

Thrombotic Markers in Plasma as Predictors of Response in Rheumatoid Arthritis Patients Treated with Baricitinib – Pilot Observation

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Abstract

Both disease and treatment carry the risk of thrombotic events in patients with rheumatoid arthritis (RA). This pilot study aimed to assess changes in thrombotic markers in plasma and their potential role as predictors of response during early baricitinib treatment. The concentrations of antithrombin III (ATIII) activity, D-dimer (DD), fibrinogen, and homocysteine (HCY) were evaluated in RA subjects before and 3 months after the treatment. At baseline, the RA group had higher DD (1472.3 ± 349.2) and fibrinogen (410.4 ± 29.5) compared with healthy controls (HC; 450.3 ± 54.5 ; $p = 0.0002$ and 334.9 ± 19.2 ; $p = 0.04$, respectively). with no differences in ATIII and HCY. After 3 months, we observed a significant increase in HCY (10.7 ± 0.6 vs. 9.1 ± 0.5 ; $p = 0.018$) and ATIII (119.7 ± 2.7 vs. 110.4 ± 3.2 ; $p = 0.004$), the latter correlated negatively with disease activity score 28 (DAS28; $r = -0.686$, $p < 0.002$). After 3 months of baricitinib therapy, the patients were divided into moderate responders (MR) and good responders (GR) groups according to EULAR criteria. At baseline, MR had higher DD (1639.2 ± 550.5 vs. 450.3 ± 54.5 ; $p < 0.0001$) and lower ATIII (105.3 ± 3.6 vs. 115.1 ± 2.7 ; $p = 0.043$) compared with HC. Thrombotic parameters in the first 3 months of baricitinib treatment were mostly in line with current findings concerning the RA population. Increased levels of DD together with low ATIII concentrations seem to predispose to a moderate response to baricitinib treatment.

Keywords

Antithrombin III • Cardiometabolic risk biomarkers • Fibrinogen • Homocystein • Rheumatoid arthritis • Baricitinib

Received: 12 September 2024 / Accepted: 28 January 2025/

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Abbreviations

ACPA, anti-citrullinated peptide antibodies; ALT, alanine aminotransferase; anti-TNF, Anti-Tumor Necrosis Factor; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; ATIII, antithrombin III; BARI, Baricitinib; cDMARD, conventional disease-modifying antirheumatic drug; CI, Confidence Interval; COVID-19, coronavirus disease 2019; CRP, c-reactive protein; DAS28, disease activity score 28; DD, D-dimer; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; GCs, Glucocorticoids; GR, good responders; HC, healthy controls; HCY, homocysteine; INR, international normalized ratio; JAKi, Janus kinase inhibitor; MACE, major adverse cardiovascular events; miRNA-19b, MicroRNA-19b; MR, moderate responders; NIGRiR, National Institute of Rheumatology, Rehabilitation, and Geriatrics; PT, prothrombin time; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, Standard Deviation; TT, thrombin time; VTE, venous thromboembolism.

1. Introduction

Patients with rheumatoid arthritis (RA) are at increased risk of venous thromboembolism (VTE) compared with the general population. The risk of VTE in RA has been shown to correlate with disease activity but is still increased, even if remission is achieved (Molander et al. 2021). There are many potential reasons responsible for that situation, including the overrepresentation of traditional risk factors, increased cytokine production, post-translational modifications derived from autoantibodies, abnormal plasma composition, continuous endothelial activation, and drug-induced reactions (Wallberg-Jonsson et al. 1997; Undas et al. 2010; Tilwawala et al. 2018; Türk et al. 2018; England et al. 2021; Molander et al. 2021; Westerlind et al. 2023).

Novel treatment options for RA include Janus kinase inhibitors (JAKi) – the group of drugs targeting one or more JAK receptors. The inhibition of the JAK signaling pathway downregulates some cytokine expression (Kotyla et al. 2020). Although JAKi have been shown to be an effective treatment for RA, there are reports that their use may increase the risk of VTE, with baricitinib (inhibitor of JAK1 and JAK2) carrying the highest risk among them (Xie et al. 2019; Kotyla et al. 2020).

JAK pathway is related to thrombosis by the regulation of platelet function, p-selectin expression, and the

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maintenance of vascular wall hemostasis (Kotyla et al. 2021). Inhibition of one JAK pathway and subsequent preference of another may cause an imbalance in its pro- and anti-thrombotic activity (Kotyla et al. 2020, 2021). It was shown that JAK pathway activation in endothelial cells is followed by increased production of prothrombotic factors (Ravid et al. 2022). These facts might result in additional growth of the VTE risk in RA patients. On the contrary, some anti-thrombotic properties of JAKi have also been noted. JAK inhibition was proved to downregulate the release of such thrombosis triggers as tissue factor expression, activity of urokinase plasminogen activator, and the expression of immunostimulatory molecules (CD80, CD86, CD83, CD40) in M1-like as well as M2-like macrophages, thus influencing their polarization (Beckman et al. 2023; Lethen et al. 2024). Furthermore, *in vitro*, anti-adhesive properties of JAKi have been demonstrated (Beckman et al. 2023). In our previous paper, we also demonstrated that circulating miRNA-19b was identified as a biomarker of disease progression and treatment response to baricitinib in RA patients (Ciechomska et al. 2023).

Thus, there is an urgent need to investigate thrombotic parameters in RA patients treated with JAKi. Antithrombin III (ATIII), homocysteine (HCY), and fibrinogen are important markers in thrombotic risk assessment in clinical practice.

ATIII is produced in endothelial cells of hepatic vessels (Meng et al. 2011). It acts as an inhibitor of thrombin and factor Xa, and its deficiency has been investigated as an early indicator for both venous and arterial thromboses (Thompson et al. 1996; Sinkiewicz et al. 2006; Meng et al. 2011; Di Minno et al. 2014; Chen-Goodspeed et al. 2023). There are studies indicating higher ATIII concentrations and activity in RA patients compared with healthy subjects; however, the exact correlation between ATIII and VTE risk in RA has not been established (Belch et al. 1984; Jones et al. 1998).

HCY is a substrate in methionine regeneration. A high concentration of HCY was observed in patients with RA (Balkarli et al. 2016). Further reports showed a strong correlation between HCY concentration and RA activity (Jensen et al. 2002; Rho et al. 2009; Katsushima et al. 2023; Popescu et al. 2023). With hyperhomocysteinemia being a known independent risk factor for atherosclerotic cardiovascular disease, it has been discussed as the potential reason for increased thrombotic risk in RA patients (Habib et al. 2023; Popescu et al. 2023). The effect of HCY on the risk of VTE in RA is also influenced by RA treatment. Methionine regeneration is downregulated by methotrexate activity (Brown et al. 2016), on the contrary, folic acid supplementation results in the reduction of HCY levels.

Fibrinogen serves as a substrate for fibrin in the blood clot formation process (Göbel et al. 2018). Hyperfibrinogenemia observed in RA affects the qualitative characteristics of blood clots and implies higher thrombotic risk (Bezuidenhout et al. 2020; Peshkova et al. 2020). Correlation with disease

activity was noticed, and the concentration was above normal levels even in subjects with well-controlled RA fibrinogen (McEntegart et al. 2001; Rooney et al. 2011). However, patients with RA in whom antibodies against post-translational fibrinogen-derived antigens were detected did not have a higher risk of VTE (Westerlind et al. 2023).

Given the complex relationship between RA activity, VTE risk, and the potential effect of baricitinib on VTE risk, we decided to conduct a study to assess the potential role of thrombotic markers as predictors of response to baricitinib treatment.

2. Materials and Methods

2.1. Study population

This observational prospective study took place at the National Institute of Rheumatology, Rehabilitation, and Geriatrics (NIGRI) in Warsaw, Poland. Patients were recruited between May 2021 and February 2022. Inclusion criteria were: the diagnosis of RA according to the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) criteria (Aletaha et al. 2010), inefficacy of at least one conventional disease-modifying antirheumatic drug (cDMARD), high disease activity (disease activity score 28-erythrocyte sedimentation rate [DAS28-ESR] >5.1), and planned baricitinib therapy in 4 mg daily dosing regimen.

Exclusion criteria were: therapy cessation before 3 months, a history of arterial or venous thrombosis, increased aminotransferases activity (aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3 x upper laboratory norm), current (<3 months) use of any anticoagulant or antithrombotic drug, surgical procedures <3 months, and pregnancy.

Patients were followed up to 24 months to report baricitinib retention and adverse events of special interest: VTE, major adverse cardiovascular events (MACE), and neoplasms.

Clinical data regarding demographics, disease duration, concomitant treatment, comorbidities, and serological status (levels of rheumatoid factor [RF] and anti-citrullinated peptide antibodies [ACPA]) were collected from electronic medical records.

Peripheral blood samples were obtained before baricitinib introduction and after 3 months of therapy. All thrombotic parameters were investigated in citrated plasma: D-dimers (DD), fibrinogen, HCY, activated partial thromboplastin time (APTT), thrombin time (TT), ATIII activity, prothrombin time (PT), and International Normalized Ratio (INR). ATIII activity, HCY, fibrinogen, APTT, PT, and TT were assessed using turbidimetric methods, and DD was assessed using the immunoturbidimetric method performed on ACL TOP 350 CTS analyzer (Werfen, Germany).

Additionally, samples of peripheral blood were obtained from 20 healthy volunteers (without RA not meeting the exclusion criteria), further termed "healthy controls" (HC).

Simultaneously with blood sampling, medical examination, interview, and disease activity assessment were performed. Disease activity was assessed using the DAS28-ESR disease activity score. Patients were termed according to EULAR response criteria (Fransen and Van Riel 2009): “good responders” (GR) (DAS28 at month 3 ≤ 3.2 and decrease by >1.2) and “moderate responders” (MR) (DAS28 at month 3 ≤ 5.1 and decrease by >0.6 and ≤ 1.2 or DAS28 at month 3 >5.1 and decrease by >1.2).

The main outcomes were: ATIII activity, DD, fibrinogen, and HCY concentrations before and 3 months after baricitinib introduction.

As secondary outcomes, we aimed to assess the potential predictors of treatment response from the above thrombotic parameters, based on clinical data and laboratory results.

2.2. Statistics

Main statistical analysis was done using Prism 4.0 (GraphPad, La Jolla, USA) software, and multivariate analysis was done using Statistica 13.3 software (StatSoft Polska, Cracow, Poland). The normality of all investigated parameters was investigated using the Shapiro–Wilk test. Comparison of results from the same patients was analyzed either by paired *t*-test or Wilcoxon test, depending on whether the normality assumption was met or not met, respectively. The differences between different groups of patients were determined using the parametric *t*-test or non-parametric Mann–Whitney test, and for categorical variables the Fisher’s exact test. Correlations between variables meeting the criteria for parametric tests were analyzed using Pearson’s correlation test, while those not meeting – by Spearman test. The significance of the results after adjusting for confounding factors was checked by linear regression. Logistic regression and odds ratio with 95% CI were used to identify predictive factors associated with good clinical response. Data are shown as mean with standard error of the mean (SEM). For all tests, a value of $p < 0.05$ was considered significant.

2.3. Ethics

The study complied with the ethical standards in accordance with the Helsinki Declaration, as well as national regulations in the field. The study was approved by the Ethics Committee of NIGRI (Approval No. KBT-6/5/2020 dated 22.12.2020). All patients gave their written consent to participate in the study.

3. Results

3.1. Clinical data

Eighteen RA patients were eligible for the study and no patient met the exclusion criteria. Patients in the RA group were

predominantly women, characterized by a long history of arthritis (mean disease duration 10.14 ± 1.5 years) and the presence of poor prognostic factors: high disease activity (DAS28-ESR: mean 5.8, SEM 0.2), or high antibody positivity (RF or ACPA ≥ 3 upper laboratory norm, $n = 16$). The detailed characteristics of RA patients and HC are shown in Table 1. Compared with HC, there were statistically more women (94% vs. 60%, $p = 0.02$) and people with dyslipidemia (72% vs. 30%, $p = 0.02$) among RA patients.

Table 1. Demographic and clinical characteristics between RA patients and HC

Characteristic	RA patients, (n = 18)	HC (n = 20)	Difference, p-value
Demographics			
Sex (female), n (%)	17 (94.4)	12 (60)	$p = 0.02$
Age (years), mean (\pm SD)	51.9 (± 3.0)	44.0 (± 3.2)	ns
BMI (kg/m ²), mean (\pm SD)	24.8 (± 3.8)	25.9 (± 3.5)	ns
CV risk factors			
Smoking ^a , n (%)	4 (22.2)	3 (15)	ns
Dyslipidemia ^b , n (%)	13 (72.2)	6 (30)	$p = 0.02$
Arterial hypertension, n (%)	5 (27.8)	5 (25)	ns
Diabetes mellitus, n (%)	1 (5.6)	0 (0)	ns
Hormonal contraception, n (%)	1 (5.6)	1 (5)	ns
RA status			
Disease duration (years), mean (\pm SD)	10.14 (± 1.5)		
ACPA (U/mL), mean (\pm SD)	312.6 (± 55.0)		
RF (IU/mL), mean (\pm SD)	160.2 (± 46.3)		
RA treatment			
Previous biologic therapy, n (%)	3 (16.7)		
GCS, n (%) dose (mg ^c), mean (\pm SD)	11 (61.1) 3.1 \pm 0.6		
cDMARDs, n (%)	14 (77.8)		
Methotrexate, n (%)	13 (72.2)		
Sulfasalazine, n (%)	5 (27.8)		
Hydroxychloroquine, n (%)	5 (27.8)		
Disease activity			
DAS28-ESR, mean (\pm SD)	5.8 \pm 0.2		
VAS pain (mm), mean (\pm SD)	75.9 \pm 2.7		
Joints (painful), mean (\pm SD)	13.1 \pm 1.4		
Joints (tender), mean (\pm SD)	7.3 \pm 1.3		
CRP (mg/L), mean (\pm SD)	22.9 \pm 7.6		
ESR (mm/h), mean (\pm SD)	26.4 \pm 5.6		

^aSmoker status: current or longtime past smoking history.

^bDyslipidemia: hypertriglyceridemia and/or hyper-LDL or normal lipid profile with current statin therapy.

^cDose in equivalent of prednisone.

ACPA, anti-citrullinated peptide antibodies; BMI, body mass index; cDMARDs, conventional disease-modifying antirheumatic drugs; CRP, C-reactive protein; CV, cardiovascular; DAS28-ESR, disease activity score 28-erythrocyte sedimentation rate; HC, healthy controls; LDL, low density lipoprotein; ns, non-significant; RA, rheumatoid arthritis; RF, rheumatoid factor; VAS, visual analog scale.

After 3 months of baricitinib therapy, a significant decrease in DAS28 (3.6 ± 0.2 vs. 5.82 ± 0.2 ; $p < 0.001$) and C-reactive protein (CRP; 8.2 ± 3.6 vs. 22.9 ± 7.6 ; $p = 0.003$) has been observed statistically. The response to treatment was classified as good in 8 patients and moderate in 10 patients. During 24 months of follow-up, five patients switched to tocilizumab due to secondary ineffectiveness. All switchers presented a moderate response at month 3. Among adverse events of special interest, we observed one non-melanoma skin cancer and one MACE (acute coronary syndrome 6 weeks after baricitinib discontinuation and 3 weeks after COVID-19 infection). No VTE was reported.

3.2. Analysis of thrombotic parameters

We investigated eight parameters connected with thrombosis risk and analyzed correlations between them and clinical criteria important for RA.

At baseline, the concentrations of DD and fibrinogen in the RA group were higher compared with HC (1472.3 ± 349.2 vs. 450.3 ± 54.5 ; $p < 0.001$ and 410.4 ± 29.5 vs. 334.9 ± 19.2 ; $p = 0.04$, respectively) (Figure 1a). After taking into account differences between groups, the difference in DD concentrations remained statistically significant, whereas fibrinogen concentrations did not. After 3 months of treatment, fibrinogen concentration in RA patients decreased to the level comparable to HC, while DD level remained elevated (although not after taking into account differences between groups). Baseline HCY and ATIII activity in RA patients were similar to HC, but after 3 months of treatment, both concentrations significantly increased (10.7 ± 0.6 vs. 9.1 ± 0.5 ; $p = 0.02$ and 119.7 ± 2.7 vs. 110.4 ± 3.2 ; $p = 0.004$ for HCY and ATIII, respectively) (Figures 1c and 1d). Also, HCY concentration after the treatment became significantly higher than in HC ($p = 0.005$).

Among other coagulation parameters (TT, APTT, PT, INR), the TT level before treatment was significantly lower (although only in univariate analysis) than in HC (11.1 ± 0.3 vs. 12.3 ± 0.3 ; $p = 0.006$; Figure 1e), but after treatment this difference was no longer statistically significant.

Parameters connected with thrombosis risk correlated significantly with some RA disease activity factors (Table S1 in Supplementary Materials). Fibrinogen level was found to correlate positively with CRP (though only at the baseline; $r = 0.721$, $p = 0.001$) and ESR ($r = 0.800$, $p = < 0.001$ and $r = 0.483$, $p = 0.042$ before and after the treatment, respectively). DD level correlated positively (and also only at the baseline) with DAS28 ($r = 0.526$, $p = 0.025$), CRP ($r = 0.650$, $p = 0.003$), and ESR ($r = 0.642$, $p = 0.004$).

ATIII activity correlated (both before and after the treatment) negatively with DAS28 ($r = -0.696$, $p = 0.003$; $r = -0.686$, $p = 0.002$), CRP ($r = -0.599$, $p = 0.013$; $r = -0.598$, $p = 0.009$), and ESR ($r = -0.572$, $p = 0.018$; $r = -0.607$, $p = 0.008$).

3.3. Analysis of the relationship between thrombotic parameters and treatment response

In the next step, we divided our patients into two groups: GR and MR for baricitinib treatment per the methods described in Materials and Methods section. The full characteristics of both groups are presented in Table S2 in Supplementary Materials. There were no statistically significant differences in clinical characteristics between GR and MR. A comparison between GR and MR in thrombotic parameters is shown in Figure 2.

The concentrations of DD at baseline were significantly higher in the MR group compared with HC (1639 ± 550.5 vs. 450.3 ± 54.5 ; $p < 0.0001$, Figure 2a), in contrast to the GR group which had the same level of DD as HC. Also, MR had at baseline a significantly lower level of ATIII (105.3 ± 3.6 vs. 115.1 ± 2.7 ; $p = 0.043$) compared with HC (Figure 2d, Table 2), in contrast to GR where no difference vs. HC in ATIII activity at baseline was observed (Figures 2d). No differences between GR and MR were observed in terms of fibrinogen or HCY concentrations (Figure 2b and 2c). Analysis of correlation and p -value of the few basic clinical parameters (CRP and ESR) with two basic thrombotic parameters (DD and ATIII) are shown as a heatmap (Figure 3). After multivariate analysis to exclude confounding factors, the differences in both DD and ATIII concentrations between MR and HC were no longer statistically significant. Finally, to identify the risk factors for MR, logistic regression was performed, but none of the factors studied predicted response to treatment.

4. Discussion

In this pioneering prospective non-intervention study, we assessed several thrombotic parameters in the early period of RA treatment with baricitinib. We observed significantly higher concentrations of baseline DD and fibrinogen in RA patients compared with HC, but not ATIII activity (Figure 1). At month 3, a significant increase in HCY level and ATIII activity was reported. All patients responded to therapy, although not all to the same extent. Interestingly, in MR but not in GR, we observed at baseline a significantly higher level of DD (1639 ± 550.5 vs. 450.3 ± 54.5 ; $p < 0.001$) together with a significantly lower level of ATIII (105.3 ± 3.6 vs. 115.1 ± 2.7 ; $p = 0.043$) as compared with HC (Figures 2a and 2d).

ATIII activity in RA patients has been the subject of previous studies, but their results are not conclusive. Some studies have described increased ATIII levels in RA patients (Jones et al. 1998), or within the norm but increased relative to HC (Belch et al. 1984), but not all (Seriolo et al. 1999; Undas et al. 2010). Studies have also been conducted to examine not only levels but also whether RA affects ATIII dysfunction. They have found no evidence for this (Ordóñez et al. 2010; Tilwala et al. 2018). High levels of citrullinated ATIII were observed in RA but without

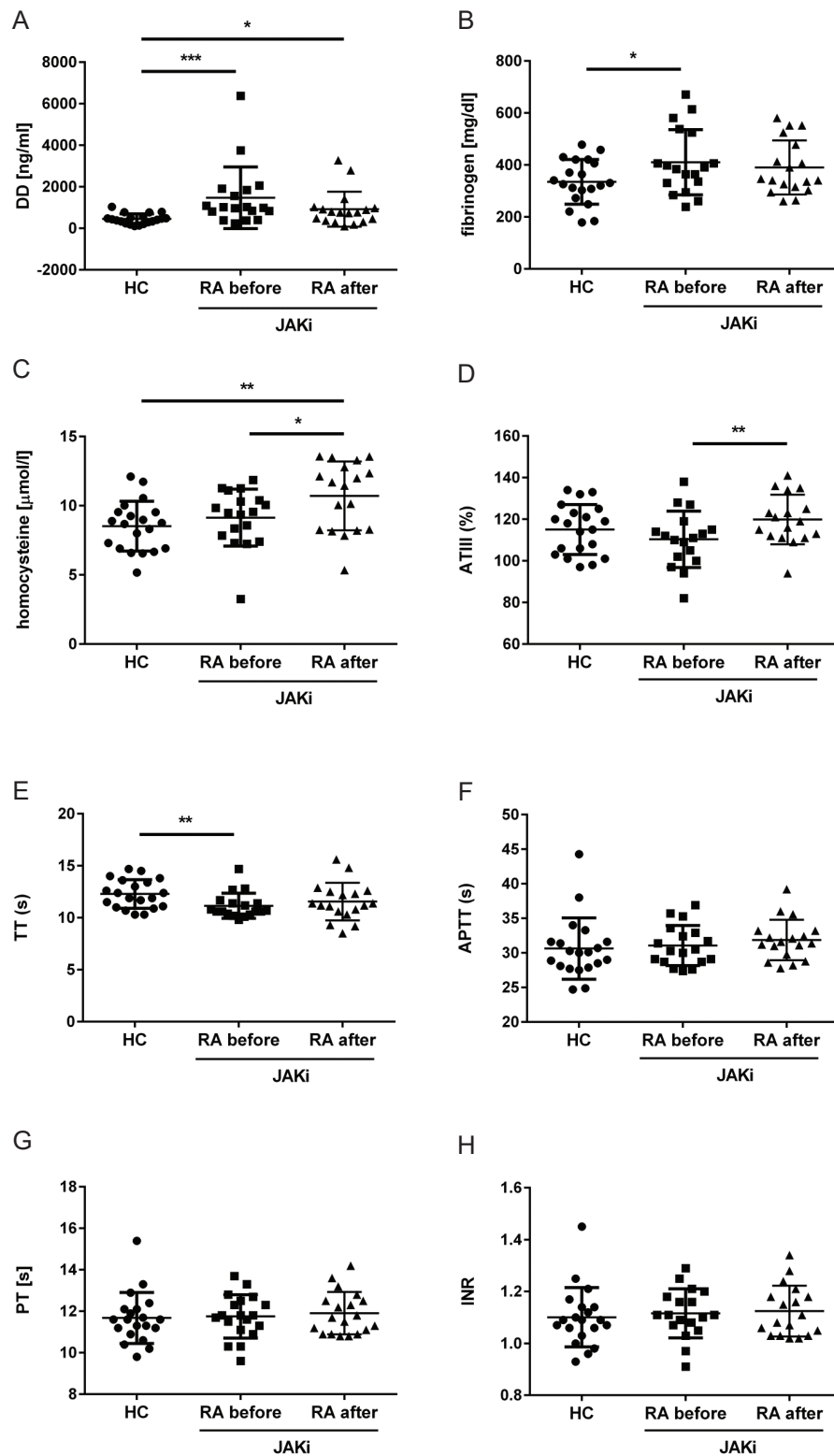


Fig 1. Thrombotic parameters DD (a), fibrinogen (b), homocysteine (c), ATIII (d), TT (e), APTT (f), PT (g) and INR (h) detected in RA patients (before and 3 months after treatment with baricitinib) in comparison with HC. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ATIII, antithrombin III; APTT, activated partial thromboplastin time; DD, D-dimer; HC, healthy controls; INR, international normalized ratio; JAKi, Janus kinase inhibitor; PT, prothrombin time; RA, rheumatoid arthritis; TT, thrombin time.

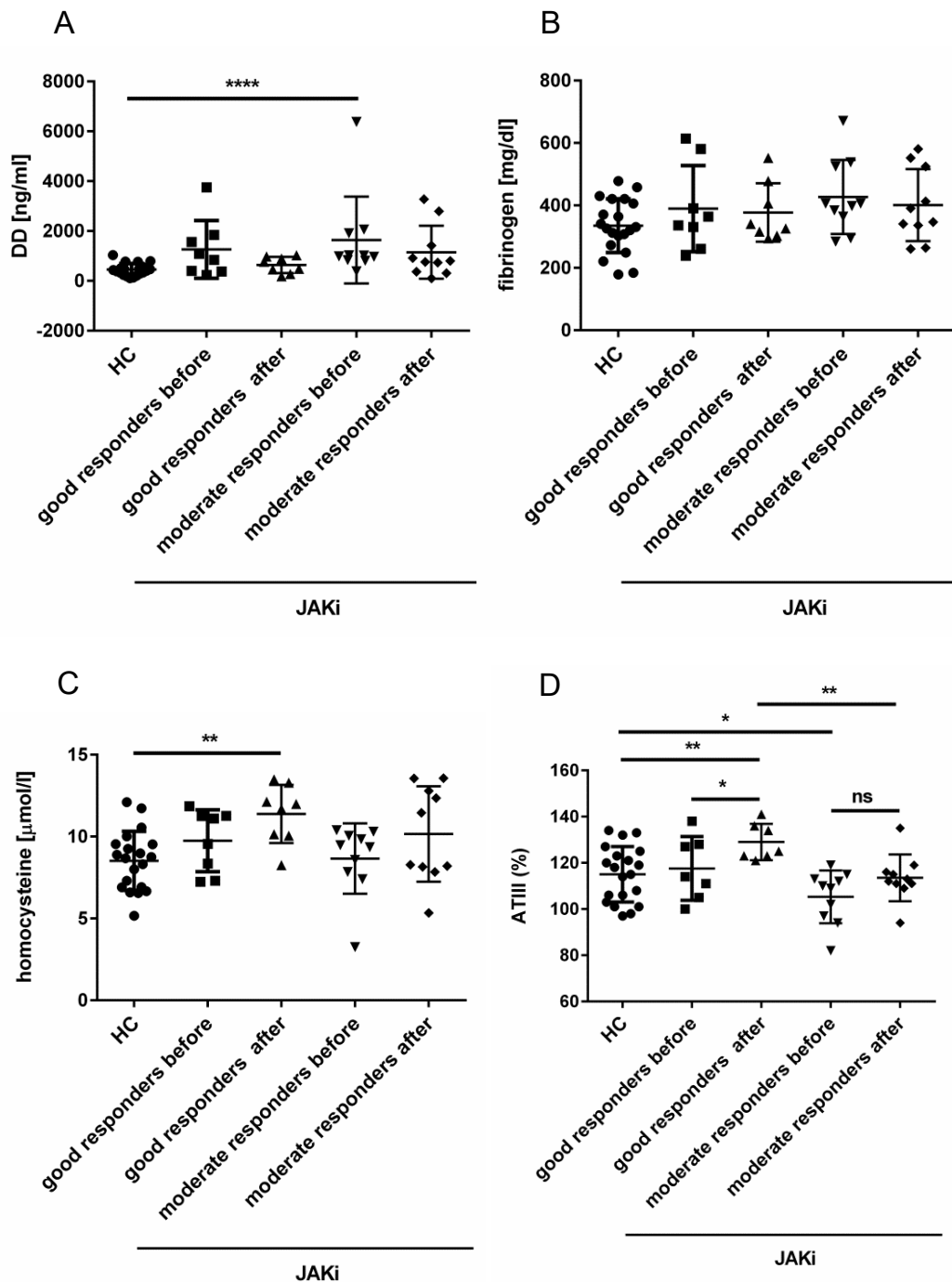


Fig 2. Thrombotic parameters DD (a), fibrinogen (b), homocysteine (c) and ATIII (d) detected in RA patients according to response to baricitinib (before and 3 months after treatment). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. ATIII, antithrombin III; DD, D-dimer; HC, healthy controls; JAKi, Janus kinase inhibitor; ns, not significant; RA, rheumatoid arthritis.

significant impact on plasma activity. In our cohort, RF but not ACPA was correlated with prothrombotic plasma composition (Table S1 in Supplementary Materials). The relationship between ATIII and RA activity is not clear but has been demonstrated in some studies on small groups of patients (Belch et

al. 1984; Jones et al. 1998; Undas et al. 2010). The ambiguity in results may be explained by different activity scores used in studies (Ritchie index or DAS28) or even different phenotypes of disease gathered in the same score range. The same DAS28 may be calculated out of high visual analog scale and low ESR,

Table 2. Clinical characteristics of patients with RA treated with baricitinib: GR and MR

	Month 0		Month 3	
	GR, n = 8	MR, n = 10	GR, n = 8	MR, n = 10
DAS28-ESR	5.52 ± 0.2 (5.1–6.9)	6.1 ± 0.3 (5.1–8.2)	2.8 ± 0.1 (2.2–3.1)	4.2 ± 0.2 (3.3–5.0)
VAS, activity (mm)	78.1 ± 4.7 (52.0–95.0)	74.1 ± 4.2 (63.0–91.0)	30.1 ± 2.6 (17.0–41.0)	50.3 ± 5.4 (28.0–82.0)
Joints, painful (n)	13.0 ± 1.7 (6–24)	13.1 ± 2.1 (6–24)	2.1 ± 0.4 (0–4)	3.0 ± 1.0 (0–10)
Joints, tender (n)	7.0 ± 1.2 (2–13)	7.5 ± 2.1 (1–22)	0.4 ± 0.2 (0–2)	1.5 ± 0.8 (0–8)
CRP (mg/dL)	12.5 ± 6.1 (1.0–45.0)	31.2 ± 11.8 (2.0–132)	3.0 ± 0.9 (0.2–7.0)	11.9 ± 5.8 (1.0–62.5)
ESR (mm/h)	18.12 ± 6.3 (5.0–60.0)	33.0 ± 7.8 (5.0–94.0)	11.9 ± 4.3 (4.0–44.0)	43.8 ± 11.0 (8.0–120.0)
DD (ng/mL)	1263.75 ± 410.1 (240.0–3757.0)	1639.2 ± 550.5 (386.0–6381.0)	636.8 ± 114.5 (105.0–2379.0)	1149.8 ± 336.8 (105.0–3279.0)
Fibrinogen (mg/dL)	389.6 ± 48.8 (239.0–614.0)	427.1 ± 37.6 (284.0–671.0)	377.13 ± 33.1 (295.0–552.0)	401.10 ± 36.5 (261.0–581.0)
HCY (μmol/L)	9.7 ± 0.7 (7.2–11.9)	8.7 ± 0.7 (3.3–10.4)	11.4 ± 0.6 (8.2–13.5)	10.2 ± 0.9 (5.4–13.6)
ATIII (%)	117.6 ± 5.2 (100.0–138.0)	105.3 ± 3.6 (94.0–119.0)	129.0 ± 3.0 (121.0–141.0)	113.50 ± 3.2 (94.0–135.0)
TT (s)	11.48 ± 0.3 (10.6–12.8)	10.88 ± 0.4 (9.8–14.7)	10.83 ± 0.5 (8.5–12.6)	12.13 ± 0.6 (9.2–15.6)
APTT (s)	31.0 ± 1.1 (27.4–35.7)	31.1 ± 0.9 (27.4–35.7)	30.25 ± 0.6 (27.8–32.4)	33.14 ± 1.0 (28.2–39.2)
PT (s)	11.7 ± 0.2 (11.1–12.5)	11.9 ± 0.4 (9.6–13.7)	11.7 ± 0.3 (10.8–13.2)	12.1 ± 0.3 (10.9–14.2)
INR	1.11 ± 0.02 (1.05–1.18)	1.12 ± 0.04 (0.91–1.29)	1.1 ± 0.03 (1.02–1.24)	1.14 ± 0.03 (1.03–1.34)

Data are presented as mean ± SEM and range.

APTT, activated partial thromboplastin time; ATIII, antithrombin III; CRP, c-reactive protein; DAS28-ESR, disease activity score 28-erythrocyte sedimentation rate; DD, D-dimer; GR, good responders; HCY, homocysteine; INR, International Normalized Ratio; MRs, moderate responders; PT, prothrombin time; RA, rheumatoid arthritis; SEM, standard error of the mean; TT, thrombin time; VAS, visual activity score.

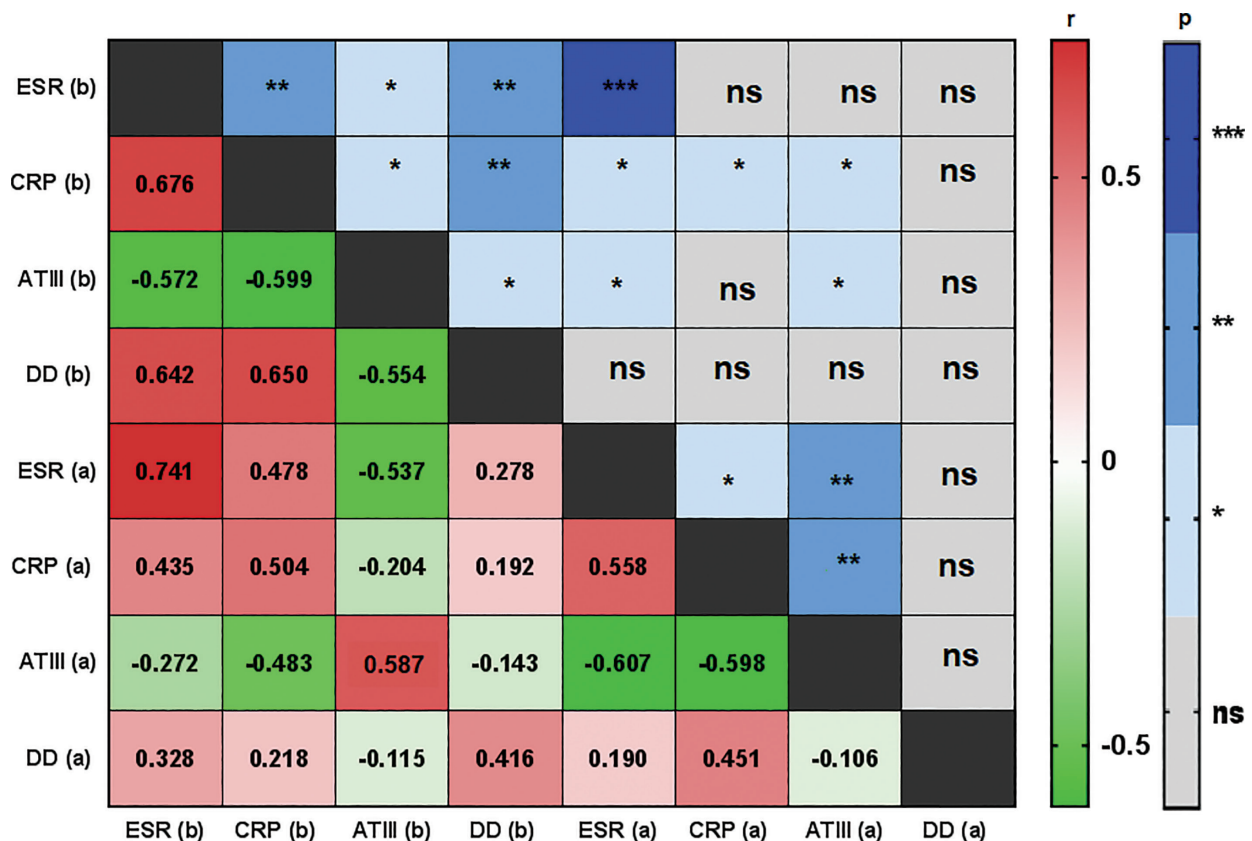


Fig 3. Correlation and p-value heatmap analysis between ESR before and after, CRP before and after, ATIII before and after, and DD before and after baricitinib therapy. p-values were expressed as follows: *0.05 > p > 0.01, **0.01 > p > 0.001, ***p < 0.001. ATIII, antithrombin III; CRP, C-reactive protein; DD, D-dimer; ESR, erythrocyte sedimentation rate; ns, not significant.

or conversely. In this study, ATIII activity correlated with disease activity parameters (Table 1 in Supplementary Materials). More importantly, in combination with DD, it predicted response at month 3, which is a unique finding in the literature. To date, the relationship between different cDMARDs and ATIII activity was not investigated. Further investigation is needed, as this might be a step forward in tailored drug choice in RA.

If we apply terms from a study investigating trajectories of response to baricitinib (Taylor et al. 2023), MR would correspond to the “gradual responders” and GR to “rapid responders” terms. We speculate that a longer timeframe could result in a subsequent increase in ATIII activity in MR – more time is needed to reach low disease activity in this group.

Another question applies to how ATIII activity influences VTE risk. Cut-off values vary between studies, but in general low activity is a predictor of cardiac events, ischemic stroke, and even death from COVID-19 infection (with a cut-off range between 88% and 210% in different studies) (Thompson et al. 1996; Meng et al. 2011; Chen-Goodspeed et al. 2023). This association could be related to excess consumption of ATIII during thrombotic events or baseline deficiency leading to thrombosis. Contrarily, mild ATIII deficiency (>70% and <80%) was not linked to primary VTE in immune-mediated disorders (Di Minno et al. 2014). Due to the short observation period and the small group of patients, the results of this study can be only used as the first step to deepen the investigation in that field.

In this study, we noticed an increase in HCY levels in RA patients after 3 months of treatment (Figure 1c), which stands in opposition to the previously described strong positive correlation with disease activity (Jensen et al. 2002; Rho et al. 2009; Katsushima et al. 2023). This discrepancy could be derived from the absence of JAKi users in the Japanese cohort. Increased HCY concentrations after treatment in this study may be an adverse effect of baricitinib and require further investigation as to whether it is one of the mechanisms by which baricitinib increases the risk of VT incidents.

Higher HCY concentrations are linked with higher cardiovascular risk (Yang et al. 2015; Habib et al. 2023; Popescu et al. 2023). The HCY concentration cut-off value for subjects at high risk for MACE, stroke, and mortality ranges according to different studies between >13.4 $\mu\text{mol/L}$ and >20.0 $\mu\text{mol/L}$ (Feng et al. 2020; Huang et al. 2021). However, the correlation between HCY and cardiovascular events in this RA patients was not studied and requires confirmation.

On the contrary, in patients responding to tofacitinib (which shares JAK1 and JAK2 receptor activity with baricitinib), HCY levels initially increased but decreased after one year of therapy (Soós et al., 2022). Therefore, the question remains whether the increase in HCY concentration observed in this study is merely temporary, and whether, with longer observation, we would see a decrease in HCY levels. Additionally, elevated HCY levels may be counterbalanced by inhibition

of the JAK2 receptor, as the atherosclerotic activity of HCY is mediated through this signaling pathway (Xu et al., 2022). This finding could help explain the low thromboembolic risk observed with selective JAK2 inhibitors (Kotyla et al., 2021). Second, high HCY levels may be counterbalanced by inhibition of the JAK2 receptor, since HCY atherosclerotic activity is mediated by this signaling pathway (Xu et al. 2022). This finding would explain the observed low thromboembolic risk in selective JAK2 inhibitors (Kotyla et al. 2021).

Certainly, further studies with active comparators are needed to evaluate the HCY level dynamics in different JAKi and their impact on adverse events.

A decrease in fibrinogen and DD in RA patients has been observed in previous studies in patients taking both JAKi and anti-TNF therapy (Hansildaar et al. 2023). In this study, we observed a decrease, but not normalization of DD and fibrinogen at month 3 (Figures 2a and 2b). This is consistent with data from a study in which even in patients achieving remission, fibrinogen, and DD levels do not normalize (McEntegart et al. 2001; Rooney et al. 2011).

The main strength of this study is the fact that, to our knowledge, this is the first study evaluating thrombotic parameters in RA patients treated with baricitinib during the first 3 months of therapy. One of the main limitations of this study is the inability to link the observed changes in coagulation parameters with the risk of VTE. This is due to the small study group and short observation time of patients. Additionally, the cohort is very homogeneous (predominantly women, all Caucasian, with low-to-moderate cardiovascular risk despite unfavorable RA course), suggesting caution in extrapolating conclusions on all patients. Also, the absence of non-responders is a spectrum bias. Therefore, the results of this pilot study should be considered as indicating a possible direction for future research.

In conclusion, baricitinib therapy in RA patients with poor prognostic factors was effective in the early period of treatment. Thrombotic parameters in the first 3 months of treatment were mostly in line with current findings. Low levels of ATIII together with increased DD seem to predispose to moderate response to baricitinib. An increase in HCY level at month 3 of unknown significance needs further investigation, as well as the role of ATIII activity and DD as potential markers of response. Altogether, we did not find data to support the evident effect of baricitinib on selected parameters related to thromboembolic risk in RA patients during the first 3 months of therapy.

Acknowledgments

We would like to acknowledge Dr M. Kosciuk-Perkowska for the helpful discussion about the clinically relevant levels of HCY.

Parts of the material were published in the form of abstracts: EULAR Congress (2024 AB0225); [European Congress of Immunology (Eur J Immunol 2024; 54(Suppl. 1): 1534)].

Statements and Declarations

AFG has speaking contracts with Pfizer, Abbvie, Biogen, Eli Lilly, Janssen, Novartis, Roche, Sandoz, and UCB. KC, PK, AK, WM, MC, and MM declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. AFG, KC, PK, AK, WM, MC, and MM contributed to

the design and implementation of the research, analysis of the results, and writing of the manuscript.

Financial Support

This work was supported by Grant No. 2020/04/X/NZ5/01820 (MINIATURA 4) from the National Science Centre, Poland for MM (consumables) and the Core Grant S99 to the National Institute of Geriatrics, Rheumatology, and Rehabilitation from the Polish Ministry of Science and Higher Education (publication costs).

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Supplementary Materials

Table S1. Correlation between the chosen parameters of thrombosis and clinical data

Before treatment		<i>r</i>	<i>p</i> -value
ATIII	DAS28	−0.696	0.003
ATIII	CRP	−0.599	0.013
ATIII	ESR	−0.572	0.018
ATIII	RF	−0.601	0.012
ATIII	DD	−0.554	0.023
DD	DAS28	0.526	0.025
DD	CRP	0.65	0.003
DD	ESR	0.642	0.004
DD	RF	0.582	0.011
DD	Fibrinogen	0.623	0.006
DD	Homocysteine	0.540	0.021
Fibrinogen	CRP	0.721	0.001
Fibrinogen	ESR	0.800	<0.001
Fibrinogen	RF	0.529	0.024
Fibrinogen	ATIII	−0.513	0.035
TT	CRP	−0.631	0.005
TT	ESR	−0.575	0.013
TT	RF	−0.574	0.013
After treatment			
ATIII	DAS28	−0.686	0.002
ATIII	CRP	−0.598	0.009
ATIII	ESR	−0.607	0.008
APTT	ESR	0.485	0.041
Fibrinogen	ESR	0.483	0.042

APTT, activated partial thromboplastin time; ATIII, antithrombin III; CRP, c-reactive protein; DAS28, disease activity score 28; DD, D-dimer; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; TT, thrombin time.

Table S2. Demographic and clinical characteristics of GR and MR to baricitinib treatment

Characteristic	GR, (<i>n</i> = 8)	MR (<i>n</i> = 10)	Difference, <i>p</i> -value
Demographics			
Sex (female), <i>n</i> (%)	7 (87.5)	10 (100)	ns
Age (years), mean (±SD)	50 (±12.9)	53.3 (±13.4)	ns
BMI (kg/m ²), mean (±SD)	25 (±3.6)	24.6 (±4.2)	ns
CV risk factors			
Smoking ^a , <i>n</i> (%)	1 (12.5)	3 (27.3)	ns
Dyslipidemia ^b , <i>n</i> (%)	6 (75)	7 (63.6)	ns
Arterial hypertension, <i>n</i> (%)	2 (25)	3 (27.3)	ns
Diabetes mellitus, <i>n</i> (%)	1 (12.5)	0 (0)	ns
Hormonal contraception	0 (0)	1 (9.1)	ns
RA status			
Disease duration (years), mean (±SD)	8.3 (±7.4)	9.5 (±9.3)	ns
RF (IU/mL), mean (±SD)	107.5 (±113)	248.2 (±231.6)	ns
ACPA (U/mL), mean (±SD)	473.2 (±59.8)	404.8 (±184.1)	ns
RA treatment			
Previous biologic therapy, <i>n</i> (%)	2 (25)	1 (9.1)	ns
GCS, <i>n</i> (%)	3 (37.5)	8 (72.7)	ns
dose (mg ^c), mean (±SD)	5 (2.5)	4.7 (±1.6)	ns
cDMARDs, <i>n</i> (%)	6 (75)	8 (72.7)	ns
Methotrexate, <i>n</i> (%)	5 (62.5)	8 (72.7)	ns
Sulfasalazine, <i>n</i> (%)	2 (25)	3 (27.3)	ns
Hydroxychloroquine, <i>n</i> (%)	3 (37.5)	2 (18.2)	ns
Disease activity			
DAS28-ESR, mean (±SD)	5.5 (±0.6)	6.1 (±1.1)	ns
VAS pain (mm), mean (±SD)	78.1 (14.2)	74.1 (±8.8)	ns
Joints (painful), mean (±SD)	13 (±5.2)	13.1 (±7)	ns
Joints (tender), mean (±SD)	7 (±3.6)	7.5 (±7.1)	ns
CRP (mg/L), mean (±SD)	12.5 (18.4)	31.2 (±39.2)	ns
ESR (mm/h), mean (±SD)	18.1 (±18.9)	33 (±25.9)	ns

^aSmoker status: current or longtime past smoking history.

^bDyslipidemia: hypertriglyceridemia and/or hyper-LDL or normal lipid profile with current statin therapy.

^cDose in equivalent of prednisone.

ACPA, anti-citrullinated peptide antibodies; BMI, body mass index; cDMARDs, conventional disease-modifying antirheumatic drugs; CRP, C-reactive protein; CV, cardiovascular; DAS28-ESR, disease activity score 28-erythrocyte sedimentation rate; GR, good responders; LDL, low density lipoprotein; MRs, moderate responders; ns, non-significant; RA, rheumatoid arthritis; RF, rheumatoid factor; VAS, visual analog scale.