Soluble vascular biomarkers in rheumatoid arthritis and ankylosing spondylitis: effects of one-year anti-TNF-α therapy

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Running head: Vascular biomarkers in arthritis

None of the authors have any potential conflicts of interest.

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Abstract

Background: Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) have been associated with cardiovascular (CV) disease. The treatment of arthritis by tumour necrosis factor α (TNF-α) inhibitors may decrease the serum concentrations of vascular biomarkers. We determined circulating levels of oxidized LDL (oxLDL)/β2 glycoprotein I (β2GPI) complexes, antibodies to 60 kDa heat shock protein (anti-Hsp60), soluble urokinase plasminogen activator receptor (suPAR) and Brain type natriuretic peptide (BNP) fragment in sera of RA and AS patients undergoing anti-TNF treatment.

Patients and methods: Fifty-three RA/AS patients were treated with etanercept (ETN) or certolizumab pegol (CZP) for one year. Circulating oxLDL/β2GPI complex (AtherOx®), anti-Hsp60 IgG and BNP8-29 fragment levels were assessed by ELISA. suPAR levels were determined by suPARnostic® Quick Triage test. Flow-mediated vasodilation (FMD), carotid intima-media thickness (IMT) and arterial pulse-wave velocity (PWV) were determined by ultrasound.

Results: One-year anti-TNF treatment significantly decreased oxLDL/β2GPI levels, as well as suPAR levels in patients with “critically” high suPAR levels at baseline. In RA, BNP levels were higher in seropositive vs seronegative patients. Serum levels of these vascular biomarkers variably correlated with lipids, ACPA, RF and CRP. IMT positively correlated with BNP, PWV with suPAR and anti-Hsp60, while FMD inversely associated with anti-Hsp60. In RM-ANOVA analysis, disease activity supported the effects of anti-TNF treatment on 12-month changes in oxLDL/β2GPI. IMT supported the effects of therapy on changes of anti-Hsp60 and suPAR.

Conclusions: These biomarkers may be involved in the pathogenesis of atherosclerosis underlying RA/AS. TNF inhibition variably affect the serum levels of oxLDL/β2GPI, suPAR and BNP.
Introduction

Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) have been associated with inflammatory atherosclerosis, increased cardiovascular (CV) morbidity and mortality (1-5). Early endothelial dysfunction and activation may precede these events already in the pre-clinical phase of arthritides (1-3, 5). Soluble vascular biomarkers, such as von Willebrand factor antigen (6) or circulating endothelial cell adhesion molecules (7) are released from the endothelial surface. Numerous proteins may serve as biomarkers of inflammatory atherosclerosis (8). The treatment of arthritis by tumour necrosis factor α (TNF-α) inhibitors may decrease the serum concentrations of these biomarkers (6, 7).

Oxidized LDL (oxLDL) and β2 glycoprotein I (β2GPI) antigens, as well as antibodies to these antigens have been implicated in atherosclerosis associated with autoimmune diseases (9-13) and atherosclerosis (10, 13-15). Unlike native LDL, oxLDL binds to β2GPI to form complexes. These complexes that are readily detectable in the blood (9, 16, 17). Macrophages uptake these complexes by scavenger receptors and this mechanism is relevant for the development of foam cells within the atherosclerotic lesions (16, 17). High circulating oxLDL/β2GPI levels have been associated with disease severity and prognosis in acute coronary syndrome (18). Serum oxLDL/β2GPI complex levels may also reflect vascular damage in SLE, and other autoimmune diseases, such as antiphospholipid syndrome and systemic sclerosis (9, 17, 19). In one study, there was a slight increase in oxLDL/β2GPI levels in RA (19). However, neither the role of these complexes in AS nor the effects of anti-TNF therapy on serum oxLDL/β2GPI complex levels have been evaluated.

Heat shock proteins (Hsp) including the human Hsp60 and the Mycobacterial Hsp65 have been implicated in the pathogenesis of atherosclerosis (10) and inflammation (20). Anti-Hsp antibodies have been detected in patients with CV disease (21, 22) and autoimmune-inflammatory diseases including RA and AS (11, 22-25). These antibodies were associated with early atherosclerotic changes (11, 22).

The urokinase plasminogen activator receptor (uPAR; CD87) is expressed mainly on immune cells, smooth muscle cells and endothelial cells, favoring extracellular matrix degradation, cell adhesion, cell proliferation and regulates cell migration (26-28). Signalling of uPA/uPAR results in the release of numerous inflammatory mediators and synovial fibroblast activation (27). We detected uPAR in the RA synovium (26). suPAR is the soluble form of the cell membrane-bound protein uPAR released by GPI-anchor cleavage from the cell surface (28, 29). Elevated serum levels of suPAR may reflect increased activation of immune system, as...
well as vascular pathology in inflammatory diseases (29-32). In RA, suPAR levels have been associated with disease activity (30, 32).

Brain type (B-type) natriuretic peptide (BNP) and its N-terminal fragment (NT-proBNP) exert a key role in cardiovascular homeostasis with biological actions including natriuresis, diuresis, vasorelaxation, and inhibition of renin and aldosterone secretion (33). BNP is synthesized as prohormone in cardiomyocytes. Upon release into the circulation, proBNP is cleaved into BNP and NT-proBNP fragments in equimolar proportions (33). A high concentration of BNP in the bloodstream is indicative of heart failure and also predicts prognosis after acute coronary syndrome (33). BNP is also a good biomarker in emergency setting (33). Increased BNP production has been associated with cardiac manifestations and may serve as a prognostic factor in various autoimmune-inflammatory diseases, such as systemic sclerosis (34, 35). In RA and AS, NT-proBNP levels were assessed and anti-TNF therapy decreased NT-proBNP in these diseases (36, 37). BNP fragment has not yet been assessed in RA and AS.

In this study, we wished to assess the effects of one-year anti-TNF therapy on vascular biomarkers described above. We also wished to associate these biomarkers with markers of disease activity, autoantibodies, lipids and vascular imaging results. Moreover, we wished to determine the predictors of these parameters at baseline, as well as the determinants on one-year change in these parameters. This study may improve our understanding of vascular biomarkers underlying inflammatory atherosclerosis.

**Patients and methods**

**Patients**

Fifty-three patients with inflammatory arthritis (36 RA and 17 AS) selected for the initiation of anti-TNF therapy were enrolled in the study. Patient characteristics are seen in Table 1. The 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. Exclusion criteria included unstable hypertension (blood pressure >140/90 mmHg), congestive heart failure, diabetes mellitus, current inflammatory disease other than RA or AS, infectious disease or renal failure (serum creatinine ≥ 117 mmol/l). None of patients received aspirin, clopidogrel, heparin or warfarin or vasoactive drugs at the time of inclusion. Patients with active disease were recruited prior to initiating a biological therapy. All patients...
started on an anti-TNF therapy at baseline and received the same biological treatment at one year. Among the 36 RA patients, 20 received etanercept (ETN) 50 mg/week subcutaneous (SC) and 16 received certolizumab pegol (CZP) (400 mg at 0, 2 and 4 weeks, and thereafter 200 mg twice weekly SC). Altogether 18 RA patients were treated with ETN and 13 with CZP in combination with methotrexate (MTX). The other patients received monotherapy. All 17 AS patients received 50 mg/week ETN monotherapy SC. RA patients did not take DMARDs other than MTX. Altogether 12 RA and 2 AS patients currently took low-dose (<6 mg/day) methylprednisolone (Table 1). Disease activity was determined by DAS28 and BASDAI in RA and AS, respectively.

The study was approved by the Hungarian Scientific Research Council Ethical Committee (approval No. 14804-2/2011/EKU). Written informed consent was obtained from each patient and assessments were carried out according to the Declaration of Helsinki.

**Laboratory measurements**

After overnight fasting, blood samples were taken from the patients for total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG). Lipids were determined using routine laboratory methods.

Serum high sensitivity C reactive protein (hsCRP; normal: ≤5mg/l) and IgM rheumatoid factor (RF; normal: ≤50 IU/ml) were measured by quantitative nephelometry (Cobas Mira Plus-Roche), using CRP and RF reagents (both Dialab). ACPA (anti-CCP) autoantibodies were detected in serum samples using a second generation Immunoscan-RA CCP2 ELISA test (Euro Diagnostica; normal: ≤25 IU/ml).

Circulating oxLDL/β2GPI complexes (U/ml) were assessed by an AtherOx® ELISA system (Corgenix, Broomfield, CO, USA) according to the manufacturer’s instructions.

Anti-human Hsp60 immunoglobulin G (IgG) levels were measured by an in-house ELISA as described previously (21, 38). In brief, plates were coated with 0.1 μg per well recombinant human Hsp60 (SPP-740; StressGen, Vicoria, Canada). After washing and blocking (phosphate-buffered saline [PBS], 0.5% gelatine), the wells were incubated with 100 μl of serum samples diluted to 1:500 (PBS, 0.5% gelatine, 0.05% Tween 20). Bound anti-Hsp60 antibodies were detected by antihuman IgG peroxidase-labeled antibodies (Sigma, St. Louis, MO, USA) and o-phenylene-diamine (Sigma). Optical Density (OD) values were assessed and concentrations expressed in AU/ml.
Circulating suPAR levels were assessed by suPARnostic® Quick Triage test (ViroGates A/S, Birkerød, Denmark) that gives fully quantitative results within the range of 2-16 ng/ml. Results were read by the suPARnostic® Quick Test Reader (ViroGates). Serum levels were expressed in ng/ml. According to the manufacturer, suPAR levels in the normal population are in the range of 2-3 ng/ml. Serum levels <4, 4-5.5, 5.5-9 and >9 ng/ml are considered as “low”, “observe”, “high” and “critical”, respectively.

BNP\textsubscript{8-29} fragment levels were assessed by commercially available ELISA kit (Biomedica, Vienna). The fragment consisted of the 8-29 C-terminal part of proBNP. Measurements were performed according to the manufacturer’s instructions.

All laboratory assessments were performed at baseline, as well as 6 and 12 months after treatment initiation.

\textit{Assessment of vascular physiology by ultrasound}

Brachial artery FMD was assessed as described before (2, 39). In brief, ultrasound examination was performed on the right arm using 10 MHz linear array transducer (ultrasound system: HP Sonos 5500) by a single trained sonographer after 30 minutes resting in a temperature-controlled room (basal value for FMD). A B-mode longitudinal section was obtained of the brachial artery above the antecubital fossa. In order to assess FMD, reactive hyperaemia was induced by release of a pneumatic cuff around the forearm inflated to suprasystolic pressure for 4.5 minutes. After deflation the maximal flow velocity and the arterial diameter was 90 seconds long continuously recorded. Flow velocities, the baseline diameter, as well as FMD were ECG gated and detected offline. FMD values were expressed as % change from baseline (resting) value.

The ccIMT measurements were carried out as described before (2, 39). Briefly, a duplex ultrasound system (HP Sonos 5500, 10 MHz linear array transducer) was used to assess the common carotid arteries by a single observer. Longitudinal high-resolution B-mode ultrasound scan were employed over both right and left common carotid arteries and were R-synchronized and recorded. The offline measurements were performed 1 cm proximal to the carotid bulb in the far wall. ccIMT was defined as the distance between the first and second echogenic lines from the lumen taking the average of 10 measurements on both sides. ccIMT values were expressed in mm.

With respect to arterial stiffness, PWV was calculated automatically by a TensioClinic arteriograph system (Tensiomed Ltd, Budapest, Hungary) as the quotient of the distance...
between the jugular fossa and symphysis as described before (39, 40). If an artery is elastic, PWV is low. With decreased arterial elasticity, PWV rises. The arteriograph assesses this parameter from the oscillometric data obtained from the 35 mmHg suprasystolic pressure of the brachial artery. In order to obtain reproducible results, the patient had to rest in a supine position for at least 10 minutes before the assessment in a quiet room. PWV is expressed in m/s.

**Statistical analysis**

Statistical analysis was performed using SPSS version 22.0 (IBM) software. Data are expressed as the mean ± SD for continuous variables and percentages for categorical variables. Continuous variables were evaluated by 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 analysis. Univariable and multivariable regression analyses using the stepwise method was applied to investigate independent associations between angiogenic biomarkers (dependent variables) and other clinical, laboratory and imaging parameters (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. Repeated measures analysis of variance (RM-ANOVA) was performed in order to determine the additional effects of multiple parameters on changes of vascular imaging markers between baseline and 12 months. The dependent variables were anti-oxLDL/β2GPI, anti-Hsp60, suPAR and BNP. Partial η² is given as indicator of effect size, with values of 0.01 suggesting small, 0.06 medium and 0.14 large effects. In all analyses, P values < 0.05 were considered significant.

**Results**

**Effects of TNF inhibition on vascular biomarkers**

First, TNF-α inhibition significantly decreased disease activity in RA and AS patients. In the RA cohort (n=36), ETN and CZP treatment resulted in significant decreases in DAS28 after 6 months (3.13±0.84; p<0.001) and 12 months of treatment (3.02±0.96; p<0.001) compared to baseline (5.00±0.86) (data not shown). In AS (n=17), BASDAI significantly decreased from
5.79±1.19 at baseline to 2.00±1.03 (p<0.001) and 1.86±1.04 (p<0.001) at 6 and 12 months, respectively (data not shown).

In the mixed cohort of 53 arthritis patients, the circulating levels of oxLDL/β2GPI significantly decreased after 12 months of anti-TNF therapy (0.20±0.11 U/ml) compared to baseline (0.24±0.10 U/ml; p=0.014) (Figure 1).

Anti-Hsp60 antibody levels did not change after 6 months (158.6±138.6 AU/ml) and 12 months (167.3±143.3 AU/ml) compared to baseline (170.3±140.4 AU/ml) (Figure 1).

suPAR levels did not change significantly after 6 months (11.3±17.7 ng/ml) and 12 months (10.3±15.3 ng/ml) versus baseline (11.5±16.4 ng/ml) (Figure 1). Among the patients, 21.2% had “low”, 36.4% “observe”, 9.1% “high” and 33.3% “critical” suPAR levels according to the classification described above (data not shown). When these four serum level categories were considered, suPAR concentrations exerted significant decrease in RA patients with “critical” suPAR levels (>9ng/ml) (p=0.04) (Figure 2).

BNP fragment levels did not change significantly after 6 months (518.2±422.4 pmol/l) and 12 months (484.1±418.2 pmol/l) versus baseline (530.8±441.8 pmol/l) (Figure 1). However, serum BNP levels at baseline and after 12 months were significantly increased in ACPA positive versus negative RA patients (baseline: 670.6±323.0 versus 138.0±436.4 pmol/l; p=0.030 and 12 months: 652.9±283.2 versus 456.5±423.1 pmol/l; p=0.021), as well as in RF positive versus negative RA patients (baseline: 680.6±381.6 versus 292.9±198.3 pmol/l; p=0.007 and 12 months: 668.9±346.5 versus 312.2±207.0 pmol/l; p=0.001) (Figure 3).

**Correlations of vascular markers with other parameters**

First, the four vascular biomarkers were correlated with each other. We found only one significant correlation: baseline BNP (BNP-0) and suPAR-0 showed positive association with each other (p=0.013) (Table S1).

When these biomarkers were correlated with other markers, oxLDL/β2GPI complex, suPAR, BNP and aHsp60 levels positively correlated with some lipids at baseline (p<0.05) (Table S1; Table 2). suPAR-0 levels also positively correlated with ACPA-0 (p<0.001) (Table S1). BNP-0 strongly correlated with RF-0 (p=0.004) and ACPA-0 (p<0.001). Moreover, BNP-12 correlated with age at disease onset (p=0.023), CRP-0 (p=0.010) and CRP-12 (p=0.014) (Table S1). None of the vascular biomarkers correlated with disease activity as determined by DAS28 or BASDAI (data not shown).
Results of the univariable and multivariable regression analyses are indicated in Table 2. In the univariable analysis, the predictor of suPAR-0 was TG-0 (p<0.05), while that of suPAR-12 was CRP-12 (p<0.05). BNP-0 was determined by TG-0 (p<0.05), while BNP-12 was determined by age at disease onset, CRP-0 and CRP-12 (p<0.05) (Table 2). The multivariable analysis confirmed the significant association of BNP-12 and CRP-0 only (p=0.028) (Table 2). Thus, most correlations found in univariable analysis, except for one, was lost during the multivariable analysis (Table 2).

When vascular biomarkers were correlated with imaging markers of vascular physiology, BNP-0 showed positive association with IMT-0 (p=0.016). aHsp-60-0 inversely correlated with FMD-12 (p=0.022) and positively with PWV-0 (p=0.040). Both suPAR-0 (p=0.045) and suPAR-12 (p=0.042) were positively associated with PWV-12 (Table S1).

Finally, GLM RM-ANOVA was performed to assess combined determinants of vascular biomarker changes over the 12-month period. The change of oxLDL/β2GPI complex levels between baseline and 12 months was determined by the anti-TNF treatment together with higher baseline disease activity (DAS28/BASDAI-0) (p=0.014). In addition, TNF inhibition and higher IMT-0 determined aHsp-60 (p=0.015) and suPAR changes over the one-year period (p=0.041) (Table 3).

Discussion

In this study, we determined changes in various vascular biomarkers upon one-year anti-TNF therapy. We also found associations of circulating levels of these biomarkers with CRP, lipids and vascular imaging markers. In the very same cohort, we have previously published the effects of anti-TNF therapy on vascular pathophysiology using imaging techniques (41). Anti-TNF therapy significantly decreased serum levels of oxLDL/β2GPI complexes. We and others suggested the involvement of both oxLDL and β2GPI, as well as anti-oxLDL and anti-β2GPI in inflammatory atherosclerosis and CV disease (9, 11, 12, 14, 15). Serum oxLDL/β2GPI levels have been associated with acute coronary syndrome (18), as well as vascular damage in various autoimmune rheumatic diseases (9, 16, 17, 19, 42). To our best knowledge, this is the first study to evaluate oxLDL/β2GPI complexes in AS and to assess the effects of anti-TNF biologics on these complexes. In addition, we found correlations between baseline levels of oxLDL/β2GPI complexes and lipids. RA and AS disease activity in combination with one-year anti-TNF treatment determined 12-month changes in oxLDL/β2GPI complexes.
oxLDL/β2GPI, as shown by RM-ANOVA. The clinical relevance of oxLDL/β2GPI levels needs to be validated.

Elevated serum levels of suPAR have been detected in various rheumatic diseases, such as RA and systemic sclerosis (29-31, 43). Moreover, according to other studies, suPAR levels may reflect disease activity in RA (30, 32), which was not confirmed in our cohort. In our cohort, baseline suPAR levels correlated with ACPA and TG levels. Again, very few studies evaluated the effects of biologics on suPAR production. We observed that one-year TNF inhibition did not change suPAR levels significantly. In another study, similarly to ours, infliximab treatment did not modify suPAR levels in pediatric inflammatory bowel disease patients (44). Interestingly, although TNF inhibition did not affect suPAR levels in the full cohort, it significantly reduced suPAR production in arthritis patients who had “critical” suPAR levels at baseline. In addition, CRP-12 may be a predictor of suPAR-12 suggesting that if, despite of one-year biologic therapy, CRP still remains higher, suPAR would also stay high. In other words, suPAR may be a marker of sustained inflammation despite anti-TNF therapy in arthritis. Finally, in another cohort, suPAR was a good predictor of adalimumab responsiveness in RA (45). We did not find any association between suPAR and clinical response to anti-TNF therapy in our cohort. Baseline suPAR correlated with BNP and anti-TNF treatment together with baseline IMT determined 12-month changes in suPAR. The clinical relevance of suPAR levels needs to be validated.

Increased production of BNP and NT-proBNP have been associated with CV disease and heart failure (36, 46). NT-proBNP may also play a role in CV comorbidities of systemic sclerosis, RA and other rheumatic diseases (34, 35, 37). Other investigators reported that TNF inhibition reduced NT-proBNP levels in RA and AS (36, 37). However, the BNP fragment has not yet been previously studied in these diseases. In this study, anti-TNF therapy did not change BNP levels significantly in the full cohort. However, circulating BNP levels were significantly elevated in seropositive RA patients compared to seronegative ones. Moreover, TG-0 was a predictor of BNP-0, while age, CRP-0 and CRP-12 were determinants of BNP-12. Thus, BNP may be associated with systemic inflammation and some lipids. Moreover, the association between CRP-0 and BNP-12 suggests that the extent of inflammation at baseline may predict how BNP levels would change after one-year TNF inhibition.

Finally, anti-Hsp60 antibodies have been detected in patients with CV disease (21, 22, 38), as well as in rheumatic diseases including RA and AS (11, 22-25). In our cohort, anti-Hsp60 levels at baseline correlated with TG. Biologics did not change anti-Hsp60 levels over time. However, TNF inhibition combined with baseline IMT determined the 12-month changes...
in anti-Hsp60 as shown by RM-ANOVA. Again, we have not found any other studies where the effects of biologics on anti-Hsp antibodies were evaluated. The clinical relevance of anti-Hsp60 levels needs to be validated.

Certainly there have been other studies that analyzed vascular biomarkers in arthritides. For example, angiopoietin-2 levels correlated with CV disease in RA (47). Anti-TNF therapy reduced resistin levels in RA (48). In AS patients, osteoprotegerin correlated with disease activity and vascular damage (49). Finally, we found that the release of angiogenic vascular biomarkers also changed upon TNF inhibition in RA and AS patients (50).

When comparing the four analyzed vascular markers, one-year anti-TNF therapy generally decreased oxLDL/β2GPI levels, suppressed suPAR in patients with “critical” levels at baseline. BNP levels were higher in ACPA and RF seropositive patients. Multiple correlations were found between any of these four markers and CRP, lipids, ACPA and RF. According to the regression analysis, CRP and TG may be important predictors of BNP and suPAR at baseline or after 12-months of treatment. Among imaging markers of vascular pathophysiology, IMT preferably correlated with BNP, FMD inversely with anti-Hsp60, while PWV with suPAR and anti-Hsp60 suggesting that various biomarkers may be associated with different pathophysiological alterations in the vasculature. Baseline disease activity or IMT may significantly influence the effects of anti-TNF treatment on changes of oxLDL/β2GPI, anti-Hsp60 and suPAR overtime.

The strength of this study is its complexity. Here we compared four vascular biomarkers with disease activity, multiple laboratory parameters, as well as imaging of vascular physiology in RA and AS. Limitations may include the relatively low number of RA and AS patients. The majority of these surrogate markers, except BNP, have not been clinically validated so it is difficult to interpret their everyday practical relevance. This study does not have a control group and power was not calculated. These may also be limitations.

In conclusion, among the four vascular markers assessed in this study, oxLDL/β2GPI was affected by anti-TNF therapy. Moreover, suPAR levels changed in patients with the highest levels at baseline. These markers are variably associated with vascular pathophysiology, CRP, ACPA, RF and lipids. oxLDL/β2GPI complexes, anti-Hsp60 antibodies, suPAR and BNP may be involved in inflammatory and vascular mechanisms underlying RA and AS. These proteins may also serve as potential biomarkers in inflammatory atherosclerosis and cardiovascular disease.

**Figure legends**
**Figure 1.** Effects of one-year anti-TNF therapy on oxLDL/β2GPI complexes (A), anti-Hsp60 (B), suPAR (C) and BNP fragments (D) in RA and AS. Asterisk indicates significant change (p<0.05).

**Figure 2.** Effects of one-year anti-TNF therapy on suPAR levels according to serum concentration quartiles. Asterisk indicates significant change (p<0.05).

**Figure 3.** Effects of one-year anti-TNF therapy on BNP levels according to ACPA and RF seropositivity vs seronegativity. Asterisk indicates significant change (p<0.05).

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References


Figure 1

338x190mm (96 x 96 DPI)
Figure 2

Mean changes in suPAR levels (ng/ml)

suPAR level categories (ng/ml)

Low: <4  Obese: 4.5-5.1  High: 5.1-9  Critical: >9

338x190mm (96 x 96 DPI)
Figure 3

338x190mm (96 x 96 DPI)
**Table 1. Patient characteristics**

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<th></th>
<th>RA</th>
<th>AS</th>
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<td>3:14</td>
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<td><strong>disease duration (mean±SD) (range), years</strong></td>
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<td>8.5±7.9 (1-44)</td>
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<td>36.4±11.6 (23-50)</td>
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<td>14</td>
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<tr>
<td><strong>RF-positivity, n (%)</strong></td>
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<tr>
<td><strong>ACPA positivity, n (%)</strong></td>
<td>21 (58)</td>
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<td><strong>DAS28 (baseline) (mean±SD)</strong></td>
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<tr>
<td><strong>BASDAI (baseline) (mean±SD)</strong></td>
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<td>5.79±1.19</td>
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<tr>
<td><strong>Treatment (ETN, CZP)</strong></td>
<td>20 ETN, 16 CZP</td>
<td>17 ETN</td>
<td>37 ETN, 16 CZP</td>
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Abbreviations: ACPA, anti-citrullinated protein antibody; AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CZP, certolizumab pegol; DAS28, 28-joint disease activity score; ETN, etanercept; RA, rheumatoid arthritis; RF, rheumatoid factor; SEM, standard error of mean.
**Table 2.** Univariable and multivariable regression analysis of vascular biomarkers

<table>
<thead>
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<th>Dependent variable</th>
<th>Independent variable</th>
<th>Univariable</th>
<th>Multivariable</th>
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<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>B</td>
</tr>
<tr>
<td>suPAR-0</td>
<td>0.382</td>
<td>0.028</td>
<td>8.314</td>
</tr>
<tr>
<td>suPAR-12</td>
<td>0.525</td>
<td>0.002</td>
<td>0.991</td>
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<tr>
<td>BNP-0</td>
<td>0.303</td>
<td>0.041</td>
<td>155.559</td>
</tr>
<tr>
<td>BNP-12</td>
<td>0.330</td>
<td>0.023</td>
<td>14.161</td>
</tr>
<tr>
<td>CRP-0</td>
<td>0.372</td>
<td>0.010</td>
<td>8.995</td>
</tr>
<tr>
<td>CRP-12</td>
<td>0.356</td>
<td>0.014</td>
<td>19.019</td>
</tr>
</tbody>
</table>

Abbreviations: BNP, B-type natriuretic peptide; CI, confidence interval; CRP, C-reactive protein; NS, non-significant; suPAR, soluble urokinase plasminogen activator receptor; TG, triglyceride.
Table 3. Significant results of general linear model (GLM) repeated measures analysis of variance (RM-ANOVA) test determining the effects of treatment and other independent variables on vascular biomarkers as dependent variables

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Effect</th>
<th>F</th>
<th>p</th>
<th>Partial η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxLDL-β2GPI 0-12</td>
<td>Treatment * DAS28/RASDAI-0</td>
<td>6.617</td>
<td>0.014</td>
<td>0.155</td>
</tr>
<tr>
<td>aHsp60 0-12</td>
<td>Treatment * IMT-0</td>
<td>6.533</td>
<td>0.015</td>
<td>0.161</td>
</tr>
<tr>
<td>suPAR 0-12</td>
<td>Treatment * IMT-0</td>
<td>4.294</td>
<td>0.041</td>
<td>0.177</td>
</tr>
</tbody>
</table>

Abbreviations: DAS28, 28-joint disease activity score; aHsp60, anti-heat shock protein 60 kDa; IMT, carotid intima-media thickness; oxLDL-β2GPI, oxidized low-density lipoprotein-β2 glycoprotein I complex; RM-ANOVA, repeated measures analysis of variance; suPAR, soluble urokinase plasminogen activator receptor.