Rheumatoid arthritis: predictors of clinical response to TNF blockade
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RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by synovitis and progressive damage to articular cartilage and subchondral bone in the majority of patients. In industrialized countries, 0.5%-1% is afflicted by RA and this disease is almost three times more common in women than men. Subjects of any age can be affected but onset is usually between the ages of 40 and 60. If not adequately treated, it is a disabling condition due to systemic inflammation and destruction of bone and cartilage, leading to loss of functioning and mobility. Patients are classified on the basis of symptoms, physical examination, and laboratory results according to the new 2010 RA criteria developed by the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR). In about 70% of RA patients, IgM Rheumatoid Factor (RF) and/or anti-citrullinated protein antibodies (ACPAs) are present. The most commonly used tests to detect ACPAs is the anti-cyclic citrullinated peptide (CCP) test.

Besides synovial inflammation and joint destruction, systemic complications may occur. Constitutional symptoms including fatigue, low-grade fever, malaise, morning stiffness, loss of appetite, and loss of weight are common systemic manifestations seen in patients with active RA. The rheumatoid nodule, which is often subcutaneous, is the cutaneous feature most characteristic of RA. But all organs can be affected by RA, most commonly seen are: interstitial lung disease, renal amyloidosis, vasculitis of the skin, episcleritis, and poly-neuropathy of multiple mono-neuropathies.

Besides these well-known systemic manifestations, during the last years it has become increasingly evident that RA patients have an increased cardiovascular (CV) risk which cannot be explained by traditional CV risk factors alone. Evidence for the pivotal role of inflammation driving increased CV risk in RA is compelling and it is thought that this risk will reduce by decreasing inflammation. RA is also a well-known risk factor for osteoporosis, not only due to the frequently used glucocorticosteroids (GC) and immobility but also due to increased osteoclastic activation. Reducing inflammation by different treatments, even including the use of low-dose GC (< 7.5 mg/day), may have a beneficial effect on bone mineral density (BMD) in RA.

Aetiology and Pathogenesis

The cause of RA is unknown. The combination of genetic susceptibility and environmental inciting events may lead to clinical signs and symptoms. Variability in both the cellular and molecular features of the inflamed synovium, as well as the heterogeneous response to treatment, suggest that RA is a clinical syndrome comprising different patho-genetic subsets. Consistent with the notion of heterogeneity, patients can be divided into autoantibody positive and autoantibody negative patients. ACPA are highly specific for RA and may be detected years before the first clinical manifestation of RA. ACPA positive RA patients have a more aggressive disease course with lower remission rates and more erosive joint damage and define a specific disease subset of RA patients. ACPAs have been shown to initiate and enhance arthritis in murine models, and human ACPAs are able to activate both Fc receptor–positive cells and the complement system, indicating that they could play a significant role in disease pathogenesis.
Heritability of RA has been estimated to be about 50%-60% for both ACPA positive and ACPA negative RA patients. Thirty gene regions are associated with RA and distinct genetic risk factors are thought to be related to ACPA negative or ACPA positive RA which also suggests different patho-physiological pathways. Apart from gender, the main known genetic factor is HLA which is associated with a 4 to 5 times increased relative risk of RA. Another allele is PTPN22 which is also associated with increased RA risk especially auto-antibody positive RA. During the last years it has become clear that smoking doubles the risk of RA, but only in autoantibody-positive RA. The combined effect of HLA shared epitope alleles and tobacco exposure has been shown to exceed the sum of their single effects, suggesting a gene–environment interaction between the HLA shared epitope alleles and tobacco exposure with regard to the development of ACPA-positive RA. Recently it has been suggested that smoking promotes nonspecific citrullination rather than citrullination of specific antigens.

Another intriguing aspect of the pathophysiology of RA is related to the question as to where does it start? Initially, the hypothesis has been that RA starts in the synovial membrane, which becomes inflamed due to several inciting events as described above. This notion is supported by the observation that activation of osteoclasts with subsequent erosions may occur in early disease. Alternatively, the disease process may start at sites other than the joint like the lymphoid tissue with local ACPA formation (in ACPA positive RA ) and the need for a second hit inducing synovitis in the joints. The initiation of synovitis is not at random but takes place at the bare area, the area where the synovium inserts the bone and where erosions are found. Conceivably, the bone marrow is important in the development of synovial inflammation. This notion is supported by recent advanced MRI studies showing bone cysts and osteitis before synovitis develops.

The Inflamed Synovium

The synovium, in normal joints, is a thin lining layer that serves as an important source of nutrients for cartilage since cartilage itself is avascular. Synovial cells synthesize joint lubricants such as hyaluronic acid, as well as collagens and fibronectin that constitute the structural framework of the synovial interstitium. In RA, this lining layer is greatly hypertrophied with fibroblasts and synovial macrophages. Besides the lining layer there is the sublining area of the synovium. Normally this area has very few cells. In RA, however, the subintimal area is infiltrated with inflammatory cells, predominantly macrophages, T lymphocytes and plasma cells, but also other cells like B lymphocytes, dendritic cells, natural killer cells, and mast cells. The intense cellular infiltrate is accompanied by new blood vessel growth, termed neoangiogenesis.

In RA, the hypertrophied synovium (at the site of invasion also called pannus) invades and erodes contiguous bone and cartilage. This inflamed synovium is associated with increased production of pro-inflammatory cytokines and chemokines by in particular macrophages and fibroblast-like synoviocytes. Synovial fibroblasts are activated, releasing matrix metalloproteinases and other degrading enzymes, leading to destruction of matrix tissues. In the synovium B-cells differentiate into plasma cells, producing polyclonal immunoglobulins, including RF and ACPA.

Between RA patients cellular and molecular features of the inflamed synovium vary widely. Also the extent and pattern of synovial lymphocyte infiltration are remarkably variable in
RA. In some tissues, a diffuse or scarce infiltration of T cells is present, while in others, B and T cells are organized in lymphocyte aggregates that may exhibit germinal center–like features. The presence of lymphocyte aggregates is not related to a local germinal center–like humoral response, but is rather thought to be secondary to the chronic inflammatory process driving RA.

Role of Adipocytokines
In recent years adipocytokines have provided a plausible link between obesity, the metabolic syndrome and inflammation. Excess fat (white adipose tissue) generates chronic low grade inflammation that eventually triggers insulin resistance and contributes to the co-morbidities associated with metabolic syndrome (hypertension, atherosclerosis, dyslipidaemia and diabetes mellitus). RA is associated with increased insulin resistance and cardiovascular risk, possibly due to increased inflammation. Adipocytokines are increased in RA and it has been suggested that they have a role in CV disease in RA although convincing data are as yet not available.

Obesity has consistently been associated with reduced radiological damage. This seems to be a paradox, as adipose tissue is associated with increased inflammation due to (adipo) cytokines produced by fat tissue. The biological mechanisms underlying this correlation is at present unclear. Especially the adipocytokines associated with increased BMI might provide the link between obesity and reduced radiological damage. At this moment data about the role of adipocytokines in relation to bone erosions are conflicting and long term prospective studies are not available.

The best known adipocytokines are leptin, adiponectin, resistin, vaspin and visfatin. Leptin may promote inflammation by inducing Th1 phenotype development. Adiponectin increases production of pro-inflammatory cytokines by synovial fibroblasts and is associated in the joint with increased inflammation. On the other hand adiponectin has anti-inflammatory effects on the vasculature and may improve insulin sensitivity. Increased serum adiponectin levels in RA patients are associated with decreased inflammation. Resistin belongs to a family of proteins found in foci of inflammation, where they may contribute to the inflammatory response. Visfatin, also known as pre-B cell colony-enhancing factor, was recently characterized as a potent pro-inflammatory mediator in RA. Vaspin is a recently recognized adipocytokine with insulin-sensitizing effects and is increased in the synovial fluid of RA patients. However, the exact role of adipocytokines in RA patho-physiology is unclear at this moment.

RA and osteoporosis
Osteoporosis is more frequent in patients with RA than in the general population and several factors may explain this increased incidence. Systemic and local inflammation activate the osteoclast, which has been identified as the predominant cell type mediating bone loss in RA. Several cytokines and growth factors involved in the inflammatory processes in RA have been demonstrated to activate osteoclasts either directly, by acting on cells of the osteoclast-lineage, or indirectly, by acting on other cell types to modulate expression of the key osteoclastogenic factor receptor activator of nuclear factor (NF) kappaB ligand (RANKL) and/or its inhibitor osteoprotegerin (OPG). Other risk factors for osteoporosis are decreased immobility due to inflamed and in later stages destroyed joints and use of GC.
bone formation mostly by inhibiting osteoblasts\textsuperscript{75}. Although in general RA patients using GC have an increased risk to develop osteoporosis and fractures, there are also data suggesting that especially use of low dose GC may have a net beneficial effect on bone due to reduced inflammation and improvement of immobility\textsuperscript{76}. Osteoporotic fractures increase morbidity and mortality especially in elderly patients\textsuperscript{77}. Preventing osteoporosis in patients with RA is important, especially in female patients who are past menopausal and thereby already at risk of developing osteoporosis.

Response to Treatment
In the end of the last century RA was mainly treated with conventional drugs like methotrexate (MTX), sulfasalazine, hydroxychloroquine, azathioprine, leflunomide and gold injections. These medications are called disease-modifying antirheumatic drugs (DMARDs) and decrease inflammation in the joints by their effects on immune cells\textsuperscript{78-81}. Most of the DMARDs are used as tablets but MTX is sometimes injected to decrease gastrointestinal side effects\textsuperscript{82}. All these treatment start to work after 6-12 weeks of treatment\textsuperscript{79}. GC are also very effective in most RA patients and may exert an effect after a few days\textsuperscript{83,84}. However, due to side effects like increased cardiovascular risk\textsuperscript{85,86}, glucose intolerance and osteoporosis\textsuperscript{87}, GC are often used temporarily as bridging therapy as most DMARDs take time to be effective. GC are also used in low doses as chronic therapy and it is debated if the side effects of these low dose GC on the cardiovascular system\textsuperscript{88} and bone are neutralized due to decreased inflammation\textsuperscript{89}. DMARD therapy is effective in about 65\% of RA patients although not always sufficient to control disease activity completely\textsuperscript{90}. At the end of the last century a new class of drugs, so called biologicals, became available for the treatment of RA\textsuperscript{91}. Biologic agents are bioengineered drugs produced by cell cultures to produce large amounts of the therapeutic protein\textsuperscript{92}. These drugs specifically target one pro-inflammatory molecule involved in the inflammatory cascade, thereby reducing inflammation\textsuperscript{92}. At this moment 5 different classes of biological drugs with different targets are available for treating RA patients, all with moderate response rates of almost 70\%\textsuperscript{93-97}. Remission is induced in only a minority of these patients. The five different biological drugs are: 1. TNF-alpha blockers (infliximab, adalimumab, etanercept, golimumab and certolizumab), 2. An -IL-6 receptor antibody (tocilizumab), 3. An inhibitor of the co-stimulatory receptors CD80 and CD86 on T-cells to prevent the interaction between T-cells and antigen presenting cells (abatacept), 4. An anti-CD20 antibody (rituximab) and 5. IL-1 receptor antagonist (anakinra)\textsuperscript{92}. IL-1 receptor antagonist is less effective in comparison with the other 4 biological classes in RA\textsuperscript{98}. Together these treatment options make RA anno 2012 a treatable disease.

In the Netherlands response to treatment is evaluated mostly by using diseases activity scores in 28 or 44 joints (DAS28 or DAS44)\textsuperscript{99,100}. Disease activity scores are computed using: a. tender joint score (TJC) and b. swollen joint core (SJC) in 28, 44 or 68 joints, c. ESR or CRP levels and d. patient’s global assessment of disease activity in the last week (for formula see: www.das-score.nl)\textsuperscript{99}. In daily clinical practice a reduction in DAS28 ≥ 1.2 over time is often used as a criterion for response\textsuperscript{101}. EULAR response criteria are often reported in clinical studies where patients are divided into three groups based on the response to treatment (good, moderate or non-response) based on the change in DAS28\textsuperscript{102,103}. ACR 20%-50%-70% criteria are also often used to report clinical response. ACR response criteria are based on respectively 20\%, 50\% or 70\% decrease of SJC and TJC and three of five other criteria namely patient’s or physician’s
assessment of disease activity, patient’s assessment of pain, disability and levels of acute phase reactants. With better treatment options, as discussed above, new remission criteria have recently been introduced as well.

Earlier initiation of treatment and more effective therapies used in a systematic way based on the treat to target principle have also significantly improved outcome parameters. It is important to reach remission shortly after diagnosis as results for the long term are better with less medication needed to maintain remission and better results in functionality and disease activity. With improving treatment of RA it has been suggested that also cardiovascular risk and risk on osteoporosis may be reduced.

At this moment treatment of RA in the Netherlands is started using conventional DMARDs (mostly MTX, sulfasalazine and/or plaquenil) in combination with GC if indicated. When two conventional DMARDs (which may have been used in combination) are ineffective and there is still active disease (DAS28 ≥ 3.2), then biological treatment can be reimbursed. TNF blockers are currently often used as a first line biological in light of the vast experience with this class of drugs. Anti-IL6 is also approved for RA in patients who used one DMARD or do not tolerate MTX. Abatacept and rituximab can be given when patients failed TNF blockade.

**TNF blockade**

TNF is a cytokine produced primarily by monocytes and macrophages but many immune and also non-immune cells can produce TNF. TNF has pro-inflammatory properties which in physiologic circumstances play a role in host defence against several danger signals in response to for instance bacterial infection or local tissue injury. In RA TNF is increased in the synovial tissue, promoting synovial inflammation and proliferation of fibroblasts as well as matrix degradation. Its synovial expression levels are significantly related to clinical signs and symptoms. In RA TNF is produced primarily by synovial macrophages. It is not exactly known which stimuli induce these synovial macrophages to produce TNF. The critical role of TNF in the pathogenesis of synovial inflammation in RA is underscored by the beneficial effects of TNF blockade. After anti-TNF treatment expression of adhesion molecules and pro-inflammatory cytokines is decreased in the rheumatoid synovium.

As described above, 5 TNF blockers are currently available. The four monoclonal antibodies targeting TNF are infliximab, golimumab, adalimumab, and certolizumab pegol. Etanercept is not an antibody, but a engineered construct consisting of the p75 receptor and the Fc part of human IgG, and also binds TNF.

Besides the positive effects on RA disease activity and protection against progressive joint destruction, side effects may occur as well. The most common side effects of TNF inhibition are infectious complications. Also a slight increase of skin malignancies may be found in patients treated with TNF blockade. In addition, biological drugs are associated with considerable costs. Due to increasing costs of health care and the current economic situation, cost-effectiveness is increasingly important. TNF blockade is an effective therapy in RA patients, but remission is induced in only a minority and almost 30% of patients do not respond. Over time response is diminished in a substantial number of RA patients, possibly in part due to formation of anti-drug antibodies. This highlights the importance of research aimed at developing personalized health care concepts to improve efficacy and cost-effectiveness.
As described above, RA is a chronic autoimmune inflammatory disease with destructive effects on bone and cartilage due to synovitis of joints which in recent years became a treatable disease due to several new treatment options. This thesis is focused on TNF inhibition in this disease, and is divided into two sections.

Section 1

Treatment of RA has improved in recent years after introduction of TNF blockade. However, almost 30% does not respond to this treatment. In general, treatment responses in RA are better when started earlier in the disease course. Recently, we found that increased TNF levels in synovium are associated with increased response rates. Also, activation of genes associated with increased inflammation is associated with a better response to anti-TNF treatment. At this moment, these associations are not strong enough to predict response to TNF blockade in the individual patient. Therefore, in this thesis we attempt to identify additional molecular or clinical biomarkers that might help to improve the prediction of response to TNF blockade. IgM RF and ACPA of the IgG isotype are well known diagnostic and prognostic biomarkers in RA. The relationship between baseline values of IgM RF and IgG ACPA and response to TNF blockade is currently not clear. IgA RF is associated with erosive disease in RA and recently found

OUTLINE OF THIS THESIS

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to be negatively associated with response to TNF\(^{128}\). No data exist about the other iso-types in relation to response in RA. Therefore, in chapter 2 we investigated if different iso-types (IgG, IgA, IgM) of RF and ACPA are related to response to infliximab in RA patients.

Some patients with RA exhibit lymphocyte aggregates in the synovium. The role of synovial lymphocyte aggregates in the pathogenesis of RA is still controversial. It has been proposed that lymphocyte aggregates may be involved in the auto-reactive humoral response\(^{50}\). On the other hand, our group recently suggested that the presence of lymphocyte aggregates is not related to a local germinal center-like humoral response, but is rather a phenomenon secondary to chronic inflammation in RA\(^{48,51}\). In chapter 3 we explored if synovial lymphocyte aggregates are an independent predictor of response in RA patients treated with infliximab. We also investigated if synovial lymphocytes are reversible after anti-TNF antibody treatment.

The E3 ubiquitin ligase synoviolin functions as an anti-apoptotic factor and its expression is associated with arthritis development in mice\(^{131}\). Recent research suggested that increased synoviolin levels in peripheral blood are associated with non-response to infliximab treatment in patients with RA\(^{132}\). In chapter 4 we studied the expression of synovial synoviolin in RA compared to patients with psoriatic arthritis and osteoarthritis. We also investigate the relationship between expression levels at baseline and the clinical response to TNF blockade.

Adipose tissue has immunomodulating effects in RA, although the exact role is at present unclear. In chapter 5 we investigated if response to infliximab, which is dosed on body weight, is related to BMI.

**Section 2**

In the second part of this thesis we investigated the role of TNF on adipocytokines and bone mineral density (BMD). In recent years adipocytokines have provided a plausible link between obesity, the metabolic syndrome and inflammation\(^{52,53}\). Adipocytokines are increased in RA\(^{57}\) and may exert pro-inflammatory and destructive effects\(^{53,61,64,70}\). It has been suggested that adipocytokines have a role in CV disease in RA although convincing data are not available yet. In Chapter 6 we examined the effects of several months of treatment with TNF blockers on adipocytokine levels and compared this effect with GC. We related these effects to clinical parameters like radiological damage, efficacy and lipid index.

Due to systemic inflammation osteoporosis is more frequent in RA patients. In chapter 7 we investigated the effects of ant TNF antibody therapy on BMD of the lumbar spine and femur neck in patients with RA.
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GENERAL INTRODUCTION


