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Interest of the Immunohistological Analysis of Architecture and Cells Distribution of the Synovial Membrane in Rheumatoid Arthritis

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Abstract

Introduction: Rheumatoid arthritis (RA) is an immune-mediated disease in which synovial inflammatory lesions resemble those found in secondary lymphoid organs. The histopathological characteristics of several synovial tissue specimens from RA patients were compared to the characteristics of synovial samples from patients with osteoarthritis (OA) in order to highlight histological features of RA synovium that could be associated with clinical phenotypes.

Methods: Synovial tissue specimens obtained from 17 patients with RA were compared to synovial samples from 9 patients with OA. Krenn's synovitis score and synovial microarchitecture were analysed. Immuno-histological analysis of synovium tissue was performed to observe the cells distribution in the inflammatory infiltrates, using CD3, CD20 and CD68 markers.

Results: Synovitis scores were of high-grade synovitis in 76.4% of the RA tissue specimens against 55.5% in the OA samples but the median synovitis score was higher in the RA group (p=0.05). The synovial tissue architecture in the RA group was characterised by the presence of lymphocyte aggregates in 70.5% of tissue specimens and diffuse infiltration in all others. Significant differences between the RA and OA samples were also observed with respect to the distribution of macrophages in the stroma.

Conclusion: This study highlights the importance of inflammatory infiltrates in the RA synovium and suggests that histopathological analysis of RA synovium can still bring data on the pathophysiological mechanisms and potential therapeutic targets in RA.

Keywords: Rheumatoid arthritis; Osteoarthritis; Synovitis; Histopathology; Tissue architecture; Infiltrate

Introduction

Rheumatoid arthritis (RA) is the most frequent chronic autoimmune rheumatism (prevalence of nearly 1% in the adult population) affecting synovial tissue in the small and large joints, leading to severe pain and disability. The synovial membrane of affected joints undergoes a series of alterations that result in the formation of a proliferative tissue (i.e. synovitis), responsible for definitive osteoarticular destructions. Although the pathological factors that initiate the inflammatory cascade responsible for rheumatoid synovitis have yet not been fully identified, the first event could be a non-specific inflammatory response leading to the activation of monocytes and the recruitment of macrophages in the synovial membrane. The cellular composition of rheumatoid synovitis includes resident hyperplastic synoviocytes, endothelial cells, T and B cells, dendritic cells and macrophages [1]. Although the process of tissue infiltration is still not fully understood, infiltrating cells in RA are often organized into distinct B-cell areas surrounded by T cells and follicular dendritic cells [2,3]. This cell organization reflects the T and B cells distribution that is found in secondary lymphoid organs, such as lymph nodes. Ectopic lymphoid structures are not specific for RA but are associated with chronic inflammatory autoimmune diseases, since they have also been identified in Sjögren's syndrome [4] or in Hashimoto thyroiditis [5]. In RA, the synovial tissue is further characterized by hyperplasia of the intimal lining cell layer and infiltration of the sublining layer (stroma) by macrophages, B and T cells, and other inflammatory cells that promote inflammation and local tissue destruction [6].

RA is a heterogeneous disease with various phenotypes: with or without erosion, autoantibodies (such as anti-citrullinated peptide antibodies (ACPA)), or extra articular manifestations. Furthermore, the clinical presentation of RA can be quite heterogeneous with a spectrum ranging from mild to severe disease [7]. This heterogeneity is also found as a feature of synovial inflammation [6-9]. The variations in response to different types of anti-rheumatic treatment in RA further support this notion [10].

RA is a systemic disease but small joints injuries are the most visible manifestations. The preferential targeting of small joints in RA does not seem to be due to specific tissue autoantigen expression [11]. In fact it seems that the rheumatoid synovium provides a breeding ground for abnormal autoimmune responses leading to tissue destructions [2]. Formation of secondary lymphoid structures in

lymph nodes shows how tissue microarchitecture determines the immune response [1,2]. This concept has been less studied in non-lymphoid tissues, while it could explain the pathogenesis of chronic inflammatory rheumatisms [2,12]. In RA, some studies have emphasized the importance of the synovium's microarchitecture responsible for the interactions between cells, leading to an immune inflammatory response [13].

In rheumatoid synovitis, cell organization is complex [14]. Indeed the inflammatory infiltrate can assume well defined yet different forms between individuals with distinct molecular and cellular signatures [7,8,15]. A synovitis score allowing the differentiation between low-grade and high-grade synovitis was developed by Krenn, and co-workers, to standardize histopathological description of the synovial membrane [16]. Three form of rheumatoid synovitis's architecture can be observed. In the first form (25% of patients), the synovitis' microarchitecture shows all characteristics of ectopic lymphoid tissue with germinal center- like formations. In this group, cells show a well-defined organization with distinct B and T cells areas and a network of follicular dendritic cells [17]. The existence of germinal center-like structure in the synovial tissue is indicative of a high level of organization allowing cell-cell contact and therefore cell activation and antibodies' production [18,19]. In the second group, representing 25% of patients, the synovium shows signs of lymphocyte aggregates without follicular dendritic cells [1]. In the most frequent type of rheumatoid synovitis, the inflammatory infiltrate is diffuse with low level of organization [17]. Finally, granuloma formation can rarely be found in the RA synovium. Several groups have studied the effect of the different types of anti-rheumatic treatment on synovium tissue architecture and showed that there are various cellular responses depending on the type of drugs, suggesting that the synovitis microarchitecture contributes to the clinical phenotype of RA and to the therapeutic effects of immunomodulatory drugs [20-26].

From all these data, the histopathological analysis of synovial membrane is the only effective way to study cell distribution and synovial microarchitecture in RA even if is not the only disease showing synovial lesions. Indeed some other diseases such as degenerative, metabolic or other autoimmune diseases can lead to synovial alterations, the majority being forms of synovitis. Although a histological analysis is not done systematically, because it is not required for RA diagnosis, it may provide complementary information, which could guide the diagnosis and influence the therapeutic decision.

In this study, histopathological features of synovial tissue specimens from RA patients were compared to the synovial samples from patients with osteoarthritis (OA) in order to (1) Highlight the histological specificities of RA synovium and (2) ind subsets of RA patients according to their clinical and histological phenotypes.

Finally we discussed the current interest of histopathological analysis of RA synovium, considering actual data on the pathophysiological mechanisms and therapeutic targets in RA.

Patients and Methods

Patients and clinical material

Synovial tissue specimens were selected randomly from a bank of specimen including biopsies from RA (n=17) and OA (n=9) patients performed between 2001 and 2013. In the OA group, synovial tissue specimens were obtained from knee or hip joints during joint replacement surgery. In the RA group, synovial tissue samples were obtained thanks to a systematic biopsy during surgical synovectomie. In two cases, synovial tissue specimens were obtained during joint replacement. Clinical diagnosis for RA was based on ACR/EULAR 2010 criteria. Tissue specimens from patients who did not fit these criteria were excluded. Age (median, range), sex and articular localisation of the biopsy were collected in both groups. In the RA group, for each patient the presence (Yes/No) of erosion was recorded at the time of the biopsy as well as autoantibodies (rheumatoid factor and/or ACPA).

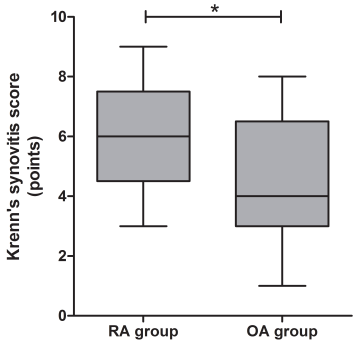

Figure 1: Box plot of the synovitis scores for RA and OA groups (*p=0.05).

Histology

Histological features of the synovial membranes were carried out on routine haematoxylin-eosin-safran–stained (HES) slides. Each synovial tissue specimen was analysed by two different observers and analysis was done at the synovial site showing the strongest histological alterations. Krenn's synovitis score was established for each sample [16]. In this score three items are analysed: the enlargement of the synovial lining cell layer, the density of the resident cells and the type of the inflammatory infiltrate. Each item is graded from 0 to 3 points, giving a maximal score of 9 points. A score between 2 to 4 points amounts to a low-grade synovitis and a score from 5 to 9 points to a high-grade synovitis. Then, we further reported synovial architecture for each synovial tissue specimen classifying tissue architecture according to the inflammatory infiltrate's organization: no infiltrate, diffuse infiltrate, infiltrate forming lymphocyte aggregates and infiltrate forming ectopic germinal center-like structures.

Immunohistochemistry

Cell distribution in the inflammatory infiltrate in synovial tissue specimens was analysed using cell lineage markers by immunostaining. We used a monoclonal mouse anti-human CD3 antibody for T cells [27] at a dilution rate of 1/50 (clone F7.2.38, DakoCytomation, Copenhagen, Denmark), a monoclonal mouse anti-human CD20 antibody for B cells [28] at a dilution rate of 1/50 (clone L26, DakoCytomation, Copenhagen, Denmark) and a monoclonal mouse anti-human CD68 antibody for macrophages [29] at a dilution rate of 1/300 (clone KP1, DakoCytomation, Copenhagen, Denmark) using staining procedures described in the DakoCytomation specification sheet of each antibody. Each sample was analysed by two observers, using a semi quantitative method. Immunostaining was graded from 0

to 3 points: 0 point, for no immunostaining, 1 point for weak immunostaining, 2 points for moderate immunostaining and 3 points for intense immunostaining. Moreover, we described the shape of the stained-infiltrate, whether diffuse or aggregates-like.

Statistics

For statistical evaluation, frequencies and median values were chosen for descriptive statistics. The Mann-Whitney test was used to compare quantitative variables between two groups. Pearson correlation was used to investigate the association between the synovitis score and clinical features of the disease in the RA group. The Fisher test was used to find an association between the type of synovial architecture and the disease (OA or RA). It was also used to find an association in the RA group between the types of synovial architecture and clinical features of the disease. A p value less than or equal to 0.05 was considered statistically significant. Statistics were calculated using GraphPad Prism version 5.01.

Results

Nine synovial tissue specimens were obtained from hip (n=3) and knee (n=6) joints from patients with a clinical diagnosis of OA. OA patients (55.5% of women) had a mean age of 65 years-old (56 to 85 years-old). In the RA group, 17 synovial tissue specimens were selected. Among them, 12 tissue specimens (70.5%) came from patients with erosive RA and 14 tissue specimens (82.3%) from patients with auto-antibodies (rheumatoid factor and/or ACPA). Synovial tissue specimens were obtained from wrist (n=11), knee (n=2) and shoulder (n=2). In 2 cases, the localisation of the biopsy was unknown. Patients (70.5% of women) in the RA group had a mean age of 59.1 years-old (38 to 78 years-old).

In the RA group, a high-grade synovitis score (≥5 points) was observed in 13/17 (76.5%) of synovial tissue specimens while in the remaining 4 samples, synovitis scores ranged from 2 to 4 points corresponding to low-grade synovitis (Table 1). In the OA group, 5/9 (55.5%) specimens were graded low, 3/9 (33.3%) high and in the last specimen there was no evidence of synovitis observed (Table 2). In the RA group, 50% of synovial tissue specimens were graded between 5 and 7 points. Despite outlier values in both groups and wide ranges (3 to 9 in RA and 1 to 8 in OA) a significant difference was observed between the 2 groups (p=0.05) (Figure 1). In the RA group, we then investigated whether synovitis score was associated with clinical features of RA such as osteoarticular destruction or auto-antibodies. We could not find significant association between Krenn's synovitis score and the erosive profile of RA or the presence of auto-antibodies; although a trend for an association between high-grade synovitis score and erosive RA was observed (p=0.07).

Tissue architecture types were not distributed as we would have expected in RA samples with 12/17 (70.5%) synovial tissue specimens showing lymphocyte aggregates, 4/17 (23.5%) specimens characterized by diffuse infiltration and 1 sample with no inflammatory infiltrate. No germinal center-like structure was observed. In 52.9% of tissue samples, the synovial lesions observed were heterogeneous in size and distribution of the infiltrate. In the OA group, 8/9 (88.8%) samples were displaying lymphocyte aggregates, which in most cases (77.7%) were homogeneous in size and distribution. We did not find any statistical difference between the 2 groups with respect to synovial architecture (p=0,34). There was also no association between synovial architecture in the RA group and the erosive profile of RA or positivity of auto-antibodies; although a trend was observed (p=0.11) between erosive RA and an inflammatory infiltrate well organized in lymphocytes aggregates.

Enlargement of the synovial lining cell layer (points)	Density of the resident cells (points)	Inflammatory infiltrate (points)	Sum (points)	Synovitis grade
3	3	3	9	high grade
1	2	1	4	low grade
1	1	3	5	high grade
3	2	3	8	high grade
2	3	3	8	high grade
1	3	3	7	high grade
1	2	0	3	low grade
3	3	1	7	high grade
3	2	2	7	high grade
1	3	3	7	high grade
3	3	3	9	high grade
1	2	1	4	low grade
1	2	2	5	high grade
1	2	3	6	high grade
1	1	2	4	low grade
3	1	2	6	high grade
3	2	1	6	high grade

Table 1: Results for Krenn's synovitis score of synovium tissue specimens in the RA group.

Enlargement of the synovial lining cell layer (points)	Density of the resident cells (Points)	Inflammatory infiltrate (points)	Sum (points)	Synovitis grade
1	1	1	3	low grade
1	1	1	3	low grade
3	3	2	8	high grade
1	1	2	4	low grade
1	2	1	4	low grade
3	3	1	7	high grade
1	1	2	4	low grade
0	1	0	1	No synovitis
2	2	2	6	high grade

Table 2: Results for Krenn's synovitis score of synovium tissue specimens in the OA group.

In the immuno-histochemistry study, we could analysed cell distribution of macrophages and T and B cells in the synovial stroma and in the synovial lining cell layer in 9 synovial tissue specimens from the RA group and in 8 specimens from the OA group (Figure 2). We observed a significant difference between the RA and OA groups regarding the distribution of macrophages in the synovial stroma (p=0.02), with an increased number of macrophages in the RA group. In contrast, no difference were observed for B and T cells' distribution, neither in the lining cell layer (B cells p=0.09, T cells p=1) nor in the synovial stroma (B cells p=0.1, T cells p=0.07).

Discussion

In this study, the analysis of synovial tissue specimens according to Krenn's synovitis score strengthens that it could be used to evaluate in a standardized way the inflammatory infiltrate lesions found in the synovium of patients with RA [16]. Indeed we found a significant statistical difference between the synovitis scores of RA and OA patients, which highlights the importance of inflammatory infiltrate in RA's pathophysiology. However, in some tissue specimens of OA patients we found the same histological synovial lesions as in RA patients. Therefore Krenn's synovitis score is not pathognomonic of RA, as described by Krenn himself who found a sensitivity of 61.7% and a specificity of 96.1% of his score [16]. One of the issues of histological analysis of synovial tissue specimens is the heterogeneity of the lesions observed on one sample, making difficult its interpretation. In this study, we chose to analyse the sites showing the strongest histopathological alterations in all tissue specimens, a method chosen by pathologists in histopathological tumour diagnosis, in order to standardize our analysis but, nevertheless, synovial inflammatory lesions can be difficult to associate with one disease [8,30]. Indeed, there are quite a number of inflammatory and non-inflammatory diseases that can give histological synovitis but very few, if not none, diseases show pathognomonic changes in the synovial membrane.

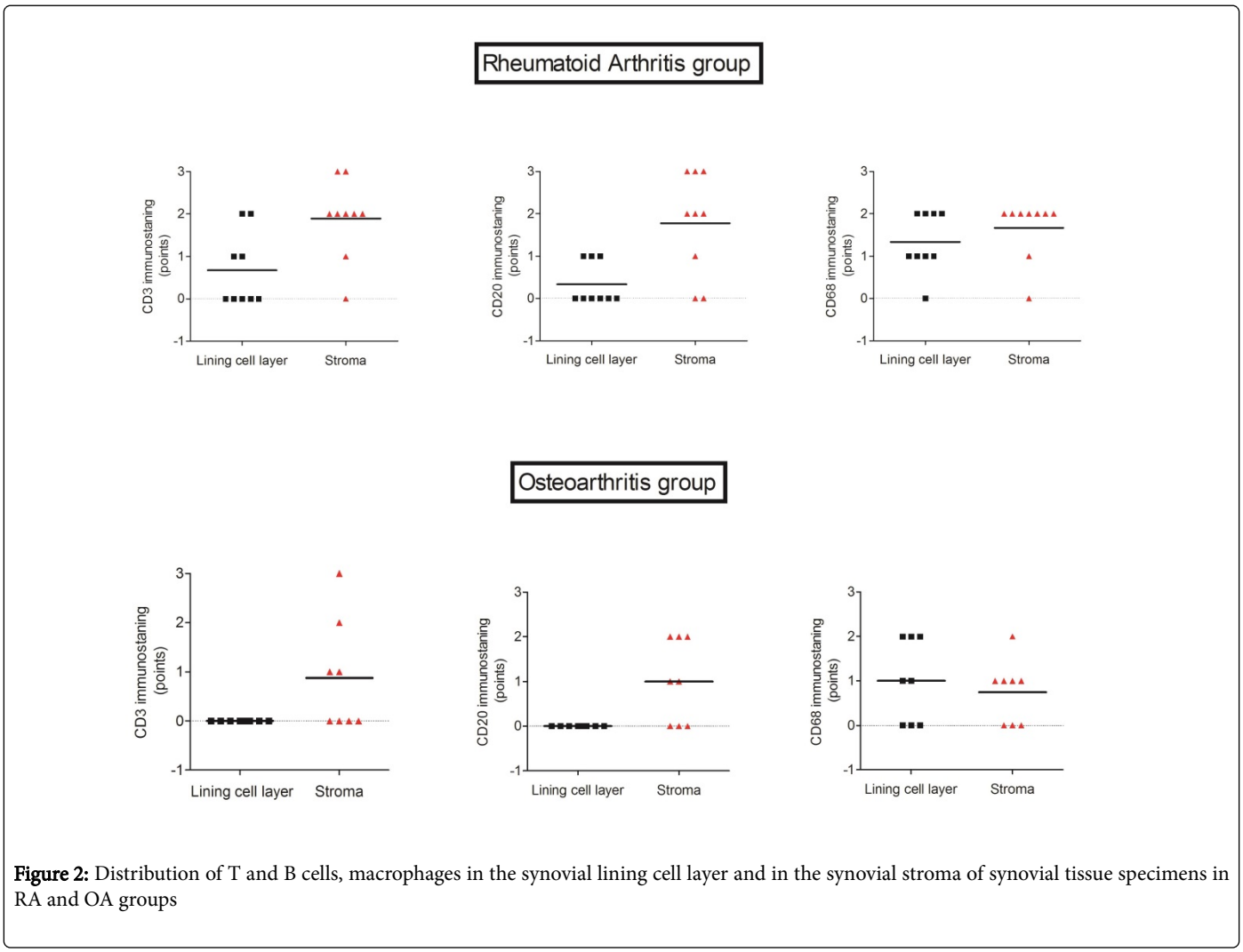

Figure 2: Distribution of T and B cells, macrophages in the synovial lining cell layer and in the synovial stroma of synovial tissue specimens in RA and OA groups

Regarding synovial architecture, our results are different from those found in the literature. Indeed, we did not find any germinal center-like structure in tissue specimens from RA patients whereas in the literature, in approximately 25% of synovium from RA patients, infiltrates of T and B cells are organized into structures resembling lymph nodes with germinal centers [17]. Furthermore, we observed in 70% of synovial tissue specimens from RA patients an inflammatory infiltrate forming lymphocyte aggregates whereas it should only represent 25% of the tissue specimens. The explanation for those differences between our cohort and other studies could be the occurrence of biological drugs in the treatment of RA in the 2000s, which could have resulted in modifications of synovial inflammatory

infiltrate in more recent tissue specimen from treated patients as the ones in this study. In support of this hypothesis, Hirohata et al. have demonstrated that TNF inhibitors alter the histological characteristics of the synovium from RA patients [21]. Another result of our study is the absence of difference in synovial architecture between RA and OA patients. This goes against the hypothesis according to which the type of synovial architecture could determine the local inflammatory response [1,11], but the small number of samples in our study does not allow us to conclude on this particular point.

We then tried to find an association between the type of synovial architecture and characteristics of the disease of RA patients. We observed a trend for association between the synovitis score and the erosive profile of RA that showed that the higher the synovitis score was the higher risk there was to have an erosive disease. However this result was not statistically significant, most probably because of our low number of patients. For many years now, other teams have tried to find a score that could correlate synovial lesions and the severity of RA [31,32]. Overall, they did not find any correlation between clinical features of RA patients and histological synovial lesions; with the exception of Koizumi et al. who found a correlation between his synovitis score and rheumatoid factor's rate and the number of affected joints [33]. In the immuno-histochemistry study, we observed a significant difference between RA and OA patients regarding the distribution of macrophages in the synovial stroma. Indeed we observed an increased number of macrophages in the RA group, hence the conclusion that macrophages are less present in OA synovial infiltrate, even in a group of inflammatory OA patients with 88.8% samples displaying lymphocyte aggregates. This result reinforces the importance of the role of macrophages in RA synovial infiltrate while other studies emphasize that synovial macrophages are appropriate biomarkers for response to treatment in RA patients [34-36].

This study reinforces our knowledge upon the specificity of synovial architecture and cell distribution of inflammatory infiltrate in synovium of patients with RA. Therefore in RA, a systematic histological analysis of synovial biopsy could be interesting for early diagnosis, to establish a prognosis and for treated patient's follow-up [21,37,38]. Indeed, even if a synovial biopsy guided by echography or arthroscopy is an invasive act, it can provide synovial tissue specimen of good quality from the most representative lesions of the joint [39,40]. Histological analysis could ease the early diagnosis of some cases in which RA diagnosis is difficult, such as in cases of no positive auto-antibody, in order to initiate a suited treatment as soon as possible. Furthermore, the immuno-histochemistry analysis of synovial tissue specimen could allow targeting more effectively the type of biological drug to initiate for each patient [8,41]. Indeed, for a patient presenting with a synovial inflammatory infiltrate formed by a majority of B cells, we would be more inclined to choose a treatment targeting B cells such as rituximab. Such data regarding the prediction of patients' response to treatment are still missing and need to be developed. The problem bound to synovium's invasive obtaining could be overlooked only if systematic histological analysis of synovial biopsy became a key element in early proactive management of patients with RA.

In conclusion, even if the histological analysis of synovial tissue is not part of the current criteria for RA diagnosis or prognosis, it may be helpful in case of difficult early diagnosis and should be further studied to better understand patients' response to treatment.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Goronzy JJ, Weyand CM (2005) Rheumatoid arthritis. Nat Rev Immunol 204: 55-73.
2. Mebius RE (2003) Organogenesis of lymphoid tissues. Nat Rev Immunol 3: 292-303.
3. Van Nierop K, De Groot C (2002) Human follicular dendritic cells: function, origin and development. Semin Immunol 14: 251-257.
4. Edwards JC, Wilkinson LS, Speight P, Isenberg DA (1993) Vascular cell adhesion molecule 1 and α4 and β1 integrins in lymphocyte aggregates in Sjögren's syndrome and rheumatoid arthritis. Ann Rheum Dis 52: 806-811.
5. Armengol MP, Cardoso-Schmidt CB, Fernandez M, Ferrer X, Pujol-Borrell R, et al. (2003) Chemokines determine local lymphoneogenesis and a reduction of circulating CXCR4+ T and CCR7 B and T lymphocytes in thyroid autoimmune diseases. J Immunol 170: 6320-6328.
6. Van der Pouw Kraan TC, van Gaalen FA, Kasperkovitz PV, Verbeet NL, Smeets TJ, et al. (2003) Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. Arthritis Rheum 48: 2132-2145.
7. Weyand CM, Klimiuk PA, Goronzy JJ (1998) Heterogeneity of rheumatoid arthritis: from phenotypes to genotypes. Springer Semin Immunopathol 11: 210-217.
8. Pitzalis C, Kelly S, Humby F (2013) New learnings on the pathophysiology of RA from synovial biopsies. Curr Opin Rheumatol 25: 334-344.
9. Van Baarsen LG, Wijbrandts CA, Timmer TC, van der Pouw Kraan TC, Tak PP, et al. (2010) Synovial tissue heterogeneity in rheumatoid arthritis in relation to disease activity and biomarkers in peripheral blood. Arthritis Rheum 62: 1602-1607.
10. Tak PP (2012) A personalized medicine approach to biologic treatment of rheumatoid arthritis: a preliminary treatment algorithm. Rheumatology 51: 600-609.
11. Kraan MC, Reece RJ, Smeets TJ, Veale DJ, Emery P, et al. (2002) Comparison of synovial tissues from the knee joints and the small joints of rheumatoid arthritis patient: implications for pathogenesis and evaluation of treatment. Arthritis Rheum 46: 2034-2038.
12. Weyand CM, Kurtin PJ, Goronzy JJ (2001) Ectopic lymphoid organogenesis: a fast track for autoimmunity. Am J Pathol 159: 787-793.
13. Brennan F, Foey A (2002) Cytokines regulation in RA synovial tissue: role of T cell/macrophage contact-dependent interactions. Arthritis Res 4: S177-S182.
14. Duke O, Panayi GS, Janossy G, Poulter LW (1982) An immunohistological analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies. Clin Exp Immunol 49: 22-30.
15. Klimiuk PA, Goronzy JJ, Björnsson J, Beckenbaugh RD, Weyand CM (1997) Tissue cytokines patterns distinguish variants of rheumatoid synovitis. Am J Pathol 151: 1311-1319.
16. Krenn V, Morawietz L, Burester GR, Kinne RW, Mueller-Ladner U, et al. (2006) Synovitis score: discrimination between chronic low-grade and high-grade synovitis. Histopathology 49: 358-364.
17. Timmer TCG, Baltus B, Vondenhoff M, Huizinga TW, Tak PP, et al. (2007) Inflammation and ectopic lymphoid structures in rheumatoid arthritis synovial tissues dissected by genomics technology. Arthritis Rheum 56: 2492-2502.
18. Wagner UG, Kurtin PJ, Wahner A, Brackertz M, Berry DJ, et al. (1998) The role of CD8+ CD40L + T cells in the formation of germinal centers in rheumatoid synovitis. J Immunol 161: 6390-6397.

19. Weyand CM, Goronzy JJ, Takemura S, Kurtin PJ (2000) Cell-cell interactions in synovitis: Interactions between T cells and B cells in rheumatoid arthritis. Arthritis Res 2: 457-463.
20. Tak PP, Taylor PC, Breedveld FC, Smeets TJ, Daha MR, et al. (1996) Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor z monoclonal antibody treatment in patients with rheumatoid arthritis. Arthritis Rheum 39: 1077-1081.
21. Hirohata S, Tomita T, Yoshikawa H, Kyogoku M (2013) TNF inhibitors induce discoid fibrosis in the sublining layers of the synovium with degeneration of synoviocytes in rheumatoid arthritis. Rheumatol Int 33: 2473-2481.
22. Revu S, Neregard P, Af Klint E, Korotkova M, Catrina AI (2013) Synovial membrane immunohistology in early-untreated rheumatoid arthritis reveals high expression of catabolic bone markers that is modulate by methotrexate. Arthritis Res Ther 15: R205.
23. Neregard P, Krishnamurthy A, Revu S, Engström M, Af Klint E, et al. (2014) Etanercept decreases synovial expression of tumor necrosis-α and lymphotoxin-α in rheumatoid arthritis. Scan J Rheumatol 43: 85-90.
24. Ducreux J, Durez P, Galant C, Toukap AN, Van den Eynde B, et al. (2014) Global molecular effects of tocilizumab therapy in rheumatoid arthritis synovium. Arthritis Rheum 66: 15-23.
25. Krann MC, Reece RJ, Barg EC, Smeets TJ, Farnell J, et al. (2000) Modulation of inflammation and metalloproteinase expression in synovial tissue by leflunomide and methotrexate in patients with active rheumatoid arthritis. Findings in prospective, randomized, double-blind, parallel-design clinical trial in thirty-nine patients at two centers. Arthritis Rheum 43: 1820-1830.
26. Vos K, Thurlings RM, Wijbrandts CA, van Schaardenburg D, Gerlag DM, et al. (2007) Early effects of rituximab on the synovial cell infiltrate in patients with rheumatoid arthritis. Arthritis Rheum 56: 772-778.
27. Alibaud L, Llobera R, Al Saati T, March M, Delsol G, et al. (1987) A new monoclonal anti-CD3 antibody reac-tive on paraffin sections. J Histochem Cytochem 48: 1609-1616.
28. Cartun RW, Coles FB, Pastuszak WT (1987) Utilization of monoclonal antibody L26 in the identification and confirmation of B-cell lymphomas. A sensitive and specific marker applicable to formalin- and B5-fixed, paraffin-embedded tissues. Am J Pathol 129: 415-421.
29. Pulford KAF, Rigney EM, Micklem KJ, Jones M, Stross WP, et al. (1989) KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 42: 414-421.
30. Tak PP, Smeets TJM, Daha MR, Kluin PM, Meijers KA, et al. (1997) Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis Rheum 40: 217-225.
31. Stiehl P (1994) Histological classification of synovial membranes of rheumatoid arthritis in dependence on the kind of cell infiltrates, destruction of synovial lining cells as well as of course of disease. Z Rheumatol Suppl 53: 50.
32. Rooney M, Whelan A, Feighery C, Bresnihan B (1989) Changes in lymphocyte infiltration of the synovial membrane and the clinical course of rheumatoid arthritis. Arthritis Rheum 32: 361-369.
33. Koizumi F, Matsuno H, Wakaki K, Ishii Y, Kurashige Y, et al. (1999) Synovitis in rheumatoid arthritis: scoring of characteristic histopathological features. Pathol Int 49: 298-304.
34. Haringman JJ, Gerlag DM, Zwinderman AH, Smeets TJ, Kraan MC, et al. (2005) Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. Ann Rheum Dis 64: 834-838.
35. Wijbrandts CA, Vergunst CE, Haringman JJ, Gerlag DM, Smeets TJ, et al. (2007) Absence of changes in the number of synovial sublining macrophages after ineffective treatment for rheumatoid arthritis: implications for use of synovial sublining macrophages as a biomarker. Arthritis Rheum 56: 3869-3871.
36. Bresnihan B, Pontifex E, Thurlings RM, Vinkenoog M, El-Gabalawy H, et al. (2009) Synovial tissue sublining CD68 expression is a biomarker of therapeutic response in rheumatoid arthritis clinical trials: consistency across centers. J Rheumatol 36: 1800-1802.
37. Smith MD, Kraan MC, Slavotinek J, Au V, Weedon H, et al. (2001) Treatment-induced remission in rheumatoid arthritis patients is characterized by a reduction in macrophage content of synovial biopsies. Rheumatology 40: 367-374.
38. Walsh CA, Fearon U, Fitzgerald O, Veale DJ, Bresnihan B (2008) Decreased CD20 expression in rheumatoid arthritis synovium following 8 weeks of rituximab therapy. Clin Exp Rheumatol 26: 656-658.
39. Goëb V, Walsh CA, Reece RJ, Emery P, Ponchel F (2012) Potential role of arthroscopy in the management of inflammatory arthritis. Clin Exp Rheumatol 30: 429-435.
40. Kelly S, Humby F, Filer A, Ng N, Di Cicco M, et al. (2015) Ultrasound-guided synovial biopsy: a safe, well-tolerated and reliable technique for obtaining high-quality synovial tissue from both large and small joints in early arthritis patients. Ann Rheum Dis 74: 611-617.
41. Teng YK, Levarht EW, Hashemi M, Bajema IM, Toes REM, et al. (2007) Immunohistochemical analysis as a mean to predict responsiveness to rituximab treatment. Arthritis Rheum 56: 3909-3918.