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ORIGINAL RESEARCH

Impaired coagulation parameters in early RA are restored by effective antirheumatic therapy: a prospective pilot study

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ABSTRACT

Objectives To assess the effect of treatment on haemostatic parameters in patients with early rheumatoid arthritis (RA).

Methods Patients with newly diagnosed RA started methotrexate and were randomised to additional conventional treatment, certolizumab pegol, abatacept or tocilizumab. Several biomarkers for haemostasis were analysed including parameters of the two global haemostatic assays—overall haemostatic potential (OHP) and endogenous thrombin potential (ETP), as well as single haemostatic factors—fibrinogen, prothrombin fragment 1+2 (F1+2), D-dimer, thrombin activatable fibrinolysis inhibitor (TAFI) and clot lysis time (CLT) in 24 patients at baseline, 12 and 24 weeks after the start of the treatment.

Results At baseline, patients had elevated levels of the following biomarkers compared with reference values: fibrinogen, F1+2, D-dimer and parameters of the two global haemostatic assays, that is, ETP and OHP. After 24 weeks we observed a significant reduction in F1+2 (p<0.01), fibrinogen (p<0.01), D-dimer (p<0.01), OHP (p<0.01), ETP (p<0.01), CLT (p<0.01), TAFI (p<0.01) and an increase of OFP (p<0.01). Tocilizumab treatment resulted in the most significant reduction of global haemostatic assays after 24 weeks, that is, a reduction of OHP 73% (p<0.01) compared with certolizumab pegol arm 32% (p<0.01), abatacept arm 24% (p=0.25) or conventional treatment arm 7% (p=0.66).

Conclusion Newly diagnosed RA patients have enhanced coagulation activation and impaired fibrinolysis as demonstrated by our results. Effective antirheumatic treatments during the first 24 weeks after diagnosis improved this haemostatic imbalance, with prominent effects of biological drugs and especially tocilizumab, compared with conventional treatment.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with rheumatoid arthritis (RA) have an increased risk of developing venous thrombosis. Reducing disease activity reduces this risk.

WHAT THIS STUDY ADDS

⇒ We found that patients with early RA had a haemostatic imbalance that improved after initiation of treatment. Biological treatment was more effective in reducing this imbalance than conventional treatment (methotrexate+prednisolone), with tocilizumab being the most effective biological agent.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Treatment of early RA patients is effective in reducing the haemostatic imbalance seen early on in the disease. Biologic disease-modifying antirheumatic drugs, especially tocilizumab, were more successful in restoring haemostasis.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common chronic autoimmune inflammatory joint disease, affecting approximately 0.5% of the worldwide population.[1] Absence or inadequate treatment may lead to permanent joint damage and subsequent physical disability.[2] RA is associated with an increased cardiovascular burden, such as atherosclerosis and venous thromboembolism (VTE) with an almost twofold higher risk compared with the healthy population.[3 4] It was recently shown that the relative risk for VTE is higher in RA patients with high disease activity, compared with patients in remission.[5] Additionally, the increased risk for VTE shown in the ORAL surveillance trial highlights the continued importance of evaluating VTE risk when initiating new treatments.[6] The increased incidence of VTE in patients with

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RA points towards the presence of a procoagulant state in these patients. There is a strong and ongoing interplay between inflammatory processes and activation of coagulation pathways, involving several pro-inflammatory cytokines. Shortly, the endothelial dysfunction induced by tumour necrosis factor (TNF) and activation of platelets by interleukin 6 (IL-6) are initial mechanisms, leading to increased expression of tissue factor on platelets and monocytes, enhanced coagulation and excessive thrombin and fibrin formation.[7 8]

Treatment of RA with disease-modifying antirheumatic drugs (DMARDs) reduces the systemic inflammatory burden and the disease activity, which contributes to reduced risk for cardiovascular comorbidities.[9] A small study showed that TNF inhibition by infliximab not only decreases inflammation, but also reduces inhibition of fibrinolysis after 14 weeks of treatment in 20 patients with high disease activity.[10] The reduction of the procoagulant condition by TNF inhibition with infliximab was later confirmed by our group.[11] Treatment with tocilizumab, a human monoclonal antibody targeting IL-6 receptor, appeared to be more beneficial compared with TNF inhibitors in reducing the levels of procoagulant biomarkers.[9 12] In addition, a study including 15 consecutive RA patients with established disease, has reported that tocilizumab reduces plasma levels of prothrombin fragment 1 and 2 (F1+2) after 4 weeks of treatment in clinically responsive patients, indicating that IL-6 signalling blockade by tocilizumab may reduce the prothrombotic state in RA.[13]

Fibrinogen is one of the most important factors involved in the coagulation process, while it is also an acute phase reactant involved in the inflammatory process. Fibrin depositions in the joints are characteristic of RA, maintaining the formation of pannus tissue and are considered pathogenic.[14] However, little is known about the predictive role of fibrinogen levels in peripheral blood in RA patients. On the other hand, D-dimer, a fibrin degradation product (FDP) is the most used indicator to reflect activation of the coagulation system. D-dimer and other FDPs might cause release of inflammatory cytokines by promoting the activation of monocytes.[15 16]

The assessment of individual haemostatic factors and/or inhibitors demonstrates only partly the complex haemostatic process. In contrast, the overall haemostatic potential (OHP) assay is a more advanced test measuring the balance between coagulation and fibrinolysis.[17] The test provides additional information on the rate of fibrin formation and breakdown, demonstrating the balance between these two pivotal and opposing haemostatic processes of coagulation and fibrinolysis. Additional parameters of the assay are overall coagulation potential (OCP), overall fibrinolytic potential (OFP) and the clot lysis time (CLT) providing more information on fibrin generation and fibrinolysis, respectively.

Comparative effects of different biologic DMARDs (bDMARDs) and conventional synthetic DMARDs (csDMARDs) on the coagulation and fibrinolytic system have not been studied yet in a randomised fashion. Therefore, the aim of our study was to investigate the coagulation and fibrinolytic systems in newly diagnosed RA patients treated with four different DMARDs, before and at 12 and 24 weeks after initiation of treatment.

METHODS

Study design

NORD-STAR is an international, multicentre, open-label, assessor-blinded, phase 4 study where patients with newly diagnosed RA started methotrexate (MTX) and were followed prospectively and randomised 1:1:1:1 to four arms: arm 1—conventional treatment (either prednisolone tapered to 5 mg/day in 9 weeks or with intra-articular corticosteroids), arm 2—certolizumab pegol, arm 3—abatacept or arm 4—tocilizumab.[18] The NORD-STAR study was performed in six countries (Denmark, Finland, Iceland, Norway, Sweden and the Netherlands). Our spin-off study was restricted to the 24 patients enrolled per protocol in Reade, the Netherlands. We selected a comprehensive blood panel consisting of both haemostatic and fibrinolytic parameters, which were measured at baseline, 12 and 24 weeks after treatment initiation.

Patient population

The most important inclusion criteria were age ≥18 years, RA according to ACR/EULAR 2010 criteria, <24 months from arthritis symptom onset, RF positive and/or anti-citrullinated protein antibody positive and/or C reactive protein (CRP) ≥10 mg/L, Disease Activity Score in 28 joints based on CRP (DAS28-CRP) >3.2, ≥2 swollen and ≥2 tender joints.[18] An extensive list of inclusion and exclusion criteria and the full study protocol is provided in online supplemental appendix. Patients who did not reach week 24 of the study (N=3) were excluded from this analysis. In this substudy the conventional treatment group consisted of five patients, the certolizumab pegol group of six patients, the abatacept group of seven patients and the tocilizumab group of six patients. Reference values as mentioned in the Results section and table 1 are measurements in healthy controls (n=20) using the same laboratory assays as mentioned. Healthy controls were free of any anticoagulant and non-steroidal anti-inflammatory drugs treatment prior to the blood sampling.

Blood sampling

Blood samples were obtained at baseline, 12 weeks and 24 weeks after treatment initiation, centrifuged for 15 min at 2563 g and platelet-poor plasma was aliquoted and stored at −80°C freezer until assessment. The frozen plasma samples were transported to the Coagulation Laboratory at the Karolinska Institute, Department of Molecular Medicine and Surgery, Stockholm, Sweden, where all analysis were performed.

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Table 1 The levels of different coagulation and fibrinolytic markers in patients with rheumatoid arthritis at baseline and during 12 and 24 weeks of treatment in all patients

	Baseline N=24	W12 N=24	W24 N=24	P value baseline vs w12	P value baseline vs w24	Reference values
F1+2 (pMol/L)	270.2 (149)	190.4 (109)	179.5 (85)	<0.01	<0.01	180–250
Fibrinogen (g/L)	4.6 (1.5)	3.6 (1.6)	2.6 (1.2)	<0.01	<0.01	2–4
D-dimer (mg/L)	2.2 (3.0)	0.3 (0.2)	0.3 (0.2)	<0.01	<0.01	0–0.25
OHP (Abs-sum)	157.4 (65)	120.6 (69)	100.5 (54)	0.01	<0.01	88–158
OCP (Abs-sum)	369.5 (59)	305.0 (102)	275.9 (83)	0.01	<0.01	213–304
OFP (%)	57.0 (13)	63.20 (13)	65.25 (11)	0.03	<0.01	45–61
Lag (s)	304.5 (71)	306.8 (72)	312.7 (65)	0.88	0.65	164–292
Slope	0.07 (0.02)	0.07 (0.03)	0.1 (0.1)	0.33	0.39	0.08–0.2
Max Abs	1.2 (0.3)	1.0 (0.3)	0.9 (0.3)	0.04	<0.01	1.1–1.6
CLT (s)	1405 (356)	1317 (377)	1231 (320)	0.13	<0.01	1182–1848
ETP (AUC)	1480 (471)	1395 (395)	1337 (429)	0.04	0.01	1431–2364
Peak	231 (78)	223 (68)	223 (74)	0.34	0.23	146–335
Lagtime (sec.)	4.1 (2.1)	3.3 (1.2)	2.8 (1.2)	<0.01	<0.01	3.9–7.5
ttPeak (s)	7.4 (2.2)	6.6 (1.5)	6.1 (1.4)	0.01	<0.01	7.24–12.0
TAFI (%)	187 (41)	168 (38)	162 (38)	<0.01	<0.01	97–159

Numbers presented as mean (SD), statistical significance (p<0.05) was determined using paired sample t-test.
CLT, clot lysis time; ETP, endogenous thrombin potential; F 1+2, prothrombin factor 1+2; OCP, overall coagulation potential; OFP, overall fibrinolytic potential; OHP, overall haemostatic potential; TAFI, thrombin activatable fibrinolysis inhibitor.

Laboratory assays

Determination of OHP

A modification of the assay described by He *et al* was employed in order to assess OHP in plasma.[19] Absorbance (Abs) at 405 nm was measured every 12 s for 60 min, and the area under the curve was calculated by summation of the Abs values (Abs-sum) and expressed as the OHP value. Two additional parameters were also analysed: OCP, determined as the area under the fibrin aggregation curve obtained without the addition of tissue plasminogen activator, and the OFP, calculated as the difference between the two areas as OFP (%)=((OCP–OHP)/OCP)×100. The intra- and inter-assay coefficients of variation for OHP were 1.6% and 6.8% and for OCP were 1.2% and 5.7%, respectively.

The turbidimetric curve for determination of OCP was used to assess fibrin clot density by the following parameters as previously described in Pruner *et al*: 'lag-time', the time-point when exponential growth of the curve begins as a measure of the clotting time; 'max absorbance', the average value of three consecutive points where the curve reached a plateau as a measure of the clot density and the 'slope' measuring the polymerisation rate of fibrin.[20]

Determination of CLT

CLT was determined based on the fibrin aggregation curve for the determination of OHP and was defined as the time from the midpoint of the clear-to-maximum turbid transition (which corresponds to the clotting time) to the midpoint of the maximum turbid-to-clear transition.

Determination of thrombin generation in plasma

Calibrated automated thrombogram assay was used according to the manufacturer's instructions (Diagnostica Stago, Asnieres, France). Thrombin generation curve (Thrombogram) was generated in recalcified plasma after the addition of tissue factor, by measuring fluorescence every 20 s and different parameters of thrombin generation were calculated by the analysing software (Thrombinoscope).[21]

From the thrombin generation curves, the following parameters were obtained: endogenous thrombin potential (ETP) (as a measure of the total endogenously generated thrombin), lag time (time to start of thrombin generation), peak of thrombin generation (the maximal concentration of thrombin generated) and ttPeak (time to peak of thrombin generation).

Other laboratory assays

CRP was analysed by using the COBAS c module, an immunotubidimetric assay. SR was determined with the Westegren method using the Starrsed Auto Compact BSE analyzer. Rheumatoid factor antibodies were measured using ELIA kit from Phadia250, anti-cyclic citrullinated peptide (aCCP) antibodies were also measured using an ELIA kit from Phadia250. CRP sedimentation rate, rheumatoid Factor and aCCP tests were performed in

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Table 2 General characteristics of the patients at baseline

	Conventional N=5	Certolizumab N=6	Abatacept N=7	Tocilizumab N=6	Total N=24
Age	51.8 (7.6)	51.8 (17.3)	51.7 (9.1)	52.0 (17.3)	51.8 (12.7)
Sex (N, % male)	2 (40)	2 (33)	3 (43)	3 (50)	10 (37)
Disease duration (days)	8.6 (5.6)	3.3 (3.2)	6.3 (4.4)	16.5 (21.2)	8.58 (11.6)
Rheumatoid factor (N, %)	5 (100)	6 (100)	6 (85.7)	6 (100)	23 (95.8)
aCCP (N, %)	5 (100)	6 (100)	6 (85.7)	6 (100)	23 (95.8)
CRP	30.8 (44.6)	13.2 (11.5)	31.6 (55.7)	19.0 (17.3)	22.3 (34.4)
ESR	35.0 (37.3)	38.8 (27.0)	45.9 (46.8)	24.8 (20.9)	34.7 (32.3)
CDAI	26.1 (4.7)	21.7 (7.7)	23.5 (12.8)	21.0 (6.6)	22 (6.5)
DAS28-CRP	5.0 (0.8)	4.5 (0.87)	4.5 (1.3)	4.4 (0.7)	4.5 (0.9)
HAQDI	1.4 (0.5)	0.8 (0.3)	1.1 (0.6)	0.9 (0.4)	1.1 (0.5)
VAS Pain	60.6 (32.0)	46.7 (12.5)	61.1 (26.4)	38.2 (22.1)	51.9 (25.9)
VAS Global	58.6 (21.8)	55.0 (10.2)	39.3 (31.0)	27.8 (27.6)	44.1 (25.6)
VAS Fatigue	58.2 (41.7)	58.8 (16.9)	47.7 (29.1)	48.0 (33.7)	52.0 (28.3)

Numbers presented as mean (SD), unless otherwise stated.
aCCP, anti-cyclic citrullinated peptide antibody; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28-CRP, Disease Activity Score of 28 joints, based on C reactive protein; ESR, erythrocyte sedimentation rate; HAQDI, Health Assessment Questionnaire Disability Index; VAS, Visual Analogue Scale.

the Clinical Laboratory, OLVG, Amsterdam, The Netherlands.

Prothrombin fragment F1+2 was analysed by the enzymatic immunoassay based on sandwich principle in the microtitre format, using Enzygnost kit, Siemens, Germany. Dade Thrombin Reagent Siemens, Germany, was used for determination of fibrinogen concentration by the Caluss method. The INNOVANCE (Siemens, Germany) D-Dimer Assay, a particle-enhanced immunoturbidimetric assay was used for the quantitative determination of cross-linked FDPs (D-dimers). Thrombin activatable fibrinolysis inhibitor (TAFI) antigen levels were assessed using ELISA Zymutest kit from Hyphen BioMed, France.

Data analysis

Statistical analysis was done using paired samples t-test and Pearsons's correlations in SPSS V.28. SD is shown where applicable.

RESULTS

Patient population

The mean age of the 24 patients was 51.8 (± 12.7) years and 58% were female. 23 (96%) patients were rheumatoid factor and/or aCCP positive. Patients had an average DAS28-CRP score of 4.6±0.9 at baseline (table 2).

Both the conventional treatment group and the abatacept group had a numerically but not statistically significant higher CRP at baseline compared with the certolizumab pegol and tocilizumab groups, respectively, while there was no difference in disease activity measured by DAS28-CRP at baseline between the four treatment arms (table 2).

Haemostatic parameters

At baseline, all patients had elevated levels of F1+2, fibrinogen and D-dimer, compared with reference values (table 1). One of the global haemostatic parameters, OCP was elevated at baseline, while OHP and parameters of thrombin generation assay were within the reference range. TAFI was increased at baseline, while other markers for fibrinolysis that is, CLT and OFP were within the normal range.

After 12 weeks of treatment, an overall significant reduction in F1+2 (29.6%, p<0.01), fibrinogen (22.2%, p<0.01), D-Dimer (84.8%, p<0.01), OHP (23.4%, p=0.01), OCP (17.5%, p=0.01) and ETP (5.7%, p=0.04) was observed (table 1). After 24 weeks of treatment, this decrease compared with baseline remained for F1+2 (33.6%, p<0.01), fibrinogen (43.3%, p<0.01), D-Dimer (86.6%, p<0.01), OHP (reduction 36.1%, p<0.01), OCP (25.3%, p<0.01) and ETP (9.7% p=0.01).

Thrombin generation measured by ETP decreased during the treatment in the whole cohort of patients, but peak of thrombin generation was unchanged. The time to peak (ttPeak) and lag time decreased significantly both in week 12 and week 24 (p=0.01 and p<0.01, respectively). The fibrinolytic parameters improved at week 24 compared with baseline, with an increase of OFP by 12.5% (p<0.01), reduction of CLT by 12.4%, (p<0.01) and TAFI by 13.4% (p<0.01), indicating a significant increase of the fibrinolytic potential during anti-rheumatic treatment.

In the whole group of investigated early RA patients. These results indicate the reduction of coagulation activation measured by the levels of D-dimer, fibrinogen, F1+2, OHP, OCP and ETP, after initiation of anti-rheumatic

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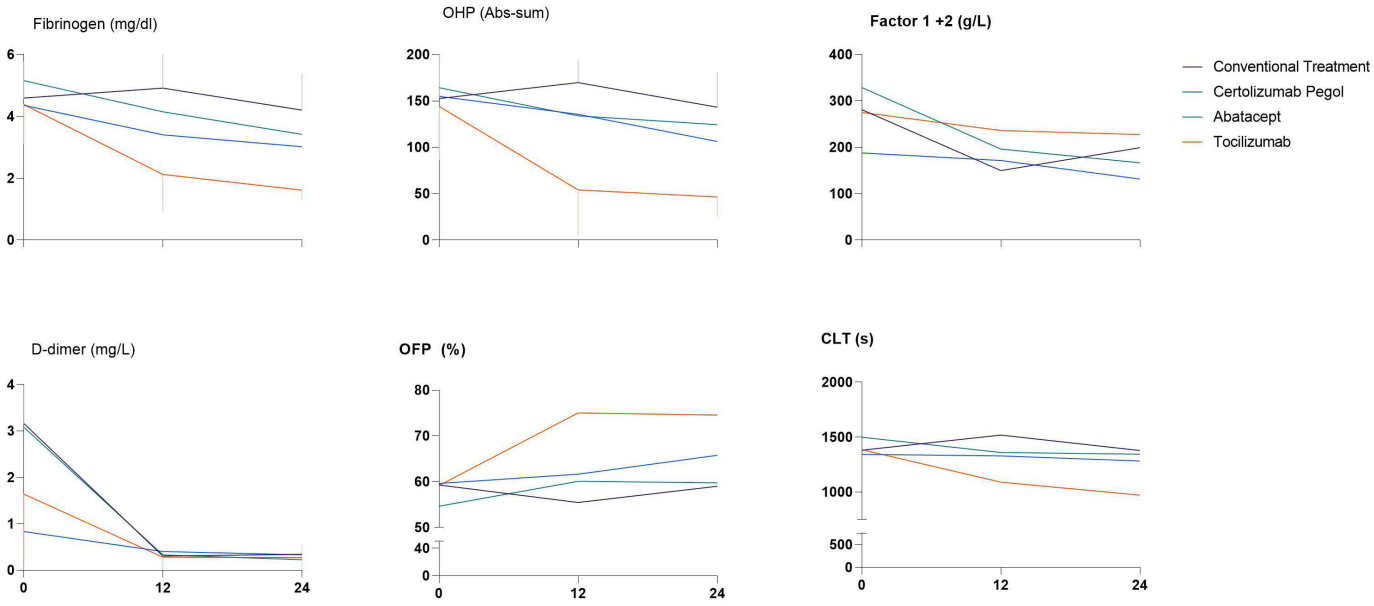

Figure 1 Coagulation markers. CLT, clot lysis time; Factor 1+2, prothrombin factor 1+2; OCP, overall coagulation potential; OFP, overall fibrinolytic potential; OHP, overall haemostatic potential.

treatment. Simultaneously, fibrinolysis is enhanced as assessed by the levels of OFP, CLT and TAFI.

Analysis of the treatment arms

The varying treatments had different effects on the haemostatic imbalance as shown in figure 1 and table 3. Tocilizumab reduced the average fibrinogen level by 63% (p<0.01) compared with 8% (ns) reduction in the conventional treatment group, 10% (p<0.01) and 34% reduction (p=0.05) in the certolizumab pegol and abatacept groups, respectively. For OHP, tocilizumab showed a similarly increased reduction of 73% at week 24 (p<0.01), whereas the other treatments led to less pronounced reductions (certolizumab pegol 32%, p<0.01, abatacept 24%, p=0.25, conventional treatment 7%, p=0.66). In terms of Factor 1+2 and D-dimer, the reductions were very similar in all groups. Interestingly, there was a large difference within the treatment groups in the ETP and peak thrombin generation. When looking at ETP the results showed a 1% increase in the conventional treatment group, a minor decrease in the certolizumab group (reduction 3.7%, p=0.42), a larger decrease in the abatacept group (decrease of 14.3%, p=0.32) and finally a statistically significant decrease of 17.7% in the tocilizumab group (table 3). In the peak thrombin generation, we observed a greater difference with the conventional and certolizumab treatment groups both resulting in an increase of 12.6% and 4.6%, respectively, while the abatacept and tocilizumab groups decreased the peak thrombin generation by 12.2% and 14.8% (p=0.021).

During the tocilizumab treatment OFP increased by 26% (p<0.01) at week 24, certolizumab pegol increased OFP by 10% (p=0.011) compared with no improvement in the conventional treatment and abatacept groups. The CLT and TAFI are in line with the remainder of the results and show more improvement in the tocilizumab group (table 3).

We investigated whether the delta in DAS-28 from baseline to week 24 was correlated to the delta in the different coagulation factors. ETP was negatively correlated with DAS28 (r=−0.48), with lagtime (r=0.65) and ttPeak showing a positive correlation (r=0.59). All the other coagulation factors were not correlated to the delta in DAS28.

DISCUSSION

In this spin-off study to the NORD-STAR trial, we demonstrated that patients with untreated early RA have significantly impaired coagulation and fibrinolysis before treatment. To the best of our knowledge, this is the first study which examines different treatment strategies on this haemostatic imbalance. We were able to demonstrate that treatment of early RA reduced the pro-coagulant state present in newly diagnosed patients, with biological treatments being the most effective, in particular IL-6 receptor inhibition. This difference was most profound in the reduction of fibrinogen as in the tocilizumab group there was a reduction of 63% compared with 8% in the conventional treatment group.

Generally, only few previous studies used global haemostatic assays investigating pro-coagulant condition in RA patients. Employing thrombin generation assay, maximum thrombin level and the rate of thrombin formation were found in samples from established RA patients, compared with sex- and age-matched healthy individuals.[22] Increased velocity of thrombin formation was seen in the samples from another cohort of RA patients, though the thrombin generating capacity was reduced, remaining thrombin assessment was not

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Table 3 The levels of different coagulation and fibrinolytic markers in patients with rheumatoid arthritis at baseline and during 12 and 24 weeks of treatment differentiated by treatment arms

	Baseline N=24	W12 N=24	W24 N=24	Change (%)	P value baseline vs w24
Factor 1+2					
Conventional treatment	281 (144)	150 (52)	199 (31)	−29	0.252
Certolizumab pegol	188 (73)	171 (95)	132 (45)	−30	0.015
Abatacept	329 (207)	196 (116)	166 (68)	−50	0.029
Tocilizumab	275 (127)	236 (150)	228 (132)	−17	0.205
Fibrinogen					
Conventional treatment	4.6 (1.2)	4.9 (1.2)	4.2 (1.2)	−8	0.463
Certolizumab pegol	3.4 (0.6)	3.4 (0.6)	3.0 (0.6)	−10	0.003
Abatacept	5.2 (2.3)	4.2 (1.9)	3.4 (0.9)	−34	0.049
Tocilizumab	4.4 (1.3)	2.1 (1.2)	1.6 (0.3)	−63	0.001
D-dimer					
Conventional treatment	3.16 (3.8)	0.30 (0.1)	0.34 (0.2)	−89	0.175
Certolizumab pegol	0.83 (0.4)	0.40 (0.3)	0.33 (0.3)	−60	0.016
Abatacept	3.08 (4.0)	0.61 (0.3)	0.23 (0.1)	−93	0.111
Tocilizumab	1.64 (2.2)	0.28 (0.3)	0.27 (0.3)	−84	0.148
OHP					
Conventional treatment	153 (65)	170 (25)	143 (38)	−7	0.66
Certolizumab pegol	155 (38)	136 (58)	106 (41)	−32	0.002
Abatacept	164 (95)	134 (75)	124 (54)	−24	0.247
Tocilizumab	156 (59)	49 (46)	42 (22)	−73	0.004
OFP					
Conventional treatment	59 (1)	55 (4)	59 (7)	−0.4	0.972
Certolizumab pegol	60 (9)	61 (11)	66 (8)	10.4	0.011
Abatacept	55 (19)	60 (15)	60 (14)	9.3	0.21
Tocilizumab	59 (13)	75 (9)	74 (9)	25.8	0.003
CLT					
Conventional treatment	1380 (375)	1518 (187)	1377 (255)	−0.2	0.898
Certolizumab pegol	1342 (225)	1328 (333)	1282 (206)	−4.5	0.324
Abatacept	1499 (476)	1359 (434)	1344 (389)	−10.3	0.091
Tocilizumab	1384 (359)	1088 (429)	970 (283)	−29.9	0.009
TAFI					
Conventional treatment	178 (43)	178 (36)	164 (47)	−8.2	0.074
Certolizumab pegol	182 (42)	178 (47)	177 (26)	−3.2	0.609
Abatacept	185 (46)	158 (38)	156 (43)	−15.4	0.349
Tocilizumab	203 (41)	161 (34)	154 (20)	−24.0	0.026
ETP					
Conventional treatment	1701 (197)	1639 (143)	1719 (211)	1.1	0.624
Certolizumab pegol	1386 (250)	1413 (226)	1335 (218)	−3.7	0.416
Abatacept	1395 (218)	1351 (135)	1195 (157)	−14.3	0.316
Tocilizumab	1489 (129)	1222 (114)	1226 (115)	−17.7	<0.01
Peak thrombin generation					
Conventional treatment	261 (25)	275 (25)	294 (27)	12.6	0.244
Certolizumab pegol	216 (38)	237 (36)	226 (34)	4.6	0.436
Abatacept	196 (34)	177 (21)	172 (28)	−12.2	0.397

Continued

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Table 3 Continued

	Baseline N=24	W12 N=24	W24 N=24	Change (%)	P value baseline vs w24
Tocilizumab	263 (21)	219 (15)	224 (18)	−14.8	0.021

Numbers presented as mean (SD), Statistical significance (p<0.05) was determined using paired sample t-test.
CLT, clot lysis time; ETP, endogenous thrombin potential; Factor 1+2, prothrombin factor 1+2; OCP, overall coagulation potential; OFP, overall fibrinolytic potential; OHP, overall haemostatic potential; TAFI, thrombin activatable fibrinolysis inhibitor.

conclusive.[23] Our results show that in patients with newly diagnosed RA, all parameters of the thrombin generation assay were within the reference range. There are two likely causes for this different, first the selection of only early RA patients could influence the thrombin generation. As there are no other studies looking into the coagulation system in early RA. The second possible cause is just randomness due to the low number of patients in the study. Notably, during tocilizumab treatment, there was a significant decline in both the ETP and the peak thrombin generation, a change not observed in the other treatment arms.

The analyses of OHP have provided additional information regarding the fibrin generation and fibrinolysis in investigated plasma samples in our study. Augmented OCP levels were found before the initiation of the antirheumatic treatments, while baseline OHP, OFP and CLT remained within the reference levels. The possible explanation for moderate thrombin and fibrin generation at baseline is the short duration of the disease before the inclusion in the study. Despite the high disease activity measured by DAS28-CRP and elevated inflammatory parameters at baseline, the effect on global haemostatic parameters was not mirrored. A significant decrease in OCP and OHP and improvement of OFP and CLT was noted along the treatments, with again strongest effect of tocilizumab.

Taken together, the parameters of overall thrombin and fibrin generation as well as fibrinolysis in plasma might reflect important aspects of the pro-coagulant state in early RA. The relatively short disease duration and acute inflammatory response do not affect the results of these assays. However, using these assays to monitor the effects of antirheumatic treatments on haemostasis appears promising.

Besides global haemostatic parameters, we have also assessed single coagulation biomarkers with the potential to predict VTE, that is, F1+2, fibrinogen and D-dimer. Baseline F1+2 levels were above the reference range, as were those of fibrinogen and D-dimer. In the recent systematic review of the studies regarding biomarkers of importance for cancer-associated VTE, F1+2 is found to be a promising predictor, compared with thrombin-antithrombin complex, and ex vivo thrombin generation.[24] Moreover, the levels of F1+2 are reported to be more valuable than D-dimer in predicting VTE after total knee arthroplasty.[25] In the clinical setting of patients with established RA and high disease activity (median DAS28 of 4.8) the levels of F1+2 were increased compared with healthy controls.[13] Reduction of F1+2 levels up to 60% was achieved during 4 weeks of tocilizumab treatment, however, non-responding patients were excluded from the analysis. F1+2 levels declined in all treatment arms in our study, without differences between treatment arms. This selection of responders to the treatment likely led to higher levels of reduction compared with our findings.

D-dimer is the most universally accepted biomarker for VTE. There are several studies linking increased D-dimer levels to increased risk of VTE.[26–28] It has been recently shown that the disease activity is a major contributor of elevated plasma D-dimer levels in patients with established RA.[29] In our study disease activity was not associated with any of the investigated haemostatic biomarkers. This is probably due to the low number of included subjects, especially when divided in different treatment arms. Still, the reduction of D-dimer levels seen in this study suggests a reduction of VTE risk in these patients after initiation of treatment.

In a study looking at patients who underwent total knee arthroplasty, patients that were treated with tocilizumab had comparable levels of fibrinogen with patients treated in the tocilizumab arm in our study.[30]

Of interest, in our study tocilizumab treatment exhibited a more pronounced decrease in fibrinogen when compared with the other bDMARDs. This phenomenon is likely attributed to the direct inhibition of IL-6 by tocilizumab, leading to subsequent reductions in fibrinogen synthesis.[31] It remains unclear whether the lower levels of fibrinogen seen in the tocilizumab arm led to lower VTE risk compared with the other treatment arms.

A limitation of our study was the relatively low number of patients. However, the prospective design enhances data robustness and offers unique insights into the effects of various initial treatments on coagulation and fibrinolysis. Furthermore, the inclusion of clinical data for these patients is invaluable for assessing correlations between disease activity and laboratory findings.

Altogether, this study shows that patients with early RA have an increased coagulation and fibrinolytic impairment, which can be improved by csDMARD or bDMARD treatment. Treatment with bDMARDs, especially tocilizumab, was most effective in improving this imbalance.

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