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

The effects of rheumatological drug treatment on cardiovascular and metabolic biomarkers in arthritides

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1 INTRODUCTION

Arthritides, such as rheumatoid arthritis (RA) and ankylosing spondylitis (AS), have been associated with atherosclerosis, increased cardiovascular (CV) morbidity and mortality, as well as metabolic changes and dyslipidemia. Increased risk of metabolic syndrome has also been associated with arthritis. The pathogenesis and comorbidities of RA have been linked to adipokines. Various metabolic markers play a role in the development of atherosclerosis both in the general population, and in patients with inflammatory rheumatic diseases. Systemic inflammation and inflammatory mediators play a key role in early atherosclerotic events in various rheumatological pathologies. Early detection of CV disorders, preferably in the preclinical phase of CV disease is important. It may be of extreme importance to search for biomarkers that could be used to predict CV risk early, even in the preclinical phase, and to confirm the pathological processes underlying different comorbidities.

Targeted therapies such as tumor necrosis factor-α (TNF-α) inhibitors may prevent secondary inflammatory atherosclerosis and major CV events (MACE), especially in patients who respond to anti-TNF therapy. The Janus kinase (JAK) inhibitor tofacitinib generally did not increase CV risk in clinical trials. In a large Phase 3 study including long-term extension studies, tofacitinib was associated with a low incidence of CV events. However, a recent study found that tofacitinib may increase the risk of MACE in RA patients with high CV risk at baseline compared to TNF-α inhibitors. Nevertheless, there was no difference between tofacitinib and anti-TNF therapy in the increase of lipid levels, and the increased risk of MACE was not associated with lipid changes.

Very few research groups have investigated the role of paraoxonase/arylesterase (PON/ARE), myeloperoxidase (MPO) and thrombospondin-1 (TSP-1) in relation to cardiovascular comorbidities in inflammatory rheumatic diseases. There is also little information on how certain targeted therapies affect these metabolic pathways, levels of lipids, adipokines, or other metabolic biomarkers.

Based on this, we have decided to examine the effects of different therapies used in rheumatology on various metabolic biomarkers, as well as their possible correlations with clinical parameters and with vascular pathophysiology. We conducted these investigation first in a mixed cohort of RA and AS patients, and secondly in a cohort of only RA patients. In addition, we also analyzed the possible relationships between these parameters. After reviewing

the literature, it should be stated that the examination of most of these metabolic biomarkers is not a novelty. Many studies have focused on adipokines, especially adiponectin, leptin and resistin, but there are fewer studies and data on newer adipokines, namely chemerin and adipsin, in RA and AS. It should also be stated that certain metabolic parameters have rarely been investigated in a complex manner, so there are fewer and often contradictory results available in relation to their correlations. Mostly data on correlations with disease activity and inflammatory parameters can be found in the literature. Also reviewing the literature on the effects of therapies, contradictory effects are reported regarding some parameters, while no or only very little data is available for several other biomarkers. In summary, it can be concluded that a complex study similar to the two studies that form the basis of my doctoral dissertation cannot be found in the relevant literature.

2 OBJECTIVES

Within the first study,

- primary objective: to investigate the effects of one year anti-TNF therapy on various metabolic parameters, including lipids (total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], triglyceride [TG]), lipid indices (TC/HDL, LDL/HDL), PON and ARE activity of PON1, MPO and adipokine (adiponectin, leptin, chemerin) levels in a mixed RA and AS cohort within the framework of a prospective study
- secondary objective: to examine the possible associations of metabolic parameters with clinical data and vascular pathophysiology (intima-media thickness [IMT], flow-mediated vasodilatation [FMD], pulse-wave velocity [PWV]) based on a previous study of our research group, using its raw data for statistical analysis
- tertiary objective: to examine the relationship between our metabolic markers

Within the second study,

- primary objective: to investigate the effects of one year tofacitinib therapy on various metabolic parameters, including lipids (TC, HDL, LDL, TG, lipoprotein(a) [Lp(a)], apolipoprotein A [APOA], APOB), lipid indices (TC/HDL, LDL/HDL), PON1, MPO, TSP-1 and adipokine (adiponectin, leptin, resistin, chemerin, adipsin) levels in a RA cohort within the framework of a prospective study
- secondary objective: to examine the possible associations of metabolic parameters with clinical data and vascular pathophysiology (IMT, FMD, PWV) based on a previous study of our research group, using its raw data for statistical analysis
- tertiary objective: to examine the relationship between our metabolic markers

3 PATIENTS AND METHODS

3.1 FIRST STUDY

3.1.1 Patients

The basis of the patient cohort examined within our fisrt study was patients with inflammatory arthritis treated at the Rheumatology Department, Faculty of Medicine of the University of Debrecen. The research was carried out after prior ethical approval (14804-2/2011/EKU), according to the Declarations of Helsinki. Patients were enrolled after obtaining their written informed consent. Patients with an active disease and a definite diagnosis of RA or AS who had clinical indication of biological therapy. Patients were enrolled before initiating the therapy. Disease activity was determined by Disease Activity Score (DAS28) in RA, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) in AS. Exclusion criteria included untreated hypertension (blood pressure >140/90 mmHg), congestive heart failure, inflammatory diseases other than RA and AS, active infection and renal failure (serum creatinine ≥117 mmol/L).

A total of 53 patients with inflammatory arthritis were included in the 12-month follow-up study, including 36 RA and 17 AS patients. None of the patients received aspirin, clopidogrel, heparin or warfarin therapy at the time of the inclusion. Hypertension was adequately controlled with antihypertensive drugs. Anti-TNF therapy was started at baseline and continued for one year. Among the 36 RA patients, 20 patients received etanercept (ETN) (50 mg/week subcutaneously [SC]), while 16 patients received certolizumab pegol (CZP) (400 mg at weeks 0, 2, and 4, then 200 mg 2 times a week SC). A total of 28 RA patients received methotrexate (MTX) therapy in combination with the anti-TNF treatment. These patients had been on MTX prior to the initiation of biological therapy, and no dose changes occured. All 17 AS patients received SC ETN treatment. Although the majority of RA patients and some AS patients had previously received corticosteroid therapy, none of the patients received such therapy at least 3 months before and during the study. Apart from MTX treatment, patients did not receive any other conventional disease-modifying anti-rheumatic drugs (DMARD). Only non-steroidal anti-inflammatory drugs (NSAIDs) were used as required. Four female RA

patients had been receiveing statins for at least 3 months prior to the study, the dose of which remained unchanged during the study.

The study cohort included 34 women and 19 men, with mean age of 52.0±12.1 (range: 24-83) years. Mean disease duration was 8.5±7.9 (range: 1-44) years, while mean age at diagnosis was 43.5±12.1 (range: 23-62) years. Mean baseline disease activity was 5.00±0.83 (DAS28) for RA and 5.79±1.19 (BASDAI) for AS patients. 72% of RA patients were rheumatoid factor (RF) positive (n=26), and 58% showed anti-citrullinated protein antibody (anti-CCP) positivity (n=21).

3.1.2 Data collection and clinical assessments

Detailed medical history was taken. We inquired for history of CV diseases, obesity (BMI), diabetes, hypertension (treated), as well as current smoking (during the last 2 years prior tot he start of this study). A total of 9 patients had a positive CV medical history (8-1 for RA-AS respectively). Hypertension was confirmed in 21 patients (17-4), diabetes in 4 patients (3-1) and smoking in 14 patients (7-7). Disease activity was determined by DAS28 in RA, and BASDAI in AS patients. BMI was calculated based on patients' height and weight. Obesity was defined as BMI>30 kg/m^2. A total of 28 patients exceeded this limit (17-11). Clinical assessments and physical examination were performed at baseline, and after 6 and 12 months.

The assessment of vascular pathophysiology, FMD, IMT and PWV, was the subject of a previous study carried out in the same patient cohort. Briefly, the FMD was determined as the percentage change from the value measured by ultrasound in the right arm after 30 minutes of rest, to the value recorded (continuously for 90 seconds) by inducing reactive hyperemia. IMT (mm) is the average of 10 measurements of the distance between the first and second echogenic lines (both left and right) from the lumen. PWV (m/s) was calculated by the arteriograph system as the ratio of the distance from the jugular fossa to the symphysis after 10 minutes of rest. In the present study, only the raw data of these parameters were used in our statistical analysis to investigate the relationship between the examined metabolic biomarkers and vascular pathophysiology. These relationships have not been published before by any other author from our research group.

3.1.3 Laboratory measurements

Blood samples were drawn from fasting patients. Serum samples were aliquted from later measurements and stored at -70ºC until use. The investigations included the determination of full blood count, various lipids, PON and ARE activity, MPO, adipokines, and C-reactive protein (CRP) at each time point, as well as the baseline RF and anti-CCP values.

Full blood count including hemoglobin, hematocrit, white blood cells, red blood cells, platelets, as well as neutrophil and lymphocyte absolute counts were determined by routine laborytory analyses. Lipid analyses including TC, LDL, HDL and TG were performed from fresh sera with Cobas c501 autoanalyzer (Roche Ltd., Mannheim, Germany).

Among adipokines, serum chemerin (ng/mL), leptin (ng/mL) and adiponectin concentrations (μg/mL) were determined by commercially available Enzyme-linked immunosorbent assay, ELISA kits (R&D Systems, Minneapolis, MN, USA). Leptin/adiponectin ratio, as well as lipid indices (TC/HDL, LDL/HDL) were calculated.

PON1 PON activity (U/L) was determined on a microtiter plate by a kinetic, semiautomated method using paraoxon (O,O-diethyl-O-p-nitrophenyl-phosphate, Sigma Aldrich) as a substrate. PON1 ARE activity (U/L) was assayed with a phenylacetate substrate (Sigma Aldrich, Merck, Darmstadt, Germany) and the hydrolysis of phenylacetate was monitored at 270 nm. Serum MPO concentration (ng/mL) were determined by a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) with 6.6-7.7 CV% intra-, and 6.5-9.4 CV% inter-assay variabilities.

CRP (mg/L) and IgM RF (IU/mL) were measured by quantitative nephelometry (Cobas Mira Plus, Roche Diagnostics, Basel, Switzerland), using CRP and RF reagents (both Dialab Ltd, Budapest, Hungary) (normal hsCRP: ≤ 5mg/L, normal RF: ≤ 50 IU/mL). Anti-CCP autoantibodies were detected in serum samples using a secound generation Immunoscan-RA CCP2 ELISA test (Euro Diagnostica, Malmö, Sweden; normal: ≤25 IU/mL). The assays were performed according tot he manufacturer's instructions.

3.2 SECOND STUDY

3.2.1 Patients

The basis of the patient cohort examined within our secound study were RA patients treated at the Rheumatology Department, Faculty of Medicine of the University of Debrecen. The research was carried out after prior ethical approval (56953-0/2015-EKL), according to the Declarations of Helsinki. Patients were enrolled after obtaining their written informed consent. Patients who met the inclusion criteria of the definitive diagnosis of RA according to the 2010 European Alliance of Associations for Rheumatology (EULAR)/American College of Rheumatology (ACR) classification criteria for RA [56]; moderate-high disease activity (DAS28 >3.2) at baseline and clinical indication of targeted therapy and had neither of the following exclusion criteria were included. The exclusion criteria included inflammatory diseases other than RA, acute/recent infection, standard contraindications to JAK inhibition, uncontrolled CV disease or hypertension, chronic renal or liver failure and malignancy within the last 10 years.

A total of 30 RA patients were included in the 12-month follow-up study. Patients were either naive to any targeted therapies (n=16) or switched to tofacitinib after stopping a biologic and an appropriate washout period had passed (n=14). Half of the patients (n=15) were randomly assigned to a 5 mg tofacitinib twice daily (bid) treatment arm; the other half (n=15) were assigned to a 10 mg tofacitinib twice daily (bid) treatment arm. Tofacitinib therapy was started at baseline and continued for one year. Supplemental therapy of either methotrexate (MTX) (n=16), sulfasalazine (n=1), leflunomide (n=4), MTX+sulfasalazine (n=1) or leflunomide+sulfasalazine (n=1) were given to patients. DMARDs were taken in stable doses at least one year prior to the present study. No dose changes of these DMARDs were allowed throughout the course of the study. Although most patients may have received corticosteroids prior to the study, none of the patients had been on corticosteroids for at least 3 months prior to or during the study.

The study cohort included 27 women and 3 men, with mean age of 52.8±10.0 (range: 27–69) years. Mean disease duration was 7.7 ± 5.0 (range: 1–21) years. Mean baseline DAS28 was 5.05±0.77 (4.80±0.69 and 5.29±0.79 in the 5 mg bid and 10 mg bid treatment arm,

respectively). Mean BMI was 29.93±6.90. 80% percent of patients (n=24) were RF positive and 80% were anti-CCP positive (n=24).

3.2.2 Data collection and clinical assessments

We inquired about the history of CVD, as well as current smoking, experience of chest pain resembling angina pectoris, hypertension and diabetes mellitus during the last 2 years prior to the start of this study. Altogether, 6 patients (3-3 on each arm) had a positive CV history. A total of 15 patients had hypertension (5-10), 2 had diabetes mellitus (1-1) and 7 patients (4-3) were current smokers at the time of inclusion. Disease activity was determined by DAS28. Obesity was defined as BMI > 30 kg/m^2; a total of 10 patients were found to exceed this limit. Clinical assessments including physical examination were performed at baseline, and after 6 and 12 months.

During the study, a total of 4 patients, 2-2 on each treatment arm, dropped out after 6 months of treatment but before the end of the study. In 2 cases, the reason was inefficacy; in one case, significantly elevated transaminases were detected; and, in the last case, the patient moved abroad. Altogether 26 patients (13-13 patients on each arm) completed the study and were thus eligible for further data analysis.

The assessment of vascular pathophysiology, FMD, IMT and PWV, was the subject of a previous study carried out in the same patient cohort. In the present study, only the raw data of these parameters were used in our statistical analysis to investigate the relationship between the examined metabolic biomarkers and vascular pathophysiology. These relationships have not been published before by any other author from our research group.

3.2.3 Laboratory measurements

Blood samples were drawn from fasting patients. Serum samples were aliquted from later measurements and stored at -70ºC until use. The investigations included the determination of full blood count, various lipids, PON1, MPO, TSP-1, adipokines, and CRP levels at each time point, as well as the baseline RF and anti-CCP values.

Lipid analyses including TC, LDL, HDL, TG, Lp(a), APOA and APOB were performed as decribed in our first study.

Among adipokines, serum chemerin concentrations (ng/mL) were determined by commercially available ELISA kits (Human Chemerin ELISA Kit, Reagent Genie) with CV<8% intra-, and CV<10% inter-assay variabilities. Adiponectin, leptin, adipsin and resistin concentrations (pg/mL) were determined by flow cytometry by a bead-based multiplex assay using sera (Human Metabolic Panel 1, 4plex, LEGEND plex, BioLegend) and analyzed by LEGENDplex software (version 8.0). The leptin/adiponectin ratio as well as different lipid ratios (TC/HDL, LDL/HDL, APOA/APOB) were calculated.

Serum PON1 (ng/mL) concentrations were determined by commercially available ELISA kits (Human PON1/Paraoxonase 1 ELISA Kit, Reagent Genie). Serum MPO (ng/mL) concentrations were determined by commercially available ELISA kits (Human Myeloperoxidase ELISA Kit, Reagent Genie). Serum TSP-1 (micrograms per milliliter, μg/mL) concentrations were determined by commercially available ELISA kits (Human Thrombospondin-1 ELISA Kit, Reagent Genie). The previous ELISA kits had CV<8% intra-, and CV<10% inter-assay variabilities.

The erythrocyte sedimentation rate (ESR) (mm/h) was determined. CRP (mg/L), IgM RF (IU/mL) and anti-CCP autoantibodies were measeures/detected as decribed in our first study. The assays were performed according to the manufacturer’s instructions.

4. STATISTICAL ANALYSIS

The statistical methods described below were used in both of our studies. Statistical analysis was performed using SPSS version 22.0 (IBM, Armonk, NY, USA) software. Data were expressed as the mean ± standard deviation (SD) for continuous variables and percentages for categorical variables. During the analyses, we used both parametric and non-parametric tests. Parametric test assume a normal distribution, while non-parametric test can be performed in the absence of normality, in case of outlier values, or asymmetric distribution. Continuous variables were evaluated by the paired two-tailed t-test and Wilcoxon test. Nominal variables were compared between groups using the chi-squared or Fisher's exact test, as appropriate. Correlations were determined by Pearson's or Spearman's analyses. Univariate and multivariate regression analysis using the stepwise method were applied to identify determinants of various dependent and independent variables. The β standardized linear coefficients showing linear correlations between two parameters were determined. The B (+95% CI) regression coefficient indicated independent associations between dependent and independent variables during changes. The general linear model (GLM) repeated measures analysis of variance (RM-ANOVA) was performed in order to determine the combined effects of several parameters affecting the change over time of each of the examied parameters. RM-ANOVA uses the F-test, resulting in the F values along with the p values. Partial η^2 is given as an indicator of effect size, with values of 0.01 suggesting small, 0.06 medium and 0.14 large effects. P values<0.05 were considered significant.

5. RESULTS

5.1 FIRST STUDY

The assessment of disease activity and inflammatory parameters in the same patient population was carried out in a previous study. Overall, TNF-α therapy significantly reduced both the disease activity (DAS28 and BASDAI) and CRP values.

5.1.1 Effects of anti-TNF therapy on circulating metabolic biomarkers

Examining the effect of TNF inhibition on various metabolic parameters, the following results were found. In the mixed cohort of 53 RA and AS patient, PON activity only numerically increased after 6 months (121.0±87.4 U/L; p=0.107) and 12 months (120.1±82.6 U/L; p=0.140) compared to baseline (114.1±79.3 U/L). PON1 ARE activity numerically decreased after 6 months (150.9±34.7 U/L; p=0.052) and significantly after 12 months (147.4±29.5 U/L; p=0.027) compared to baseline (171.4±56.6 U/L). MPO also numerically decreased after 6 months (703.0±738.1 ng/mL; p=0.097) and significantly after 12 months (255.5±137.3 ng/mL) versus baseline (758.6±651.4 ng/mL; p<0.001). Among adipokines, leptin levels did not change after 6 (31.4±37.4 ng/mL; p=0.642) or 12 months (31.0±35.9 ng/mL; p = 0.918) versus baseline (30.6±38.7 ng/mL). Adiponectin levels did not change after 6 months (9.54±5.29 µg/mL; p=0.405) but significantly decreased after one year (8.85±4.53 µg/mL; p=0.013) compared to baseline (9.74±4.75 µg/mL). Leptin/adiponectin ratios did not change over time. Chemerin levels significantly decreased both after 6 months (82.6±20.6 ng/mL; p<0.001) and 12 months (86.7 ± 19.9 ng/mL; p<0.001) versus baseline (111.3±34.7 ng/mL).

Lipid fractions (TC, HDL, LDL, TG), as well as lipid indices (TC/HDL, and LDL/HDL) did not change between baseline and 12 months upon anti-TNF therapy.

5.1.2 Associations of metabolic biomarkers with clical parameters, vascular pathophysiology and other parameters

In the simple correlation analysis, several correlations were found between lipids, adipokines other metabolic parameters and clinical parameters and vascular pathophysiology. The data will not be presented in detail due to content limitation. In general, lipids and lipid indices variably correlated with age, disease duration, disease activity, CRP, IMT, and PWV ($p<0.05$). Interestingly, IMT showed negative correlations with LDL and lipid ratios. PON and ARE activity, as well as MPO showed variable associations with age, disease duration, CRP, IMT and PWV ($p<0.05$). PON and ARE activity exerted positive correlations with FMD, but negative associations with disease duration and activity, CRP, IMT, and PWV. MPO showed positive correlations with CRP, IMT, and PWV. Among adipokines, adiponectin correlated with age and PWV, leptin with age, disease activity, CRP, IMT, and PWV, leptin/adiponectin ratios with CRP and PWV, while chemerin only with CRP ($p<0.05$).

When the metabolic markers were correlated with categorical (binary) variables, obesity was associated with lower HDL, higher TC/HDL ratio, higher leptin, lower adiponectin levels, and higher leptin/adiponectin ratio ($p<0.05$). Positive CV history was associated with lower ARE and PON activities, as well as higher leptin levels ($p<0.05$).

Univariable and multivariable regression analyses were performed in order to determine independent metabolic determinants of IMT, PWV, and FMD, as well as independent determinants of the various metabolic parameters. In the univariable analysis, IMT positively correlated with TG and leptin and negatively with MPO ($p<0.05$). PWV was variably, positively associated with TC, LDL, TG, leptin, and leptin/adiponectin ratio and inversely with ARE activity ($p<0.05$). FMD inversely correlated with TG ($p<0.05$). The multivariable analysis confirmed the above associations of IMT with TG, MPO, and leptin and that of PWV with TC, ARE activity, and leptin ($p<0.05$).

Similarly, in the univariable analysis, among lipids, TC, and LDL were associated with PWV. HDL positively correlated with age, CV history, disease duration, and IMT, while inversely with CRP and obesity ($p<0,05$). TG at baseline negatively correlated with disease activity, but after treatment, it was positively associated with CRP ($p<0,05$). TG also inversely correlated with FMD ($p<0.05$). PON activity negatively correlated with age, CV history and IMT, while ARE activity was inversely associated with age, CV history, and PWV ($p<0.05$).

Among adipokines, leptin positively correlated with age, CV history, obesity, disease activity, CRP, IMT, and PWV ($p<0,05$). Adiponectin was positively associated with age and negatively with obesity. The leptin/adiponectin ratio correlated with obesity and PWV. Finally, chemerin was only associated with CRP ($p<0.05$). Among these associations, multivariable analysis confirmed those of HDL with age, obesity, disease duration and CRP. LDL correlated with PWV, TG with disease activity, PON activity with age, ARE activity with age and CV history, leptin with obesity and IMT. Adiponectin and leptin/adiponectin ratio also correlated with obesity ($p<0.05$).

Finally, RM-ANOVA analysis was performed in order to look for combined determinants of biomarker changes between baseline and 12 months. TC change overtime was associated with treatment and increased baseline leptin levels ($p=0.039$). HDL changes correlated with treatment and lower baseline CRP ($p=0.016$). TG changes were associated with treatment and higher adiponectin levels at baseline ($p=0.014$). Changes in ARE activity correlated with treatment and lower baseline disease activity ($p=0.046$). Finally, IMT changes were associated with treatment and baseline chemerin ($p=0.003$). Regarding other analyzed parameters, the analysis did not show significant results.

5.2 SECOND STUDY

Simularly tot he first study, the assessment of disease activity and inflammatory parameters in the same patient population was the subject of a previous study. Overall, JAK inhibitor tofacitinib significantly reduced both the disease activity and the CRP values (within the whole RA cohort and in the investigated treatment groups as well).

5.2.1 Effects of tofacitinib therapy on circulating metabolic biomarkers

Examining the effects of tofacitinib therapy, we obtained the following results. In our cohort of RA patients, TC levels significantly increased after 6 months (5.95±1.15 mmol/L; p=0.003) and 12 months (5.95±1.20 mmol/L; p=0.007) compared with the baseline (5.49±0.92 mmol/L). HDL significantly decreased after 6 months (1.62±0.58 mmol/L; p=0.047), but significantly increased after 12 months (1.66±0.51 mmol/L; p=0.004) compared with the baseline (1.64±0.95 mmol/L). LDL significantly increased after 6 months (3.67±0.95 mmol/L; p=0.039) and 12 months (3.90±1.12 mmol/L; p=0.003) compared with the baseline (3.43±0.83 mmol/L), and there was also a significant increase between 6 months and 12 months (p=0.035). APOA also significantly increased after 6 months (1.76±0.42 g/L; p=0.024) and 12 months (1.79±0.39 g/L; p=0.001) compared with the baseline (1.65±0.42 g/L). APOB also showed a significant increase after 6 months (1.19±0.33 g/L; p=0.022) and 12 months (1.21±0.31 g/L; p=0.006) compared with the baseline (1.09±0.25 g/L). Lp(a) showed a significant decrease after 6 months (147.53±190.27 mg/L; p=0.013) and 12 months (150.80±202.29 mg/L; p=0.024) compared with the baseline (181.20±237.81 mg/L). TG and lipid ratios (TC/HLDL, LDL/HDL) showed no significant changes between baseline and one year upon tofacitinib therapy. In the treatment group of 5 mg bid tofacitinib, we found no significant changes; however, in the treatment group of 10 mg bid tofacitinib we found similar changes to that of the whole cohort.

Among adipokines, only adiponectin numerically increased after 6 and 12 months compared with the baseline. Leptin showed a significant increase after 6 months (36,196.97±21,952.19 pg/mL; p=0.001) and 12 months (36,467.00±18,219.75 pg/mL; p=0.003) compared with the baseline (26,071.51±13,592.91 pg/mL). Adipsin significantly increased after 6 months (1,995,901.98±1772,069.37 pg/mL; p=0.030), but only numerically increased after 12 months (1,680,141.92±390,567.84 pg/mL; p=0.124) compared with the

baseline (1,447,195.50±463,232.82 pg/mL). Resistin only numerically decreased after 6 and 12 months compared with the baseline. Chemerin showed a significant decrease after 6 months (173,193.33±54,900.17 ng/mL; p=0.024) and 12 months (172965.38±48647.12 ng/mL; p=0.040) compared with the baseline (191,196.67±57,747.70 ng/mL). In the treatment group of 5 mg bid and 10 mg bid tofacitinib, we found similar changes to that of the whole cohort.

Regarding other investigated parameters, PON1 numerically decreased after 6 months (193.79±35.71 ng/mL; p=0.079) and significantly decreased after 12 months (194.10±19.86 ng/mL; p=0.040) compared with the baseline (203.21±36.05 ng/mL). MPO significantly decreased after 6 months (73.27±31.54 ng/mL; p=0.028) and numerically after 12 months (66.80±31.84 ng/mL; p=0.058) compared with the baseline (94.46±39.89 ng/mL). Finally, TSP-1 significantly increased after 6 months (3.02±0.77 μg/mL; p=0.009), but only numerically after 12 months (2.99±0.82 μg/mL; p=0.182) compared with the baseline (2.77±0.78 μg/mL). In the treatment group of 5 mg bid and 10 mg bid tofacitinib, we found similar changes to that of the whole cohort.

5.2.2 Associations of metabolic biomarkers with clinical parameters, vascular pathophysiology and other parameters

Several correlations were found between metabolic, clinical and vascular parameters in a simple correlation analysis performed by Pearson's correlation analysis. The data will not be presented in detail due to content limitation. Correlation analysis was performed for the whole study population, as well as for each treatment arm. Not including all the correlations, baseline BMI correlated positively with disease activity, CRP, ESR, FMD, PWV, leptin, resistin, PON1 and MPO, while inversely with TC, HDL and APOA ($p<0.05$). In general, in our study cohort, lipids and lipid ratios variably correlated with other lipids, lipid ratios, adipokines, other metabolic parameters, and clinical and vascular parameters ($p<0.05$).

Among adipokines, adiponectin, adipsin, leptin and chemerin correlated with age ($p<0.05$). Leptin and resistin correlated positively, while adiponectin inversely with CRP ($p<0.05$). Leptin and resistin correlated with BMI ($p<0.05$). Adiponectin correlated positively, while chemerin inversely with IMT ($p<0.05$). Adipsin and resistin correlated with PWV, leptin correlated with FMD, while adipsin also correlated with FMD ($p<0.05$). Adiponectin, leptin and resistin correlated positively with adipsin ($p<0.05$). Leptin also correlated with resistin,

while adipsin correlated with adiponectin, leptin and resistin ($p<0.05$). Adiponectin correlated with TSP-1 and PON1, adipsin with TSP-1, PON1 and MPO, resistin with PON1 and MPO, leptin only with PON1, and chemerin only with TSP-1 ($p<0.05$). Adiponectin also correlated positively with HDL, APOA and APOA/APOB, while inversely with TG, TC/HDL, LDL/HDL and anti-CCP ($p<0.05$). Leptin correlated inversely with TC, adipsin correlated inversely with RF. Resistin correlated positively with disease activity, ESR, RF and leptin, but inversely with TC and APOA. Chemerin correlated positively with LDL, APOB and LDL/HDL ($p <0.05$).

Among other parameters, TSP-1 correlated positively with HDL, APOA, adiponectin, adipsin, chemerin and PON1, while inversely with disease activity, TG, TC/HDL, LDL/HDL, RF and ESR ($p<0.05$). MPO correlated with BMI, CRP, Lp(a), adipsin, resistin, PON1 and PWV ($p<0.05$). PON1 correlated positively with age, BMI, adiponectin, adipsin, leptin, resistin, TSP-1 and MPO, while inversely with TC/HDL, LDL/HDL, anti-CCP and RF ($p<0.05$).

IMT correlated with age, TC, LDL, adiponectin, chemerin and PWV ($p<0.05$). FMD correlated with BMI, CRP, TG, TC, Lp(a), HDL, APOA, TC/HDL, LDL/HDL, leptin and adipsin ($p<0.05$). PWV showed positive correlations with age, BMI, TC, LDL, APOB, LDL/HDL, adipsin, resistin, MPO and IMT, and inverse correlation with HDL ($p<0.05$). The correlation analysis found similar associations for the treatment arms as well.

In order to determine independent determinants of FMD, IMT and PWV, as well as independent determinants of our studied metabolic parameters, univariable and multivariable regression analyses were performed for the whole study population, as well as for each treatment arm. In the univariable analysis, FMD and BMI, TG, TC, APOB, Lp(a), TC/HDL, LDL/HDL, adipsin and leptin; IMT and age, TC and adiponectin; and PWV and age, BMI, adipsin, resistin, MPO and LDL showed a positive correlation ($p<0.05$). While FMD and APOA; and IMT and chemerin showed an inverse correlation ($p<0.05$). The multivariable analysis confirmed the abovementioned associations of FMD with TC/HDL and leptin; IMT with age; and PWV with age, BMI, resistin and LDL ($p<0.05$).

Regarding metabolic parameters the following results were found by univariate regression analysis. BMI and disease activity, CRP, ESR and PWV; TC and FMD, PWV; LDL and PWV; TG and FMD; APOB and disease activity, ESR, RF, FDM and PWV; Lp(a) and age, and FMD; TC/HDL and disease activity, CRP, ESR, RF and FMD; and LDL/HDL and disease activity, We, RF and PWV; adiponectin and age, and IMT; leptin and age, BMI, CRP, and FMD;

adipsin and age, PWV, and FMD; resistin and BMI, CRP, ESR, and PWV; eptin/adiponectin and anti-CCP, and FMD; MPO and BMI, CRP, disease activity, and PWV; and PON1 and age, and BMI were positively correlated ($p<0.05$). On the other hand TC and BMI, CRP, and disease activity; HDL and BMI, disease activity, CRP, ESR, anti-CCP, RF, FMD and PWV; APOA and BMI, disease activity, ESR, CRP, RF and FMD; APOA/APOB and disease activity, ESR and RF; adiponectin and CRP, anti-CCP and RF, adipsin and RF; chemerin and age and IMT; leptin/adiponectin and age, IMT and PWV; PON1 and CRP and RF; and TSP-1 and disease activity, RF and ESR were inversely correlated ($p<0.05$).

A number of these associations were also confirmed by multivariable analysis. These included positive associations of BMI with CRP and PWV; APOB with FMD and PWV; Lp(a) with age and FMD; TC/HLD with RF; LDL/HDL with disease activity, RF and PWV; adiponectin with age; leptin with age, BMI and FMD; adipsin with PWV; resistin with CRP; leptin/adiponectin ratio with FMD; MPO with disease duration and PWV; PON1 with BMI; and ($p<0.05$). On the other hand inverse association of TC with CRP; HDL with ESR, FMD and PWV; APOA with ESR, CRP and RF; APOA/APOB with ESR; adiponectin with anti-CCP; chemerin with IMT; leptin/adiponectin ratio with age and IMT; and PON1 with age and RF; TSP-1 with RF ($p<0.05$). Similar associations were found in the treatment arms.

Finally, RM-ANOVA analysis was performed to identify combined determinants of changes between baseline and 12 months. The analysis was performed in relation to adipokines, and TSP-1, PON1 and MPO.

Among adipokines, adiponectin changes overtime were associated with treatment and PWV ($p=0.023$). Leptin changes correlated with treatment and age ($p=0.043$), as well as treatment and CRP ($p=0.005$). Resistin changes were associated with treatment and BMI ($p=0.005$). Among other investigatied parameters, changes in TSP-1 correlated with treatment and CRP ($p=0.029$). PON1 changes were associated with treatment and CRP ($p=0.032$), as well as treatment and ESR ($p=0.022$). Finally, MPO changes correlated with treatment and disease duration ($p=0.038$), as well as with treatment and anti-CCP ($p=0.046$).

6. DISCUSSION

6.1 FIRST STUDY

During 12-month follow-up study in a mixed RA and AS cohort, our primary objective was to investigate the effects of one year of anti-TNF therapy (ETN, CZP) on various metabolic parameters, including lipids (TC, HDL, LDL, TG), lipid indices (TC/HDL, LDL/HDL), PON and ARE activity, MPO and adipokine levels (adiponectin, leptin, chemerin). Metabolic syndrome has been associated with arthritis. In our study, TNF-α inhibition resulted in significantly decreased ARE activity, while disease activity showed a significant improvement. This may be because of the mixed cohort or the relatively small sample size, but could also be caused by an external, unexamined factor. There are no previous reports on the effects of biologics on ARE activity. In our study, the therapy resulted in decreased MPO level, which is as expected, since this probably reduced the role of MPO in inflammatory oxidation in tissue. Similarly, there are no data available on the effects of biologics on MPO in the literature. Decreased adiponectin levels were observed after 12 months. In addition, the therapy resulted in decreased chemerin levels after 6 and 12 months. In other studies, biologics also inhibited chemerin production. However, no significant changes were observed in lipid levels (TC, HDL, LDL, TG) and PON activity. In the case of PON activity, only a numerical increase was observed, while PON activity is known to be impared in RA. Thus, TNF inhibition was able to maintain PON activity and prevent its further impairment. Regarding leptin, no significant changes were observed either. In previous studies, the effects of biologics on leptin and adiponectin levels were controversial. Lipid levels showed no changes during 12 months of therapy. Other studies have described similarly unchanged lipid levels with anti-TNF therapy. The favorable effects of targeted therapies, especially IL-6 and JAK inhibitors, are reflected in transient increase in leptin levels, including TC, LDL, HDL, and TG. TNF inhibitors can also temporarily increase lipid levels, although to a lesser extent than IL-6 or JAK inhibitors. All these results were accompanied by clinical efficacy, improved FMD, decreased PWV and unchanged IMT values.

As a secondary objective, the associations of different examined metabolic parameters with clinical data and vascular pathophysiology were described, based on a previous study of our working group, using its raw data. In close relation to this objective, the relationships

between the metabolic markers were defined as a tertiary objective. The regression analysis on the one hand showed a correlation between vascular pathophysiology and ARE activity, MPO, leptin and lipids. On the other hand, the investigated metabolic parameters showed various significant relationships with disease duration, CRP, obesity, PWV and IMT.

Among metabolic pathways, PON and ARE exert antioxidant and atheroprotective effects, while MPO plays a role in both inflammation and CV diseases. PON and ARE activity are impared in arthritis and it may be related to inflammation, since it is known that PON1 may also have anti-inflammatory effects based on literature data. These physiological and pathophysiological effects may also be present in inflammatory rheumatic diseases, such as RA or AS, but their exact location and role during the course of the disease is unclear. Various inverse correlations were observed between PON and ARE activity and disease duration, disease activity, and CRP. One study also showed a negative correlation between PON and disease activity, however, our study had a longer duration and the patient group also included AS patients. In our study, PON activity was positively correlated with FMD and inversely with CV history and IMT, while ARE activity was negatively correlated with CV history and PWV. Changes in ARE activity over time were determined by one year of anti-TNF therapy and lower baseline disease activity. These results support that PON1 is indeed involved in the maintenance of vascular pathophysiology, acts against atherosclerosis and inversely regulated by inflammation in arthritis. This is also supported by the correlations between PON and IMT shown in previous studies in AS patiens. MPO was positively correlated with CRP, IMT, and PWV, supporting its role in RA-related inflammation and atherosclerosis. MPO has shown a correlation with disease activity in other RA studies. In the general population, MPO levels and PON activity were inversely correlated, however, no such correlation was found in our arthritis cohort. We found no correlation between MPO and atherosclerosis. In other studies performed in non-inflammatory studies, MPO was associated with IMT in metabolic syndrome, but not in type 2 diabetes.

Among adipokines, leptin and chemerin have pro-inflammatory and pro-atherogenic effects in both inflammatory and non-inflammatory conditions. In the present study, leptin was associated with disease activity, CRP, obesity, IMT and PWV, indicating that leptin indeed forms a bridge between inflammation and atherosclerosis. Others also found that higher leptin levels were correlated with more severe RA, obesity and CV disease in RA. The leptin/adiponectin ratio showed a correlation with PWV and obesity. This ratio has been associated with atherosclerosis and seems to be a good marker of arterial stiffness. Chemerin

showed a correlation with CRP, supporting its role as a pro-inflammatory adipokine. In addition, treatment and baseline chemerin levels determined changes in IMT over time, suggesting a role of chemerin in arthritis-associated atherosclerosis.

Finally, the "lipid paradox", where lipid levels are inversely correlated with systemic inflammation has been identified in inflammatory rheumatic diseases. Lipids showed a positive correlation with PWV and CV history and an inverse correlation with FMD, suggesting their role in vascular pathophysiology. A negative correlation between lipids and CRP, or disease activity, was observed at baseline, thus reflecting the lipid paradox. On the other hand, the correlation between these parameters became positive after the therapy, which suggests that the biological therapy reduced the level of inflammation and thus the lipid paradox is probably no longer present after one year of treatment. Finally, changes in TC over time were determined by treatment and baseline leptin level, while changes in HDL over time were determined by treatment and baseline CRP.

This first presented study has certain advantages and limitations. A complex follow-up study was carried our which examined several metabolic parameters together with raw data on clinical efficacy and vascular pathophysiology from previous investigations in the same cohort. A similarly complex study was not found in the literature. Several new results were presented. The effects of TNF-α therapy on ARE activity and MPO levels have not been investigated before. Based on the results of this study, a number of previously not described associations were presented, such as the relationship between adiponectin and PWV, leptin and IMT, PVW, and leptin/adiponectin ratio and PWV. Moreover, our analyses also confirmed several previously described effects and correlations, such as effects regarding TNF-inhition on chemerin, or correlations between certain adipokines and obesity, disease activity, or CRP.

The relatively small sample size may mask potentially significant results. However, the examinations and measurements would have been much more difficult to carry perform, would have required much more time and resources in the case of a larger sample. Patients with potentially positive CV history were also included in our study, however, these data were indicated and analyzed. RA and AS patients were not analyzed separately due to the relatively small number of patients and their unequal distribution within the cohort. In conclusion, our data represent a starting point, but at the same time, the performance of further studies with a larger number of patients is recommended in order to gain more experience, get a clear view of the correlations, identify biomarker combinations and, possibly, to determine limit values that can also be used in the daily clinical practice.

6.2 SECOND STUDY

During the secound follow-up study in a RA cohort, our primary objective was to investigate the effects of 12-months of tofacitinib therapy on various metabolic parameters, including lipids (TC, HDL, LDL, TG, Lp(a), APOA, APOB), lipid indices (TC/HDL, LDL/HDL), PON1, MPO, TSP-1 and adipokine levels (adiponectin, leptin, resistin, adipsin, chemerin). One-year tofacitinib treatment increased lipids and lipoproteins, including TC, HDL, LDL, APOA and APOB, but decreased the pro-atherogenic Lp(a). TG and lipid ratios (TC/HDL, LDL/HDL) did not change overtime. Similar to our findings, tofacitinib therapy in combination with DMARDs or as a monotherapy was previously reported to increase LDL and HDL levels. In our study, lipid ratios (TC/HDL and LDL/HDL) showed no significant changes indicating that lipid elevations observed upon tofacitinib therapy may not have important clinical relevance for CV disease. Only very slight change in the LDL/HDL ratio was previously reported. In our treatment group of 5 mg bid tofacitinib, no significant changes were found; however, in the treatment group of 10 mg bid tofacitinib, similar changes to that of the whole cohort have been observed. We may speculate that it is due to the smaller mean BMI in the group of 5 mg bid (28.82±5.63) compared with that of the 10 mg bid (31.03±8.01).

Among adipokines, tofacitinib treatment significantly increased leptin and adipsin levels, while it decreased chemerin levels. Adiponectin and resistin showed no significant changes overtime. There are no previous reports on the effects of tofacitinib on adipokines. The JAK inhibitor baricitinib therapy, was previously found th decrease adiponectin, but increase leptin, resistin and adipsin levels increased. Regarding other parameters, PON1 and MPO levels significantly decreased. However, another study reported increasing PON1 levels in response to tofacitinib therapy. It may be speculated that in our study the observed decrease is only temporary, and it normalizes at a later timepoint. In this study, TSP-1 increased significantly during therapy. However, due to the pro-atherogenic and pro-inflammatory properties of this parameter and based on previos findings of elevated TSP-1 levels in RA, a decrease was expected. Since there are no previous reports on the effects of tofacitinib therapy, we may also speculate that this is only a temporary condition, or that some external factor may have an influence on this change, no clear conclusion can be drawn.

As a secondary objective, the associations of different examined metabolic parameters with clinical data and vascular pathophysiology were described, based on a previous study of our working group, using its raw data. In close relation to this objective, the relationships

between the metabolic markers were defined as a tertiary objective. In the correlation analysis, BMI correlated positively with disease activity, CRP, ESR, FMD, PWV, leptin, resistin, PON1 and MPO, while inversely with TC, HDL and APOA. Univariable regression analysis found a possible association of BMI with disease activity, CRP, ESR and PWV, while multivariable analysis confirmed association with CRP and PWV.

Lipids and lipid ratios variably correlated with other lipids, lipid ratios, adipokines, and other metabolic parameters, as well as clinical and vascular parameters. Univariable regression analysis found various possible associations of lipids and lipid ratios, while multivariable analysis confirmed inverse associations of TC with CRP; HDL with ESR, FMD and PWV; APOA with ESR, CRP and RF; and APOA/APOB with ESR; and positive associations of APOB with FMD and PWV; Lp(a) with age and FMD; TC/HLD with RF; and LDL/HDL with disease activity, RF and PWV.

Among adipokines, adiponectin correlated positively with age, HDL, APOA, APOA/APOB, IMT, adipsin, TSP-1 and PON1, while inversely with CRP, TG, TC/HDL, LDL/HDL and anti-CCP. Univariable regression analysis showed a possible positive association with age and IMT, but an inverse association with CRP, anti-CCP and RF. Multivariable analysis confirmed a positive association with age and inverse association with anti-CCP. Adiponectin was previously positively associated with age and disease activity, while inversely associated with BMI. In our study, adiponectin changes overtime were associated with treatment and PWV. Leptin correlated positively with age, CRP, BMI, FMD, adipsin, resistin and PON1, while inversely with TC. Univariable regression analysis showed possible positive association with age, BMI, CRP and FMD, while multivariable analysis confirmed association with age, BMI and FMD. Leptin was previously correlated with BMI, disease duration, disease activity, ESR and CRP. In our study, leptin changes correlated with treatment and age, as well as treatment and CRP. Adipsin showed positive correlation with age, PWV, FMD, adiponectin, leptin, resistin, TSP-1, PON1 and MPO, but an inverse correlation with RF. Univariable regression analysis showed a possible positive association with age, PWV and FMD, and inverse association with RF. Multivariable analysis confirmed association with PWV. Adipsin was previously reported to correlate with BMI and disease activity. Resistin correlated positively with BMI, disease activity, CRP, ESR, RF, PWV, adipsin, leptin, PON1 and MPO, while inversely with TC and APOA. Univariable regression analysis showed a possible positive association with BMI, CRP, ESR and PWV, while multivariable analysis confirmed association with CRP. Resistin was previously associated with CRP and disease

activity. In our study, resistin changes were associated with treatment and BMI. Chemerin showed positive correlation with age, LDL, APOB, LDL/HDL and TSP-1, while inverse correlation with IMT. Univariable regression analysis showed a possible inverse correlation with age and IMT, while multivariable analysis confirmed association with IMT. Chemerin was previously correlated with disease activity, BMI, CRP, RF, ESR and anti-CCP.

MPO showed a positive correlation with BMI, CRP, Lp(a), adipsin, resistin, PON1 and PWV. Univariable regression analysis showed a possible positive association with BMI, CRP, disease duration and PWV. Multivariable analysis confirmed association with disease duration and PWV. Similar to our findings, others also reported on positive correlation with CRP, but also with disease activity. In our study, MPO changes correlated with treatment and disease duration, as well as with treatment and anti-CCP.

TSP-1 correlated positively with HDL, APOA, adiponectin, adipsin, chemerin and PON1, while inversely with disease activity, TG, TC/HDL, LDL/HDL, RF and ESR. Univariable regression analysis showed a possible inverse association with disease activity, RF and ESR. Multivariable analysis confirmed inverse association with RF. TSP-1 was previously associated with disease activity and ESR. Changes in TSP-1 correlated with treatment and CRP.

Serum PON1 correlated positively with age, BMI, adiponectin, adipsin, leptin, resistin, TSP-1 and MPO, while inversely with TC/HDL, LDL/HDL, anti-CCP and RF. Univariable regression analysis showed possible positive association with age and BMI, while inverse association with CRP and RF. Multivariable analysis confirmed positive association with BMI, while inverse association with age and RF. Previous studies reported an inverse association with RF, anti-CCP and disease activity. Finally, PON1 changes were associated with treatment and CRP, as well as with treatment and ESR.

This second presented study also has certain advantages and limitations. A complex follow-up study was carried our which examined several metabolic parameters together with raw data on clinical efficacy and vascular pathophysiology from previous investigations in the same cohort. Similar to the first study, there was no similarly complex study found in the literature regarding this therapy and parameters. Several new results were presented. The effects of tofacitinib therapy on adipokines, MPO and TSP-1 levels have not been investigated before. Based on the results of this study, a number of previously not described associations were presented, such as the relationship between adiponectin and IMT, or TSP-1; leptin and FMD; adipsin and PWV, FMD; resistin and PWV; chemerin and IMT; MPO and PWV; and TSP-1

and RF. Moreover, our analyses also confirmed several previously described correlations, such as correlations between certain adipokines and obesity, disease activity, or CRP.

The relatively small sample size may mask potentially significant results. However, the examinations and measurements would have been much more difficult to carry perform, would have required much more time and resources in the case of a larger sample. Patients with potentially positive CV history and diabetes were also included in our study, however, these patients presented no complaints of these nature on inclusion, their condition was controlled. Similarly to the first study, further studies with a larger number of patients are recommended in order to gain more experience, get a clear view of the correlations, identify biomarker combinations and, possibly, to determine limit values that can also be used in the daily clinical practice.

6.3 NEW FINDINGS

1st study

- We were the first to assess the effects of anti-TNF therapy on ARE activity and MPO levels, during which we found decreased ARE activity and decreased MPO levels.
- Several new correlations were described in the complex follow-up study:
    - the relationship between adiponectin and PWV,
    - the relationship between leptin and IMT, PVW,
    - the relationship between leptin/adiponectin ratio and PWV.

2nd study

- We were the first to assess the effects of tofacitinib therapy on various adipokine levels (adiponectin, leptin, chemerin, resistin, adipsin), where we found unchanged adiponectin and resistin, increased leptin and adipsin, and decreased chemerin levels.
- We were the first to assess the effects of tofacitinib therapy on MPO and TSP-1, regarding which we found decreased MPO levels and increased TSP-1 levels.
- Several previously not discussed connections were described:
    - the relationship between adiponectin and IMT, TSP-1,
    - the relationship between leptin and FMD,
    - the relationship between adipsin and PWV, FMD,
    - the relationship between resistin and PWV,
    - the relationship between chemerin and IMT,
    - the relationship between MPO and PWV,
    - the relationship between TSP-1 and RF.

7 SUMMARY

Arthritides, such as RA and AS, have been associated with atherosclerosis, increased cardiovascular morbidity and mortality, metabolic changes and dyslipidemia. In patients with inflammatory rheumatic diseases different metabolic factors play a role in the development of atherosclerosis. In the everyday patient care it would be important to select biomarkers or biomarker groups that could indicate cardiovascular changes occuring together with the inflammatory activity of the underlying disease or persistent disease, preferably in the preclinical phase. For this purpose, while also monitoring the changes during the therapy, we have choosen the complex assessment of different metabolic markers. No similarly complex studies have been published to this point in time. In case of many parameters, missing data or contradictory results can be found in the literature. For example, there are fewer studies available on the effects of tofacitinib on various metabolic parameters, and for some of the investigated parameters there was no previous data to be found in the literature. Therefore, conducting further studies and possibly contributing to future metaanalysis supported the topic selection.

In the first presented study, one year of anti-TNF therapy (ETN, CZP) significantly reduced MPO and chemerin levels in a mixed RA and AS patient cohort. These parameters could be relatively easily accessible and testable for a longer follow-up and a larger patient population. In the second study, twelve months of tofacitinib therapy among RA patients resulted in a significant increase in TC, HDL, LDL, APOA, APOB, leptin, adipsin and TSP-1 levels, while Lp(a), chemerin, PON1 and MPO levels showed a significant decrease. Therefore, the previously mentioned MPO and chemerin levels may be worthy of similar follow-up here as well. Although the increase in HDL and LDL levels has already been reported, several presented results with tofacitinib can be considered new. Without detailing our extensive correlation results, the thesis presents previously unpublished correlations within both studies.

Based on these studies, it can be cautiosly concluded that various correlations and associations may exist between metabolic parameters and clinical parameters and vascular pathophysiology, thus the joint evaluation of lipids, adipokines and other metabolic parameters with disease activity, CRP and ultrasound-based techniques can help in establishing and monitoring cardiovascular status during therapy, as well as monitoring the effects of therapy on preclinical vascular pathophysiology, and determining cardiovascular burden. Considering that most of the

metabolic biomarkers we have chosen have proinflammatory and proatherogenic effect, but their exact participation in the pathogenesis of inflammatory rheumatological diseases, their location, time and involvement in the early accelerated arteriosclerosis associated with the diseases is not known, it is very difficult to select an optimal biomarker. The determination of a biomarker pattern may probably be helpful in this regard for which performance of further studies with a larger number of patients is recommended.

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Registry number: DEENK/36/2023.PL
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Candidate: Monika Bodoki (name at birth: Monika Czókolyová)
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Czókolyová, M.**, Hamar, A. B., Pusztai, A., Tajti, G., Végh, E., Pethő, Z., Bodnár, N., Horváth, Á., Soós, B., Szamosi, S., Szentpéteri, A., Seres, I., Harangi, M., Paragh, G., Kerekes, G., Bodoki, L., Domján, A., Hódosi, K., Seres, T., Panyi, G., Szekanecz, Z., Szűcs, G.: Effects of One-Year Tofacitinib Therapy on Lipids and Adipokines in Association with Vascular Pathophysiology in Rheumatoid Arthritis.
Biomolecules. 12, 1-22, 2022.
DOI: http://dx.doi.org/10.3390/biom12101483
IF: 6.064 (2021)

2. **Czókolyová, M.**, Karancsiné Pusztai, A., Végh, E., Horváth, Á., Szentpéteri, A., Hamar, A. B., Szamosi, S., Hódosi, K., Domján, A., Szántó, S., Kerekes, G., Seres, I., Harangi, M., Paragh, G., Szekanecz, É., Szekanecz, Z., Szűcs, G.: Changes of Metabolic Biomarker Levels upon One-Year Anti-TNF-α Therapy in Rheumatoid Arthritis and Ankylosing Spondylitis: associations with Vascular Pathophysiology.
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3. Soós, B., Fagyas, M., Horváth, Á., Végh, E., Karancsiné Pusztai, A., **Czókolyová, M.**, Csongrádi, A., Hamar, A. B., Pethő, Z., Bodnár, N., Kerekes, G., Hódosi, K., Szekanecz, É., Szamosi, S., Szántó, S., Szűcs, G., Papp, Z., Szekanecz, Z.: Angiotensin Converting Enzyme Activity in Anti-TNF-Treated Rheumatoid Arthritis and Ankylosing Spondylitis Patients.
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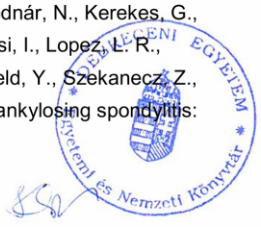

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Total IF of journals (all publications): 47,566
Total IF of journals (publications related to the dissertation): 12,128

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.

09 February, 2023

DEBRECENI EGYETEM
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