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Clinical science

Effects of 1-year tofacitinib therapy on angiogenic biomarkers in rheumatoid arthritis

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Abstract

Objectives: Cardiovascular (CV) morbidity and mortality, and perpetuated synovial angiogenesis have been associated with RA. In our study we evaluated angiogenic factors in relation to vascular inflammation and function, and clinical markers in RA patients undergoing 1-year tofacitinib therapy.

Methods: Thirty RA patients treated with either 5 mg or 10 mg twice daily tofacitinib were included in a 12-month follow-up study. Eventually, 26 patients completed the study and were included in data analysis. Levels of various angiogenic cytokines (TNF-α, IL-6), growth factors [VEGF, basic fibroblast (bFGF), epidermal (EGF), placental (PlGF)], cathepsin K (CathK), CXC chemokine ligand 8 (CXCL8), galectin-3 (Gal-3) and N-terminal prohormone brain natriuretic peptide (NT-proBNP) were determined at baseline, and at 6 and 12 months after initiating tofacitinib treatment. In order to assess flow-mediated vasodilation, common carotid intima-media thickness (ccIMT) and carotid-femoral pulse-wave velocity, ultrasonography was performed. Synovial and aortic inflammation was also assessed by ^{18}F-fluorodeoxyglucose-PET/CT.

Results: One-year tofacitinib therapy significantly decreased IL-6, VEGF, bFGF, EGF, PlGF and CathK, while it increased Gal-3 production ($P < 0.05$). bFGF, PlGF and NT-proBNP levels were higher, while platelet-endothelial cell adhesion molecule 1 (PECAM-1) levels were lower in RF-seropositive patients ($P < 0.05$). TNF-α, bFGF and PlGF correlated with post-treatment synovial inflammation, while aortic inflammation was rather dependent on IL-6 and PECAM-1 as determined by PET/CT ($P < 0.05$). In the correlation analyses, NT-proBNP, CXCL8 and Cath variables correlated with ccIMT ($P < 0.05$).

Conclusions: Decreasing production of bFGF, PlGF or IL-6 by 1-year tofacitinib therapy potentially inhibits synovial and aortic inflammation. Although NT-proBNP, CXCL8 and CathK were associated with ccIMT, their role in RA-associated atherosclerosis needs to be further evaluated.

Keywords: rheumatoid arthritis, tofacitinib, angiogenesis, growth factors, PET/CT.

Rheumatology key messages

- Cardiovascular morbidity and mortality, and perpetuated synovial angiogenesis have been associated with RA.
- In our study, 1-year tofacitinib therapy decreased the production of angiogenic factors including basic fibroblast growth and placental growth factors (bFGF, PlGF) and IL-6.
- Interestingly, TNF-α, bFGF and PlGF correlated with post-treatment synovial inflammation, while aortic inflammation was dependent on IL-6 and platelet-endothelial cell adhesion molecule 1.

Introduction

Janus kinases (JAK) are involved in cytokine signalling [1, 2]. Up to now, four JAK inhibitors, tofacitinib, baricitinib, upadacitinib and filgotinib, have been approved for the treatment of RA [1, 2].

RA has been associated with both inflammatory angiogenesis [3–7] and increased cardiovascular (CV) risk [8–12]. The perpetuation of endothelial activation and angiogenesis has been described in the RA synovial tissue [3–6, 13]. Numerous angiogenic mediators including growth factors, cytokines,

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chemokines, proteases, extracellular matrix macromolecules, cell adhesion molecules and others promote synovial neovascularization in RA [5, 7, 13–15]. The hypoxia-inducible factor (HIF)–VEGF–angiopoietin 1 (Ang1) axis plays a key role in regulating RA-associated angiogenesis. Ang2 is a partial agonist to the receptor tyrosine kinase-2 (Tie2) and competes with Ang1 for Tie2 binding [3, 5, 16, 17]. Other angiogenic growth factors include basic fibroblast (bFGF), epidermal (EGF) and placental (PlGF) growth factors [7, 13, 18, 19]. Among pro-inflammatory cytokines, both TNF-α and IL-6 promote synovial neovascularization [7]. Among several other molecules, platelet-endothelial cell adhesion molecule 1 (PECAM-1; CD31), the CXC chemokine ligand 8 (CXCL8) chemokine (IL-8), the cathepsin K (CathK) protease, galectins (Gal) and N-terminal prohormone brain natriuretic peptide (NT-proBNP) have also been implicated in inflammatory angiogenesis [3, 5, 7, 20, 21]. Most of these angiogenic markers are readily detectable in the sera and synovial fluids of RA patients and thus they might serve as potential biomarkers of inflammatory angiogenesis [5, 7, 15, 21, 22].

Among these biomarkers, all growth factors (bFGF, EGF, VEGF, PlGF), as well as galectin-3 (Gal-3) and IL-6 utilize JAK-signal transducer and activator of transcription (STAT)-dependent pathways during signalling, while angiopoietin 1 (Ang1), Ang2, CathK, CXCL8, NT-proBNP, PECAM-1 and TNF-α do not (Table 1).

Abnormal CV pathophysiology can be observed very early, even in RA patients without overt CV disease [9, 12, 23]. US-based imaging is suitable to detect preclinical vascular pathophysiology [24]. Endothelial dysfunction of the brachial artery, carotid atherosclerosis and increased arterial stiffness are indicated by impaired endothelium-dependent, flow-mediated vasodilation (FMD), increased common carotid intima-media thickness (ccIMT) and carotid-femoral pulse-wave velocity (cfPWV), respectively [24, 25]. These preclinical abnormalities might predict subsequent CV events in RA [24, 25].

In order to link these two processes, angiogenesis has been implicated in the pathogenesis of atherosclerosis [15, 26, 27]. Moreover, systemic inflammation, which is also associated with RA, is the major driver of both inflammatory angiogenesis and atherosclerosis [7, 9, 15, 23, 27]. Various cytokines that utilize the JAK-STAT signalling pathways including IL-2, IL-6, IL-12, IL-22, IL-23, G-CSF, GM-CSF and IFNs have been implicated in the pathogenesis of RA-related angiogenesis and atherosclerosis [5, 28–31]. The control of inflammation by targeted therapies including JAK inhibitors may dampen these two processes [31, 32]. Indeed, in an experimental model of RA, tofacitinib inhibited vessel formation *in vitro*, as well as VEGF and Ang2 production *in vivo* [31]. Moreover, another JAK inhibitor, filgotinib, suppressed the release of several disease-associated biomarkers including those of angiogenesis [33]. However, there have been very few studies on the effects of JAK inhibition on angiogenesis and angiogenic markers.

In this study, we assessed the effects of 1-year tofacitinib therapy on the production of various angiogenic biomarkers. Our research aims and hypotheses were:

- targeted synthetic DMARD treatment suppresses the production of these markers indicating that JAK inhibition might also dampen RA-related angiogenesis;
- changes in angiogenic marker release upon tofacitinib therapy would also be correlated with changes in vascular pathophysiology;
- changes in biomarker levels might also correlate with disease activity and autoantibodies during tofacitinib therapy;
- eventually we wished to further understand the complexity of angiogenesis regulation during JAK inhibitor treatment.

Table 1. Biomarkers assessed in this study

Biomarker	Abbreviation	JAK/STAT utilization
Angiopoietin 1	Ang1	–
Angiopoietin 2	Ang2	–
Basic fibroblast growth factor	bFGF	+
Cathepsin K	CathK	–
CXC chemokine ligand 8/interleukin 8 chemokine	CXCL8/IL-8	–
Epidermal growth factor	EGF	+
Galectin-3	Gal-3	+
Interleukin 6	IL-6	+
N-terminal prohormone brain natriuretic peptide	NT-proBNP	–
Platelet-endothelial cell adhesion molecule 1	PECAM-1	–
Placental growth factor	PlGF	+
Tumour necrosis factor α	TNF-α	–
Vascular endothelial growth factor	VEGF	+

Patients and methods

Patients and study design

Thirty patients with active RA were recruited for this tofacitinib interventional study. Patient characteristics are presented in Table 2. Inclusion criteria included definitive diagnosis of RA according to the 2010 EULAR/ACR classification criteria for RA [34], moderate–high disease activity score (DAS28 >3.2) at baseline and clinical indication of targeted therapy. Patients were either naïve to any targeted therapies ($n = 16$) or initiated tofacitinib after stopping a biologic followed by an appropriate washout period ($n = 14$). 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 10 years.

Table 2. Patient characteristics

	Tofacitinib-treated patients
Number of patients (*n*)	26
Female:male ratio	23:3
Age (years), mean ± SD. (range)	51.9 ± 9.7 (27–69)
BMI (kg/m^2), mean ± SD. (range)	30.3 ± 7.4 (20.8–51.4)
Positive CV history, *n* (%)	6 (23.1)
Positive history of hypertension, *n* (%)	13 (50.0)
Positive history of diabetes mellitus, *n* (%)	2 (7.7)
Smoking (current), *n* (%)	7 (26.9)
Disease duration (years), mean ± SD. (range)	7.5 ± 4.8 (1–21)
RF positivity, *n* (%)	22 (84.6)
Anti-CCP positivity, *n* (%)	22 (84.6)
DAS28 (baseline), mean ± SD.	5.12 ± 0.82

CV: cardiovascular; DAS28: 28-joint DAS.

The 30 enrolled patients received either 5 mg or 10 mg tofacitinib twice daily in the two treatment arms. All patients received tofacitinib in combination with either MTX ($n = 23$) or LEF ($n = 7$). MTX and LEF had been taken in stable dose for at least 1 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 CS prior to the study, none of the patients had been on CS for at least 3 months prior to and during the study.

Clinical assessments were performed at baseline, and after 6 and 12 months of therapy. Four patients (two on each arm) completed the 6-month follow-up but did not complete the 1-year treatment. Twenty-six patients completed the 1-year treatment period and were included in the data analysis.

The study was approved by the Hungarian Scientific Research Council Ethical Committee (approval No. 56953-0/2015-EKL). Written informed consent was obtained from each patient and assessments were carried out according to the Declaration of Helsinki and its amendments.

Clinical assessment

First, a detailed medical history was taken. We inquired about history of CV disease 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 by a questionnaire (Table 2). Further clinical assessments including physical examination were performed at baseline, and after 3, 6 and 12 months of tofacitinib therapy.

Assessment of vascular physiology by US

US-based functional vascular assessments, such as brachial artery FMD [35], cfPWV [36] and ccIMT [37] measurements have been carried out at baseline and during the follow-up. Details of investigations were thoroughly described and published previously [24, 25]. In the present study, FMD, ccIMT and cfPWV data are only used in the correlation analysis.

^{18}F-fluorodeoxyglucose-PET/CT assessment

In our previous study, synovial and vascular inflammation was simultaneously assessed by PET/CT [38]. In brief, for the quantification of synovial and aortic inflammation, mean and maximum standardized uptake values (SUV) were determined in five predefined articular regions (hand/wrist, elbow, shoulder, hip and knee) on both sides, as well as in five predefined aortic segments (ascending aorta, aortic arch, descending thoracic aorta, suprarenal and infrarenal abdominal aorta), respectively. Mean and maximum synovial or aortic target-to-background ratios (TBR) were also calculated by dividing synovial SUV values by liver SUV values or aortic SUV values by superior vena cava SUV values, respectively [38]. In this study, synovial (TBR-SYN) and aortic PET/CT data (TBR-VASC) are only used in the correlation analysis.

Laboratory measurements and disease activity

ESR was determined by a standard procedure. Serum high sensitivity CRP (hsCRP; normal $\leq$5 mg/l) and IgM RF (normal $\leq$50 IU/ml) were measured by quantitative nephelometry (Cobas Mira Plus, Roche Diagnostics, Basel, Switzerland), using CRP and RF reagents (both Dialab Ltd, Budapest, Hungary). ACPA (CCP) autoantibodies were detected in serum samples using a second-generation Immunoscan-RA CCP2 ELISA test (Euro Diagnostica, Malmö, Sweden; normal $\leq$25 IU/ml). The assays were performed according to the manufacturer's instructions. Disease activity of RA was calculated as DAS28-CRP (three variables).

Angiogenic biomarkers

Serum concentrations of TNF-α (normal <136.3 pg/ml), IL-6 (normal <3.7 pg/ml), Ang1 (normal <5.36 ng/ml), Ang2 (normal <1.18 ng/ml), VEGF (normal <204.2 pg/ml), EGF (normal <149.6 pg/ml), bFGF (normal <556.4 pg/ml), PlGF (normal <389.1 pg/ml), CXCL8 (normal <120.1 pg/ml) and PECAM-1 (normal <26.9 ng/ml) were determined by flow cytometry using a bead-based multiplex assay (Human Angiogenesis Panel 1, 10-plex, LEGENDplex, BioLegend, San Diego, CA, USA) and analysed by LEGENDplex software. Normal values were determined by validation using normal human serum samples.

Serum CathK (ng/ml) concentrations were determined by commercially available ELISA kits (Human Cathepsin K ELISA Kit, Reagent Genie, AssayGenie, Dublin, Ireland) (range 0.156–10 ng/ml, sensitivity 0.094 ng/ml) with CV <8% intra-assay and CV <10% inter-assay variabilities by sandwich ELISA method.

Serum Gal-3 (ng/ml) levels were measured using commercially available ELISA kits (Human Galectin-3 ELISA Kit, ALPCO, Salem, NH, USA) (sensitivity 0.29 ng/ml) with CV <7.5% intra-assay and CV <5.4% inter-assay variabilities by sandwich ELISA method.

Serum NT-proBNP (pmol/l) concentrations were detected by commercially available ELISA kits (NT-proBNP ELISA, Biomedica, Vienna, Austria) (standard range 0–640 pmol/l or 0–5.424 pg/ml, sensitivity 3.0 pmol/l or 25.4 pg/ml) with CV $\leq$4% intra-assay and CV $\leq$7% inter-assay variabilities by sandwich ELISA method. Kit specificities showed median value of 5.8 pmol/l or median value of 49.1 pg/ml, with a range of 1.1–541.5 pmol/l in serum samples of healthy donors. The assays were performed according to the manufacturer's Instructions.

Statistical analysis

Statistical analysis was performed using SPSS version 26.0 (IBM, Armonk, NY, USA) software. Data are expressed as the mean ± S.D. for continuous variables and percentages for categorical variables. The distribution of continuous variables was evaluated by Kolmogorov–Smirnov test. 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 analysis using the stepwise method were applied to investigate independent associations between synovial and vascular pathology as determined by US and PET/CT parameters (dependent variables) and angiogenic biomarkers (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.

General linear model repeated measures analysis of variance (RM-ANOVA) was performed in order to determine the additional effects of angiogenic markers together with the effects of treatment on 12-month changes in vascular pathophysiology (dependent variable) as determined by US or PET/CT. Two-way RM-ANOVA analysis was also conducted in order to determine the additional effects of angiogenic marker

(independent variable) level changes between baseline and 12 months together with the effects of treatment on 12-month changes in vascular pathophysiology (dependent variable). In the RM-ANOVA and two-way RM-ANOVA analyses, partial η^2 is given as indicator of effect size, with values of 0.01 suggesting small, 0.06 medium and 0.14 large effects [39]. *P*-values <0.05 were considered significant.

The reliability of the vascular US measurements was tested by inter-item correlation and two-way, mixed, single-rater intraclass correlation as published before [25, 40, 41].

Results

Characteristics of patients

These data have been published before based on other studies emerging from the same tofacitinib cohort [38, 42]. In brief, eventually a total of four patients dropped out after 6 months of treatment but before the end of the study. Thus, 26 patients completed the study and were eligible for further data analysis [38, 42]. Characteristics of the 26 patients used for this study are included in Table 2. We confirm that the 6-month results of the four dropouts do not significantly differ from those of the 26 remaining patients (data not shown).

Effects of tofacitinib therapy on disease characteristics and vascular pathophysiology

As published before, during the 1-year observation period, CRP (from 5.05 ± 0.77–3.32 ± 1.12) and DAS28 (from 14.8 ± 14.9 mg/l to 7.4 ± 7.7 mg/l) significantly decreased after 12 months compared with baseline [38, 42]. ACPA- and/or RF-seropositive patients had significantly higher DAS28 (5.33 ± 0.67 *vs* 4.76 ± 0.88; $P = 0.002$) and CRP levels (19.7 ± 17.5 mg/l *vs* 12.5 ± 11.6 mg/l; $P = 0.023$) at baseline compared with seronegative patients. Regarding vascular function, in brief, FMD and cfPWV did not change, while ccIMT increased over time [42]. Here we only used these data in order to correlate the angiogenic biomarkers with them. Thus, none of the data presented below has been published.

Effects of tofacitinib therapy on circulating angiogenic biomarkers

Among the pro-inflammatory angiogenic cytokines, tofacitinib treatment significantly decreased IL-6 levels after 6 months (16.1 ± 9.3 pg/ml; $P = 0.001$) and 12 months (16.0 ± 9.1 pg/ml; $P = 0.001$) compared with baseline (41.4 ± 46.2 pg/ml) (Fig. 1A). On the other hand, TNF-α production did not change significantly over time (baseline: 10.1 ± 17.5 pg/ml; 6 months: 8.3 ± 13.7 pg/ml; 12 months: 11.7 ± 28.2 pg/ml; $P > 0.05$) (Fig. 1B).

With respect to growth factors, VEGF production significantly decreased after 6 months (318.1 ± 191.7 pg/ml; $P = 0.001$) and 12 months (326.0 ± 180.9 pg/ml; $P = 0.019$) compared with baseline (390.1 ± 247.5 pg/ml) (Fig. 1C). Other growth factors exerted different patterns. bFGF levels did not change after 6 months (878.7 ± 615.3 pg/ml; $P = 0.459$) but then significantly decreased after 12 months (847.8 ± 653.6 pg/ml; $P = 0.046$) compared with baseline (902.4 ± 645.0 pg/ml) (Fig. 1D). Similarly, EGF concentrations remained unchanged after 6 months (352.7 ± 104.7 pg/ml; $P = 0.469$) but decreased after 1 year (321.0 ± 115.4 pg/ml; $P = 0.038$) in comparison with baseline (338.1 ± 102.1 pg/ml) (Fig. 1E). Finally, PlGF concentrations remained similar after 6 months (38.4 ± 25.0 pg/ml; $P = 0.295$) but then significantly decreased after 12 months (34.1 ± 19.8 pg/ml; $P < 0.001$) compared with baseline (41.4 ± 23.1 pg/ml) (Fig. 1F). The levels of Ang1 (Fig. 1G) and Ang2 (Fig. 1H) included in the VEGF pathway only numerically, but not significantly, decreased over time ($P > 0.05$).

Levels of the proteolytic enzyme CathK significantly decreased after 6 months (62.4 ± 20.2 ng/ml; $P = 0.001$) and 12 months of treatment (64.6 ± 25.3 ng/ml; $P = 0.040$) compared with baseline (75.3 ± 20.7 ng/ml) (Fig. 1I). CXCL8/IL-8 chemokine (Fig. 1J), soluble PECAM-1 (Fig. 1K) and NT-proBNP (Fig. 1L) levels did not change significantly during the period of treatment ($P > 0.05$).

Interestingly, the levels of Gal-3 did not change significantly after 6 months (7.49 ± 3.87 ng/ml; $P = 0.069$) but then increased after 12 months (9.96 ± 4.27 ng/ml; $P = 0.03$) in comparison with baseline (8.96 ± 4.65 ng/ml) (Fig. 1M).

There were no major differences between the 5 mg and 10 mg twice daily tofacitinib-treated subsets (data not shown).

Association of angiogenic markers with serological status

We compared angiogenic biomarker levels in RF-positive *vs* -negative, as well as anti-CCP-positive *vs* -negative RA patients at baseline. bFGF, PlGF and NT-proBNP levels were higher, while soluble PECAM-1 levels were lower in RF-positive compared with RF-negative patients. In addition, PlGF levels were also higher in anti-CCP positive *vs* negative patients (Table 3). Other angiogenic biomarkers did not correlate with serological status. In addition, we did not find associations between serological status and angiogenic biomarkers at the 6- and 12-month time points.

Associations of angiogenic biomarkers with vascular pathophysiology

In the simple Pearson's correlation analysis, several correlations were found between angiogenic and vascular parameters (data not presented). Therefore, we performed uni- and multivariable regression analyses using vascular imaging (US, PET/CT) parameters as dependent, and angiogenic biomarkers as independent factors. Data are presented in Table 4. In the univariable regression analysis, among the US-based vascular parameters, only post-treatment ccIMT correlated with pre- and post-treatment NT-proBNP (6 months: $\beta = 0.462$, $P = 0.017$; 12 months: $\beta = 0.415$, $P = 0.035$). cfPWV and FMD were not associated with any analysed angiogenic biomarker (data not presented). With respect to PET/CT parameters, synovial inflammation indicated by $SUV\text{-}SYN_{mean}$ after 12 months of tofacitinib treatment correlated with post-treatment TNF-α ($\beta = 0.502$, $P = 0.029$). Baseline $TBR\text{-}SYN_{mean}$ and PECAM-1 also correlated with each other ($\beta = 0.528$, $P = 0.020$). Mean post-treatment synovial inflammation indicated by $TBR\text{-}SYN_{mean}$-12 were determined by baseline ($\beta = 0.527$, $P = 0.021$), 6-month ($\beta = 0.536$, $P = 0.018$) and 12-month TNF-α ($\beta = 0.633$, $P = 0.004$), baseline ($\beta = 0.528$, $P = 0.020$), 6-month ($\beta = 0.564$, $P = 0.012$) and 12-month bFGF ($\beta = 0.626$, $P = 0.004$), as well as baseline ($\beta = 0.536$, $P = 0.018$), 6-month ($\beta = 0.516$, $P = 0.024$) and 12-month PlGF levels ($\beta = 0.639$, $P = 0.003$). Mean baseline aortic inflammation indicated by $TBR\text{-}VASC_{mean}$-0 correlated with baseline IL-6 ($\beta = 0.588$,

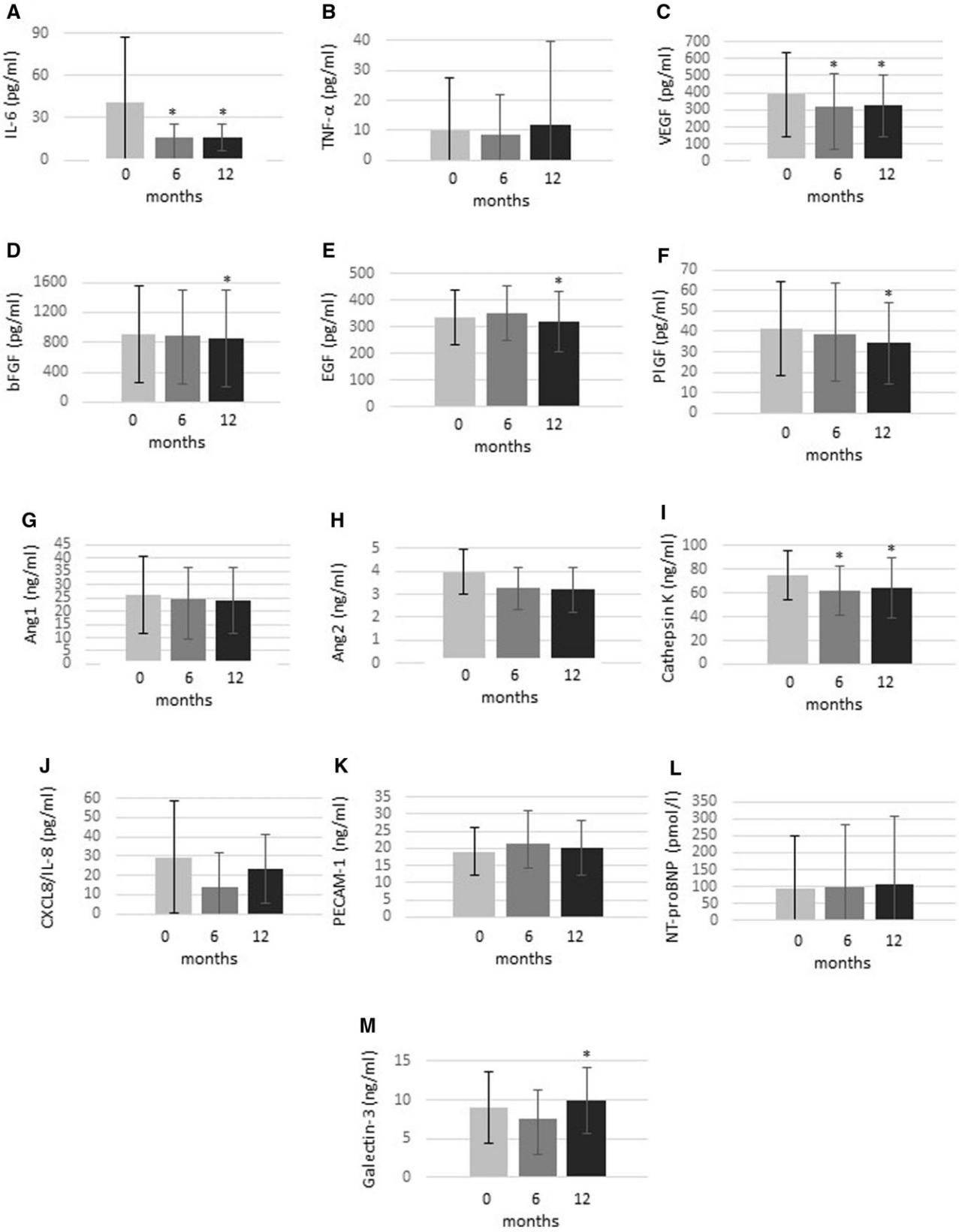

Figure 1. Angiogenic biomarker levels at baseline (0), and after 6 and 12 months of treatment. We measured IL-6 (**A**), TNF-α (**B**), VEGF (**C**), basic fibroblast growth factor (bFGF) (**D**), epidermal growth factor (EGF)l), placental growth factor (PlGF) (**F**), angiopoietin 1 (Ang1) (**G**), angiopoietin 2 (Ang2) (**H**), cathepsin K (**I**), CXC chemokine ligand 8 (CXCL8)/IL-8 (**J**), platelet-endothelial cell adhesion molecule 1 (PECAM-1) (**K**), NT-proBNP (**L**) and galectin-3 (**M**). Values are expressed as mean ± S.D. $^*P < 0.05$

$P = 0.008$). TBR-VASC$_{mean}$-12 was determined by baseline ($\beta = 0.754$, $P < 0.001$) and 12-month IL-6 ($\beta = 0.634$, $P = 0.004$), as well as baseline ($\beta = 0.466$, $P = 0.044$), 6-month ($\beta = 0.670$, $P = 0.002$) and 12-month PECAM-1 ($\beta = 0.657$, $P = 0.002$). Finally, maximum post-treatment vascular inflammation indicated by TBR-VASC$_{max}$-12 also correlated with baseline ($\beta = 0.564$, $P = 0.012$) and 12-month IL-6 ($\beta = 0.742$, $P = 0.039$), as well as 12-month PECAM-1 ($\beta = 0.456$, $P = 0.049$) (Table 4). We also performed a multivariable analysis of these data, but this analysis did not show that multiple factors together would influence the model in any way (data not shown).

RM-ANOVA and two-way RM-ANOVA analyses were performed in order to determine the effects of tofacitinib treatment in combination with angiogenic biomarker levels on changes in vascular parameters over time, as well as the effects of 0–12 month changes in angiogenic biomarkers together with treatment on changes in vascular parameters over time, respectively (Table 5). When performing the RM-ANOVA analysis, baseline CXCL8 together with treatment determined changes in ccIMT from baseline to 12 months ($P = 0.02$). Baseline IL-6 levels and treatment determined aortic inflammation by PET/CT. Both mean and maximum TBR-VASC were dependent on baseline IL-6 ($P = 0.012$ and $P = 0.040$, respectively). Synovial inflammation as determined by PET/CT was associated with baseline VEGF ($P = 0.017$) and Gal-3 ($P = 0.019$) (Table 5).

With respect to two-way RM-ANOVA, US and PET-CT imaging parameters were dependent, while angiogenic biomarkers were independent variables. Changes of CathK and ccIMT over time correlated with each other ($P = 0.041$). When correlating angiogenic biomarkers and PET/CT parameters, CathK ($P = 0.002$), IL-6 ($P = 0.003$) and VEGF ($P = 0.003$) changes over time together with treatment determined TBR-VASC$_{max}$ changes between baseline and 12 months (0–12). Regarding synovial inflammation, VEGF changes over time and treatment determined 0–12 changes of SUV-SYN$_{mean}$ ($P = 0.022$) and TBR-SYN$_{mean}$ ($P = 0.003$). Similarly, 0–12 CathK ($P = 0.027$), IL-6 ($P = 0.017$) and Gal-3 ($P = 0.045$) changes together with treatment also correlated with 0–12 changes of TBR-SYN$_{mean}$. Finally, 0–12 months changes in PlGF together with treatment determined TBR-SYN$_{max}$ changes over time ($P = 0.046$) (Table 5). Changes in disease activity did not correlate with changes in any biomarkers overtime (data not shown).

Table 3. Effects of serological status on angiogenic biomarkers at baseline

	RF positive	RF negative	*P*
bFGF (pg/ml)	1004.79 ± 668.79	472.55 ± 278.96	0.034
PlGF (pg/ml)	51.54 ± 30.45	19.48 ± 4.87	0.001
PECAM-1 (ng/ml)	17.65 ± 6.30	25.43 ± 6.85	0.041
NT-proBNP (pmol/l)	112.25 ± 169.68	7.86 ± 8.47	0.015
	Anti-CCP positive	Anti-CCP negative	*P*
PlGF (pg/ml)	51.47 ± 31.45	25.05 ± 12.39	0.011

bFGF: basic fibroblast growth factor; PECAM-1: platelet-endothelial cell adhesion molecule 1; PlGF: placental growth factor; NT-proBNP: N-terminal prohormone brain natriuretic peptide.

Table 4. Univariable analysis of significant associations between angiogenic biomarkers and vascular pathology

Dependent variable	Independent variable	Univariable analysis			
		β	*P*	B	95% CI
ccIMT-6	NTproBNP-0	0.462	0.017	0	0–0.001
ccIMT-12	NTproBNP-12	0.415	0.035	0	0–0.001
SUV-SYN$_{mean}$-12	TNFα-12	0.502	0.029	0.048	0.006–0.091
TBR-SYN$_{mean}$-0	PECAM1-0	0.528	0.020	0	
TBR-SYN$_{mean}$-12	TNFα-0	0.527	0.021	0.006	0.001–0.011
	TNFα-6	0.536	0.018	0.007	0.001–0.013
	TNFα-12	0.633	0.004	0.27	0.01–0.044
	bFGF-0	0.528	0.020	0	
	bFGF-6	0.564	0.012	0	
	bFGF-12	0.626	0.004	0	
	PlGF-0	0.536	0.018	0.004	0.001–0.006
	PlGF-6	0.516	0.024	0.003	0–0.006
	PlGF-12	0.639	0.003	0.005	0.002–0.008
TBR-VASC$_{mean}$-0	IL6-0	0.588	0.008	0.004	0.001–0.007
TBR-VASC$_{mean}$-12	IL6-0	0.754	<0.001	0.003	0.002–0.005
	IL6-12	0.634	0.004	0.022	0.008–0.035
	PECAM1-0	0.466	0.044	0	
	PECAM1-6	0.670	0.002	0	
	PECAM1-12	0.657	0.002	0	
TBR-VASC$_{max}$-12	IL6-0	0.564	0.012	0.004	0.001–0.006
	IL6-12	0.742	0.039	<0.001	0.021–0.056
	PECAM1-12	0.456	0.049	0	

The numbers 0, 6 and 12 represent baseline, 6-month and 12-month results. bFGF: basic fibroblast growth factor; ccIMT: common carotid intima-media thickness; NT-proBNP: N-terminal pro-brain natriuretic protein; PECAM-1: platelet-endothelial cell adhesion molecule 1; PlGF: placental growth factor; SUV-SYN$_{mean}$: mean synovial standard uptake value; TBR-SYN$_{mean}$: mean synovial target-to-background ratio; TBR-VASC$_{max}$: maximum aortic target-to-background ratio; TBR-VASC$_{mean}$: mean aortic target-to-background ratio.

Table 5. RM-ANOVA and two-way RM-ANOVA results of the effects of angiogenic biomarkers at baseline and across three time points during treatment on vascular pathology

Dependent variable	Effect	*F*	*P*	Partial η^2
RM-ANOVA				
ccIMT (0–12)	CXCL8-0	4.673	0.020	0.289
TBR-VASC$_{mean}$ (0–12)	IL-6-0	7.727	0.012	0.312
TBR-VASC$_{max}$ (0–12)	IL-6-0	4.220	0.040	0.199
TBR-SYN$_{mean}$ (0–12)	VEGF-0	5.313	0.017	0.399
	Gal-3-0	5.160	0.019	0.392
Two-way RM-ANOVA				
ccIMT (0–12)	CathK (0-12)	4.224	0.041	0.150
TBR-VASC$_{max}$ (0–12)	CathK (0-12)	9.273	0.002	0.322
	IL-6 (0-12)	8.490	0.003	0.500
	VEGF (0-12)	8.123	0.003	0.489
SUV-SYN$_{mean}$ (0–12)	VEGF (0-12)	6.333	0.022	0.271
TBR-SYN$_{mean}$ (0–12)	CathK (0-12)	4.513	0.027	0.347
	IL-6 (0-12)	5.261	0.017	0.382
	VEGF (0-12)	11.683	0.003	0.407
	Gal-3 (0-12)	4.670	0.045	0.216
TBR-SYN$_{max}$ (0–12)	PlGF (0-12)	4.636	0.046	0.214

The numbers 0 and 12 represent baseline and 12-month results. CathK: cathepsin K; Gal-3: galectin-3; ccIMT: common carotid intima-media thickness; CXCL: CXC chemokine ligand; PlGF: placental growth factor; RM-ANOVA: repeated measures analysis of variance; SUV-SYN$_{mean}$: mean synovial standard uptake value; TBR-SYN$_{mean}$: mean synovial target-to-background ratio; TBR-SYN$_{max}$: maximum synovial target-to-background ratio; TBR-VASC$_{mean}$: mean aortic target-to-background ratio; TBR-VASC$_{max}$: maximum aortic target-to-background ratio.

Discussion

To our best knowledge, this is the first 1-year, prospective study assessing various angiogenic biomarkers in relation to clinical parameters and US- and PET/CT-determined vascular pathology function in RA patients undergoing tofacitinib therapy.

Previous studies suggested that efficient disease control by biologics (bDMARDs) might also suppress the production of angiogenic biomarkers studied in the present project, as well as other biomarkers. Both anti-TNF bDMARDs and bDMARDs of different mode of action might suppress angiogenesis and the production of angiogenic markers in arthritis [5, 22, 43].

Tofacitinib treatment significantly decreased IL-6, VEGF, bFGF, EGF, PlGF and CathK, while it increased Gal-3 production. IL-6, growth factors and CathK are pro-inflammatory mediators also associated with angiogenesis [7, 21, 22, 28]. IL-6 signals through JAK1 and JAK2, so it is evident that tofacitinib suppresses IL-6 production [1, 44]. On the other hand, TNF-α signalling does not involve JAK-STAT pathways [1, 44]. Indeed, in our study, tofacitinib did not alter the level of TNF-α. Among growth factors, VEGF might signal through JAK2-STAT5 [45]. The release of pro-inflammatory growth factors and CathK involves multiple cytokines that are associated with JAK-STAT signalling [1, 44]. Thus, tofacitinib might suppress the production of these angiogenic mediators either directly via JAK inhibition or indirectly. The mechanism by which tofacitinib might increase Gal-3 production is unknown. Pharmacologic JAK2 blockade resulted in the inhibition of Gal-3-induced pro-inflammatory pathways [46]. As tofacitinib is mainly a JAK1 and JAK3 inhibitor, theoretically, the blockade of these JAK isoforms might induce counter-regulatory mechanisms resulting in increased Gal-3 production.

With respect to potential mechanisms, among the above-mentioned angiogenic factors, all growth factors, as well as IL-6 and Gal-3, signal through JAK/STAT pathways. The other mediators interact with JAK-dependent cytokines and thus might indirectly influence JAK-dependent angiogenesis [1, 44]. Cytokine-dependent angiogenesis might also be different in microvessels and larger vessels [47].

bFGF and NT-proBNP were associated with RF seropositivity, PlGF with RF and anti-CCP seropositivity, while PECAM-1 was correlated with RF seronegativity. Seropositive RA has been considered as more inflammatory and progressive than seronegative RA [48]. In addition, angiogenesis might differ between seropositive and seronegative arthritides [49, 50]. In another RA cohort, we also demonstrated higher BNP levels in seropositive patients [50]. Thus, some angiogenic biomarkers might be associated with RF and/or anti-CCP seropositivity in RA.

^{18}F-fluorodeoxyglucose-PET/CT is able to simultaneously detect joint (synovial) and vascular (aortic) inflammation in RA patients [38]. We have shown previously that 1-year tofacitinib therapy was able to dampen synovial and vascular inflammation as detected by PET/CT [38]. Here we showed that TNF-α, bFGF and PlGF at different time points correlated with post-treatment synovial inflammation detected by PET/CT. On the other hand, post-treatment aortic wall inflammation was dependent on IL-6 and PECAM-1 at various time points. Thus, it seems that different angiogenic biomarkers are involved in synovial and vascular inflammation as demonstrated by PET/CT. Synovial inflammation might be mostly TNF-α- and growth-factor-dependent, while vascular inflammation rather involves IL-6- and PECAM-1-mediated mechanisms. Indeed, TNF-α, bFGF and PlGF have been implicated in synovial inflammation and angiogenesis in RA [4, 5, 7, 21, 28, 29]. IL-6 is involved in both angiogenesis and atherosclerosis [4, 8, 29]. PECAM-1 mediates homotypic endothelial adhesion and angiogenesis in RA [7, 51]. The two-way RM-ANOVA analysis also revealed that VEGF, IL-6, CathK, PlGF and Gal-3 might support tofacitinib therapy in dampening synovial inflammation over time as monitored by PET/CT. Both growth factors and Gal-3 exert pro-inflammatory and pro-angiogenic actions [3, 5, 13, 15, 45, 46, 49].

With respect to the correlation analyses, among the angiogenic biomarkers, only NT-proBNP correlated with carotid atherosclerosis as indicated by ccIMT at baseline and after

12 months. When combined with the effects on 1-year tofacitinib therapy, also baseline CXCL8 (IL-8) and the 0–12 months change in CathK correlated with 1-year changes in ccIMT. None of the studied angiogenic factors showed any correlations with endothelial dysfunction (FMD) or arterial stiffness (cfPWV). Thus, NT-proBNP, CXCL8 and CathK might be associated with the development of carotid atherosclerosis. In addition, the determination of CXCL8 and CathK levels might reflect the influence of tofacitinib treatment on ccIMT. In the very same cohort, we demonstrated that 1-year tofacitinib treatment was unable to affect ccIMT progression over time [42]. It is likely that this lack of efficacy might depend on various pro-inflammatory and pro-angiogenic mediators.

This study has certain strengths and limitations. It is a complex follow-up study assessing a number of angiogenic biomarkers together with previous findings of clinical efficacy and vascular pathophysiology. To our best knowledge this is the first time that the effects of JAK targeting on angiogenic biomarkers, PET/CT- and US-based vascular pathophysiology assessments have been analysed in the same study. As a limitation, the relatively small sample size and the lack of a comparison group should be mentioned. Patients with positive CV history could also be included, however, the CV status of these patients was well-controlled.

Conclusions

During 1-year tofacitinib therapy the production of IL-6 with VEGF, bFGF, EGF, PlGF and CathK decreased, TNF-α, CXCL-8/IL-8, PECAM-1 and NT-proBNP did not change and Gal-3 level increased. Our PET/CT study suggests that TNF-α, bFGF and PlGF are involved in synovial inflammation while vascular inflammation mediated by IL-6 and PECAM-1. Both inflammatory processes might be inhibited by tofacitinib. Although NT-proBNP, CXCL-8/IL-8 and CathK might be associated with ccIMT, their role in RA-associated atherosclerosis needs to be further evaluated. Thus, tofacitinib therapy might be useful not only to dampen inflammation and disease activity in RA patients, but also to suppress angiogenesis and the production of angiogenic biomarkers.

Data availability

Original data are available upon request to the corresponding author.

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