Clinical and molecular classification of very early arthritis patients
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GENERAL DISCUSSION AND SUMMARY
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Relatively little is known about the etiology of rheumatoid arthritis (RA). Previous studies have shown that from an immunological perspective early RA already presents chronic inflammation. Increased insight into the processes active during the preclinical course of RA, the phase before the onset of arthritis, might help to identify individuals with a high risk of developing RA and could offer opportunities to explore preventive treatment.

Until preventive treatment strategies will become available, the treatment goal in RA is directed at early and aggressive treatment with a tight control of disease activity aiming for a state of disease remission [1] to improve outcome. It is well-known that RA, as we define it today, comprises different patient subsets based on clinical as well as molecular parameters. Supposedly, these patient subsets may require different treatment regimens. Besides, several patients presenting in early arthritis clinics do not fulfill the diagnostic criteria for RA but may still develop persistent or even destructive arthritis requiring DMARD treatment. Therefore, there is a need for a better understanding of the processes active in these different disease subsets by profiling based on molecular features and for identification of new potential diagnostic and prognostic biomarkers that could be used in early arthritis clinics to guide treatment decisions in the individual patient.

Chapter 2 describes the currently used procedures for synovial tissue sampling and processing and introduces an international guideline for arthroscopic synovial tissue sampling and processing. This technique is applied in chapters 3, 5, 6, 7, and 8.

Preclinical arthritis

To date it is unknown where in the body the immune response is initiated that will finally lead to full blown RA. In chapter 3 we studied synovial tissue characteristics by DCE-MRI and phenotypic and vascular marker expression in the synovium, the primary target of RA, in individuals with an increased risk of developing RA defined by elevated serum levels of IgM rheumatoid factor and/or anti-citrullinated peptide antibodies (ACPA) compared to healthy individuals. We found that individuals with elevated levels of RA-specific auto-antibodies have comparable numbers of CD3+ T cells, CD22+ B cells, CD138+ plasma cells, CD68+ intimal lining and sublining macrophages, tryptase positive mast cells and vWF expression as healthy individuals. Similarly, DCE-MRI parameters in the knee joint were comparable between the preclinical RA patients and the healthy individuals. This shows that the emergence of RA-specific autoantibodies does not coincide with synovial inflammation. These findings suggest that the immune response is initiated in another compartment of the body. The lungs, gingiva and lymph nodes have been proposed as candidate organs for immune activation.[2-5] We propose that after initiation of the immune response at a distant site leading to production of ACPA, a second “hit” like a trauma or a viral infection, leading to inflammation in the synovium, may result in expression of citrullinated antigens in the joint, epitope spreading, and autonomous disease progression, finally resulting in full-blown RA.

Cardiovascular risk

In RA chronic systemic inflammation is associated with an increased risk of cardiovascular co-morbidity. It is, however, not clear from which point onwards RA patients are subjected to an increased risk of cardiovascular disease (CVD). As early RA represents already chronic
inflammation, and in preclinical arthritis patients elevated serum levels of CRP and sPLA2 have been found (in healthy individuals known to predict future coronary artery disease [6;7]), we hypothesized that the risk might already be present in the very early stages of RA. Therefore, in chapter 4 we investigated the atherogenicity of the very early stages of RA as well as the role of RA-associated antibodies by measuring intima media thickness in preclinical and early RA patients as well as in age- and gender-matched controls.

We found a positive correlation between serum ACPA levels and IMT in individuals with RA-associated antibodies. This finding is in line with previous studies, but more studies are needed to better understand the potential effects of autoantibodies on atherogenesis. We found no evidence of accelerated atherosclerosis in preclinical and recent-onset arthritis. This finding lends further support to the concept of active treatment of inflammation and and treat CVD risk factors according to national guidelines in RA patients from the onset of clinically manifest arthritis in order to decrease morbidity and mortality from CVD in more advanced RA.

The 2010 ACR/EULAR RA criteria
Recently, novel RA criteria have been formulated by ACR and EULAR aimed at early identification of RA patients.[8;9] With these criteria more early arthritis patients are correctly diagnosed with RA. However, cohort studies showed that although the 2010 ACR/EULAR criteria have a higher sensitivity, not only patients with persistent or even destructive arthritis are identified but also patients who develop self-limiting disease.[10-12] This may lead to a perhaps slightly more heterogeneous RA population. In chapter 5 we observed that the patients classified according to the 2010 ACR/EULAR criteria have on average similar expression of inflammatory cells such as T cells, B cells, macrophages, plasma cells compared to patients fulfilling the 1987 criteria for RA. This is of importance for future studies studying the synovial tissue of patients selected based on the novel 2010 ACR/EULAR RA criteria.

Stratification of early arthritis patients
It is known that neovascularisation, the influx of inflammatory cells and products of inflammatory cells are all involved in the pathogenesis of RA and other forms of arthritis. Since RA primarily involves the synovium, it can be anticipated that synovial tissue analysis may provide insight into the pathogenesis of RA and may help to identify different patient subsets associated with persistence of synovitis and degradation of the integrity of the joint.

The inflamed synovium is infiltrated by mainly macrophages and lymphocytes but also dendritic cells, mast cells and natural killer cells are present. The lymphocyte infiltration is organized in different profiles. In some patients lymphocytes are diffusely spread throughout the tissue, whereas in others lymphocytes are organized in clusters of B cells and T cells. [13;14] Even lymphoid neogenesis characterized by segregation of B and T cell clusters which are surrounded by follicular dendritic cells and high endothelial venules are present.[15;16] Previously it has been suggested that the lymphocyte infiltration pattern might be patient-specific with stable profiles in various joints and a stable profile over time,[17] although prospective studies addressing this issue have as yet not been published. Conceivably, patients expressing these different profiles represent different clinical and pathogenetic subsets.[13;18] Recent cross-sectional studies, however, have suggested that lymphoid neogenesis is not correlated with specific diagnoses, clinical phenotype, or pathogenetic entities but rather with
the level of synovial inflammation. In chapter 6 we studied lymphocyte infiltration patterns in relationship to diagnosis and outcome in an early arthritis patient cohort. We showed that lymphocyte neogenesis was present in about 30% of all patients, and FDCs were present in 15% of the patients with lymphoid neogenesis. There was no relationship between the presence of lymphocyte aggregates at baseline and definite diagnosis or development of persistent or erosive disease after follow-up. The presence of lymphocyte aggregates differed over time. Previously it was suggested that lymphoid neogenesis in the synovial merely reflects a high inflammatory state and might not be functional. Also in our cohort we observed that lymphoid neogenesis was associated with the level of inflammation.

Angiogenesis is one of the characteristic features of synovial inflammation. Newly formed vessels provide nutrients and allow inflammatory cells to invade the synovium. Angiopoietins and VEGF regulate vessel remodelling and are together with their receptors TIE-2 and VEGFR expressed in the inflamed synovium.[19-22] Angiogenesis has been shown to play a role in initiation and perpetuation of synovial inflammation and development of erosions in RA. In chapter 7 we studied expression of the angiopoietins Ang-1, Ang2, their receptor TIE-2, and VEGF and its receptor VEGFR. We observed that expression of the angiogenic factors was comparable between patients fulfilling RA criteria already at baseline and those who fulfilled criteria of RA during follow up. In contrast to serum, synovial tissue expression of Ang-1, Tie2 and pTie2 was increased in RA compared to SpA patients, whereas Ang-2 expression was increased in SpA. The Ang-1/pTie2 axis is specifically activated in patients with undifferentiated arthritis at baseline who have persistent disease after follow up. In RA the ratio of pTie2/Tie2 expression is related to development of erosive disease. With this study we showed for the first time that synovial tissue changes can be observed even before a diagnosis of RA is made. As the Ang-1/pTie2 axis plays an important role in the initiation, progression and development of joint destruction in RA these factors might be used as biomarkers predicting diagnosis and outcome.

Inflammatory cells in the inflamed synovium receive various extracellular stimuli. These stimuli initiate signal transduction cascades within the cell, thereby regulating gene expression. Mitogen-activated protein kinases (MAPK) play an important role in these signal transduction cascades. Members of the MAPK family, such as the p38 kinases, extracellular signal regulated kinases (ERKs) and c-Jun-N-terminal kinases (JNKs) are expressed and activated in inflamed synovial tissue.[23-25] Little is known about their contribution to the onset, persistence and development of joint destruction in RA. In chapter 8 we studied the relationship between synovial tissue MAPK expression and activation and diagnosis and outcome in early arthritis. We observed an increased expression of activated ERK and JNK in RA patients compared to other inflammatory joint diseases. JNK was even increased in patient with undifferentiated arthritis at baseline, who fulfilled criteria for RA after follow up; JNK predicted fulfilment of criteria for RA. Both ERK and JNK activation was associated with development of erosive disease. These observations suggest that JNK activation in the synovial tissue might be used as a novel biomarker predicting diagnosis and outcome in early arthritis patients. However, at the individual level JNK expression and the angiogenic factors comprising the Ang-1/pTie2 axis do not have enough power as biomarkers to guide treatment decisions in the individual patient.

Imaging of the synovium may provide other parameters that might be used as diagnostic or prognostic biomarkers in early arthritis patients. MRI is one of the most sensitive imaging modalities to study the synovium.[26;27] In chapters 9 and 10 we studied the synovial tissue
with dynamic-contrast-enhanced (DCE) MRI. With DCE-MRI uptake of a contrast agent in tissue over time is analyzed. Previously, different methods have been applied to quantify the uptake of contrast agent in the tissue.[28-31] Recently, we have developed a novel analysis technique, which allows analysis of the dynamics of the uptake of contrast agent in whole tissue lesions by color-coded time intensity curve (TIC) shape mapping.[32;33] With this technique the uptake of the contrast agent in time is visualized in a curve, the TIC. Depending on various tissue characteristics, such as vascularity, vessel permeability an interstitial space, this curve can have different shapes. With our novel technique these TIC shapes are analyzed pixel-by-pixel in a 3 dimensional volume.

In chapter 9 we showed that this technique is sensitive in detecting synovial inflammation. We observed a significant increase in TIC shape type 4 expression, which is characterized by a fast initial enhancement an a rapid wash-out phase, between RA patients and healthy controls. In oncology, TIC shape type 4 is associated with malignant, invasive growing tumors. As RA synovial tissue shares some clinical features with malignant tumors and TIC shape type 4 is increased in inflamed RA synovial tissue as shown in chapter 9, we hypothesized that DCE-MRI TIC shape type expression might be different in early arthritis patient groups. In chapter 10 we showed that TIC shape type 4 is significantly more seen in RA synovium compared to non-RA. However, there was still a fair overlap between RA and non-RA patients in the level of TIC shape type 4 expression, indicating that it has not enough power to be used as a single diagnostic biomarker.

Concluding remarks/Future directions
The preclinical phase, before the onset of arthritis, is a growing field of investigation. Since it is known that RA-specific autoantibodies can be detected in the serum up to 14 years before onset of arthritis,[34] we have rapidly increased our knowledge of the processes that are active in this preclinical phase of RA. Studies in retrospective and prospective cohorts of preclinical RA patients and their relatives have shown that the antibody response evolves over time. Both the immunoglobulin antibody isotypes and the epitopes against which the antibody response is directed increase over time. Similarly, the serum levels of IgM rheumatoid factor and ACPA increase in the phase preceding the onset of arthritis.[35-37] Future studies will be aimed at elucidating the question as to where in the body the initial immune response is initiated and which factors are associated with a break of tolerance resulting in an autoimmune response. As mentioned above, the lymph nodes, gingiva and lungs have been suggested as potential organs in which the response is initiated. Studying these tissues with various techniques might increase our insight into the processes active in the body in the preclinical phase.

In this thesis we also studied the clinical and immunohistochemical biomarkers in the earliest stages of clinically manifest RA in relationship to outcome, and provide initial proof of concept that new diagnostic and prognostic biomarkers may be identified based on synovial tissue analysis. Future studies will include gene arrays, proteomics and other high throughput techniques, which might eventually result in a subset of markers that can be used and developed into a diagnostic or prognostic tool with value for the individual patients.[38] In general, synovial tissue arthroscopic biopsy sampling is well tolerated (de Hair et al. submitted). With the development of ultrasound examination of the joints as a routine procedure in the outpatient clinic, ultrasound-guided synovial tissue biopsy may become widely available.
However, until the test characteristics of diagnostic and prognostic synovial biomarkers can be further improved to reliably guide treatment decisions in the individual patient, synovial tissue biopsy studies will mainly be used to increase our knowledge of the disease processes during onset and perpetuation of arthritis and to guide development of novel therapeutics.

Together, the studies described above provide insight into the earliest stages of RA, and may eventually lead to the development of novel diagnostic as well as preventive and therapeutic strategies.

REFERENCES


