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Chronic prothrombotic tendency in patients with granulomatosis with polyangiitis (GPA)

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Short title: Prothrombotic state in GPA

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What’s new?

Granulomatosis with polyangiitis (GPA) is an antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), primarily associated with ANCA directed against proteinase 3 (PR3-ANCA). Patients in an active phase of AAV are at increased risk of venous thromboembolic events. We assessed thrombin generation in patients with GPA using calibrated automated thrombography (CAT) and their plasma fibrinolytic activity measured by clot lysis time (CLT) both in active phase and during remission of their disease. We have shown that the prothrombotic tendency in GPA patients extends beyond the acute phase of the disease to its remission phase, which might persistently increase the risk of thromboembolism.
Abstract

**Introduction.** Granulomatosis with polyangiitis (GPA) patients show increased tendency to thromboembolic phenomena in the active phase of their disease.

**Objectives.** To evaluate thrombin generation potential and fibrinolytic plasma activity in GPA patients, both in the active phase and in GPA remission.

**Patients and methods.** Thirty-eight GPA patients were studied; 18 with active GPA and 20 in remission. Control group consisted of 39 healthy subjects similar in age and sex. Plasma thrombogenic potential was assessed using calibrated automated thrombography. Plasma fibrinolytic potential was studied by clot lysis time (CLT). In all subjects, inflammatory markers, thrombomodulin and fibrinolysis proteins were also measured.

**Results.** In the whole group of GPA patients endogenous thrombin potential was higher by about 25% ($P < 0.001$), while the CLT was lower by about 20% ($P = 0.02$) as compared to controls. Higher ETP and lower CLT together with elevated levels of thrombomodulin and inflammation markers (C-reactive protein, fibrinogen, factor VIII) were also seen between controls and exacerbation and remission subgroups separately, but not between subgroups. The only parameter which differentiated patients with GPA exacerbation from those in remission was D-dimer (1151 ng/mL; IQR 597.2-2468.7 vs. 340.4 ng/mL; IQR 255.1-500.7; $P < 0.001$), a marker of lysis of intravascularly formed fibrin.

**Conclusions.** GPA patients show increased prothrombotic state, both at exacerbation and remission phase of disease. This is probably related to ongoing low grade inflammation and endothelial injury. Large, clinical studies would be required to address the need for, and appropriate type of antithrombotic prophylaxis during the course of GPA.
Introduction

Granulomatosis with polyangiitis (previously known as Wegener’s granulomatosis; GPA) is a necrotizing granulomatosis, characterized by granulomata of the respiratory tract and systemic necrotizing vasculitis that mainly affects small and medium vessels. GPA is an antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), primarily associated with ANCA directed against proteinase 3 (PR3-ANCA). Symptoms of GPA include mainly granulomatous inflammation of the upper and lower respiratory tract, necrotizing vasculitis and glomerulonephritis. It usually starts as granulomatous disease affecting the respiratory tract and then progressing to the generalized vasculitis [1]. PR3-ANCA, neutrophils, neutrophil extracellular traps (NETs), T and B cells and vascular endothelial cells are intimately involved in the GPA pathogenesis [2]. It has been reported that patients with active AAV are at increased risk of venous thromboembolic events (VTE); about 7 cases per 100 person-years, compared to 0.15-0.31 in the general population [3]. Consequently, several other reports have confirmed a prothrombotic tendency, especially during the early and active GPA phase [4-7]. Moreover, an increased risk of arterial events in AVV has also been reported with an estimated prevalence of between 3.1% and 18.7% [8-9]. In 2011 the European Vasculitis Study Group (EUVAS) proposed a prognostic tool to define the 5-year cardiovascular risk for AVV patients. Using this tool, it has been shown that about 12% of newly diagnosed GPA patients reported at least one cardiovascular event (CVE), defined as cardiovascular death, myocardial infarction, coronary bypass/percutaneous coronary intervention or stroke [10]. All of these observations are in line with a more general observation showing an increased tendency of venous thromboembolism in hospitalized patients with various acute systemic rheumatic diseases [11]. Data on the possible prothrombotic mechanisms in GPA are scarce. Endothelial cell dysfunction, a feature of AVV, can lead to an interaction between neutrophils
(activated by TNFα and ANCA) and endothelial cells, with consequent massive oxidative stress finally leading to atherothrombotic complications [12].

Neutrophils are able to release extracellular nucleic acids along with histones and granular proteins capable of entrapping bacterial agents. These NETs have been recently also been implicated in thrombotic events bridging autoimmunity with the hemostatic system [13,14]. In active AAV, neutrophils have also been shown to release tissue factor-expressing NETs [15].

Association between the active phase of the disease and thromboembolism suggests the involvement of mechanisms at the crossroads of thrombosis and inflammation – thromboinflammation [16]. Thrombin is a key component propagating not only thrombosis, but also inflammation.

For this reason, we decided to assess thrombin generation using calibrated automated thrombography (CAT) together with plasma fibrinolytic activity measured by plasma clot lysis time (CLT) in patients with GPA. Plasma hemostatic parameters together with markers of inflammation and endothelial injury were also measured. We attempted to evaluate whether a prothrombotic tendency in GPA extends beyond the acute phase of the disease.

**Patients and Methods**

**Patients**
The study included 38 consecutive adult GPA patients recruited from the Outpatient Clinic for Autoimmune Diseases, Department of Internal Medicine, Jagiellonian University Medical College, Kraków, Poland. The patients were enrolled in the study when they fulfilled the 2012 Chapel Hill Consensus Conference GPA nomenclature criteria [17]. Activity of the disease was assessed according to the Birmingham Vasculitis Activity Score version 3 (BVAS v.3) [18], and chronic organ damage by the Vasculitis Damage Index (VDI) [19]. The exclusion
criteria included: cancer, severe hepatic injury (Child-Pugh class 3), heart failure (NYHA class III or IV), and current anticoagulant therapy. Patients were considered to be in the remission phase if their BVAS score was 0.

This study was approved by the local Ethics Committee, and informed consent was obtained from all patients according to the Declaration of Helsinki.

**Laboratory investigations**

Venus blood samples were drawn in 3.2% (0.109 mol/L) sodium citrate tubes (one part sodium citrate to nine parts venous blood), then centrifuged at 2000 x g for 10 min within 30 minutes after drawing, and stored in aliquots at -80°C for further analysis.

Blood from patients in the acute phase of the disease was drawn before the start of the induction immunosuppressive therapy with cyclophosphamide and/or rituximab. They were receiving small to median doses of glucocorticosteroids (GKS), at a median daily dose of 18.2 mg (range from 0 to 80.0 mg) in the acute phase and 3.8 mg (range from 0 to 14.0 mg) in remission.

Complete blood counts, biochemical parameters (glucose, creatinine) and basic coagulation tests (prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen) were determined using routine laboratory assays. C-reactive protein (CRP), C3 and C4 were measured by nephelometry (Siemens, Marburg, Germany). Antineutrophil cytoplasmic antibodies (ANCA) were measured by indirect immunofluorescence (Euroimmun, Lubeck, Germany). The identification of specific anti-proteinase (PR3) and anti-myeloperoxidase (MPO) antibodies was performed by the immunoenzymatic assays (ELISA anti-PR3 and ELISA anti-MPO, Euroimmun, Lubeck, Germany).

Commercially available immunoenzymatic assays were used to determine plasma tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) antigens (both
Hyphen Biomed, Neuville, France), thrombin-antithrombin complexes (TAT, Enzygnost TAT micro, Siemens, Marburg, Germany), thrombomodulin (Human Thrombomodulin Elisa Kit, Biorbyt LLC, San Francisco, USA). Activity of plasma α2-antiplasmin (α2AP) and plasminogen were measured by chromogenic assays (Berichrom α2-antiplasmin and Berichrom plasminogen; Siemens, Marburg, Germany). Concentrations of D-dimer were assessed using turbidimetric method (Innovance D-dimer; Siemens, Marburg, Germany). Factor VIII activity was measured using a coagulometric assay with FVIII deficient plasma (Siemens, Marburg, Germany).

The activity of antithrombin and protein C were determined with chromogenic methods (Innovance Antithrombin, Berichrom Protein C; Siemens, Marburg, Germany).

Concentrations of free protein S was assessed using turbidimetric method (Innovance Free Protein S; Siemens, Marburg, Germany).

Lupus anticoagulant (LA) was determined in a three-step procedure according to the guidelines of the ISTH [20]. Anticardiolipin (aCL) and anti-β2-glycoprotein I (aβ2GPI) antibodies of IgG and IgM isotype were assessed using enzyme-linked immunosorbent assays QUANTA Lite® aCL and β2GPI (Inova Diagnostics, Saint Louis, USA) according to ISTH guidelines [21].

Genotyping for factor V Leiden R506Q (mutation rs6025) and prothrombin G20210A (mutation rs1799963) was performed as previously described [22,23] or by using TaqMan assays (Applied Biosystems). All the tests were performed in both, patients and controls.

**Plasma thrombogenic potential**

Plasma thrombogenic potential was assessed according to the manufacturer’s instructions using calibrated automated thrombography (CAT) with a computational model of thrombin dynamics (Thrombinoscope BV, Maastricht, the Netherlands). Duplicate plasma samples
were analyzed in the 96-well plate fluorimeter (Ascent Reader, Thermo Lab Systems OY, Helsinki, Finland) equipped with the 390/460 filter at 37°C. Briefly, 80 µl platelet-poor plasma was diluted with 20 µl of a tissue factor (TF)-based activator (Diagnostica Stago, Asnieres, France) and 20 µl of FluCa solution (Diagnostica Stago, Asnieres, France). We assessed peak thrombin generation (maximum concentration of thrombin formed during the recording time), time to peak, lag time and endogenous thrombin potential (ETP; area under curve) [24,25].

**Clot lysis time (CLT).**

Clot lysis time was measured as previously described [26]. Briefly, citrated plasma was mixed with 15 nM calcium chloride, human thrombin (Merck, Kenilworth, NJ, USA) at a final concentration of 0.5 U/ml, 10 µM phospholipid vesicles and 18 ng/ml recombinant t-PA (Boehringer Ingelheim, Ingelheim, Germany). The mixture was transferred to a microtitre plate and its turbidity was measured at 405 nm at 37°C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition, which represents clot formation, to the midpoint of the maximum–turbid-to-clear transition. Intra-assay and inter-assay coefficients of variation were 6-8 %. The assay was performed in triplicates.

**Statistical analysis**

The results were obtained using STATISTICA Tibco 13.3 software. Data distribution was evaluated by the Shapiro-Wilk test. All continuous variables were non-normally distributed, thus were presented in the manuscript as a median with first and quartile range and compared by Mann-Whitney U-test, Kruskal-Wallis and we can use multiple comparison tests with the Bonferroni correction, as appropriate. Categorical variables were compared by the Chi² test. To evaluate the relationship between continuous variables a Spearman rank correlation test
was utilized. Results that presented p value of less than 0.05 were considered statistically significant.

**Results**

A total of 38 patients with GPA were studied, including 18 subjects in the exacerbation phase and 20 patients in remission of their disease (BVAS=0). Demographic and laboratory characteristics of the patients are presented in table 1. The median disease duration was 1.5 years (IQR: from 1 month to 8 years since diagnosis). The control group was comprised of 39 healthy volunteers of similar age and sex who gave no history of any thromboembolic complications and were not taking any antithrombotic drugs. Their BMI and smoking status was not different from GPA patients.

All patients in remission had completed the induction immunosuppressive therapy at least 6 months before the blood drawing and were receiving small doses of GKS, 3 subjects were on maintaining therapy with methotrexate (MTX), and 3 patients received azathioprine (AZA). Low dose aspirin was taken by 6 (15.8%) subjects (3 in acute phase and 3 in remission phase of the disease). None of them suffered from any thromboembolic complications in the past nor was treated with anticoagulant drugs.

Among 18 exacerbated patients, 11 (61.1%) presented with a generalized form of GPA and 7 (38.9%) with its limited form. The most frequently affected organs were ear/nose/throat (n=13; 72.2%), lungs (n = 13; 72.2%), and kidneys (n = 11; 61.1%). Among 20 patients in remission 7 (35.0%) presented with the generalized form of the disease, while 13 (65.0%) with its limited form. The most frequently chronically damaged organs were lungs (n=13; 65.0%) and kidneys (n=11; 55.0%).
Positive PR3-ANCA were detected in all patients in the exacerbation phase (median: 40.0 I.U./mL, IQR 17.0-71.0 I.U./mL) and in 13 (65.0%) of the patients in remission (median: 7.5 I.U./mL, IQR 1.6-52.5 I.U./mL).

Patients with vasculitis, both in the exacerbation and the remission phase showed typically lower values of hematological parameters as compared to healthy controls. Parameters of kidney function were slightly but insignificantly higher in both groups of patients. As expected, all patients presented with elevated serum inflammatory markers (CRP, fibrinogen, factor VIII activity) as compared to healthy subjects. All of those parameters were understandably higher in the acute phase of the disease.

**Thrombin generation assay and fibrinolytic activity**

GPA patients showed significantly altered thrombin generation profile compared to the control group (Table 2; Supplementary material, *Figure S1*), only slightly less evident for the “time to peak” parameter. Higher thrombin generation parameters clearly differentiate GPA patients from control subjects (p<0.001 for all parameters) but could not significantly differentiate between exacerbation and remission phases of the disease. Nevertheless, a certain uniform trend towards higher values for endogenous thrombin potential (ETP), peak thrombin and TAT during exacerbation phase could be seen, as compared to the patients in remission. CAT parameters in individual patients correlated with TAT, a plasma thrombin generation marker. In the entire GPA group there was a negative association between TAT and the time to peak, and lag time (rho= -0.55, P<0.001 and rho= -0.47, P=0.003, respectively). In the exacerbation group negative association was found between TAT and ETP, and time to peak (rho= -0.56, P=0.02 and rho= -0.50, P=0.04). In the remission group a significant positive correlation was observed between TAT and the peak of thrombin generated (rho=0.47, P=0.04). In addition, ETP was positively correlated with fibrinogen levels: rho=0.50; P< 0.001. Otherwise we did not find any significant associations between
parameters describing thrombin generation and the remaining hemostatic parameters measured, including disease activity (BVAS) or measures of organ damage (VDI) – a result which is most probably related to the small number of subjects in the groups studied.

Among plasma components of the fibrinolytic system, only plasminogen and t-PA antigen levels were significantly higher in GPA patients as compared to healthy controls \((P=0.001\) and \(P=0.008\), respectively). These changes also influenced at least in part, the results of the test which generally assessed in vitro plasma fibrinolytic potential, namely clot lysis time (CLT). Impaired fibrinolysis in GPA patients was reflected by a significantly prolonged CLT both in the exacerbation phase \((p=0.02)\) and in remission \((p=0.04)\), but again with no difference between GPA patients during exacerbation and in the disease remission (Table 2; Supplementary material, Figure S2). In the entire GPA group CLT showed positive correlation with all plasma components of the fibrinolytic system measured: alfa2-antiplasmin, t-PA, PAI-1 and plasminogen \((\rho=0.54, \rho=0.45, \rho=0.66, \rho=0.42\) respectively; \(P<0.001\) for all). These associations were even stronger in the remission group \((\rho=0.58, \rho=0.57, \rho=0.71, \rho=0.57\) respectively; \(P<0.001\) for all). Interestingly, in the exacerbation group CLT positively associated only with alfa2-antiplasmin and with PAI-1 \((\rho=0.52, P=0.003\) and \(\rho=0.80, P<0.001\)), both inhibitors of fibrinolysis. These findings were accompanied by elevated levels of D-dimers, especially marked in patients in the exacerbation phase of the disease. D-dimer levels showed a positive association with one of the main inflammation markers – CRP \((\rho=0.51, P<0.001)\).

**Endothelial dysfunction**

Thrombomodulin (TM), an indirect marker of endothelial injury, was significantly elevated in both the exacerbation \((p<0.001)\) and remission phase \((p=0.002)\) of GPA as compared to controls but with no difference between these subgroups (Table 1). Thrombomodulin levels positively correlated with D-dimers and activity of factor VIII in all GPA patients \((\rho=0.63\)
and \( \rho = 0.53 \), respectively; \( P < 0.001 \) for both) and in exacerbation subgroup (\( \rho = 0.75 \) and \( \rho = 0.64 \), respectively; \( P < 0.001 \) for both). In the remission subgroup positive correlation of thrombomodulin levels with D-dimer was maintained (\( \rho = 0.63; P < 0.001 \)) and, in addition, a new association was shown with plasma fibrinogen levels (\( \rho = 0.54; P < 0.001 \)). In the exacerbation phase there was also a weak association between thrombomodulin and the BVAS score (\( \rho = 0.48; P = 0.04 \)), paralleled by a similar association with the VDI score in remission (\( \rho = 0.52; P = 0.02 \)).

Among 38 GPA patients 11 (28.9%) had eGFR ≤ 30 ml/min. There were no significant differences in the majority of thrombotic and fibrinolytic parameters measured between patients with eGFR <30 ml/min and the remainder. Only levels of thrombomodulin, D-dimer, and factor VIII were significantly higher in patients with eGFR ≤30 ml/min (\( P < 0.001 \), \( P = 0.008 \), \( P = 0.01 \), respectively).

Testing for hereditary thrombophilia showed heterozygosity for prothrombin mutation (G20210A) in three patients and no factor V Leiden mutation (G1691A). Natural anticoagulants (AT, PC, PS) were within normal limits. Antiphospholipid antibody testing (LA, aCL and aβ2GPI) was negative in all patients. There were no thrombophilic defects in any of the control subjects.

**Discussion**

To the best of our knowledge, our study is the first to show that in patients with GPA thrombin generation is elevated in parallel with impaired fibrinolysis, both in the active phase of the disease and in its remission. Those two phenomena may explain, at least in part, increased prothrombotic tendency and higher incidences of thromboembolic complications in GPA patients.
In comparison with healthy controls our patients were also characterized by increased levels of inflammation markers and indicators of endothelial dysfunction (see tab.1), with an insignificant though visible trend to higher values in the active phase of GPA. Increased thrombin generation parameters and decreased fibrinolytic activity in GPA patients showed some significant associations with markers of inflammation and endothelial dysfunction (see infra).

**Thrombin generation potential**

Plasma thrombogenic potential assessed using calibrated automated thrombogram is recommended to provide a comprehensive insight into prothrombotic plasma properties [27]. In the only previous study Hilhorst et al. [28] using CAT method have shown increased thrombin potential in AAV patients, all in the remission phase of their disease. The majority of their patients were diagnosed with GPA. The authors were also able to show significant correlation of ETP with the plasma activity of factor VIII, interpreted as a marker of endothelial cell activation and dysfunction. In our study, ETP showed positive correlation with fibrinogen level – one of the acute phase reactants.

**Plasma fibrinolytic potential**

To assess overall plasma fibrinolytic activity, clot lysis time was used, showing clearly prolonged lysis time in all GPA patients as compared to healthy controls. The only other study in the literature examining clot lysis in AAV involved patients with eosinophilic granulomatosis with polyangiitis and showed denser fibrin clots and prolonged lysis time in vasculitic patients [29]. In GPA such prothrombotic clot phenotype with impaired clot lysis may be, at least partially, explained by the presence of anti-plasminogen autoantibodies in antiPR-3 ANCA patients [30,31]. These antibodies were shown to delay conversion of plasminogen to plasmin and to increase lysis time of fibrin clots [29]. At the same time,
however, elevated circulating levels of D-dimer indicate constant on-going fibrin lysis in GPA patients, significantly more pronounced in the acute phase of the disease. It may indicate increased fibrin deposition and turnover with resulting hypercoagulable state, most pronounced in GPA exacerbation, as already suggested by Ma et al. [32].

**Endothelial dysfunction**

Patients with antineutrophil cytoplasmatic antibody-associated vasculitis have an increased risk of developing venous thromboembolism especially when the disease is in an active phase [3-7]. Such an increased risk for arterial events in AVV has also been reported [8,9]. Our findings may explain major prothrombotic mechanisms leading to thrombosis in GPA involving both increased thrombin generation and impaired fibrinolysis. Exact mechanisms probably involve multiple pathways, activated by autoimmune inflammation and tightly related to the activation and dysfunction of endothelial cells with an initiation and propagation of thrombosis [16]. In keeping with the concept of immunothrombosis, our results indirectly indicate endothelial dysfunction in GPA patients (e.g., increased factor VIII activity and thrombomodulin levels), both in the exacerbation phase and in remission of their disease. Positive associations shown between TM levels and D-dimers, activity of factor VIII, fibrinogen and BVAS, indicate an interplay between endothelial dysfunction, disease activity, inflammation, and hypercoagulability all typical for the process of thromboinflammation [16].

It also concurs with findings presented in our previous study [33] and those of others [34] supporting activation and disturbed endothelial function in patients with GPA.

**Limitations of study**

Our study has several limitations. First, the number of patients included in the study was low in both the exacerbation and remission phases of the disease. Secondly, both subgroups slightly differed in their clinical characteristics. Increasing the number and clinical uniformity of patients studied would make conclusions more solid. Particularly, more convincing
evidence could have been offered for the correlation between hypercoagulability and activity of the disease and other possible associations between parameters of thrombin generation and fibrinolysis, and inflammation markers or other biochemical markers. It is also impossible to assess the real risk of VTE associated with the increase in thrombin generation and impairment of fibrinolysis measured as there was no follow-up of the patients studied. In addition, the possible influence of the patients’ treatment on parameters measured has not been analyzed. These limitations arise mainly from the fact that GPA is a rare disease. For this reason, the Polish registry for AAV has been created [35] to facilitate research in this field. Regardless of all these limitations results of the present study clearly show prothrombotic and hypofibrinolytic changes associated with inflammation and endothelial dysfunction in patients with GPA.

In summary, our study shows that in patients with granulomatosis with polyangiitis there is evidence of hypercoagulability which extends from the acute phase of the disease to its more prolonged remission phase, which might persistently increase the risk of thromboembolism. It may call for more extended antithrombotic prophylactic measures.

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**Contribution statement:** TI contribution to concept and design, analysis and interpretation of data: MCL, KWA, JKW, KW clinical evaluation of the patients. LZ interpretation of data and statistical analyses. MZ analysis and interpretation of data. JM critical writing and revision the intellectual content; final approval of the manuscript.
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Table 1. Characteristics of patients with granulomatosis with polyangiitis and the control group.

| Variable         | GPA (all patients, n=38) | GPA (exacerbation, n=18) | GPA (remission, n=20) | Controls (n=39) |
|------------------|---------------------------|---------------------------|------------------------|-----------------|
| Female/Male      | 21/17                     | 10/8                      | 11/9                   | 19/20           |
| Age, years       | 55.0 (39.5-61.7)          | 53.0 (39.0-64.7)          | 56.0 (40.5-61.2)       | 44.0 (40.0-58.5) |
| BVAS v. 3        | 18.0 (16.0-25.0)          |                           |                        | N.A.            |
| VDI              |                           |                           | 5.0 (4.0-6.0)          | N.A.            |

Basic laboratory tests

| Variable         | GPA (all patients, n=38) | GPA (exacerbation, n=18) | GPA (remission, n=20) | Controls (n=39) |
|------------------|---------------------------|---------------------------|------------------------|-----------------|
| RBC x 10^6/µl    | 4.3³ (3.8-4.7)            | 4.1² (3.6-4.5)            | 4.4 (4.2-5.1)          | 4.7 (4.5-5.0)   |
| Hgb, g/dl        | 12.5² (10.9-13.9)         | 11.5³ (10.2-13.5)         | 13.1⁴,⁵ (12.1-14.6)    | 14.3 (13.8-14.7) |
| Hct, %           | 37.5³ (33.6-42.3)         | 35.3⁴ (31.6-40.5)         | 39.8⁴,⁵ (35.6-43.5)    | 43.1 (41.6-43.8) |
| WBC x10^³/µl     | 8.4² (6.1-10.9)           | 10.0² (6.5-11.7)          | 7.1³ (5.8-9.6)         | 5.8 (4.6-7.0)   |
| Platelets x10^³/µl | 260.0² (226.0-334.0)     | 278.5² (253.0-445.0)     | 232.0 (206.0-295.0)    | 224.0 (208.0-247.0) |
| Glucose, mmol/l  | 5.3³ (5.0-6.4)            | 5.7³ (5.1-6.4)            | 5.3³ (4.9-6.4)         | 4.6 (4.4-5.4)   |
| Urea, mmol/l     | 6.8³ (4.9-12.5)           | 8.4³ (4.6-16.3)           | 5.4 (5.0-10.2)         | 5.1 (4.3-5.4)   |
| Creatinine, µmol/l | 79.7³ (70.4-205.3)       | 104.3³ (70.4-285.6)      | 77.7 (70.3-163.6)      | 71.1 (67.2-82.3) |
| Aspartate transaminase, U/l | 16.0 (14.0-21.0) | 15.0 (12.0-21.0) | 17.5 (14.5-21.0) | 16.4 (13.7-18.8) |
| Alanine transaminase, U/l  | 19.0 (13.0-25.0)        | 14.5 (12.0-29.0)         | 21.5 (16.0-24.0)       | 14.7 (12.6-22.3) |
| PT, sec          | 11.4 (10.9-12.2)          | 12.1³ (11.4-12.9)         | 11.3³ (10.7-11.7)      | 11.1 (10.7-12.0) |
| aPTT, sec        | 26.1 (23.1-28.0)          | 27.1 (23.5-30.0)          | 25.6 (22.9-27.6)       | 26.6 (24.9-28.4) |
| Fibrinogen, g/l  | 4.6³ (3.9-5.7)            | 5.0³ (3.8-5.8)            | 4.3³ (3.9-5.2)         | 2.4 (2.2-3.3)   |
| Factor VIII, %   | 202.4³ (140.2-291.0)     | 257.1³ (205.4-310.7)     | 159.4³,⁴ (131.5-218.8)| 113.4 (93.9-139.1) |
| CRP, mg/l        | 6.5 (3.5-14.3)            | 12.1 (5.6-84.8)           | 4.3³ (2.4-8.9)         | ND³⁶        |
| C3, g/l          | 1.21                      | 1.23                      | 1.21                   | ND³⁶        |
|                | (1.13-1.35) | (1.15-1.32) | (1.12-1.36) | ND<sup>f</sup> |
|----------------|-------------|-------------|-------------|----------------|
| **C4, g/l**    | 0.26        | 0.24        | 0.27        |                |
|                | (0.23-0.34) | (0.21-0.30) | (0.24-0.34) |                |
| **Thrombomodulin, pg/ml** | 7321.5<sup>a</sup> | 8480.5<sup>b</sup> | 6528.9<sup>c</sup> | 5204.5         |
|                | (5618.1-11252.7) | (5349.6-11682.0) | (5618.1-11078.7) | (4752.0-5987.3) |

aPTT – activated partial thromboplastin time, BVAS - Birmingham Vasculitis Activity Score, CRP - C-reactive protein, GPA - granulomatosis with polyangiitis, Hct – hematocrit, Hgb-hemoglobin, PT – prothrombin time, RBC – red blood cells, WBC – white blood cells, VDI - Vasculitis Damage Index.

Data are presented as median with lower and upper quartiles; <sup>a</sup> significant differences between the whole GPA group vs controls; <sup>b</sup> significant differences between GPA exacerbation vs controls; <sup>c</sup> significant differences between GPA remission vs controls; <sup>d</sup> significant differences between GPA exacerbation vs GPA remission; <sup>e</sup> N.A. – not applicable, <sup>f</sup> N.D. – not determined.
Table 2. Comparison of thrombin generation and fibrinolytic parameters between patients with granulomatosis with polyangiitis and the control group.

| Variable                  | GPA (whole group, n=38) | GPA (exacerbation, n=18) | GPA (remission, n=20) | Controls (n=39) |
|---------------------------|--------------------------|--------------------------|-----------------------|-----------------|
| ETP, nM/min               | 1994.7a (1700.2-2261.4)  | 2027.9b (1792.0-2338.9)  | 1878.0c (1676.8-2156.3) | 1600.7 (1397.1-1714.0) |
| Lag time, min             | 3.5a (3.1-4.1)           | 3.4b (3.1-3.7)           | 3.7c (3.1-3.7)         | 3.0 (2.7-3.6)   |
| Peak TG, nM               | 382.0a (338.6-466.5)     | 423.6b (367.3-494.5)     | 369.0c (331.4-432.8)   | 303.7 (260.5-339.9) |
| Time to peak, min         | 5.7 (5.3-6.7)            | 5.7 (5.3-6.1)            | 6.2c (5.4-7.0)         | 5.3 (5.1-6.7)   |
| TAT, µg/l                 | 5.1a (3.4-9.7)           | 7.8b (3.7-12.6)          | 4.7 (3.4-8.0)          | 4.2 (3.1-5.2)   |
| CLT, min                  | 119.0a (89.0-173.0)      | 119.0b (92.0-173.0)      | 115.9c (87.9-195.2)    | 95.8 (85.9-109.9) |
| D-dimer, ng/ml            | 550.6* (309.0-1260.4)    | 1151.0b (597.2-2468.7)   | 340.4c,d (255.1-500.7) | 231.0 (178.2-362.0) |
| α2-antiplasmin, %         | 106.5 (102.6-115.2)      | 105.4 (101.8-115.2)      | 107.7 (103.8-113.9)    | 109.7 (102.1-115.1) |
| Plasminogen, %            | 109.9a (98.3-125.5)      | 109.1b (102.1-125.4)     | 111.6c (93.9-124.5)    | 98.9 (88.2-105.6) |
| t-PA:Ag, ng/ml            | 9.7a (6.3-11.9)          | 8.4 (6.7-11.7)           | 10.3c (5.9-11.9)       | 7.0 (4.0-9.6)   |
| PAI-1:Ag, ng/ml           | 18.3 (11.2-61.8)         | 14.6 (11.3-74.6)         | 19.1 (13.3-32.8)       | 15.4 (8.9-23.6)  |

CLT – clot lysis time, ETP – endogenous thrombin potential, GPA – granulomatosis with polyangiitis, PAI-1 – plasminogen activator inhibitor-1, peak TG – peak thrombin generation, TAT – thrombin-antithrombin complex, t-PA – tissue-type plasminogen activator.

Data are presented as median with lower and upper quartiles; *significant differences between the whole GPA group vs controls; a significant differences between GPA exacerbation vs controls; b significant differences between GPA remission vs controls; c significant differences between GPA exacerbation vs GPA remission.