# The 2018 OMERACT Synovial Tissue Biopsy Special Interest Group Report on Standardization of Synovial Biopsy Analysis

Mihir D. Wechalekar, Aurélie Najm, Douglas J. Veale, and Vibeke Strand

**ABSTRACT.** Objective. The Outcome Measures in Rheumatology (OMERACT) synovial tissue biopsy (STB) working group initiated an international effort to standardize STB analyses, define consensual items to inform treatment choices, and predict responses in rheumatoid arthritis (RA).

*Methods.* (1) A Delphi survey to determine items for STB analyses. (2) A multicenter retrospective study of STB data in patients with RA posttreatment with biological disease-modifying antirheumatic drugs.

*Results*. The Delphi survey identified 18 STB analyses items. Consensus on histological markers was achieved in the OMERACT 2018 SIG.

*Conclusion.* Six markers were identified for examination in a multicenter study designed to define an OMERACT-endorsed set of STB markers to predict responses to treatment. (First Release January 15 2019; J Rheumatol 2019;46:1365–8; doi:10.3899/jrheum.181062)

*Key Indexing Terms:* OMERACT

**SYNOVIUM** 

Despite current therapeutic advances in rheumatoid arthritis (RA), predicting and achieving adequate treatment responses and remission remains elusive for a substantial proportion of patients. Indeed, even with biological disease-modifying antirheumatic drugs (bDMARD), rates of remission and improvement in function are suboptimal<sup>1,2</sup>. RA is a heterogeneous disease clinically and pathologically<sup>3,4</sup>, so one explanation for low response rates may be the current nontargeted, "trial and error" use of conventional DMARD (cDMARD) and bDMARD. Although there have been several attempts to identify biomarkers in peripheral blood

From the Rheumatology Unit, Flinders Medical Centre, Bedford Park and College of Medicine and Public Health, Flinders University, Adelaide, Australia; Rheumatology Department, Centre Hospitalier Universitaire (CHU) de Nantes, and INSERM UMR 1238, Faculty of Biology of Nantes, Nantes, France; The Centre for Arthritis and Rheumatic Diseases, St. Vincent's University Hospital and Dublin Academic Medical Centre, University College Dublin, Elm Park, Dublin, Ireland; Division of Immunology and Rheumatology, Stanford University, Palo Alto, California, USA.

M.D. Wechalekar, PhD, Rheumatologist, Flinders Medical Centre and Senior Lecturer, Flinders University; A. Najm, MD, Rheumatology Department, CHU de Nantes, and INSERM UMR 1238, Faculty of Biology of Nantes; D.J. Veale, PhD, Director of Translational Research, Dublin Academic Medical Centre, Adjunct Professor of Medicine, University College Dublin, Fellow of The Conway Institute of Biomedical and Biomolecular Research, and Consultant Rheumatologist, St. Vincent's University Hospital; V. Strand, MD, Biopharmaceutical Consultant.

Address correspondence to Professor D.J. Veale, St. Vincent's University Hospital, Elm Park, Dublin, D4, Ireland.

E-mail: douglas.veale@ucd.ie

Accepted for publication November 20, 2018.

to guide therapeutic choices for individual patients<sup>5,6,7</sup>, they have generally proven inconclusive and inconsistent.

Because the synovium is the major target of disease and a robust measure of treatment response in RA<sup>8</sup>, it is probable that the search for biomarker(s) to guide therapy and predict response to treatment may best be addressed by synovial tissue biopsy (STB) analyses<sup>9,10,11,12</sup>. Indeed, despite current therapeutic approaches, 30–40% of patients are resistant to therapy, having persistent synovitis and erosion progression<sup>11</sup>. An alternative "pathobiological" approach<sup>13</sup> basing treatment selection upon individual patient factors such as STB analyses could provide a better rationale for the selection of appropriate bDMARD, resulting in better individual patient responses.

STB are not currently considered standard of care in randomized controlled trials or for choice of therapy for a variety of reasons <sup>14,15</sup>, including relative lack of access and technical expertise in their performance and evaluation <sup>16</sup>. An important step advancing their use occurred at the OMERACT 2016 STB SIG, establishing the feasibility, reliability, and validity of minimally invasive ultrasound (US)-guided synovial biopsy techniques according to the OMERACT Filter 2.0<sup>14,17,18</sup>.

Regarding evaluation and interpretation of STB, considerable heterogeneity remains across centers. Before addressing more advanced techniques to interpret STB, such as RNA sequencing and transcriptomics<sup>19</sup>, standardization of standard histologic and immunohistochemistry (IHC) analysis is critical. Given the relative ease of performing

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2019. All rights reserved.

histological semiquantitative analysis (SQA), it is likely to be the most feasible technique to direct treatment choices, facilitating rapid decision making from the bench to the bedside.

However, before proposing routine use of standardized STB histologic and IHC techniques to guide therapy, several factors must be satisfied, including (1) uniformity of biopsy handling and analyses across centers, (2) creation of a uniform quality score, (3) validation of the score to identify the relationship between immunopathology and therapeutic responses, and (4) identification of novel STB markers of disease phenotype and outcomes and to match these with circulating biomarkers and imaging techniques. Hence, the strategic aims and objectives of the OMERACT STB SIG were to initially conduct a Delphi survey regarding STB handling and analytic procedures, followed by analysis of STB histological markers to assess whether they can (1) guide choices of appropriate therapeutic agents, (2) predict responses to treatment, and thereby (3) define a consensual set of histological items to be used for prediction of therapeutic responses in RA.

#### MATERIALS AND METHODS

The initial Delphi survey (Table 1 and Supplementary Data, available with the online version of this article) invited participation from relevant experts in performance and analysis of STB. The survey was conducted in 3 stages. The first stage included 44 items in 2 distinct clinical parts: clinical practice and translational research. Each participant rated each item on a scale of 1 to 5 (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree). The second round constituted iterative refinements from the earlier round.

Based on the consensual set of items that emerged from this Delphi, the OMERACT STB SIG decided to perform a study to determine whether STB histological markers can be used to guide treatment choices, predict responses, and define a consensual set of histological items for future use.

Table 1. The key areas assessed in the Delphi survey for performance and analysis of synovial tissue biopsy.

Biopsy sampling

No. samples retrieved per large or small joint and from different areas of the joint

Biopsy processing

Duration of time biopsies should be kept in formalin prior to being processed

Histological criteria

Biopsy area

Specific morphological criteria including presence of lining layer and preservation of tissue architecture

Staining and immunohistochemistry

Requirement for H&E and staining for specific mandatory or optional immunohistochemical cellular markers (CD68, CD3, CD19, CD20, etc.)

Biopsy interpretation

Requirement for synovitis score, pathotype, presence of lymphoid follicles

Type of analysis: semiquantitative or quantitative, and no. areas of biopsy to be assessed

RNA analysis

Pooling of biopsies for RNA extraction

This analysis of STB histological markers will be performed retrospectively on collected STB at baseline and followup ≥ 6 months in biologic treatment–naive patients with RA [fulfilling the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 criteria for diagnosis] following initiation of treatment with bDMARD. Synovial biopsies will be stained for H&E, CD68+, CD3+, CD19+, or CD20+, presence of lymphoid aggregates, and staining of blood vessels.

Following initial quality control, all slides will be independently scored (using SQA) by 2 observers at each center who are blinded to the design of the study and responses to the treatment. Each observer will be asked for a percent likelihood of response to each of the following bDMARD: interleukin 6 blockade, T cell costimulation inhibition, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) blockade, and anti-CD20, depending on histological features.

Statistical analyses will include descriptive analyses, logistic regression, and  $\kappa$  coefficients for each histological marker.

## RESULTS

Delphi exercise: first round. Twenty-seven experts from 19 centers were contacted by e-mail. Twenty participants (85%) from 18 centers responded; of these, 19 (95%) were rheumatologists, and 1 (5%) was a pathologist. Twenty-three out of 44 items (52.3%) from the clinical practice portion of the first questionnaire were selected for the second round based on their score and percentage of agreement. Of these, 5 remained unchanged, 16 were modified, and 2 were added according to participants' comments. Nineteen out of 43 items (44%) from the translational research component of the first questionnaire were selected for the second round; of these, 10 remained unchanged and 9 were modified according to participants' comments; no new item was proposed.

Delphi exercise: second round. From the clinical practice component of the second round, 20/23 (87%) and 18/19 items (95%) from the clinical practice and translational research components, respectively, were selected for the third round.

Face-to-face meeting. Results of the second round were disseminated through participating members and orally presented to the task force at the EULAR Synovitis Study Group meeting in June 2017. All task force members agreed on the final set of items. Items with a median score > 3.5 and percentage of agreement > 70% were selected for further rounds. Statistics were performed using GraphPad Prism 6.0. Results of synovial biopsy study. The planned multicenter (n = 5 per center) study will analyze STB histological markers for macrophages, T cells, B cells, lymphoid aggregates, and blood vessels to determine likelihood of response to treatment with bDMARD (tocilizumab, abatacept, TNF inhibitor, or rituximab). These results will be presented once the planned study has been completed.

Results from discussion at OMERACT 2018. At the OMERACT STB SIG, results of the Delphi survey and methodological details of the next component (analysis of histological markers on retrospectively collected STB) were presented and discussed.

A consensus was reached regarding quality control for histology and SQA and IHC markers to be used in STB analyses. It was decided that the next steps would be the

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2019. All rights reserved.

finalization of participating centers and a uniformly agreed-upon protocol, to establish an atlas of SQA to ensure uniformity across centers and provide a reference standard for future studies. A qualitative study on participant experiences with arthroscopy and US-guided STB was also proposed.

#### DISCUSSION

Standardization of synovial tissue analysis as a predictor to guide therapy and response to treatment. Although there is considerable literature on responsiveness to changes in RA disease activity following treatment with c/bDMARD<sup>16</sup>, there are limited data, despite a strong pathobiologic rationale that demonstrates the usefulness of STB guiding treatment choices<sup>13</sup>. This is particularly relevant in the current context of availability of bDMARD with diverse mechanisms of action, but relatively similar responses across treatments<sup>13</sup> and may likely reflect synovial pathotype heterogeneity as shown in a recent prospective study that reported higher levels of B cell infiltrates and lymphoid aggregates in anticitrullinated protein antibody-positive patients<sup>20</sup>. Another notable study by Dennis, et al<sup>9</sup> revealed better responses to TNF inhibitors with an underlying "myeloid" as opposed to "lymphoid" or other synovial phenotypes.

Another important factor contributing to the heterogeneity of results is the varying methodology assessing STB across centers. Although analysis of IHC by SQA has been used as one of the fastest quantification techniques, it is subject to observer error. Despite attempts to reduce this error by observer training and standardization and using 2 observers and a consensus score, none of these methods have been thoroughly validated <sup>16</sup>.

Data and insights following discussion at OMERACT 2018. Following the OMERACT STB SIG, further steps are to finalize a staining protocol, and formally invite STB centers of excellence to participate in the study, which will be undertaken through the EULAR/ACR STB SIG. A quality-control exercise will follow, which will result in an atlas of SQA as the reference standard for this and future studies. Following patient selection across centers and appropriate IHC staining, slides will be uploaded onto a central server for blinded analyses, followed by formal statistical analyses, including histopathological correlations with clinical and imaging outcomes.

Following this exercise, it is expected that the OMERACT STB working group will be able to create and validate an acceptable uniform quality score, gain insights into histological markers that will help identify relationships between synovial immunopathology and treatment responses (or lack thereof), and facilitate identification of novel STB biomarkers of disease phenotype and outcomes and match these with circulating biomarkers and imaging techniques.

# ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

## REFERENCES

- Hetland ML, Christensen IJ, Tarp U, Dreyer L, Hansen A, Hansen IT, et al; All Departments of Rheumatology in Denmark. Direct comparison of treatment responses, remission rates, and drug adherence in patients with rheumatoid arthritis treated with adalimumab, etanercept, or infliximab: results from eight years of surveillance of clinical practice in the nationwide Danish DANBIO registry. Arthritis Rheum 2010;62:22-32.
- Callhoff J, Weiß A, Zink A, Listing J. Impact of biologic therapy on functional status in patients with rheumatoid arthritis—a meta-analysis. Rheumatology 2013;52:2127-35.
- van der Pouw Kraan TC, van Gaalen FA, Kasperkovitz PV, Verbeet NL, Smeets TJ, Kraan MC, et al. Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the Stat-1 pathway between rheumatoid tissues. Arthritis Rheum 2003;48:2132-45.
- Weyand CM, Klimiuk PA, Goronzy JJ. Heterogeneity of rheumatoid arthritis: from phenotypes to genotypes. Springer Semin Immunopathol 1998;20:5-22.
- Lequerré T, Gauthier-Jauneau AC, Bansard C, Derambure C, Hiron M, Vittecoq O, et al. Gene profiling in white blood cells predicts infliximab responsiveness in rheumatoid arthritis. Arthritis Res Ther 2006:8:R105.
- Koczan D, Drynda S, Hecker M, Drynda A, Guthke R, Kekow J, et al. Molecular discrimination of responders and nonresponders to anti-TNF alpha therapy in rheumatoid arthritis by etanercept. Arthritis Res Ther 2008;10:R50.
- Sekiguchi N, Kawauchi S, Furuya T, Inaba N, Matsuda K, Ando S, et al. Messenger ribonucleic acid expression profile in peripheral blood cells from RA patients following treatment with an anti-TNF-alpha monoclonal antibody, infliximab. Rheumatology 2008;47:780-8.
- Bresnihan B, Pontifex E, Thurlings RM, Vinkenoog M, El-Gabalawy H, Fearon U, et al. Synovial tissue sublining CD68 expression is a biomarker of therapeutic response in rheumatoid arthritis clinical trials: consistency across centers. J Rheumatol 2009;36:1800-2.
- Dennis G Jr, Holweg CT, Kummerfeld SK, Choy DF, Setiadi AF, Hackney JA, et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. Arthritis Res Ther 2014:16:R90.
- Lindberg J, af Klint E, Catrina AI, Nilsson P, Klareskog L, Ulfgren AK, et al. Effect of infliximab on mRNA expression profiles in synovial tissue of rheumatoid arthritis patients. Arthritis Res Ther 2006;8:R179.
- Pitzalis C, Kelly S, Humby F. New learnings on the pathophysiology of RA from synovial biopsies. Curr Opin Rheumatol 2013;25:334-44.
- Orr C, Vieira-Sousa E, Boyle DL, Buch MH, Buckley CD, Canete JD, et al. Synovial tissue research: A state-of-the-art review. Nat Rev Rheumatol 2017;13:463-75.
- Astorri E, Nerviani A, Bombardieri M, Pitzalis C. Towards a stratified targeted approach with biologic treatments in rheumatoid arthritis: role of synovial pathobiology. Curr Pharm Des 2015;21:2216-24.
- 14. Humby F, Kelly S, Bugatti S, Manzo A, Filer A, Mahto A, et al. Evaluation of minimally invasive, ultrasound-guided synovial biopsy techniques by the OMERACT filter—determining validation requirements. J Rheumatol 2016;43:208-13.
- 15. van de Sande MG, Gerlag DM, Lodde BM, van Baarsen LG, Alivernini S, Codullo V, et al. Evaluating antirheumatic treatments using synovial biopsy: a recommendation for standardisation to be used in clinical trials. Ann Rheum Dis 2011;70:423-7.
- Smith MD, Baeten D, Ulfgren AK, McInnes IB, Fitzgerald O, Bresnihan B, et al; OMERACT synovial special interests group.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2019. All rights reserved.

- Standardisation of synovial tissue infiltrate analysis: how far have we come? How much further do we need to go? Ann Rheum Dis 2006;65:93-100.
- Boers M, Kirwan JR, Tugwell P, Beaton D, Bingham CO III, Conaghan PG, et al. The OMERACT Handbook. [Internet. Accessed May 17, 2017.] Available from: https://omeract.org/resources
- Boers M, Kirwan JR, Wells G, Beaton D, Gossec L, d'Agostino MA, et al. Developing core outcome measurement sets for clinical trials: OMERACT filter 2.0. J Clin Epidemiol 2014;67:745-53.
- Guo Y, Walsh AM, Canavan M, Wechalekar MD, Cole S, Yin X, et al. Immune checkpoint inhibitor PD-1 pathway is down-regulated in synovium at various stages of rheumatoid arthritis disease progression. PLoS One 2018;13:e0192704.
- Orr C, Najm A, Biniecka M, McGarry T, Ng CT, Young F, et al. Synovial immunophenotype and anti-citrullinated peptide antibodies in rheumatoid arthritis patients: Relationship to treatment response and radiologic prognosis. Arthritis Rheumatol 2017;69:2114-23.