SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Assessment of cardiovascular risk and rapid radiological progression of inflammatory arthritis

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UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2023

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The Examination takes place at Building B, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 1st February 2024, 11:00 am.

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The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 1st February 2024, 01:00 pm

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disease affecting primarily the joints. It is most commonly characterised by symmetric arthritis affecting the small joints of the hands and feet, leading to joint destruction and severe joint deformities if left untreated. There are several radiological damage scoring systems to monitor progression, such as the van der Heijde modified Sharp score (vdHSS/SHS). In addition to joint involvement, the disease may also present with various extra-articular manifestations (e.g. interstitial lung disease) and co-morbidities need to be controlled, as they collectively increase morbidity and worsen quality of life. In RA, cardiovascular (CV) disease is by far the most important comorbidity, due to its prevalence and adverse impact on survival. Other inflammatory arthritic conditions, such as ankylosing spondylitis (SPA), are also associated with a similar phenomenon. SPA belongs to the group of spondylarthritic disorders (SpA), which is characterised by axial joint involvement, sacroiliitis and spondylitis, progressive development and spinal stiffness.

In both inflammatory conditions, delay or failure to provide adequate treatment has serious consequences, both in terms of musculoskeletal damage and the rapid onset of CV disease. The treatment strategy in both diseases is staged, with different conventional synthetic disease-modifying therapies (csDMARDs) followed by targeted therapies targeting key molecules identified in the pathogenesis of the disease. Among the targeted therapies, tumour necrosis factor α (TNF-α) inhibitors are currently the first line of choice among bDMARDs in both RA and SPA.

1.1. The pathogenesis of rheumatoid arthritis

The pathogenesis of RA is complex, with many factors at play. In addition to genetic and environmental influences, various immunological processes are responsible for the onset and development of the disease. More than 100 genetic loci are associated with the risk of disease development and progression, mainly involving products of immune effector and regulator genes. The most important are genes encoding MHC class II molecules (mainly *HLADR01/04*). These molecules are located on the surface of antigen-presenting cells and are involved in antigen presentation. The T helper (Th) cell recognises this MHC class II antigen on the surface of the antigen-presenting cell (APC) and the combination of peptides bound to it and previously degraded by the APC, and thus activates this T cell subtype and initiates further immunological processes. Other RA-prone genes encode proteins involved in e.g. costimulatory pathways (*CD28, CD40*, cytokines, interleukins e.g. *IL6*), intracellular signalling (e.g. *PTPN22, STAT4, TNFAIP3*) or post-translational modifying enzymes (e.g. *PADI*). Against this genetic background, environmental factors may induce the disease. Such provoking factors may include smoking, vitamin D deficiency and obesity. Altered gastrointestinal microbiome may also affect immune regulation through *Porphyromonas gingivalis in* the oral mucosa, which may provoke disease development through tissue cytrullination.

PADI, activated early in the disease in response to some environmental stimulus, contributes to the formation of citrullinated antigens and stimulates the production of various autoantibodies (anti-citrullinated protein/peptide antibodies [ACPA], rheumatoid factor [RF]). Disease-specific ACPA and RF form an immune complex with citrullinated proteins and activate macrophages, resulting in the release of various inflammatory cytokines (e.g. IL-6, TNF-α). In addition, Th17 cells stimulated by their own antigen produce IL-17. This cytokine-mediated pathway is at the core of the pathogenesis of RA. Proinflammatory cytokines, such as TNF-α, IL-6, IL-17, activate synovial fibroblasts, which produce additional pathogenic molecules, cytokines (e.g. IL-6, granulocyte-macrophage colony stimulating factor [GM-CSF]) and enzymes (e.g. matrix metalloproteinases [MMPs]). The amount of these mediators increases continuously during inflammation.

In addition to these, various signals activate innate lymphoid cells (ILC2), notably IL-2 from Th17 cells, IL-7 from inflamed tissue and IL-33 from endothelium or synovial fibroblasts. ILC2 eventually produces GM-CSF, which plays a role in the chronic phase of RA. These stimuli activate receptor activator nuclear factor kappa B ligand (RANKL) on fibroblasts and induce the formation of osteoclasts from extrinsic and intrinsic macrophages. RANKL binds to the RANK receptor and activates MAPK via c-Jun signalling and NF-κB via c-Fos signalling. Finally, NFATc1 induces the differentiation of osteoclasts involved in bone resorption. Recent analyses have shown that macrophages migrating from outside the synovium (extrinsic, bone marrow-derived) have a pathogenetic role by secreting proinflammatory cytokines, whereas intrinsic macrophages present in the synovium from birth have an anti-arthritogenic effect.

1.2. TNF-α and its role in the pathogenesis of rheumatoid arthritis

TNF-α is the key cytokine in the pathogenesis of RA at many points, but is also present in many other inflammatory arthritic diseases (e.g. SpA). TNF-α belongs to the TNF superfamily. Among the members of this family, TNF-α, lymphotoxins (LTα and LTβ) and RANKL are involved in lymphoid tissue development and maintenance of the inflammatory state, among other things. They may be involved in costimulation during the immune response (CD27, CD30), while others are involved in the process of apoptosis (e.g. Fas ligand, TRAIL). TNF-α is predominantly produced by monocytes/macrophages. This cytokine is produced as a transmembrane protein (memTNF), which is cleaved from the cell surface by a metalloproteinase, the enzyme TNF convertase (TACE), and thus converted to a soluble form. There are two TNF receptors, one 55kDa (TNFR1) and one 75 kDa (TNFR2), which mediate intracellular signalling. TNFR1 is found on all cells, whereas TNFR2 is found only on T cells, myeloid and endothelial cells. The signaling pathway that passes through TNFR1 leads to the upregulation of proinflammatory cytokines in RA, while the other signaling pathway acts against inflammation, arthritis. TNF-α is indeed the master regulator of inflammatory processes and is involved in almost all basic mechanisms. It stimulates the expression of many adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), E-selectin, intercellular adhesion molecule 1 (ICAM-1) and others on both the vascular endothelium and leukocyte surface. Thus, it stimulates the egress of inflammatory cells from the vasculature and their accumulation in inflamed tissues. TNF-α also stimulates the production of IL-6, IL-17, GM-CSF, leukaemia inhibitory factor (LIF), several chemotactic chemokines (IL-8, MCP-1, MIP-1, ENA-78) and prostaglandin E_2 , among others. A relationship between TNF-α and IL-1 is also suggested, as IL-1 production by synovial fibroblasts is reduced by anti-TNF-α treatment. Angiogenesis is a very important mechanism, as both the number of new small vessels and the total endothelial surface area increase during inflammation and thus may further increase the tissue accumulation of inflammatory leukocytes. TNF-α is also a major mediator of this process. TNF-α inhibitor treatment reduces vascular endothelial activation.

In terms of joint destruction, this cytokine plays a central role in both cartilage and bone damage. On the one hand, it enhances the production of cartilage-degrading MMPs such as collagenase, elastase, stromelysin, and on the other hand, it stimulates the production of RANK and RANKL, which play a major role in bone degradation, as well as IL-17 and PGE_2 , while inhibiting the release of tissue inhibitor of metalloproteinase (TIMP). In terms of bone metabolism, the RANK-RANKL system is mainly involved in osteoclast activation and destruction, while the Wnt-β-catenin system is involved in osteoblast activation and bone formation. The counter-regulatory mechanisms of the RANK-RANKL and Wnt axes play a fundamental role in the balance of bone formation and breakdown, and in the disruption of this balance. The production of DKK-1 protein can be enhanced by TNF-α, an important counter-regulator of Wnt, and thus acts in the direction of bone resorption. TNF-α therefore plays an important role in both local bone destruction (erosions) and the development of generalised osteoporosis. A beneficial effect is observed with the use of anti-TNF biologics, with a reduction in the formation of erosions and generalised bone loss.

1.3 Role of TNF-α in the pathogenesis of ankylosing spondylitis

The pathogenesis of SPA is less well understood than that of RA. Here, too, several genetic variations have been associated with the disease (e.g. *IL-23R* gene), but the best known is the association with HLA-B27. A number of environmental influences (gut inflammation, dysbiosis, smoking) are also implicated in the development of the disease, and these also seem to be essential for the development of the disease. Regarding the cytokine network, the IL-23/IL-17 axis plays a major role in SPA. IL-23 is a heterodimeric molecule belonging to the IL-12 superfamily and is primarily produced by antigen-presenting cells (macrophages, intestinal epithelial cells, dendritic cells). At sites of immune barrier function (gut, skin) where dendritic cells are activated, IL-23 is transported and migrates to disease sites. It mainly stimulates Th17 cells to produce additional inflammatory or regulatory cytokines (IL-17, TNF-α, IL-22). Mesenchymal cells act as a kind of connecting link between bone metabolism and immune homeostasis. These cells partly induce a pathological osteogenesis and promote monocyte migration and polarisation of proinflammatory macrophages through various chemokines (CCL2) and increase their production of TNF-α, causing local inflammation. TNF-α provoked inflammation eventually leads to bone destruction and is responsible for synovitis in peripheral joints. Mechanical stress, which is also considered a key factor in the pathogenesis of SPA, activates stromal cells, which attract monocytes by releasing chemokines and also generate TNF-α production. In the bone marrow and synovium, the interaction between mesenchymal and T cells is sufficient for IL-17 production and monocyte appearance without IL-23. This suggests that IL-23 may have a different role in the development of inflammation in different tissues and that IL-17 production is possible without IL-23 (e.g. via mucosa-associated invariant T cells [MAIT], ILC3 cells, γδ T cells). These findings suggest that the key cytokine in the pathogenesis of SPA is IL-17 rather than IL-23, the latter playing a crucial role in the initial inflammatory phase of the disease. In patients with SPA, IL-17 is produced by innate immune actors (neutrophils, mast cells), γδ T cells, MAIT cells, CD8+ T cells and Th17 cells. IL-17 has a complex effect on bone metabolism, promoting increased osteoclast activity, but IL-17A also has an effect on osteoblast differentiation.

1.4 Role of TNF-α in atherosclerosis

In inflammatory arthritis, including RA and SPA, accelerated (inflammatory) atherosclerosis leads to an increase in CV events independent of classical risk factors (e.g. smoking, diabetes). Underlying this are immune activation and systemic inflammation. Persistently elevated acute phase proteins (e.g. CRP), proinflammatory cytokines (e.g. TNF-α, IL-6) play a direct role in increased atherogenesis and are closely associated with CV events. It has been confirmed for several decades that inflammation plays an important role at all points in the atherosclerosis process. This has been observed in endothelial activation, leukocyte-endothelial interaction, modification of lipid particles, plaque formation, metalloproteinase-induced plaque rupture and its repair. T cells are involved in both arthritis and atherosclerosis. Specific autoreactive T cells enhance atherosclerosis in RA, with a primary role of Th1 type cells and Th17 cells. Oxidized LDL is a key factor in the development of inflammatory Th17 cells in atherosclerosis. Treg cells counteract this atherogenic and inflammatory effect. In addition to the different subtypes of T cells, cell adhesion molecules also play an important role in both processes. Among the soluble mediators, TNF-α and IL-6 cytokines are those directly involved in vascular inflammation and atherosclerosis. TNF-α is produced by inflammatory leukocytes, vascular endothelium and smooth muscle cells. TNF-α promotes the appearance of cell adhesion molecules on the endothelium thereby facilitating leukocyte migration. TNF-α, in addition to IL-1, partially reduces the production of natural inhibitors of the coagulation system (e.g.

antithrombin III, protein C, thrombomodulin, tissue factor pathway inhibitor [TFPI]) and is partially degraded by neutrophil elastase, also resulting in a prothrombotic state. TNF-α also has an effect on fibrinolysis: it inhibits fibrin degradation by increasing plasminogen activator inhibitor-1 (PAI-1) levels. These alterations in secondary hemostasis increase the incidence of arterial events. Adipose tissue is an organ with a high immuno-inflammatory activity and produces a number of proteins (adipokines). The best known adipokines involved in arthritis are adiponectin, resistin, leptin, visfatin and chemerin, some of which have pro-inflammatory and pro-atherogenic effects. Resistin and chemerin are induced by TNF-α, their levels correlate with joint damage, and are also good indicators of atherosclerosis and CV events. Visfatin formation is also induced by TNF-α, which induces proinflammatory cytokines and MMPs on synoviocytes. Insulin resistance may also be induced by TNF-α or adipokines, which is associated with coronary artery calcification and carotid plaque formation.

1.5. Inflammatory joint diseases and cardiovascular risk

The risk of CV disease was increased in RA and SPA compared to the general population. In RA patients, this risk is similarly increased as in diabetic patients: about twice that of the general population. Atherosclerosis and consequent cardiovascular disease are closely associated with chronic inflammatory processes, and this is also observed in RA. Optimal control of inflammation leads to a reduction in the concomitant CV risk. Major clinical trials (e.g. CANTOS, CIRT) investigating the effect of immunomodulator therapies in CV have shown conflicting results. In addition to these, considerable evidence has now accumulated on the potential beneficial effects of bDMARDs, particularly in rheumatoid arthritis. Most of the data on bDMARDs are for TNF-α inhibitors. The proinflammatory TNF-α is largely involved in the pathogenesis of RA-related atherosclerosis.

Early detection of CV disorders is key. In addition to clinical and laboratory biomarkers, non-invasive ultrasound-based techniques may also be useful for the evaluation of preclinical vascular pathophysiology in RA and SPA. Early endothelial dysfunction, overt atherosclerosis and increased arterial stiffness are predicted by abnormal brachial artery endothelium-dependent flow-mediated vasodilation (FMD), carotid artery carotid communis intima-media thickness (ccIMT) and carotid plaque, and arterial pulse wave velocity (PWV). These preclinical abnormalities also predict subsequent CV events in inflammatory joint disease. Anti-TNF biologics may improve or at least stabilize vascular morphology and function, including FMD, ccIMT and PWV.

1.6 Assessment of rapid radiological progression in rheumatoid arthritis

Early disease detection is crucial both to prevent joint destruction and to prevent comorbidities. The classic predictors of poor prognosis are listed in the 2019 European Alliance of Associations for Rheumatology (EULAR) therapeutic recommendations. Such factors include persistent moderate or high disease activity despite csDMARD therapy; persistently elevated CRP; high swollen joint count; persistent RF, ACPA levels, mainly detected in high titres; presence of early erosions; and inadequate response to two or more csDMARDs.

X-ray evidence of joint damage may be one of the most important and objective outcome indicators of RA. We therefore need to identify patients at high risk of rapid radiological progression (RRP) early and this should influence our treatment strategy. In such patients, effective therapy may reduce the chance of progression. Early, intensive treatment can slow the rate of radiological progression. Various clinical and biological markers have been identified as initial risk factors for radiological progression. Optimally, a combination of several markers can improve the predictive value. Recommendations for the management of RA (e.g. EULAR, Hungarian national guidelines) also emphasise the importance of prognostic markers in RA treatment decisions, referring to the matrix risk model developed by Vastesaeger et al. These recommendations also suggest the early introduction of biological therapy in high-risk RRP patients.

In order to determine whether early use of biological therapy is necessary in everyday clinical practice, it is essential to estimate which patients would benefit most from early aggressive therapy. The matrix risk model developed by Vastesaeger et al. is an evidence-based, easy-to-use tool for assessing patients' RRP risk using a specific combination of readily available variables. This model was developed by sub-analysing data (ASPIRE, ATTRACT) from patients with active RA who had not previously received biological therapy.

A single-centre retrospective study in Hungary evaluated data from 100 patients with RA. Twenty-one percent of patients with active RA had a high (≥40%) risk of RRP (vdHSS ≥5/year), and response to methotrexate (MTX) therapy was a key parameter in determining RRP risk. In other published prediction models, ACPA positivity, baseline erosions and smoking may also be predictors of RRP.

There have also been studies that have criticised the applicability of this type of matrix model in everyday clinical practice. De Cock and colleagues tested six matrices in 74 early RA patients and followed up the results with radiographs of the patients' hands and feet. In the end, they found these matrices not to be fully reliable for predicting RRP in daily practice.

2. Objectives

Based on the literature, higher CV risk associated with inflammatory joint disease and RRP are two factors that determine the odds of CV events and joint damage. Prevention of these is essential by performing various screening tests, which is what we focused on in our research in our care patients.

2.1 Study I: Effect of TNF-α inhibitor treatment on vascular parameters in RA and SPA

We assessed the vascular effects of one year of TNF-α inhibitor treatment using a non-invasive ultrasound-based technique in patients with RA and SPA.

We investigated:

- the effect of TNF-α inhibitor treatment on clinical activity;
- the effect of treatment on early endothelial dysfunction (FMD), manifest atherosclerosis (ccIMT), and the parameter indicating increased arterial stiffness (PWV);
- the relationship between vascular parameters, clinical parameters of patients and response to therapy.

2.2 Study II: Assessing the risk of radiological progression in RA patients with RA in a nationwide survey

Our aim was to determine the risk of RRP using a matrix risk model developed by Vastesaeger et al. in a selected population of RA patients in Hungary who have not yet received bDMARD treatment. Although the original matrix model was developed for use in clinical trials to determine treatment efficacy, we aimed to apply this model to select candidates for biological therapy among patients who were biologic therapy naïve. This was a non-interventional, cross-sectional, retrospective, population-based, nationwide survey based on hospital registry data. Theoretical prediction was performed, as prospective follow-up of radiological progression was not performed.

We have defined:

- the prevalence of high-risk (≥40%) RRP in a cross-sectional, retrospective, population-based, nationwide survey of non-interventional, biologic therapy-naive patients;
- the difference in prevalence of high-risk RRP between MTX-responders (cR) and non-responders (cNR);

- characteristics of patients at high risk of RRP and their association with other parameters not included in the model.

3. Patients and methods

3.1. Study I

3.1.1. Patients with RA and SPA

We included 53 patients with inflammatory arthritis (36 RA and 17 SPA) who were selected for initiation of anti-TNF-α therapy. Inclusion criteria were patients with RA and SPA diagnoses, unresponsive to csDMARD and with inflammatory arthritic activity, according to national recommendations. CV disease was not an exclusion factor for inclusion. Exclusion criteria included untreated, unstable hypertension (blood pressure >140/90 mm Hg), diabetes, preexisting inflammatory disease other than RA and SPA, infectious disease, and renal failure (serum creatinine ≥ 117 mmol/l). None of the patients were receiving aspirin, clopidogrel, anticoagulant therapy, or vasoactive drugs at inclusion.

The cohort consisted of 34 women and 19 men, with an average age of 52.0 ± 12.1 (range: 24-83) years. Mean duration of disease was 8.5 ± 7.9 (range: 1-44) years, while mean age at diagnosis was 43.5 ± 12.1 (range: 23-62) years. At baseline, patients with RA had a mean activity index, DAS28, of 5.00 ± 0.86, while the mean SPA activity index, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), was 5.79 ± 1.19. Of the 36 patients with RA, 20 patients received etanercept (ETN) 50 mg subcutaneously (sc) weekly and 16 patients received certolizumab pegol (CZP) (400 mg every 0, 2 and 4 weeks, followed by 200 mg once every two weeks). In total, 18 patients received MTX in addition to ETN and 13 patients received MTX in addition to CZP. The remaining patients received monotherapy. All 17 SPA patients received 50 mg/week ETN sc monotherapy. Patients with RA were not taking any other csDMARDs besides MTX in addition to biological therapy. A total of 12 patients with RA and 2 SPA were taking low-dose (<6 mg/day) methylprednisolone. The study was approved by the Research and Research Ethics Committee of the Health Scientific Council (ETT-KEB) (approval number 14804-2/2011/ECU). All patients were informed orally and in writing, after which they provided written informed consent. The assessments were performed in accordance with the Declaration of Helsinki.

3.1.2. Methods

3.1.2.1 Patient selection method

First we took a detailed medical history. A questionnaire was used to assess CV history and current smoking, experience of chest pain suggestive of angina pectoris in the 2 years before the study, and the presence of hypertension and diabetes mellitus. Additional clinical assessments, including physical examination, were performed at baseline and 3, 6 and 12 months after treatment initiation. At baseline, patients were started on anti-TNF-α therapy and received the same biological therapy for one year. Disease activity was assessed by the three-variable DAS28 calculator and the BASDAI index in RA and SPA, respectively. Response to therapy (cR vs. cNR) status was determined after 12 months of ETN or CZP treatment according to the EULAR response criteria originally published by Van Gestel et al. and our previously described criteria. Clinical status was assessed at baseline and at 6 and 12 months.

3.1.2.2 Laboratory methods

High-sensitivity C-reactive protein (hsCRP; normal: ≤ 5mg/l) and IgM rheumatoid factor (RF; normal: ≤ 50 IU/ml) were measured in serum by quantitative nephelometry (Cobas Mira Plus-Roche) using CRP and RF reagents (Dialab). ACPA (anti-CCP) autoantibodies were detected in serum samples using a second generation Immunoscan-RA CCP2 ELISA test (Euro

Diagnostica; standard: ≤ 25 IU/ml) according to the manufacturer's instructions at the Institute of Laboratory Medicine, University of Debrecen. Laboratory tests were performed at baseline and at 6 and 12 months.

3.1.2.3. Methods for testing vascular parameters

Brachial artery FMD was assessed as described in previous studies. In brief, ultrasound examination was performed on the patient's right arm using a 10 MHz linear transducer (ultrasound system: HP Sonos 5500) by a single trained sonographer after the patient had rested for 30 min in a temperature-controlled room (basal FMD value). A B-mode longitudinal section of the brachial artery above the antecubital fossa was obtained. To assess FMD, reactive hyperaemia was induced by releasing a pneumatic cuff around the forearm, which was inflated to suprasystolic pressure for 4.5 min. After deflation, maximum flow rate and artery diameter were recorded continuously for 90 seconds. Flow rates, baseline diameter, and FMD ECG were recorded offline in a gated fashion. FMD values were expressed as % change from baseline (resting) value.

In brief, ccIMT measurements were performed using a duplex ultrasound system (HP Sonos 5500, 10 MHz linear transducer) to evaluate the common carotid artery by a single observer. Longitudinal high-resolution B-mode ultrasound imaging was applied to both the right and left common carotid arteries and R-synchronization and data acquisition were performed. Offline measurements were taken proximal to 1 cm from the carotid bulb in the peripheral wall. We defined ccIMT as the distance of the first and second echogenic line from the lumen, taking the average of 10 measurements on each side. The ccIMT values were expressed in mm.

For arterial stiffness, PWV was automatically calculated by a TensioClinic arteriographic system (Tensiomed Ltd., Budapest, Hungary) as the ratio of the distance between the jugular fossa and the symphysis as described previously. If the artery is flexible, the PWV is low, so in the case of a stiff arterial wall, the PWV value will increase. The arteriograph evaluates this parameter from oscillometric data obtained from the brachial artery pressure of 35 mm Hg suprasystoles. In order to obtain reproducible results, the patient had to rest in a supine position in a quiet room for at least 10 minutes before the test. PWV values were expressed in m/s. Pathophysiological studies were performed at baseline and after 6 and 12 months.

3.1.2.4. Statistical analysis

Statistical analysis was performed using SPSS version 22.0 (IBM) software. Data were expressed as mean ± SD for continuous variables and as number of cases or percentage for categorical variables. The distribution of continuous variables was tested using Kolmogorov-Smirnov test. Independent and paired two-sample t-tests were used to assess differences. Nominal variables were compared between groups using the chi-square test and Fisher's exact test. Correlations were determined using Pearson's test. Univariate and multiple regression analyses using stepwise methods were used to examine independent associations between angiogenic biomarkers (dependent variables) and other clinical, laboratory and imaging parameters (independent variables). Standardized linear coefficients β showing linear correlation between two parameters were determined. The regression coefficient B (+ 95% CI) indicated independent correlations between the dependent and independent variables across the changes. Repeated-measures analysis of variance (RM-ANOVA) was performed to determine the further effects of different parameters on changes in vascular imaging markers between baseline and 12 months. The dependent variables were FMD, ccIMT and PWV. The partial $\eta2$ value is given as an indicator of effect size, where 0.01 represents a small effect, 0.06 a medium effect and 0.14 a large effect. The Friedman test was used to compare three matched samples. Reliability was tested using positive inter-item correlation and intraclass correlation (ICC). For the FMD, ccIMT and PWV tests, ICC = 0.470; F-test value: 1.887; $p = 0.001$. Power was estimated using G-Power software. Values of $p < 0.05$ were considered significant.

3.2. Study II

3.2.1. Patients with RA

In study II, patients with RA treated in a biological therapy centre in our country were randomly recruited. Exclusion criterion was age younger than 16 years, thus excluding juvenile arthritis.

3.2.2. Methods

In the model of Vastesaeger et al., RRP was defined as the change in the threshold of the modified Sharp/van der Heijde score (SHS), which is > or = 5 U/year. We conducted our analysis in this way. The developed and validated risk matrix model allows the RRP to be determined without actual radiographs, based on three simple variables. In this model we used 28 swollen joint count (SJC), RF and CRP level as trichotomous variables. These three variables were equally weighted.

3.2.2.1. Sample element number determination and patient selection process

Precision-based sample element number estimation was used to calculate the minimum sample element number using the formula $\pi (1-\pi)/e^2$, where π is the expected ratio and e is the size of the standard error. The precision was defined as the ± range around the estimated ratio and was defined as $\pm 1.96\times e$ (70). If the expected ratio $\pi = 0.2$ and the required precision ± 0.02, then the minimum sample size required was 1537. Assuming that the estimated proportion of patients with incomplete data would be 15%, the adjusted minimum sample size was 1537/0.85, corresponding to 1808 patients. This was the number of patients needed for the estimate with the precision ± 0.02.

A multistage sampling method was used to ensure equal probability of selection of the targeted patients. In order to obtain a population-based sample, 20 regional rheumatology centres (stage 1 sampling units) evenly distributed in our country were invited to participate in the study. At the start of the study, the number of cases (n) per centre was allocated according to the number of RA patients treated at each centre. Subsequently, investigators collected patient data until the allocated sample size (n) was reached, thus ensuring the probability of sampling in proportion to the size of the stage 1 sampling units (i.e. the 20 rheumatology centres).

Patients were then randomly selected (stage 2 sampling units) to avoid selection bias. A list of currently treated RA patients (sampling frame) was prepared at each centre. The number of allocated patients was selected from the sampling frame by simple random sampling (random number tables) to ensure that all patients had an equal chance of selection. Based on these calculations, 1843 patients were selected for data analysis.

3.2.2.2.2. Data recording process

We collected patient data from the most recent visit, which took place just before the patient was selected. For patients already on biologic therapy, we retrospectively recorded the last data before the biologics were initiated. Thus, only data from biologically naive patients were evaluated. We used clinical data obtained from hospital records and evaluated baseline radiographs for the presence or absence of erosion. A standardised electronic spreadsheet (Microsoft Excel) was used to record the data.

Based on hospital records, the following data were collected:

- age

- not
- Duration of RA
- the history of the use of DMARD:

  (MTX and other; currently/previously/never used)
- Response to MTX (cR/cNR)
- SJC, CRP, RF (to calculate the matrix-based RRP risk)
- ACPA status (positive/negative)
- DAS28 activity score
- presence of baseline erosions on baseline radiographs (yes/no)
- smoking (current/previously/never)

To define RRP, we used the three traditional variables, SJC, CRP and RF, as defined by Vastesaeger et al. However, we added some binary variables as described above to search for additional denominators.

3.2.2.3 Statistical analysis

MS Excel was used to record, aggregate and clean the data. Statistical analyses were performed using IBM SPSS 20. Continuous variables were defined as mean and standard deviation. Distribution was defined by the number and percentage of cases. The distribution was analyzed by Kolmogorov-Szmirnov test. Differences between groups were analysed using Mann-Whitney test and Chi^2 test. Independent predictive factors were identified using univariate and multivariate regression analysis. Associations were considered significant at a p-value less than 0.05.

4. Results

4.1. Study I

4.1.1 Effect of anti-TNF-α therapy on clinical activity, inflammation and clinical response in RA and SPA

In RA (n = 36), one year of ETN or CZP therapy significantly improved DAS28 (12 months: 3.02 ± 0.96; baseline: 5.00 ± 0.86; $p < 0.001$). Similarly, in SPA (n = 17), BASDAI was significantly reduced from 5.79 ± 1.19 (baseline) to 1.86 ± 1.04 after 12 months of ETN treatment ($p < 0.001$). With respect to inflammation, CRP in the mixed arthritis cohort was significantly reduced (7.57 ± 12.02 mg/l) after 3 months of treatment compared to baseline (14.88 ± 17.09 mg/l; $p <0.001$). This effect was even more pronounced after 6 months (6.79 ± 9.52 mg/l; $p <0.001$) and 12 months of treatment (6.49 ± 7.60 mg/l; $p <0.001$).

As described above, the cR and cNR status of patients was also determined. In total, 34 patients (64%) were cR and 19 (36%) were cNR.

4.1.2. Effect of TNF-α inhibition on vascular pathophysiology

It is important to note that FMD, ccIMT and PWV do not have a "normal value", but it was clearly seen that baseline FMD was lower, while ccIMT and PWV were higher in RA compared to SPA, suggesting a more pronounced pathology in the RA patient group.

In a mixed arthritis cohort of 53 patients, FMD measured on brachial artery was significantly improved after 6 months of anti-TNF-α therapy (10.01 ± 5.26%) compared to baseline (7.67 ± 4.40%; $p = 0.004$) (Figure 2). This improvement, although not significant, was also observed at 12 months (9.70 ± 4.91%; $p = 0.065$) (Figure 2). ccIMT remained stable, showing no significant progression from baseline (0.540 ± 0.087 mm) at 6 months (0.59 ± 0.11 mm; $p = 0.09$) or 12 months (0.59 ± 0.12 mm; $p = 0.240$). PWV, which is closely related to arterial stiffness and rigidity, showed a slight improvement after 6 months (7.32 ± 1.63 m/s; $p = 0.866$)

compared to the start of therapy (7.48 ± 1.98 m/s). This improvement was significant after 12 months (7.17 ± 2.59 m/s; p = 0.034). We also examined the effects of ETN and CZP separately, but found no differences in FMD, ccIMT and PWV at baseline and after 6 and 12 months.

4.1.3. Correlation of markers of vascular pathophysiology with clinical response and other parameters

When evaluating nominal variables, patients with a history of CV had significantly higher ccIMT after 6 months (0.60 ± 0.07 mm vs. 0.55 ± 0.09 mm; p = 0.045) and after 12 months (0.61 ± 0.12 mm vs. 0.54 ± 0.10 mm; p = 0.033), and higher PWV at baseline (8.34 ± 2.29 m/s vs. 6.62 ± 1.13 m/s; p = 0.005) and after 6 months (7.94 ± 1.86 m/s vs. 6.66 ± 1.23 m/s; p = 0.012) compared to patients with no history of CV. Furthermore, patients currently experiencing chest pain had significantly higher baseline PWV (8.64 ± 2.47 m/s vs. 7.59 ± 2.00 m/s; p = 0.017) compared to those with no previous history of *chest* pain. Patients with hypertension had higher 12-month ccIMT (0.62 ± 0.11 mm vs. 0.55 ± 0.11 mm; p = 0.050) and baseline PWV (8.36 ± 2.24 m/s vs. 6.88 ± 1.56 m/s; p = 0.016) compared with those with normal blood pressure.

When comparing vascular pathophysiology and clinical response to therapy, cNR patients had significantly higher baseline ccIMT (0.60 ± 0.08 mm vs. 0.52 ± 0.08 mm; p = 0.009), 6-month ccIMT (0.64 ± 0.14 mm vs. 0.56 ± 0.09 mm; p = 0.023) and baseline PWV (8.47 ± 2.62 m/s vs. 7.08 ± 1.55 m/s; p = 0.038) compared to cR patients.

When we examined the relationship between vascular imaging parameters, baseline ccIMT correlated with baseline PWV and 12-month ccIMT correlated with 12-month PWV.

Examining simple correlations of vascular imaging and other parameters, baseline and 12-month ccIMT and 12-month PWV were significantly correlated with age. Baseline ccIMT and

PWV and 12-month ccIMT and PWV correlated with age at diagnosis of RA. Baseline FMD and baseline and 12-month PWV were positively correlated with baseline CRP.

In a univariate analysis of the mixed RA + SPA population, baseline FMD was determined by baseline CRP ($p = 0.040$). Predictors of baseline ccIMT were age, age at diagnosis and cNR vs. cR status ($p < 0.05$). after 12 months of treatment, ccIMT was predicted by age, age at diagnosis and CV history ($p < 0.05$). Age at diagnosis, history of CV and current hypertension, current chest pain and cNR vs cR status predicted baseline PWV ($p <0.05$). Finally, PWV at 12 months was determined by age and age at diagnosis ($p <0.001$).

Multivariate analysis of the RA + SPA population confirmed a significant association of baseline ccIMT with age ($p = 0.003$) and cNR vs cR status ($p = 0.009$). ccIMT after 12 months of treatment, was also associated with age ($p < 0.001$). Baseline PWV was determined by age at diagnosis ($p = 0.022$) and current chest pain ($p = 0.004$), while 12-month PWV was associated with age ($p < 0.001$).

When patients with RA and SPA were analysed separately, univariate analysis showed that disease (RA vs SPA) had a significant effect on ccIMT and PWV values. However, we could not detect this in the multivariate analysis, so disease is presumably not an independent prognostic factor for vascular pathophysiology.

Finally, in the RA + SPA mixed population, RM-ANOVA analysis was performed to assess the determinants of 12-month changes in vascular imaging parameters. Anti-TNF-α treatment determined the changes in FMD ($p = 0.020$), ccIMT ($p = 0.024$) and PWV ($p = 0.007$) between baseline and 12 months. In addition, treatment with cR ($p = 0.045$) had a combined effect on changes in FMD. Furthermore, treatment and age jointly influenced changes in PWV ($p = 0.003$).

4.2. Study II

4.2.1. Descriptive results

We initially included 1843 patients in our study. Four patients were excluded at the start because they were under 16 years old. 222 patients were not included due to missing DAS28 scores, while a further 261 patients were not included due to missing data for RRP calculation. Finally, data from 1356 patients with RA were included in the analysis, for whom all necessary data were available. The mean age of the patients was 55.5 ± 13.3 years (range: 17-89) and the mean duration of disease was 8.4 ± 8.8 years (range: 0-62). A total of 1148 patients (85%) were female. Patients not responding to MTX were defined as those who had ever taken MTX and had a DAS28:5.1 or those who had never taken MTX (but had taken other csDMARDs) and had a DAS28:5.1.

4.2.2. Association of different parameters with RRP risk

First, the risk of RRP was calculated for all 1356 patients with RA according to the matrix model. The risk of 40% was the threshold between high and low risk patients. In total, 247 patients had a RRP risk ≥40% (18.2%) and 1109 patients had a low risk (81.8%).

Among the continuous variables other than the data required to calculate RRP risk, it was notable that RA patients with an RRP risk ≥40% (n = 247) had a significantly lower age compared to those with an RRP risk <40% (n = 1109) (53.33 ± 12.31 vs. 56.02 ± 13.50 years; p = 0.001). These two groups of patients showed no significant difference in duration of disease (7.89 ± 9.31 vs. 8.56 ± 8.70 years; p = 0.104).

Binary variables included gender, smoking and ACPA status, presence of erosions on baseline radiographs and status of response to MTX. For the binary variables, a risk of RRP ≥40% was significantly associated with lack of response to MTX (OR: 17.82), male sex (OR:

1.53), ACPA positivity (OR: 2.11), presence of erosions (OR: 1.37) and current smoking status (OR: 1.66).

Multivariate logistic regression analysis showed that lack of response to MTX (OR: 16.84), male sex (OR: 1.67), baseline erosion (OR: 1.50) and ACPA positivity (OR: 2.18) were independent predictors of high risk (≥ 40%) of RRP.

4.2.3. Factors associated with non-response to MTX

Based on our results, it appeared that lack of response to MTX may be the most strongly associated with high risk of RRP, so we also analysed in detail the factors significantly associated with MTX response.

Patients who did not respond to MTX therapy were defined as those who had joint activity on MTX (or other csDMARDs) administered for at least 6 months at a stable dose, i.e. a DAS 28 >5.1, which is the national threshold for initiation of biological therapy. Of 1356 cases analysed 691 cases were identified as lack of response to MTX (51%) as defined. Patients with lack of response to MTX had significantly lower age ($p < 0.001$), higher RF level ($p = 0.002$), CRP level ($p < 0.001$), DAS28 score ($p < 0.001$) and SJC ($p < 0.001$) than patients with no response to MTX. Similarly, more patients who did not respond to MTX had erosions ($p = 0.033$) and more of these patients were smokers at the time of the study ($p = 0.03$) compared to MTX responders. The mean risk of RRP was also significantly higher in patients not responding to MTX (37.8 ± 6.6% versus 15.3 ± 8.9%, $p < 0.001$). However, the risk of RRP was not different between MTX non-responders and MTX responders when considering patients who would have started infliximab treatment (either because they did not respond to MTX treatment or because they had become active again after the initial response). The risk of RRP in these two subgroups was quite low (6.5 ± 4.2% and 5.8 ± 2.0%, respectively).

Subgroups of MTX non-responders and MTX responders were analysed separately for RRP risk and associated factors. Among the continuous variables, there was no difference in age or duration of disease between the RRP ≥40% and RRP <40% subgroups in the MTX non-responders group. Univariate regression analysis of binary parameters showed a significant association of high risk of RRP with ACPA positivity (OR: 1.96) and current smoking (OR: 1.56) in MTX nonresponders, but not with gender and presence of erosions.

Multivariate logistic regression analysis showed that ACPA positivity (OR: 1.925) was an independent predictor of a risk of RRP ≥40% in the subgroup of patients not responding to MTX.

Similarly, for patients (n = 665) who responded to MTX, there were no differences in age and duration of disease between subgroups with ≥40% RRP (n = 18) and <40% RRP (n = 647). Among the binary variables, high risk of RRP was significantly associated with male sex (OR: 3.98), but not with ACPA status, presence of erosion, or current smoking. These associations were defined by p values and confidence intervals.

Multivariate logistic regression analysis, however, confirmed that male sex (OR: 5.20), ACPA positivity (OR: 4.57) and the presence of erosions (OR: 7.98) were independent predictors of high risk of RRP in the subpopulation of patients responding to MTX.

5. Discussion

5.1. Study I

As expected, anti-TNF-α therapy was clinically effective in both RA and SPA, as indicated by significant reductions in DAS28 and BASDAI values. Two-thirds of patients responded to treatment (cR).

Ultrasound-based imaging can raise awareness of CV risk subgroups in RA and SPA, and EULAR recommends that it can be used for this purpose. Literature suggests that ultrasound may be preferable to coronary calcium assessment in CV risk assessment. In RA and SPA, "standard values" for FMD, ccIMT and PWV are not known due to heterogeneity in patient populations and methodology. However, in different studies FMD was 4.6-7.7% in active patients, 8.6-13.5% in anti-TNF-α treated arthritic patients and 8.3-14.9% in healthy controls. Similarly, the ccIMT was 0.63-0.76 mm in active patients, 0.62-0.68 mm in patients on biologic treatment and 0.54-0.62 mm in controls. In a meta-analysis of 22 studies of 1384 patients with RA and 1147 controls, the mean ccIMT values were 0.71 mm and 0.62 mm, respectively. Furthermore, when comparing RA patients with and without CV events, most CV events occurred in patients with ccIMT > 0.91 mm. Finally, PWV was 8.3-8.6 m/s in active patients, 7.5-7.7 m/s in anti-TNF-α treated patients and 7.5-8.0 m/s in controls. In a meta-analysis of 208 patients and 10 trials, anti-TNF-α treatment improved PWV by an average of 0.53 m/s.

The magnitude of FMD, ccIMT and PWV before and after treatment were similar to those previously described. In our study, TNF-α inhibition resulted in a transient significant improvement in FMD by month 6 and a trend towards improvement was observed after one year. Based on the literature and our own data, biologics may inhibit the development and progression of atherosclerosis and reduce the incidence of CV events in arthritis. Among biologics, most of the data are available for TNF inhibitors, with infliximab resulting in an increase in FMD in several studies. This increase was transient in only a few studies, while only a few long-term studies longer than one year have been performed. The improvement in FMD was generally related to the clinical response to biologics.

The ccIMT did not change significantly with biological therapy in our study. Other groups also reported similar results for RA and SPA, while one group reported an improvement in ccIMT after one year of treatment with various biologics. In previous studies by our group, we found that anti-TNF-α therapy can improve ccIMT in early RA, and in the results of others, improvement in carotid atherosclerosis was associated with clinical efficacy. It is also known that carotid atherosclerosis in arthritis may progress over time without treatment.

Our own results also showed a beneficial response to treatment, with PWV significantly reduced from baseline at month 12. Similarly, other investigators have reported improvement in PWV in RA in response to anti-TNF-α therapy. The results are not clear in early RA. In our own study, TNF-α inhibition improved PWV in early RA, while others found no such improvement, especially in the long term. One group that found no change in vascular wall stiffness assessed the augmentation index (AIx) rather than PWV, so the different result may have resulted from using a different test method. However, a meta-analysis of 10 studies also suggested a beneficial effect of biologics on arterial stiffness. Interestingly, although there are some reports on the effect of ETN on these parameters, no such data were found for CZP. However, it has been described that CZP alters endothelial cell gene expression, including cell adhesion molecules and attenuates the inflammatory state, endothelial cell activation and adhesion.

Among the vascular imaging markers, ccIMT and PWV correlated with each other both at baseline and after 12 months in our study group. We have also previously found correlations between carotid atherosclerosis and vessel wall stiffness, while other research groups have not found such correlations. It is possible that ccIMT and PWV are not independently related and that other factors are involved in this relationship.

Higher ccIMT and PWV values were correlated with CV history and current hypertension. Both carotid atherosclerosis and arterial wall stiffness are known to be associated with CV disease. Furthermore, because hypertension correlates with 12-month ccIMT, hypertension may exacerbate consequent carotid atherosclerosis. It is also important to note that early, non-radiological SPA was not associated with increased ccIMT, suggesting that early treatment has a role in preventing subsequent atherosclerosis by exploiting the window of opportunity. In our patients, baseline PWV was correlated with most CV parameters studied, including CV history, current chest pain, hypertension and cR, and assessment of arterial stiffness may be a good screening method. Administration of this tool is simpler than FMD or ccIMT assessment in routine clinical care.

The clinical response to biologics may be related to vascular pathophysiology. In this study, cNR patients had significantly higher baseline and 6-month ccIMT values and baseline PWV values. The cNR patients may reflect a more severe, difficult to treat subgroup of arthritic patients. Persistent inflammation and clinical activity, associated with lack of response to treatment, accelerates atherosclerosis in RA and SPA. A reduction in the risk of myocardial infarction was mainly observed among those responding to anti-TNF-α therapy in parallel with a reduction in inflammation.

Baseline FMD and baseline PWV were correlated with CRP, highlighting the impact of systemic inflammation and acute phase proteins on vascular pathophysiology. Also important for patient follow-up is that baseline CRP correlated with 12-month PWV. CRP higher than baseline may drive vascular pathophysiology, resulting in higher arterial stiffness after one year despite anti-TNF-α treatment. These results were confirmed by univariate analysis.

Multiple analyses confirmed the association of baseline ccIMT with age and cNR, where age was also found to be a predictor of 12-month ccIMT value. Age also determined baseline and 12-month PWV. Similar to our results, in studies by other working groups, age was correlated with carotid atherosclerosis, improvement in carotid atherosclerosis was associated with clinical efficacy, and baseline PWV was determined by current chest pain, also supporting the impact of arterial stiffness on CV disease. Considering that among arterial stiffness parameters, PWV was a better predictor of CV disease than AIx, assessment of PWV rather than measurement of AIx is recommended in patients with arthritis. When patients with RA and SPA were analyzed separately, disease had an effect on atherosclerosis and vessel wall stiffness in univariate analysis. However, this was not confirmed in the multivariate analysis. Therefore, disease type is probably not an independent predictor of vascular pathology.

In RM-ANOVA analysis, TNF-α inhibition determined the one-year changes in FMD and PWV. As described above, anti-TNF-α agents have been shown to have beneficial effects on endothelial function and arterial wall stiffness in several studies. FMD clearly improved, although sometimes only transiently, in most studies, whereas PWV showed long-term improvement in this and some other studies. In addition, treatment with cR had a combined effect on changes in FMD. Thus, not only treatment but also cR may predict changes in FMD. As discussed above, improvements in FMD in other studies were associated with cR, not with biologics. The clinical efficacy of a TNF-α inhibitor is usually assessed after the first 12 weeks and in this FMD is the earliest indicator of vascular pathophysiology. In addition, anti-TNF-α treatment had a more pronounced effect on the incidence of CV morbidity among those responding to therapy. Based on what we know so far, treatment and age jointly influenced changes in PWV. In several studies, including our own, age was also an important determinant of baseline ccIMT and PWV.

Our investigation has certain limitations. The relatively small sample size may mask potentially significant results. In addition, we included patients with a history of CV disease as a potential cause. Patients with RA and SPA were not analysed separately due to the relatively small number of patients. In conclusion, ultrasound-based non-invasive techniques, as recommended by the EULAR, represent an additional value in determining CV risk and monitoring the effects of anti-TNF-α agents on vascular pathophysiology in terms of clinical efficacy.

5.2. Study II

Our analysis was the first study to assess the risk of RRP in a real-world setting in a national multicentre RA cohort. We found that 18.2% of patients with RA had RRP risk ≥40% based on a model using 3 variables as input parameters. This rate was considered significant with a high risk of radiographic deterioration and joint damage. The high risk of RRP in the analysed population was significantly associated with male sex, ACPA positivity, presence of erosions and lack of response to MTX. About half of the patients analysed did not respond to MTX. In patients who did not respond to MTX, RRP was significantly associated with ACPA positivity, whereas in patients who did respond to MTX, male sex, ACPA positivity and baseline erosions predicted RRP. In our study subgroups, we found that if TNF-inhibitor treatment had been adjusted, the risk of RRP was low regardless of the initial response to MTX, thus treatment with a biological agent was beneficial in preventing further radiological progression.

Our cohort consisted of biologically naive RA patients. We assessed the risk of RRP only theoretically, as we did not use prospective assessment of radiographic progression. There have been other studies using similar matrix-based prediction models, but some of them had a different study design. After the introduction of the model used by Vastesaeger et al. in our study, Durnez et al. validated the same matrix in their observational study using radiographs based on the ASPIRE early RA study. The model was useful for determining the predictive value of different treatment strategies in early RA. Visser et al. used a slightly different model in the BeSt study, where autoantibodies, CRP, erosion score and treatment group were used as predictors to determine RRP. This model included an assessment of radiographs and was able to identify differences in RRP between the four treatment strategies used in the BeSt study. Vanier et al. recently developed an updated matrix model using baseline erosions as predictors in addition to RF positivity, SJC and CRP. Data were collected from several large early RA cohorts, including registries and clinical trials. This model can determine the probability of RRP with high accuracy. Although the matrix had moderate sensitivity and specificity, the authors found its application in everyday practice useful. Conversely, when Lillegraven and colleagues compared three matrix-based models, including the model used in our present study, they found that these models were likely to have limited predictive value for RRP. However, unlike ours, this study was conducted in patients with early and not yet definite RA, and the study by Lillegraven et al. included patients who had already received biologics. Still, the majority of studies, like ours, have found matrix-based predictive models useful for simple prediction of RRP in everyday practice.

In our study, we analysed the risk of RRP in terms of the absence of baseline erosion. The use of erosions to predict further radiographic progression may be specific, however, as mentioned above, others have used baseline erosions in their predictive models and it is known that erosions already detected at diagnosis may also be predictive of further destructive lesions.

6. New findings

1. One year of anti-TNF-α treatment significantly but transiently improved FMD, improved PWV and stabilized ccIMT in a mixed cohort of patients with RA and SPA with a favorable clinical response.
2. Based on simple correlation analysis, the assessment of arterial stiffness may be an appropriate screening method. CRP-indicated systemic inflammation may define FMD, an early indicator of vascular pathophysiology. Age, CV history, hypertension or chest pain may be more associated with arterial stiffness and carotid atherosclerosis.
3. Biological treatment alone or in combination with other factors determines the change in FMD and PWV over 12 months.
4. This is the first biologically naïve domestic RA cohort in which the risk of RRP has been assessed. In this study, 18% of patients were found to be at high risk of RRP. Lack of response to MTX, male sex, baseline erosion and ACPA seropositivity were independent predictors of high risk of RRP. In MTX non-responders, RRP was significantly associated with ACPA positivity, whereas in MTX-responders, male sex, ACPA positivity and baseline erosion

predicted RRP. In our study subgroups, we found that if TNF-inhibitor treatment had been adjusted, the risk of RRP would have been low regardless of the initial response to MTX.

Our data, together with other studies, suggest that these models may be useful in predicting radiographic progression in everyday practice.

7. Acknowledgements

I would like to thank the staff of the Department and the Clinic for all the help and support they have given me over the years, who have been by my side like family. Among them I would also like to highlight my supervisor, **Gabriella Szűcs,** Head of the Clinic, and Professors **Zoltán Szekanecz,** Head of the Department, who have been my role models and I am proud to be their colleague.

I would like to thank **all the staff of** the **Department of Rheumatology** and the patients we care for who helped me to complete this thesis.

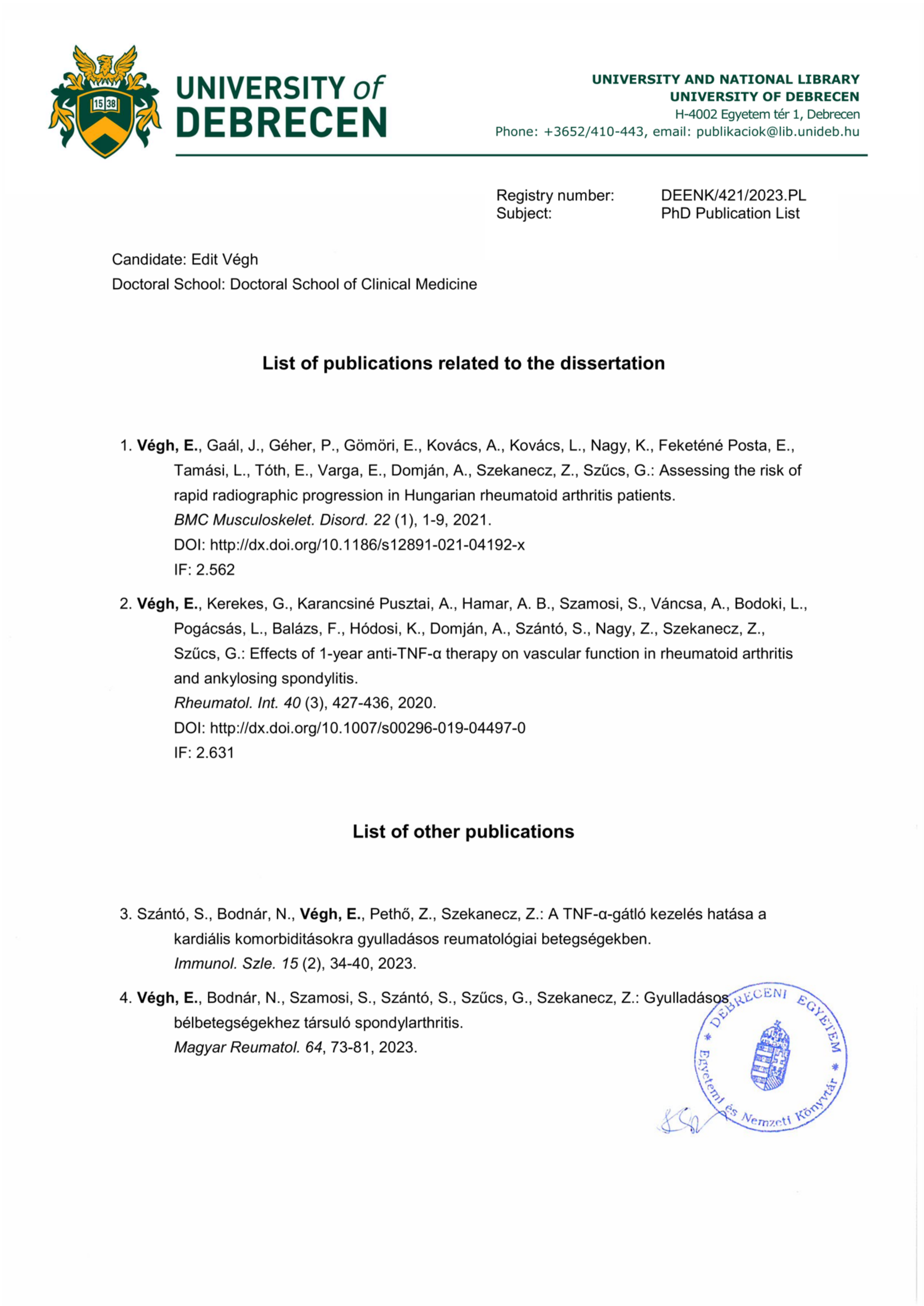
Thank you to the staff of the Arthritis Centres in Hungary.

I would like to thank **György Kerekes, Adjunct Professor,** who introduced and taught me how to perform non-invasive ultrasound examinations of peripheral blood vessels.

Thank you to the staff of the In Vitro Diagnostic Laboratory for their help in carrying out the tests.

I am grateful to **Katalin Hodosi** for the statistical analysis.

I thank my husband, children, family and friends for the loving support they have given me.



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Registry number: DEENK/421/2023.PL
Subject: PhD Publication List

Candidate: Edit Végh
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Végh, E.**, Gaál, J., Géher, P., Gömöri, E., Kovács, A., Kovács, L., Nagy, K., Feketéné Posta, E., Tamási, L., Tóth, E., Varga, E., Domján, A., Szekanecz, Z., Szűcs, G.: Assessing the risk of rapid radiographic progression in Hungarian rheumatoid arthritis patients.
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Total IF of journals (all publications): 105,47
Total IF of journals (publications related to the dissertation): 5,193

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

13 September, 2023