SCOPE OF THIS THESIS

In this thesis effects of anti-inflammatory treatment on cardiovascular risk factors in patients with rheumatoid arthritis (RA) or ankylosing spondylitis (AS) are investigated against the background of the intertwined mechanisms involved in inflammation and atherosclerosis. We have studied traditional cardiovascular risk factors such as dyslipidemia, insulin resistance and thyroid dysfunction. Additionally, other factors potentially influencing or mediating cardiovascular risk, such as microvascular function, surrogate markers of cardiovascular disease, such as carotid intima media thickness and possible new markers for monitoring disease activity in AS are examined. Eventually, since the increased risk of cardiovascular disease in rheumatic diseases also encourages more prompt and intensive treatment of the disease itself, the effects of tight control therapy is evaluated in an early inflammatory arthritis cohort with mild disease activity. Finally, the role of cell derived microparticles in the inflammatory process in early RA is explored.

CARDIOVASCULAR RISK AND MONITORING OF DISEASE ACTIVITY IN PATIENTS WITH ANKYLOSING SPONDYLITIS

Although literature about cardiovascular risk is AS is limited, inflammation in AS seems to act independently or synergistically with other cardiovascular risk factors in the pathogenesis of atherosclerosis. There is increasing evidence that early functional and structural vascular abnormalities (surrogate markers) such as endothelial dysfunction, intima media thickness and arterial stiffness predict cardiovascular risk independently of “classical” cardiovascular risk factors.

In chapters 1.1 and 1.2 we observed impairment of different surrogate markers in patients with active AS. Microvascular endothelium-dependent vasodilatation, capillary recruitment in skin, as well as intima media thickness of the carotid artery were impaired as compared to control subjects. These differences remained after adjustment for established cardiovascular risk factors, which supports a potential role of these markers as a tool for identifying patients at elevated cardiovascular risk. In addition, microvascular function as well as capillary recruitment improved after TNF blocking therapy, which supports a role for inflammation deteriorating the microcirculation.

One of the most established risk factors for atherosclerosis is dyslipidemia. Inflammation deteriorates the lipid profile, which is also observed in several inflammatory rheumatic diseases. However, besides or even beyond focusing solely on lipid levels, in particular HDL-c levels, the actual composition of the HDL and thereby its functional characteristics seems to be important, in order to learn more about its effects on the vascular system and
cardiovascular risk. Nowadays, HDL protein profiling is increasingly used to determine the biochemical composition of HDL. In chapter 1.3 we demonstrate, that during anti-TNF treatment for AS, along with favourable changes in lipid profile, HDL composition is actually altered. Serum amyloid A (SAA), which is an acute phase reactant, transported mainly in HDL as an apolipoprotein and is associated with increased cardiovascular risk, was present on HDL during active disease. However, after anti-TNF treatment SAA disappeared from the HDL particle, rendering it more atheroprotective. This observation underlines the importance of understanding the role of functional characteristics of HDL cholesterol in cardiovascular diseases, related to chronic inflammatory conditions such as AS.

Disease activity in AS is generally measured with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Despite the fact that the BASDAI is a validated instrument used in many clinical trials as an outcome parameter for disease activity, it remains a subjective parameter that is based on a patient questionnaire. In recent years TNF blocking therapy has become widely available for patients with active AS. Since anti-TNF therapy is not without risks and is also very costly, it is of great importance to identify patients likely to (non)respond to this type of drug. Similar as in RA, there is need for more objective disease activity markers such as ESR and CRP. In AS, however, the sensitivity of these inflammatory markers as biomarkers of disease activity is controversial. Therefore, in chapter 1.4, we explored the usefulness of ESR, CRP, hsCRP, and SAA for monitoring inflammation in AS patients treated with anti-TNF along with the association between these inflammatory markers and the BASDAI over time. The data demonstrated that, unlike BASDAI, in particular CRP and SAA, served as a powerful tool not only for monitoring the efficacy of anti-TNF therapy, but also for the selection of AS patients with a high likelihood of responding to anti-TNF treatment.

CARDIOVASCULAR RISK IN PATIENTS WITH RHEUMATOID ARTHRITIS

Cardiovascular disease has been recognized as the major cause of excess morbidity and mortality in patients with RA. This increased cardiovascular risk in RA may partly be due to traditional cardiovascular risk factors, i.e. an atherogenic lipid profile and hypertension, but chronic inflammation is also thought to be important.

The "metabolic syndrome" is the co-occurrence of metabolic risk factors for both type 2 diabetes and cardiovascular disease (abdominal obesity, hyperglycemia, dyslipidemia (low HDL cholesterol and high triglycerides (TG) levels), and hypertension) and appears to be more prevalent in patients with RA compared to controls. The presence of the metabolic syndrome increases the risk for a future cardiovascular event. Another disease in which a
relationship with metabolic syndrome has been described is hypothyroidism. Hypothyroidism is a well established risk factor for cardiovascular disease and a common comorbidity in RA. Recent studies demonstrated that hypothyroidism is associated with cardiovascular disease in RA. Therefore we hypothesized that the prevalence of metabolic syndrome in patients with both RA and hypothyroidism could well be increased. Indeed, in chapter 2.1 it is demonstrated that the prevalence of metabolic syndrome in hypothyroid RA patients is substantially elevated compared to patients with RA alone. Since having metabolic syndrome increases the risk of a cardiovascular event, this may be a possible explanation for the observation that RA with concomitant hypothyroidism are at even more pronounced cardiovascular risk than patients with RA alone.

Another important mechanism at the basis of the development of atherosclerosis is endothelial dysfunction. Microvascular endothelial dysfunction is important not only in the development of target-organ damage in the heart and kidney, but also in the development of cardiovascular risk factors such as hypertension and insulin resistance. In longstanding RA impaired microvascular function has been previously demonstrated. An intriguing question is at what point in the inflammatory disease course abnormalities in (micro)vessel function occur. In chapter 2.2 we study microvascular function in early DMARD-naive RA-patients with low systemic inflammatory activity, since it was unknown whether microvascular dysfunction is already present in very early stages of the disease. We observed a preserved microvascular endothelium-dependent vasodilatation and capillary recruitment during reactive hyperemia in this patient group, suggesting that systemic inflammatory activity is necessary to cause microvascular dysfunction.

In chapter 2.3 we describe two cases of RA patients with concomitant diabetes in which glycaemic control parameters beneficially changed after initiation of anti-TNF therapy. Beneficial clinical effects of treatment of RA with TNFα-antagonists on concomitant diabetes had not been previously described. TNFα had been closely linked to obesity and insulin resistance and it was suggested that TNFα-antagonists, in addition to their known powerful anti-inflammatory effects, may have a beneficial effect on insulin resistance in rheumatic diseases. Although our results are observational, the findings presented in this chapter suggest that TNFα-blockade causes better glycaemic control in RA patients with concomitant diabetes, probably by improving insulin resistance.

EARLY INFLAMMATORY ARTHRITIS

Early and aggressive treatment of patients with RA has increasingly been shown successful, particularly with combinations of disease modifying antirheumatic drugs (DMARD), also
containing anti-TNF therapy. Among the results are percentages of sustained (drug-free) remission of around 40%, excellent functional status and nearly complete arrest of radiological damage progression. In an attempt to explain these better results than had been attained before in RA of longer duration, the concept of a “window of opportunity” was proposed, suggesting that early suppression of active inflammation produces long-term benefits. **Chapter 3.1** Investigates whether the approach of striking fast and hard, which has been shown useful in active RA, is also effective in arthritis patients presenting with only moderately active disease, i.e. in those patients who would not meet the usual inclusion criteria for trials in active RA. In this randomized trial of a “fast and hard” versus a “go low, go slow” approach to early oligoarthritis, most patients had an excellent outcome with respect to disease activity, with remission rates exceeding 50%, functionality and radiographic damage regardless of the treatment group they were randomized to. Based on this data we cannot recommend aggressive therapy in all patients presenting with two to five swollen joints. This study shows that the benefit of tight control treatment in early inflammatory arthritis is not as evident as it is in polyarthritis. Since a minority of patients in both groups experienced radiographic progression despite treatment with higher-dose methotrexate and despite being in remission at most time points it may be suggested that the widely used DAS-driven therapy in reducing structural joint damage may be less useful than for example a radiographic-driven therapy.

Recently, cell derived microparticles (MP) have emerged as a new pro-inflammatory mediator. MP were shown to be associated with complement activation, inflammation and coagulation in various diseases, including inflammatory diseases. In fact, they are thought to amplify or disseminate inflammation. MP probably trigger inflammation by several processes such as activation of endothelial cells and leukocytes, triggering production and release of chemokines and cytokines, and by activating the complement cascade which is thought to play a key role in the pathogenesis of RA. On the other hand, inflammation may trigger MP formation. For instance, in vitro studies showed that MP are released from cells incubated with TNFα or IL-1, and a study in mice showed that the number of platelet-derived MP in plasma markedly increased upon injection with TNFα. However, although inflammation causes release of MP and in turn MP may induce or enhance inflammation, it remains unknown whether circulating MP merely reflect ongoing inflammation or whether MP actually contribute to the disease development. In **chapter 3.2** we determine whether circulating MP numbers are associated with inflammatory activity in RA patients, by comparing MP in very early yet untreated arthritis patients and healthy controls. Additionally, we determined the effects of changes in disease activity upon intense anti-inflammatory therapy with the COBRA strategy on MP numbers and composition. This study demonstrates that MP
exposing complement components (C1q) or activator molecules (CRP or SAP) are elevated in early active RA. Although the earlier mentioned strong anti-inflammatory combination DMARD therapy strongly suppressed inflammatory activity, circulating MP were unaffected. These observations may suggest that inflammation is not the underlying cause of MP generation in these patients. Our main finding that powerful inhibition of inflammation did reduce disease activity but did not disturb the association between circulating MP and complement activation, suggests that both MP and complement actively contribute to the development of and/or the chronic character of inflammatory diseases such as RA.

**GENERALISIBILITY**

This thesis contributes to the concept that inflammatory diseases such as RA and AS increase the risk of cardiovascular disease. By demonstrating that inflammation deteriorates lipid profile, and that hypothyroidism elevates the risk of metabolic syndrome in RA patients, this thesis shows that the worsening of cardiovascular risk is, at least partially, caused by deterioration of traditional risk factors. In addition this thesis addresses the possibility of a direct pro-atherogenic effect of inflammation, the key feature of rheumatic diseases, on cardiovascular risk. Harmful effects of inflammation are supported by demonstrating impaired microvascular function, increased intima media thickness and increased arterial stiffness in patients with AS. Moreover signs of impaired glucose handling in patients with RA, partly reversible by anti-inflammatory therapy, are demonstrated.

Our findings may have implications for screening and management of cardiovascular comorbidity in patients with inflammatory diseases. Their increased risk should encourage active treatment for the rheumatic disease itself, since lowering the inflammatory activity seems to slow down the progression of atherosclerosis. This thesis contributes to this notion by testing an aggressive treatment strategy in a very early stage of the inflammatory arthritis. The data show that results of both tight-control therapy and conventional therapy are excellent. However, it also shows that, until now, full disease control including radiographic arrest in all patients remains an elusive target even in moderately active early arthritis. The fact that the majority of the patients with radiographic progression were in DAS remission most of the time points out that especially in early and less active arthritis patients other markers may be preferred to monitor disease activity.
FURTHER RESEARCH

Inflammation may well be the missing link between inflammatory rheumatic conditions and excess cardiovascular risk. Future research should further elucidate the mechanisms by which such inflammation enhances cardiovascular risk. Some studies report associations between cardiovascular surrogate markers and time-specific inflammatory parameters, but not with chronic inflammatory parameters, whilst other studies report opposite results. Hence, it is interesting to investigate whether the contribution of ‘acute’ versus ‘chronic’ inflammation to cardiovascular risk is different. In addition, an intriguing question is if, and if so at what stages of the disease, the adverse effects of inflammation on the vascular system are still reversible. Observational studies demonstrate that arthritis patients, who respond to effective anti-inflammatory therapy (i.e. TNF blockade or methotrexate therapy), are associated with both a lower cardiovascular risk and improved cardiovascular risk profile. These observations strongly support further research in this area to unravel pathways through which inflammation mediates cardiovascular disease and effective anti-inflammatory drugs may have (anti-) atherogenic effects. Taken together, appreciation of shared inflammatory mechanisms in atherosclerosis and chronic inflammatory conditions, like inflammatory arthritis, is mandatory for better understanding of the cardiovascular burden in this population.

Regarding the early arthritis trial presented in this thesis, in which a minority of patients in both inflammatory arthritis groups experienced radiographic progression despite treatment with higher-dose methotrexate and despite being in remission at most time points, it may be suggested that the widely used DAS-driven therapy in reducing structural joint damage may be less useful than for example a radiographic-driven therapy. More research is needed to answer these intriguing questions and particularly to further refine early prediction and thus guide therapy at the individual level.

CONCLUSIONS / PRACTICAL IMPLICATIONS

This thesis demonstrates that treatment of early arthritis patients results in high remission rates, low bone damage and excellent functional status. However, full disease control including radiographic arrest in all patients remains an elusive target even in moderately active early arthritis. The fact that the majority of patients with radiographic progression were in DAS remission most of the time, points out that especially in early and less active arthritis patients other markers may be preferred to monitor disease activity.

A better disease control enhances the importance of identifying and treating co-morbid conditions associated with the underlying inflammatory disease. This thesis provides a
rationale for increased attention for cardiovascular risk in patients with RA and AS. Clinically, rheumatologists should be aware of the increased cardiovascular risk in their patients and should be encouraged to actively screen their population. Although still under debate, this thesis supports the idea of primary prevention of cardiovascular disease in patients with inflammatory diseases (by agents such as aspirin, statins or by early treatment of high blood pressure). In addition, cardiologists should recognize that rheumatic inflammatory disease is an important risk factor for cardiovascular disease. Eventually, patients with an inflammatory rheumatic disease should be made aware of the excess cardiovascular risk factor they run, and should be encouraged to improve their modifiable cardiovascular risk factors.