The complex relationship between clinical disease activity, synovial inflammatory profile and treatment response in RA

Clément Triaille, Ph.D.[1-3], Pr. Patrick Durez[1,3], M.D., Francesco Natalucci[1,3], M.D., Pr Rik Lories[4,5], Ph.D., Pr Peter C. Taylor[6], Ph.D.

1: Pôle de pathologies rhumatismales systémiques et inflammatoires, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium

2: Department of Pediatric Hematology, Oncology, Immunology and Rheumatology, Cliniques Universitaires Saint-Luc, Brussels, Belgium

3: Department of Rheumatology, Cliniques Universitaires Saint-Luc, Brussels, Belgium

4: Development and Regeneration Dep., Skeletal Biology and Engineering Research Centre, KU Leuven, Leuven, Belgium

5: University Hospitals Leuven - UZ Leuven, Division of Rheumatology, Leuven, Leuven, Belgium

6: Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom

Corresponding authors:

Dr Clément Triaille

Pôle RUMA, Institut de Recherche Expérimentale et Clinique, avenue Hippocrate 10, B-1200 Bruxelles, Belgique
email : clement.triaille@uclouvain.be

Pr Peter C. Taylor

Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Headington, Oxford OX3 7LD, UK

email: peter.taylor@kennedy.ox.ac.uk

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Abstract

The synovial tissue (ST) is widely considered as a strong candidate in the development on individualized therapeutic strategies in rheumatoid arthritis (RA). Recently, several factors have contributed to major developments in ST analysis: (*i*) improvement in synovial tissue biopsy techniques, (*ii*) availability of powerful biotechnologies with ever-increasing granularity, (*iii*) recruitment of larger cohorts of patients, (*iv*) development of recommendations to standardize ST analysis, and (*v*) an expanded therapeutic armamentarium of targeted therapies. While some recent studies have suggested the existence of RA subtypes based on the ST inflammatory profile, with potential therapeutic implications, other works have yielded different results.

In this *Viewpoint*, we discuss and put in context the findings of the recent major studies in the field of ST. We highlight how disease activity, ST inflammatory burden, and response to therapy are interdependent features in RA, both in early RA and later in the disease course. From there, we discuss how this multidirectional relationship has impacted (and potentially influenced the interpretation of) the findings of ST-based studies. Finally, we discuss the different hypotheses explaining the link between ST, clinical features and therapeutic response.

Rheumatoid arthritis (RA) is an idiopathic inflammatory condition associated with systemic autoimmune features and alterations/damage in target tissues, predominantly the synovial joints. Synovial pathology has long been of interest to rheumatologists but in recent years, there has been a resurgence of research focussed upon synovial tissue (ST) analysis as new technologies have emerged that allow detailed cellular and molecular interrogation of tissue such as bulk and single-cell RNA-sequencing and spatial transcriptomics. Furthermore, improvements in advanced analytic and bioinformatics approaches, and in study design (*i.e.* recruitment of significantly larger cohorts, randomized controlled trials including ST analysis, investigation of early and untreated patients) have contributed to an impressive new body of literature. ST analysis has greatly contributed to the understanding of RA pathophysiology. In view of the striking inter-patient heterogeneity in synovial features, it has also been proposed to have the potential to contribute to individualized patient care through prognostic or theranostic prediction (**Figure 1**). This latter concept has led to much hope and optimism with respect to informing a rational choice of pharmacotherapeutic intervention on an individual basis from a range of treatment mechanisms of very similar efficacy at a cohort level (1). But in reality, does the collective evidence thus far live up to expectations of ST-based precision medicine? In this viewpoint article we will discuss some important knowledge gained regarding the complex relationships and interdependency between clinical disease activity, the synovial inflammatory pattern and treatment response, first in early untreated RA (ERA), then in patients under therapy with long disease duration.

ST research originally focused on advanced RA, with most historical investigations performed on donated synovium harvested at the time of joint replacement surgeries and therefore acquired in the long-established phase of disease. It has since been recognized that disease duration and treatment exposure impact the ST profile and, as such, represent potential confounding factors when comparing patients (2-4). More recently, large cohorts analyzing ST obtained by biopsy in early, untreated RA (ERA) have been published (**Table 1**). In an attempt to define predictors of outcomes, it has been proposed in the pioneer PEAC cohort to classify ERA patients according to their synovial cellular and transcriptomic profiles (the latter being defined by the expression of predefined sets of genes measured using bulk RNA-seq): the so-called pathotypes (5). Thus, three subgroups were initially suggested in a cohort of 144 ERA patients: lympho-myeloid, diffuse

myeloid and pauci-immune. The expression of lympho-myeloid and diffuse myeloid gene sets showed a positive correlation with most clinical indices of disease activity; the lympho-myeloid gene expression pattern was also associated with progressive erosive disease and seropositivity. Furthermore, the expression of these 2 genesets showed a correlation with a larger absolute decrease in the DAS28CRP disease activity score after 6 months of csDMARD therapy (mainly methotrexate). Another analysis of this cohort (with genome-wide RNAseq) confirmed that a more

inflammatory synovial phenotype at baseline correlated with a higher baseline disease activity, and a greater fall in DAS28-CRP after 6 months of csDMARD treatment (6). The authors consequently hypothesized that these subgroups represent endotypes – subgroups of RA driven by distinct mechanisms, which could be preferentially targeted by different therapies to increase response rate.

While these findings and hypotheses have been widely documented and discussed, some limitations should be considered. First, glucocorticoid usage was not controlled, meaning that patients with more active disease would likely be more susceptible to receive glucocorticoids. More crucially, another potential confounding factor, namely the intrinsic correlation between baseline disease activity and amplitude of response, has not been corrected for, an issue we will discuss in the following paragraphs.

In studies from the UCLouvain team, RNAseq was performed on ST of 74 treatment-naïve ERA using unbiased approaches. We found that disease activity was the major correlate of transcriptomic heterogeneity across patients (7). Rather than distinct subgroups, we found a continuum of samples spanning from patients with lower disease activity and lower expression of inflammatory pathways, to more active disease and strong expression of inflammation-associated genes (both from lymphoid and myeloid lineages). As expected, patients with a higher synovial inflammatory burden (and more active disease) were more likely to present with erosive disease at diagnosis. The strong correlation between global synovial inflammation and baseline disease activity has been replicated in a large ERA cohort using Krenn synovitis score (n=240 ERA) (3).

These studies thus support a different concept than the earlier described phenotypic paradigm. A reason for this resides in the complex relationship linking the overall inflammatory burden

indicated in the ST sample, the clinical disease activity, and the response to therapy in early arthritis (**Figure 2A**). A first critical point to consider is how *treatment response* is defined across studies. Indeed, higher baseline disease activity in clinical trials is associated with (i) a greater range of possible absolute improvement (presented as the Delta, Δ) which is therefore easier for the physician and patient to detect, yet (ii) lower chance to achieve a state of remission/low-disease activity, suggesting that there is a floor-effect in disease activity scoring of treatment response (determined by biological factors or by the way we measure disease activity) (**Figure 3A**) (8). Observational real-life data from the UCLouvain cohort of 280 DMARDs-naïve RA patients also corroborate this correlation between baseline disease activity and absolute or relative treatment response at 6 months (**Figure 3B-D**). This relationship, shown both in treatment-naïve and methotrexate-resistant RA, is critical to consider when comparing the improvement in disease activity between two groups with different baseline histomorphologies (e.g. lympho-myeloid *vs* pauci-immune). Thus, it can be easily appreciated that a subgroup of ERA with higher ST inflammation will have higher baseline clinical disease activity, and it will consequently be easier to measurably detect absolute improvement in disease activity on therapy (but be less likely to reach clinical remission). In fact, the most relevant question for the clinician is whether ST can identify predictors of therapeutic response (to a specific therapy such as MTX or to any treatment) *independently* of baseline disease activity.

RA can exhibit features of both systemic and local inflammation, both of which contribute to composite scores of disease activity. However, while synovial tissue inflammatory burden correlates with most indices of clinical disease activity, the direction of causality between tissue inflammation and clinical activity remains unclear. This open question deserves to be addressed in future research by innovative approaches (9).

In long-standing RA, the correlation between histopathological and/or transcriptomic analysis of ST inflammation and disease activity is far less clear. Two pioneering biopsy-driven trials stratified long-standing RA patients into ‘B cell–rich’ and ‘B cell–poor’ synovial phenotypes (based on CD20 immunostaining or a B-cell gene signature) (10, 11). These large trials, R4RA (n=164, TNF-inhibitor inadequate responders) and STRAP/STRAP-EU (n=223, csDMARD inadequate responders), enrolled patients on stable background therapy (glucocorticoids and/or csDMARDs) to test biologic treatments guided by synovial phenotype. There was no difference

in baseline disease activity across these two groups, although an elevated B cell infiltration is expected to reflect a global higher ST inflammation (at least in early disease (7)). Of note, the clinical features of groups based on transcriptomic classification were not reported. The primary endpoint initially planned in this study was to assess differences between therapeutic responses to rituximab or tocilizumab in "B-cell-poor" patients as defined by synovial histomorphology, but this did not reveal a statistically significant difference. However, when using an alternative definition of "B-cell poor" based on RNA-sequencing, better clinical responses to tocilizumab (two thirds of the patients) were observed than to rituximab (one third of the patients). It may be that the transcriptomic definition of "B-cell poor" is a more biologically meaningful measure, but this was not a planned primary outcome and further prospective studies will be necessary to confirm these findings. In the STRAP/STRAP-EU trial, B cell rich and poor groups did not display difference in response to rituximab *vs* tocilizumab or etanercept (10).

In retrospective cohorts, transcriptomic profiling of 123 long-standing RA patients revealed higher CRP and ESR values in the groups with higher synovial inflammation, but not higher swollen joint counts (12). In another cohort of 41 long-standing RA patients undergoing joint surgery, three distinct clusters were proposed based on the global ST transcriptomic profile (using bulk RNAseq) (13). Disease activity indices were not significantly different across patient clusters, although a trend for higher disease activity in the group with more proinflammatory synovium was observed. Yet, a significant correlation between CDAI and the proportion of autoimmune-associated B cells was found. These latter were defined based on scRNAseq from a previous study (14).

More recently, scRNA-seq analysis has greatly enhanced the granularity of data obtainable from ST samples albeit with limitations in gene expression profiling depth and potential cell selection bias. In addition to highlighting the existence of numerous cell states/and subtypes within the synovium, scRNAseq has been used to search for synovial prognostic/theranostic factors. Thus, another way of classifying RA patients was suggested, based on synovial cell proportions (endothelial, fibroblast, myeloid and T/B cells): six groups of cell-type abundance phenotypes (CTAPs) were defined based on the analysis of 70 patients (28 ERA) (14). While the CTAPs did not significantly differ in disease activity, the relative proportion of T cells (no matter the

CTAPs) did correlate with DAS28CRP in another analysis of the same cohort (15), reminiscent of previous observations (7).

Altogether, it seems that the relationship between disease activity and synovial inflammation is less evident in long-standing, treated RA patients, or is less consistently observed. This was also confirmed in a study specifically comparing these relationships in 165 treatment-naive ERA *vs* 164 with an established phase disease under therapy (PEAC and R4RA cohorts) (16). This is likely due to the fact that patients sometimes receive multiple combinations or cycles of therapies, and can experience joint damage accumulation, both factors potentially impacting the synovial profile, and blurring this correlation (4) (**Figure 2B**). Alternatively, RA could also be a dynamic process characterized by different pathogenic mechanisms in early *vs chronic or refractory* stages.

Refractory RA, a corollary of treatment response, has also been specifically investigated in some of the previously discussed studies. Thus, in a re-analysis of the R4RA cohort, the fibroid/pauci-immune ST profile (~20% of patients) displayed a similar response (≥50% CDAI improvement) to the first non TNFi targeted therapy than other subgroups. Nevertheless, the ST of patients refractory to both tocilizumab and rituximab (n= 32) was enriched in genes reminiscent of a fibroblast signature (compared to those who responded to the second drug) (17). In another analysis of this cohort using the CTAP classification, the predicted fibroid CTAP (18.9% of patients) was associated with a lower chance to achieve ≥50% decrease in CDAI (14). However, the clinical description of this fibroid subgroup was limited, impairing the interpretation of these findings. In a subsequent re-analysis of the STRAP trial, the ST expression profile of patients not responding to the first targeted therapy (defined as failure to reach DAS28-ESR < 3.2) was not enriched with a fibroid/pauci-immune signature, rather with several metabolic pathways of uncertain relevance (18). However, it should be stressed here that different patient populations are investigated in these two cohorts: refractory patients in the R4RA cohort have failed ≥3 targeted therapies, while those in the STRAP cohort have only failed one. Interestingly, another work has shown that synovial fibroblasts in low inflammatory synovium might directly interact with nociception mechanisms, suggesting that pain can be directly elicited through ST fibroblasts in absence of inflammatory cells (19). This observation is reminiscent of the recently proposed concept of “non-inflammatory refractory RA” (20). Altogether, it remains unclear whether low-

inflammatory/pauci-immune ST represents a separate, more refractory RA entity or the extremity of the same common spectrum, with lower objective measures of disease activity, but more pain burden (21).

We propose an alternative model to explain the heterogeneity in RA ST, namely that interpatient variation in ST features may rather represent *differences of degree and not of kind*. Instead of dissecting RA into distinct subgroups, we suggest a continuous spectrum, along which synovial inflammation and ongoing disease activity go hand in hand (at least in early stages) (1). The latter also determines the room for improvement under therapy. It is tempting to consider that the slope of the continuous spectrum includes a window of opportunity for full and partial remission. Notwithstanding its capacity to enhance our understanding of RA synovial pathophysiology, the potential of ST analysis to provide clinically useful predictors of outcomes *independent* from disease activity remains unclear.

We acknowledge that our model remains hypothetical and based on indirect evidence. The existence of distinct, genuine endotypes remains indeed biologically and clinically plausible (and even desirable for both patients and clinicians). However, current evidence does not permit clear-cut conclusions as to which hypothesis is the most likely (distinct endotypes *vs* continuous spectrum), underlining the importance of an open debate in the field. In our view, if RA is to be divided into distinct subgroups based on ST, the quantitative inflammatory load, rather than qualitative assessment remains the main driver of the ST characteristics. Therefore, a more robust classification might be “high inflammatory” vs “fibroid/low-inflammatory”, rather than the originally described pathotypes. Finally, another credible possibility is that response to a specific therapy is *partially* dependent on the presence /activation of the cells and pathways targeted, amongst many other independent factors (1). This hypothesis would differ from the endotypes definition *per se* and diminish the potential of ST analysis to make accurate prediction (22).

Future work aiming to define patient subgroups for likelihood of response to therapies based on ST analysis are ongoing and results are eagerly awaited (23). To avoid previously encountered limitations, these approaches should (i) include more objective measures of inflammatory response, such as quantitative imaging, in addition to composite scores of disease activity, in order to better differentiate between “persistent inflammatory refractory RA” vs “non

inflammatory refractory RA mechanisms (20); (ii) strictly control glucocorticoids use, (iii) provide longer clinical follow-up data to evaluate the persistence of the response/refractory states, (iv) carefully report, and if possible account for baseline disease activity when measuring improvement. In this regard, the use of *relative* response to treatment (such as percentage of improvement compared to baseline) might be more relevant, although it still remains dependent on baseline disease activity measure (24). Finally, it will also be informative to evaluate how these biomarker-discovery approaches perform in the context of drugs with a broader range of targeted mechanisms, such as Janus-kinase inhibitors.

Reference	Study design	Patients and samples	N of patients analyzed	Disease (or symptoms) duration	Disease activity at biopsy	Ongoing DMARD	Technique	Findings on the relationship between clinical DA, ST profile and treatment response
Humby, *et al.* (PEAC cohort) (5)	Multicentric, retrospective observational study	SB in untreated RA	129	<12 months	DAS28: 5.6 ± 1.5	No	IHC; Custom Nanostring panel	- ERA patients can be classified into three subgroups based on ST histology: lympho-myeloid, diffuse myeloid and pauciimmune-fibroid, with differences in baseline disease activity - The expression of lympho-myeloid and diffuse myeloid gene sets showed a positive correlation with most clinical indices of disease activity. -The expression of lympho-myeloid genes is associated with progressive erosive disease and seropositivity. - The expression of both lympho-myeloid genes and diffuse-myeloid genes correlate with a larger absolute decrease in the DAS28CRP disease activity score after 6 months of csDMARD therapy (mainly methotrexate).
Alivernini, *et al.* (3)	Monocentric, retrospective observational study	SB in untreated treated RA	545 (240 untreated, 213 active RA on DMARD, 92 in remission)	<3 months to >10 years	DAS28: 5.46 ± 0.08 (untreated); 5.47 ± 0.08 (active); 2.22 ± 0.03 (remission)	Variable	H&E	-Krenn synovitis score strongly correlates with baseline disease activity - Patients with lower Krenn synovitis score are more likely to reach remission at 6 months
Triaille, *et al.* (7)	Monocentric, retrospective observational study	SB in untreated RA	74	<12 months	DAS28CRP : 5.26 ±1.25	No	Bulk RNAseq, multiplex IF	- The ST transcriptome of ERA patients is distributed on a continuous spectrum reflecting disease activity - The expression of various inflammatory pathways (e.g. B-cell mediated immunity, TCR signaling, M2/M1-like macrophages ratio) shows a continuous correlation with clinical disease activity

								-Creating distinct patients subgroups based on ST transcriptome is possible using unsupervised clustering. However, this results in dividing patients into 2 clusters: one with high inflammatory ST and disease activity *vs* the second with low inflammatory ST and less active disease. - The absolute decrease in DAS28CRP is correlated with the baseline DAS28CRP
Humby, *et al*. (R4RA) (11)	Randomized control trial	SB in treated RA, unresponsive to TNFi	164	9 years (median)	DAS28CRP : 5.31 ± 1.2	Yes	IHC, RNAseq with focus on a custom geneset related to B cells	-Patients with B cell-poor *vs* B cell-rich ST (defined by IHC) have comparable baseline disease activity -Patients with B cell-poor ST (defined by RNAseq) have a better response to tocilizumab than rituximab at 16weeks (CDAI≥50% improvement of 36% for RTX *vs* 63% for TCZ) -Patients with B cell-rich ST (defined by RNAseq or IHC) have a comparable response to tocilizumab and rituximab at 16weeks (CDAI≥50% improvement of 50 % for RTX *vs* 48% for TCZ)
Rivellese, *et al.* STRAP-RA (10)	Randomized control trial	SB in treated RA, unresponsive to csDMARD	226	3 years median (1.0-8.0)	DAS28CRP : 5.9 ± 1.2	Yes	IHC, RNAseq with focus on a custom geneset related to B cells	-Patients with B cell-poor *vs* B cell-rich ST (defined by IHC) have comparable baseline disease activity -Patients with B cell-poor ST (defined by IHC) have a comparable response to tocilizumab than rituximab/etqnercept at 16weeks
Orange, *et al*. (12)	Monocentric, retrospective observational study	Arthroplasty in treated RA	123	14 years (median)	DAS28: 3.8	Yes	H&E, RNAseq	- Patients can be divided in three subgroups of ST (high inflammatory, low inflammatory and mixed). - The high inflammatory subgroup was associated with higher CRP, ESR and seropositivity
Nakajima, *et al.*(13)	Retrospective observational study	Arthroplasty in treated RA	41	Median ±SD: 20 ±13 years	DAS28CRP : 3.4 ± 1	Yes	Bulk RNAseq	- Cell deconvolution based on scRNAseq data from (14) - Patients can be divided in three subgroupswith different predicted proportion of cell populations - These subgroups seem to reflect a spectrum

								of biological process ranging from "inflammatory" to "degenerative" synovium - No robust clinical difference across subgroups - Correlation between the predicted proportion of "autoimmune associated B cells" and disease activity (CDAI)
Zhang *et al* (14), Weisenfeld, *et al.(15)* (AMP RA/SLE cohort)	Multicentric, retrospective observational study	SB in a mixed cohort of treated and untreated RA	70 (n=28 untreated RA; n=27 MTX-inadequate responders; n=15 TNFi-inadequate responders)	Mean in years (SD) for each of the 3 groups: -2.64 (8.33) -7.42 (8.80) - 12.58 (8.79)	DAS28 for each of the 3 groups: -4.84 ± 1.38 -5.08 ± 1.57 -4.55 ± 1.15	Yes (in 42/70)	scRNASeq, H&E, multiplex IF	- Patients can be divided into six subgroups of ST based on abundance of synovial cells subtypes (CTAPs): (i) endothelial, fibroblast and myeloid, (ii) fibroblasts, (iii) T cells and fibroblasts, (iv) T and B cells, (v) T and myeloid cells, (vi) myeloid cells -CTAPs do not associate with disease activity or other clinical features (beside anti-CCP being lower in myeloid group) -Application of CTAPs classification method to the R4RA cohort (based on bulk RNAseq) suggested that patients in the Fibroblast subgroups have lower chance of response (>50%CDAI improvement) to treatment with TCZ/RTX (without apparent differential effect for one or the other molecule)

Table 1: Summary of the characteristics of major recent selected studies on synovial tissue discussed in this Viewpoint, with a focus on the relationship between clinical disease activity, synovial tissue profile and treatment response.

Abbreviations: CDAI: clinical disease activity index; csDMARD: conventional synthetic disease modifying antirheumatic drugs; DAS28: disease activity score 28 joints; DMARD: disease modifying antirheumatic drugs; H&E: hematoxylin eosin; IF: immunofluorescence; IHC: immunohisto chemistry; RA: rheumatoid arthritis; RNAseq: RNA sequencing; scRNAseq: single cell RNA sequencing SB: synovial biopsy, SD: standard deviation.TNFi: TNFα inhibitor

Legends

Figure 1: inter-patient variations in synovial cells composition between patients with untreated rheumatoid arthritis, assessed by multiplex immunofluorescence. The patient 1 (upper panels) show a high infiltration of $CD45^+$ cells in the sublining, and a lining rich in "M2 macrophage-like" ($CD68^+CD206^+$) cells. The patient 2 (lower panels) show a moderate infiltration of $CD45^+$ cells in the sublining, and a lining rich in "M1 macrophage-like" ($CD68^+CD206^-$) cells. Nuclei in dark blue, CD45 in light blue, CD68 in green, and CD206 in orange. See reference (7) for full methods.

Figure 2: Schematic representation of relationships between clinical disease activity, synovial tissue profile and response to therapy in treatment-naive RA (**A**), and in established, treated RA (**B**).

Figure 3: **A.** Schematic representation of the association between baseline disease activity (BL DA) and different definitions of treatment response. High baseline disease activity is associated with increased probability to observe a large absolute improvement (Δ), but lower chance to reach a threshold of remission. The dashed line represents the maximal possible improvement. Patients close to this line can be defined as favorable response, while patients further from it have a unfavorable response. **B.** Patient's characteristics (n=280 DMARDs-naïve patients) in the UCLouvain cohort. Results are shown as percentages or median [25^{th} percentile – 75^{th} percentile]. **C-D.** Correlation between baseline SDAI (simplified Disease Activity Index) and absolute (**C**) or relative (**D**) improvement at 6 months of therapy. On the lower panel, a 100% improvement in SDAI represents a complete resolution of SDAI components. Spearman's correlation and corresponding p-value are shown on the graph. Dots are colored according to SDAI level of activity at 6 months (green: remission; yellow: low disease activity; orange: moderate disease activity; red: high disease activity). Of note, similar strong correlations between baseline values and absolute improvement were found in all components of the SDAI score (not shown). Abbreviations: BL DA: baseline disease activity; BL SDAI: baseline simplified disease activity index; Δ: delta/improvement in disease activity compared to baseline; DA: disease activity; LDA; low disease activity; R: remission.

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References

1. Triaille C, Quartier P, De Somer L, Durez P, Lauwerys B, Verschueren P, et al. Patterns and determinants of response to novel therapies in juvenile and adult-onset polyarthritis. Rheumatology (Oxford). 2023.
2. Smeets TJ, Barg EC, Kraan MC, Smith MD, Breedveld FC, Tak PP. Analysis of the cell infiltrate and expression of proinflammatory cytokines and matrix metalloproteinases in arthroscopic synovial biopsies: comparison with synovial samples from patients with end stage, destructive rheumatoid arthritis. Ann Rheum Dis. 2003;62(7):635-8.
3. Alivernini S, Tolusso B, Gessi M, Gigante MR, Mannocci A, Petricca L, et al. Inclusion of Synovial Tissue-Derived Characteristics in a Nomogram for the Prediction of Treatment Response in Treatment-Naive Rheumatoid Arthritis Patients. Arthritis Rheumatol. 2021;73(9):1601-13.
4. Triaille C, Durez P, Sokolova T, Tilman G, Meric de Bellefon L, Galant C, et al. Common Transcriptomic Effects of Abatacept and Other DMARDs on Rheumatoid Arthritis Synovial Tissue. Front Immunol. 2021;12:724895.
5. Humby F, Lewis M, Ramamoorthi N, Hackney JA, Barnes MR, Bombardieri M, et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. Ann Rheum Dis. 2019;78(6):761-72.

6. Lewis MJ, Barnes MR, Blighe K, Goldmann K, Rana S, Hackney JA, et al. Molecular Portraits of Early Rheumatoid Arthritis Identify Clinical and Treatment Response Phenotypes. Cell Rep. 2019;28(9):2455-70 e5.
7. Triaille C, Tilman G, Sokolova T, Loriot A, Marchandise J, De Montjoye S, et al. Disease activity drives transcriptomic heterogeneity in early untreated rheumatoid synovitis. Ann Rheum Dis. 2023.
8. Capelusnik D, Aletaha D. Baseline predictors of different types of treatment success in rheumatoid arthritis. Ann Rheum Dis. 2021.
9. Orange DE, Yao V, Sawicka K, Fak J, Frank MO, Parveen S, et al. RNA Identification of PRIME Cells Predicting Rheumatoid Arthritis Flares. N Engl J Med. 2020;383(3):218-28.
10. Rivellese F, Nerviani A, Giorli G, Warren L, Jaworska E, Bombardieri M, et al. Stratification of biological therapies by pathobiology in biologic-naive patients with rheumatoid arthritis (STRAP and STRAP-EU): two parallel, open-label, biopsy-driven, randomised trials. The Lancet Rheumatology. 2023;5(11):e648-e59.
11. Humby F, Durez P, Buch MH, Lewis MJ, Rizvi H, Rivellese F, et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. Lancet. 2021;397(10271):305-17.
12. Orange DE, Agius P, DiCarlo EF, Robine N, Geiger H, Szymonifka J, et al. Identification of Three Rheumatoid Arthritis Disease Subtypes by Machine Learning Integration of Synovial Histologic Features and RNA Sequencing Data. Arthritis Rheumatol. 2018;70(5):690-701.
13. Nakajima S, Tsuchiya H, Ota M, Ogawa M, Yamada S, Yoshida R, et al. Synovial Tissue Heterogeneity in Japanese Patients With Rheumatoid Arthritis Elucidated Using a Cell-Type Deconvolution Approach. Arthritis Rheumatol. 2023;75(12):2130-6.
14. Zhang F, Jonsson AH, Nathan A, Millard N, Curtis M, Xiao Q, et al. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. Nature. 2023;623(7987):616-24.
15. Weisenfeld D, Zhang F, Donlin L, Jonsson AH, Apruzzese W, Campbell D, et al. Associations Between Rheumatoid Arthritis Clinical Factors and Synovial Cell Types and States. Arthritis Rheumatol. 2024;76(3):356-62.
16. Rivellese F, Humby F, Bugatti S, Fossati-Jimack L, Rizvi H, Lucchesi D, et al. B Cell Synovitis and Clinical Phenotypes in Rheumatoid Arthritis: Relationship to Disease Stages and Drug Exposure. Arthritis Rheumatol. 2020;72(5):714-25.
17. Rivellese F, Surace AEA, Goldmann K, Sciacca E, Cubuk C, Giorli G, et al. Rituximab versus tocilizumab in rheumatoid arthritis: synovial biopsy-based biomarker analysis of the phase 4 R4RA randomized trial. Nat Med. 2022;28(6):1256-68.
18. Lewis MJ, Cubuk C, Surace AEA, Sciacca E, Lau R, Goldmann K, et al. Deep molecular profiling of synovial biopsies in the STRAP trial identifies signatures predictive of treatment response to biologic therapies in rheumatoid arthritis. Nat Commun. 2025;16(1):5374.
19. Bai Z, Bartelo N, Aslam M, Murphy EA, Hale CR, Blachere NE, et al. Synovial fibroblast gene expression is associated with sensory nerve growth and pain in rheumatoid arthritis. Sci Transl Med. 2024;16(742):eadk3506.
20. Buch MH, Eyre S, McGonagle D. Persistent inflammatory and non-inflammatory mechanisms in refractory rheumatoid arthritis. Nat Rev Rheumatol. 2021;17(1):17-33.
21. Nerviani A, Di Cicco M, Mahto A, Lliso-Ribera G, Rivellese F, Thorborn G, et al. A Pauci-Immune Synovial Pathotype Predicts Inadequate Response to TNFalpha-Blockade in Rheumatoid Arthritis Patients. Front Immunol. 2020;11:845.
22. Triaille C, Durez P, Smolen JS, McInnes IB, Lauwerys B, Limaye N. Precision medicine and the chaos theory in rheumatoid arthritis. Ann Rheum Dis. 2025.

23. Pitzalis C. Advances in Targeted Therapies (ATT) What's cooking in the academic's kitchen? 3TR (IMI). Semin Arthritis Rheum. 2024;64S:152317.
24. Capelusnik D, Aletaha D. Baseline predictors of different types of treatment success in rheumatoid arthritis. Ann Rheum Dis. 2022;81(2):153-8.