasma cells are reported to be similar in biopsy samples (78), one study did find a difference for macrophages (73), but other studies did not confirm this finding (78).

Thus, it is still a matter of debate on the best location from which to obtain synovial tissue samples within a given joint. Recently, an international expert consensus stated that a minimum of four synovial tissue specimens from small joints and six from large joints have to be retrieved for reproducible research studies (79–81), while a previous study showed that using US-guided biopsy of hand joints 12 different samples are recommended to have a valid immunohistochemical assessment (82). During the disease course, the immunohistological features vary when consecutive synovial biopsies from the same joint are analyzed. In the 80s, it has been shown that synovial biopsy of the affected knee in RA patients changes in terms of T and B cells infiltrates according to disease activity when pre- and post-treatment were assessed (39, 51). For this reason, synovial biopsy has also been proposed as a biomarker to evaluate drug response (19, 21, 52).

HOW TO PERFORM A SYNOVIAL BIOPSY

Synovial tissue samples can be retrieved by using different techniques (**Table 4**) (1). Tissue samples are commonly collected using blind-needle, ultrasound-guided, or arthroscopic-assisted biopsy procedures, but in specific cases, larger synovial samples can also be obtained during an open surgical procedure. In particular, ultrasound-guided needle biopsy (from small and large joints), ultrasound-guided portal and forceps procedures, and arthroscopy are equally successful in sampling synovial tissue and they yield sufficient tissue quantity for transcriptomic studies (83). Moreover, these techniques do not differ in safety or patient tolerability (84). Needle techniques are less invasive for the patients and permit obtaining good-quality synovial tissue in most cases.

Blind-Needle Biopsy

Blind-needle methods were described in 1950 by Polley et al. with the use of 5-mm-large needles, resulting in a practical but invasive procedure for the modern standards considering that the needle size is similar to the new arthroscopy instrument size. In the years, new and thinner needles have been introduced in

TABLE 4 | Characteristics of the different techniques used for obtaining a synovial sampling from different joints.

	Standard arthroscopy	Needle arthroscopy	Ultrasound needle biopsy	Blind needle biopsy
Technical issues				
Synovial sampling success rates	+++	++	++	+
Technically simple	+	++	++	+++
General issues				
Acceptability	++	+++	+++	+++
Serial biopsies	+++	+++	+	+
Costs	+++	+++	++	+
Target joints				
Large Joints	+++	+++	+++	+++
Small Joints	++	+++	+++	+

the market, simplifying the histologic investigation of articular rheumatic diseases and allowing to perform these procedures without significant pain for the patients, reducing the risk of post-procedural complications (85). Parker and Pearson were the first clinicians to propose a new technique using a composite of two standard items, a 14-gauge thin-walled needle with matching stylet and a 15-gauge aspirating needle with a hook-like beveled tooth that can catch the tissue (40). This instrumentation has been improved in the years to make it more effective, and many semiautomatic guillotine biopsy needles are available on the market to perform a needle biopsy. After disinfection, the skin, subcutaneous tissue, and joint capsule are anesthetized. Anatomical references for the specific joint can be identified with a marking pen to recognize the entry point correctly. Injection of 10–20 cc of isotonic saline into the joint can help the clinicians obtain some material if there is no clinical evidence of effusion. In standard technique, the larger needle is inserted into the joint without a skin incision, and the smaller needle is slipped snugly through it. The needle tip is then entered into the synovial tissue, and its specific hook-design allows to obtain selected tissue when it is withdrawn from the larger needle. Multiple tissue samples can be obtained by changing the direction of the needle (42). This painless procedure gained significant popularity and is considered the basis of modern synovial biopsy techniques due to its numerous advantages like minimal trauma for joint tissues, the possibility of obtaining several samples in one procedure, or performing serial synovial biopsies from the same joint at different times in an outpatient setting. This technique can be performed quickly and with good results in larger joints as the knee; smaller needles can also obtain samples in smaller joints such as the wrist and the ankle. By contrast, the operator cannot have real-time control of the biopsy site. It has been shown that the blind-needle method is less reliable than ultrasound-guided procedures for sampling synovial tissue from the small and large joints (83).

Ultrasound-Guided Needle Biopsy

Using a blind-needle biopsy technique, the clinicians achieve the procedure without a direct or indirect view, and it is not always possible to have an adequate tissue sample, especially in joints lacking a significant effusion. Some authors described a technique of synovial biopsy under fluoroscopy visualization with a semiautomatic needle. This technique allows the performance of multisite biopsies such as in the hips, shoulders, elbows, ankles, and wrists but requires a complex setting, exposing the patients to x-ray irradiation. Performing harvesting with the aid of ultrasounds could combine the low morbidity of a needle biopsy and the instrumental support's accuracy without ionizing radiation exposure. In recent years, many authors have described good results of ultrasound-guided needle biopsy (43, 44). The skin disinfection and the local anesthesia can be achieved as described in the blind-needle technique; the transducer has to be covered with sterile gel and sterile sheath. The procedure is similar to the already described technique, with all the passages performed under ultrasound control. Authors that published results of this technique described a high success rate of the procedure, with only rare and minor complications (44). Ultrasound-guided needle biopsy and arthroscopic methods are equally successful when sampling synovial tissue from large joints (83).

Arthroscopic-Guided Synovial Biopsy

Arthroscopic-assisted synovial biopsy is a surgical technique in which the tissue is harvested under the direct view of the suspected pathological area's region, dramatically reducing the risk of mistakes. The technique is a standard joint arthroscopy, with a second portal required to harvest the material of interest using specific instrumentation. This approach's advantages are obtaining more significant macroscopic evaluation pieces, with better sampling from interest areas. Also, arthroscopic synovial biopsy techniques allow biopsies from sites adjacent to the cartilage (47). This area differs quantitatively and qualitatively from the synovium, and collecting tissue with a standard needle technique can be challenging and, in some cases, impracticable. The arthroscopic-assisted technique limits are related to the fact that it is a proper surgical procedure: the need for at least two skin incisions, a longer "learning curve," and the requirement of a sterile area and operation theater facilities. These procedures are performed in many hospitals by trained orthopedic surgeons, requiring teamwork among different specialists.

Needle Arthroscopy

A new impressive field of research is needle arthroscopy, where clinicians can use in local anesthesia small modern devices, which permits an exploration of the joint in an outpatient setting and obtain tissue samples under direct view. This well-tolerated procedure allows good macroscopic evaluation of synovial inflammation and selective sampling of the synovial membrane (76).

Finally, surgeons can obtain samples of synovial tissue during an open surgical procedure as a total joint replacement. This technique permits obtaining a relevant quantity of tissue and can help obtain synovial tissue useful for clinical and histological studies in rheumatic patients.

CONCLUSION

Within the past decades, several considerable advances have been made in synovial tissue research. Synovial tissue represents the target tissue of many rheumatic and non-rheumatic diseases, and its analysis is crucial in the assessment of many infective, malignancy, and infiltrative disorders. Retrieving synovial tissue samples of good quality using affordable and safe methods from large and small joints is now a realistic desirable objective. Thus, clinical practice pathobiology might be a key component in the management and treatment decision-making process, even in rheumatology.

AUTHOR CONTRIBUTIONS

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