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Low factor XIII levels and altered fibrinolysis in patients with multiple myeloma

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ABSTRACT

Background: Acquired factor FXIII (FXIII) deficiency can be immune- or non-immune mediated and may cause severe bleeding symptoms. The incidence of acquired FXIII deficiency and its etiology in patients with multiple myeloma (MM) are poorly understood.
Objectives: To assess FXIII levels and the balance of fibrinolysis in newly diagnosed, untreated MM and monoclonal gammopathy of undetermined significance (MGUS) patients.
Methods: FXIII activity, mixing studies, FXIII-A_2B_2 antigen, total FXIII-B antigen were measured in platelet-poor plasma from 17 untreated MM patients, 33 untreated MGUS patients, and 30 age and sex-matched healthy controls. Besides routine laboratory measurements, the balance of coagulation and fibrinolysis was evaluated using quantitative fibrin monomer (FM) test, thrombin-antithrombin assay, α2-antiplasmin activity, plasmin-α2-antiplasmin (PAP) complex, D-dimer, plasmin generation assay, clot lysis assay, and ClotPro-TPA test.
Results: FXIII-A_2B_2 levels were significantly lower in MM patients compared to controls [median (IQR):14.6 (11.2–19.4) vs. 21.8 (17.1–26.4) mg/L, $p = 0.0015$], whereas total FXIII-B did not differ between groups. Decrease in FXIII activity was parallel to the decrease in FXIII-A_2B_2. An immune-mediated inhibitory mechanism was ruled out. Free/total FXIII-B was significantly higher in MM patients compared to MGUS and healthy controls, suggesting an etiology of FXIII-A consumption. In MM and MGUS patients, FM, D-dimer, and PAP complex were significantly elevated compared to controls, indicating hypercoagulability and ongoing fibrinolysis.
Conclusions: Low FXIII levels due to consumption were observed in MM patients at diagnosis. Hypercoagulability and ongoing fibrinolysis were detected in MM and MGUS, indicating that a disturbed hemostasis balance is already present in the latter benign condition.

1. Introduction

Plasma cell dyscrasias (PCDs) are a heterogeneous group of disorders characterized by the proliferation of monoclonal bone marrow plasma cells that produce monoclonal immunoglobulins [1]. The spectrum of PCDs includes multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS), among other disorders (smoldering MM, amyloidosis, PC leukemia, etc.). PCDs increase the risk of thrombotic complications and bleeding [2,3]. Venous thromboembolism (VTE) has previously been reported in patients with MM [4,5] and

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asymptomatic MGUS [6–10]. Treatment with immunomodulatory drugs including thalidomide, lenalidomide and pomalidomide increases VTE in MM by up to 26 % [11]. Thrombosis in MM and MGUS has been associated with high levels of factor VIII (FVIII), von Willebrand factor (VWF), decreased protein S activity, and increased thrombin generation (TG) [12–16]. We recently reported that, in contrast to patients with MGUS, APC cannot inhibit TG in newly diagnosed MM patients, which may increase their prothrombotic tendency [12]. Reduced plasma fibrinolytic potential has been described as a risk factor for the development of thrombosis [17,18], however, alterations of fibrinolysis in patients with MM or MGUS have been only partially revealed. In MM, prothrombotic fibrin clot phenotype and alterations in fibrin clot properties by monoclonal immunoglobulins [19,20] or antimyeloma therapy [21] have been described. On the other hand, little is known about the individual levels of important regulators of fibrinolysis, e.g., factor XIII (FXIII) and its effect on the balance of fibrinolysis in patients with PCDs [22–24]. Few contradictory reports on highly increased (up to 600 %) levels and markedly decreased FXIII levels have been published, but these early reports used a methodology that is now obsolete for the detection of FXIII activity or antigen levels [22,24].

The clinical significance of such investigations lies in the fact that while overt bleeding is rare in patients with PCDs [2], bleeding complications are still a frequent cause of morbidity and mortality [25]. Bleeding complications have been reported in 13 % of IgG and 33 % of IgA myeloma patients, respectively, and 36 % of macroglobulinemia cases [2,26–28].

Factor XIII (FXIII) is a plasma protransglutaminase that after activation by thrombin, stabilizes the fibrin clot and protects it from degradation by the fibrinolytic system [29]. FXIII is of tetrameric structure (FXIII-A_2B_2) consisting of two, potentially active A subunits and two protective/regulatory B subunits. The B subunits (FXIII-B) are present in plasma in ~50 % excess. By cross-linking fibrin α and γ chains and cross-linking α2-plasmin inhibitor to fibrin, activated FXIII (FXIIIa) is one of the most important regulators of clot firmness and stability [30]. Acquired FXIII deficiency is a rare bleeding disorder in the general population that may be associated with severe bleeding symptoms. Causes of acquired FXIII deficiency include immune-mediated inhibition (autoantibody against FXIII), as well as non-immune mediated consumption or decreased synthesis [31–34]. Whereas immune-mediated FXIII deficiency is generally associated with a potentially life-threatening bleeding phenotype, the clinical presentation of FXIII deficiency associated with consumption or hyposynthesis may vary significantly, depending on many factors including FXIII levels. In any case, recognition of low FXIII levels and the underlying etiology is imperative, as treatment options and management vary depending on FXIII levels and pathophysiology.

The goal of this study was to test the possibility of acquired FXIII deficiency in newly diagnosed MM and MGUS patients, to determine the etiology and its potential effects on the balance of fibrinolysis.

2. Materials and methods

2.1. Study population

Newly diagnosed, untreated MM or MGUS patients were recruited from two medical centers: the Department of Internal Medicine at the University of Debrecen and the Department of Hematology at the Jósa András Teaching Hospital in Nyíregyháza, Hungary. The international myeloma working group (IMWG) criteria were used to diagnose MM and MGUS [35]. MM patients were staged using the International Staging System (ISS) [36]. As a few hemostasis or fibrinolysis markers investigated in the study are known to be influenced by age-, sex, or blood group [37,38], the control group consisted of age-, sex-, and blood group-matched healthy blood donors. Both patients and controls were subjected to exclusion criteria, including inherited thrombotic or hemorrhagic disorders, antiphospholipid syndrome (APS), liver disease, history of other malignancies, exposure to anticoagulant or antiplatelet drugs (<1 month to enrollment), major surgery or trauma within 1 month before enrollment, thrombocytopenia (with a platelet count of $<100 \times 10^9$/L), hypofibrinogenemia (fibrinogen concentration < 1.5 g/L), increased cardiovascular risk, acute illnesses, and end-stage renal failure. Notably, moderate hypertension was not a reason for exclusion. All patient samples underwent serum protein electrophoresis (SPE) and immunofixation using routine methods according to the manufacturer's instructions (Hydrasys 2 analyzer, Hydragel IF kits, Sebia, Lisses, France). Paraproteins were detected as homogeneous fractions in the gamma (γ) or beta (β) globulin region and classified as IgG, IgM, IgA, or IgD kappa (κ) or lambda (λ). No paraproteins were detected by SPE analysis of control samples.

2.2. Informed consent

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Debrecen (approval number: DE RKEB/IKEB 5906-2021). All participants provided written, informed consent voluntarily.

2.3. Blood sampling and processing

Peripheral blood samples were drawn from all participants into vacutainer tubes containing ethylene diamine tetra-acetic acid (K_3 EDTA, 3.2 %, 0.105 M), sodium citrate, or serum separator (Becton Dickinson, San Jose, CA). Complete blood count (CBC) and ClotPro measurements were performed on the EDTA and citrated whole blood, respectively. Lactate dehydrogenase (LDH), total protein, and total calcium were measured in frozen sera. For the hemostasis and fibrinolysis assays, plasma was separated from citrated whole blood by centrifugation at 1500*g*, 15 min, room temperature (RT). Depending on the assay, platelet-poor plasma (PPP) was obtained by consecutive centrifugation at either 1500*g*, 15 min or 10,000*g*, 10 min at RT. Plasma samples were aliquoted and stored at −70 °C until analysis.

2.4. Laboratory investigations

CBC and chemistry tests were performed on an ADVIA-2120i analyzer (Siemens Healthcare Diagnostics, Marburg, Germany) and a Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany), respectively, according to the manufacturer's instructions. Screening tests of hemostasis (prothrombin time, activated partial thromboplastin time, and thrombin time) and fibrinogen concentration by Clauss were determined by standard methods on a BCS-XP coagulometer (Siemens Healthcare Diagnostic Products, Marburg, Germany). Plasma levels of FXIII activity were determined by an ammonia release assay using commercially available reagents (Technochrome FXIII, Technochlone, Austria). To evaluate the presence of an inhibitor, FXIII activity assay was repeated in all cases where FXIII activity was below the lower limit of reference (69 %), performing a 1:1 mixing study of the patient and healthy controls' plasma. A sandwich enzyme-linked immunosorbent assay was used to determine the levels of FXIII-A_2B_2 antigen levels [39] as well as the total FXIII-B subunits [40]. Free FXIII-B subunit levels were calculated based on FXIII-A_2B_2 and total FXIII-B subunit levels according to the following formula: free FXIII-B = (total FXIII-B)-[FXIII-A_2B_2 × (0.49)]. Quantitative fibrin monomer (FM) was measured using the Liatest FM assay (Diagnostica Stago, Asnieres, France). Commercially available ELISA kits were used to determine plasmin-α2-antiplasmin (PAP) complex (Technozym PAP complex ELISA kit, Technoclone, Vienna, Austria), and thrombin-antithrombin (TAT) complex levels (Enzygnost TAT micro, Siemens Healthcare Diagnostic Products, Marburg, Germany). Quantitative D-dimer levels were measured using a particle-enhanced, immuno-turbidimetric assay (Innovance D-dimer) on a BCS-XP coagulometer (Siemens Healthcare Diagnostic Products, Marburg, Germany). α2-plasmin inhibitor (α2-PI)

activity and plasminogen activity were determined by commercially available methods (Siemens Healthcare Diagnostic Products, Marburg, Germany).

2.5. Viscoelastometry

The ClotPro point-of-care device (Enicor GmbH, Munich, Germany) and its consumables were used for viscoelastometric tests. Briefly, clot formation is initiated by electronically pipetting 340 μL of citrated whole blood into an 'active tip' filled with activator reagents [41,42]. The sample is mixed with the reagent during pipetting and immediately transferred to a cylindrical cup and pin. An elastic element rotates the cup alternately in a clockwise and anticlockwise direction during the measurement, while the pin remains stationary. As a clot forms, the analyzer records the restriction of movement in the cup. The data is used to generate thromboelastographic amplitude values that are plotted against time. The measurements were performed at 37 °C for 40 min and automatically terminated by the software. Quality control measurements were routinely performed as recommended by the manufacturer. The TPA-Test is a tissue factor (TF)-activated coagulation test that also contains recombinant tissue plasminogen activator (rtPA, 650 ng/mL). The following ClotPro parameters were evaluated: clotting time [CT (s); the time from the start of the test until a clot amplitude of 2 mm is reached], maximum clot firmness [MCF (mm); the maximum amplitude reached during the test], and lysis time [LT (s); the time from CT until 50 % of fibrinolysis is detected].

2.6. In vitro clot lysis assay (CLA)

CLA was performed as previously described [43]. Briefly, plasma samples were thawed in a water bath at 37 °C. In the wells of a 96-well microtiter plate (Greiner Bio-One International GmbH, Kremsmünster, Austria), a clot induction and lysis mix was prepared in HEPES buffer (10 mM HEPES, 150 mM NaCl, 0,05 % Tween20, pH 7.4), where citrated plasma was mixed with 1000-fold diluted human TF (Innovin, Siemens, Marburg, Germany) and 100 ng/mL rtPA (Alteplase, Boehringer Ingelheim, Ingelheim, Germany). The plasma was diluted with buffer 1.2 times. Clotting and subsequent lysis were induced by pipetting HEPES buffer containing $CaCl_2$ (21 mM) into each sample well automatically. All concentrations are given as final concentrations in a final well volume of 100 μL. Turbidity was monitored at 340 nm using a TECAN Infinite m200 microplate reader (TECAN Trading AG, Mannedorf, Switzerland) every minute for 300 min at 37 °C. Samples were tested in quadruplicate. The Shiny App software tool [44] was used to analyze curves. Clot formation and lysis were defined using the following variables calculated from the turbidimetric curves: maximum absorbance, time to maximum absorbance, various clot lysis time (CLT) points: 10% CLT, 50%CLT, 90%CLT, and area under the curve (CLA AUC). Clot lysis times were defined as the time from the 10 %, 50 %, or 90 % point, from clear to maximum turbidity, to the 10 %, 50 %, or 90 % point in the transition from maximum turbidity to the final baseline turbidity, respectively.

2.7. Plasmin generation (PG) assay

PG was assessed in plasma using a calibrated automated method, as published earlier, with some modifications [45]. Briefly, 70 μL of reagent containing 5 pM TF, 4 μM phospholipids, and 1.25 μg/mL rtPA (Synapse Research Institute, Maastricht, The Netherlands) or calibrator (Synapse Research Institute, Maastricht, The Netherlands) was pipetted into a 96-well black plate (Greiner Bio, One North America Inc., Monroe, MI, USA). Thirty microliters of freshly thawed PPP was added and incubated at 37 °C for 10 min. PG was started by the automatic dispensation of 20 μL of a solution containing plasmin substrate (Boc-Glu-Lys-Lys-AMC) and $CaCl_2$ final concentrations, (500 μM and 16.7 mM respectively) using the Fluoroskan Ascent FL fluorimeter, (Thrombinoscope BV, Maastricht, The Netherlands). The following PG parameters were evaluated using the Thrombinoscope software (Thrombinoscope BV, Maastricht, The Netherlands): lag time, peak plasmin, time to peak, and endogenous plasmin potential (EPP). All measurements were carried out in triplicate.

2.8. Statistical analysis

The data were analyzed using GraphPad Prism (GraphPad Prism Inc., La Jolla, CA, USA). The Kolmogorov-Smirnov test was used to test for normality. Differences between categorical variables were assessed by the $\chi 2$ test and expressed as frequency (%) of subjects in each category.

Table 1
Baseline characteristics of the study population.

Parameters	HC ($n = 30$)	MGUS ($n = 33$)	MM ($n = 17$)	*p*-Value
Age, yr	65 (61–71)	67 (65–74)	67 (64–76)	0.0877
Female, *n*(%)	23 (76.7)	19 (57.6)	12 (70.6)	0.2586
Hemoglobin, g/L	141.1 ± 11.5	134.5 ± 17.4	102.8 ± 16.2	<0.0001 *<0.0001 #<0.0001
RBC, $x10^{12}$/L	4.7 (4.5–5.1)	4.4 (4.1–5.0)	3.1 (2.9–3.8)	<0.0001 *<0.0001 #<0.0001
WBC, $x10^{9}$/L	6.4 (5.3–7.8)	6.1 (4.8–8.1)	7.0 (5.8–7.9)	0.4763
PLT, $x10^{9}$/L	245.6 ± 56.5	235.5 ± 67.6	205.0 ± 66.5	0.1203
LDH, U/L	111.5 (79.5–133.3)	188.0 (161.0–209.0)	158.5 (137.8–197.3)	<0.0001 +<0.0001, *0.0065
Total protein, g/L	70.6 ± 10.2	76.5 ± 7.0	101.6 ± 28.2	<0.0001 *<0.0001 #<0.0001
Total calcium, mmol/L	2.4 ± 0.3	2.4 ± 0.1	2.5 ± 0.3	0.2990
PT, s	8.4 (7.9–8.7)	8.3 (8.0–8.7)	9.0 (8.1–10.2)	0.0631
APTT, s	27.5 ± 2.5	29.4 ± 4.0	28.6 ± 4.4	0.1409
TT, s	17.9 (17.2–18.7)	17.6 (17.0–18.7)	18.4 (17.6–22.5)	0.1555

Mean ± SD, median (interquartile range), or frequency (%) are shown. *p*-values without annotation represent the overall ANOVA or Kruskal-Wallis test results for the indicated parameters, while *p*-values with annotation show the outcome of the post-hoc analysis between two groups only where significant: comparison between +HC vs. MGUS, *HC vs. MM, and #MGUS vs. MM. HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; RBC, red blood cell; WBC, white blood cell; PLT, platelet; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time.

Continuous variables were expressed as mean ± SD or median and interquartile range. Ordinary one-way ANOVA, followed by Tukey's post hoc test or Kruskal-Wallis with Dunn's post hoc-test were used to compare differences between groups with Gaussian and non-Gaussian distributions, respectively. Pearson's or Spearman's correlation coefficient was used to determine the strength of the correlation between continuous variables. For all comparisons, a p-value <0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics of the study cohort

The study included 80 subjects: 17 newly diagnosed MM patients, 33 newly diagnosed MGUS patients, and 30 age- sex-, and blood group-matched healthy controls. There were 10 patients (59 %) with IgG MM, 1 (6 %) with IgA, 1 (6 %) with IgD, and 5 (29 %) with light chain disease (LCD). Twelve of the myeloma patients had kappa, while 5 had lambda light chains. Twenty-one (64 %) MGUS patients had IgG, 5 (15 %) had IgA, and 7 (21 %) had IgM paraproteins. Of them, 18 had kappa, while 15 had lambda light chains. The frequency of the ISS stages in MM patients was 23 % ($n = 4$), 12 % ($n = 2$), and 65 % ($n = 11$) for stages I, II and III respectively. Table 1 shows the baseline characteristics of the study population. The median age of patients was 67 years. Hemoglobin concentration and red blood cell (RBC) count were significantly lower in MM patients compared to healthy controls and MGUS patients, whereas total protein was higher in this group. Platelet (PLT) count did not differ significantly between groups. Lactate dehydrogenase concentrations (LDH) were higher in MM and MGUS patients compared to controls. Screening tests of hemostasis did not differ significantly between groups. At the time of enrollment, none of the patients had bleeding symptoms and all had negative venous or arterial thrombotic history.

3.2. FXIII antigen and activity levels in the study population

FXIII-A_2B_2 antigen levels were markedly reduced in MM patients compared to healthy controls and MGUS patients [median: 14.6 (IQR: 11.2–19.4) mg/L vs. 21.8 (17.1–26.4)] mg/L and 21.8 (IQR:18.2–27.5) mg/L, respectively, $p = 0.0015$] (Fig. 1A). In 38 % ($n = 6$) of MM patients, FXIII-A_2B_2 levels were below the lower limit of reference, indicating an acquired deficiency of FXIII. FXIII activity showed parallel results to FXIII-A_2B_2 (Fig. 1B). FXIII-A_2B_2 and FXIII activity showed good correlation in all groups, including MM patients (healthy controls: $r = 0.6780$, 95%CI: 0.3688–0.8520, $p = 0.0004$; MGUS: $r = 0.8200$, 95%CI: 0.6226–0.9193, $p < 0.0001$; MM: $r = 0.6061$, 95%CI: 0.0827–0.8674, $p = 0.0281$). Two patients with MGUS had FXIII activity and FXIII-A_2B_2 levels slightly below the reference range. In all cases with FXIII activity below the reference range, mixing studies using 1:1 ratio of patient and healthy control plasma did not suggest the presence of an FXIII inhibitor. Total FXIII-B did not differ between healthy controls and patients with MM or MGUS (Fig. 1B), but interestingly, median FXIII-B levels were at the upper limit of reference in all investigated groups in this cohort. Based on the results of mixing studies and that of FXIII-B levels, an immune-mediated inhibitory mechanism was ruled out. Free/total FXIII-B was significantly higher in MM patients compared to MGUS patients and healthy controls (Fig. 1C), suggesting that the underlying pathomechanism of low FXIII levels in MM patients is most likely due to increased FXIII activation and consumption.

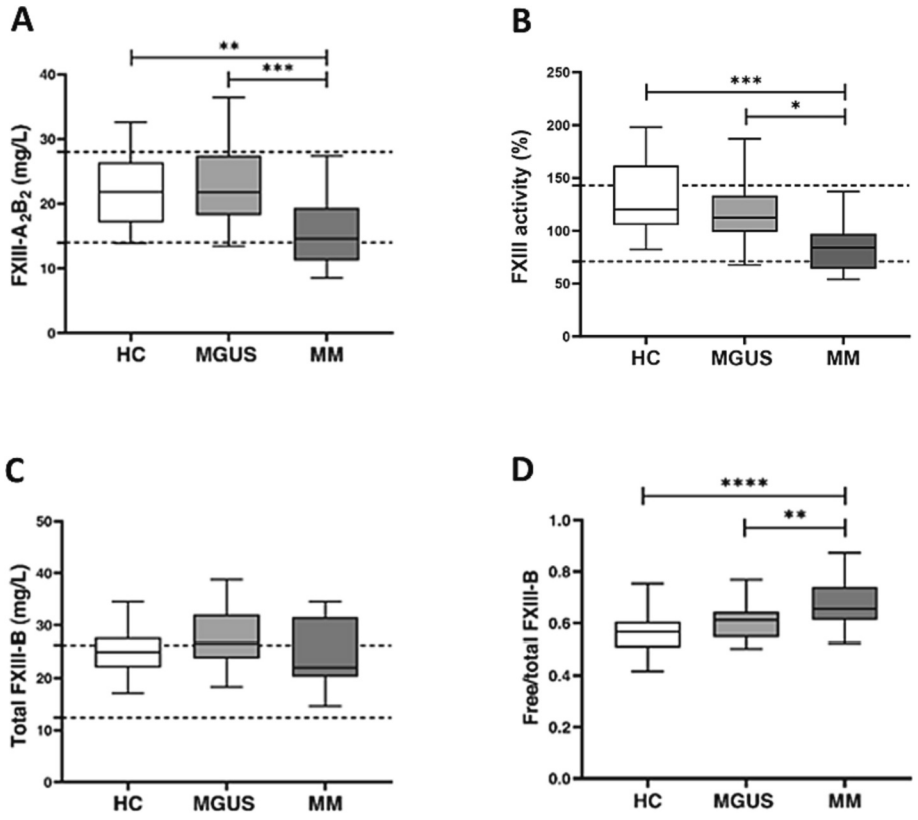

Fig. 1. FXIII-A_2B_2 antigen levels, FXIII activity, total FXIII-B, and free/total FXIII-B in healthy individuals (white boxes), MGUS (light grey boxes), and MM (dark grey boxes) patients. The lower and upper box boundaries represent the 25th and 75th percentiles, respectively, horizontal solid lines represent the median, and whiskers indicate range. Horizontal dashed lines indicate reference ranges (FXIII-A_2B_2: 14–28 mg/L; FXIII activity: 69–143 %; FXIII-B:12.4–26.3 mg/L). HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; FXIII A_2B_2, factor XIII antigen; FXIII-B, factor XIII-B. $^{**}p < 0.01$, $^{***}p < 0.001$ (A), ordinary one-way ANOVA with Tukey's post-hoc test; $^{**}p < 0.01$, $^{****}p < 0.0001$ (C) ordinary one-way ANOVA with Tukey's post-hoc test.

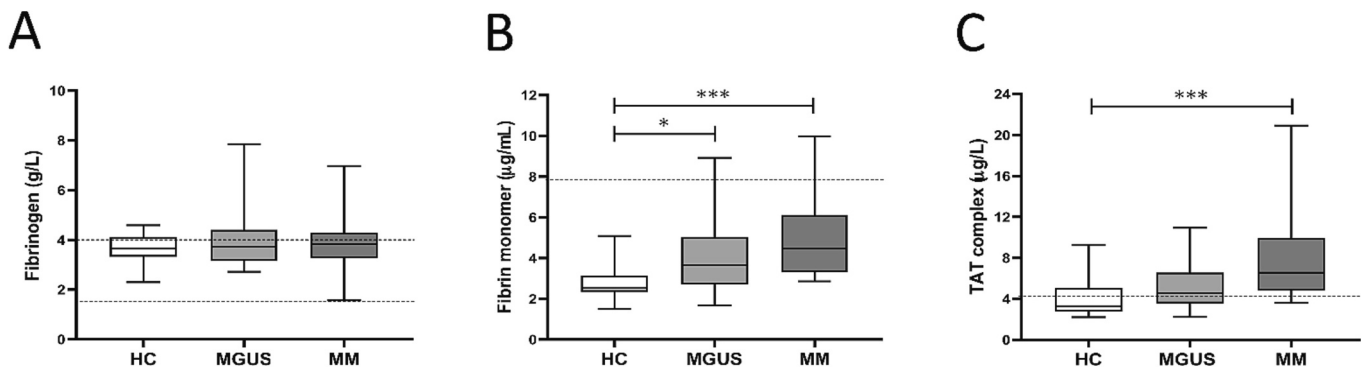

Fig. 2. Fibrinogen, quantitative fibrin monomer, and TAT complex levels in healthy individuals (white boxes), MGUS (light grey boxes) and MM (dark grey boxes) patients. The lower and upper box boundaries represent the 25th and 75th percentiles, respectively, horizontal solid lines represent the median, and whiskers indicate range. Horizontal dashed lines indicate reference ranges or limits of reference (fibrinogen: 1.5–4.0 g/L; quantitative fibrin monomer: <7.8 µg/mL; TAT complex: <4.2 µg/L). HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; TAT, thrombin-antithrombin. *$p < 0.05$, ***$p < 0.001$ (B, C), Kruskal-Wallis with Dunn's post-hoc test.

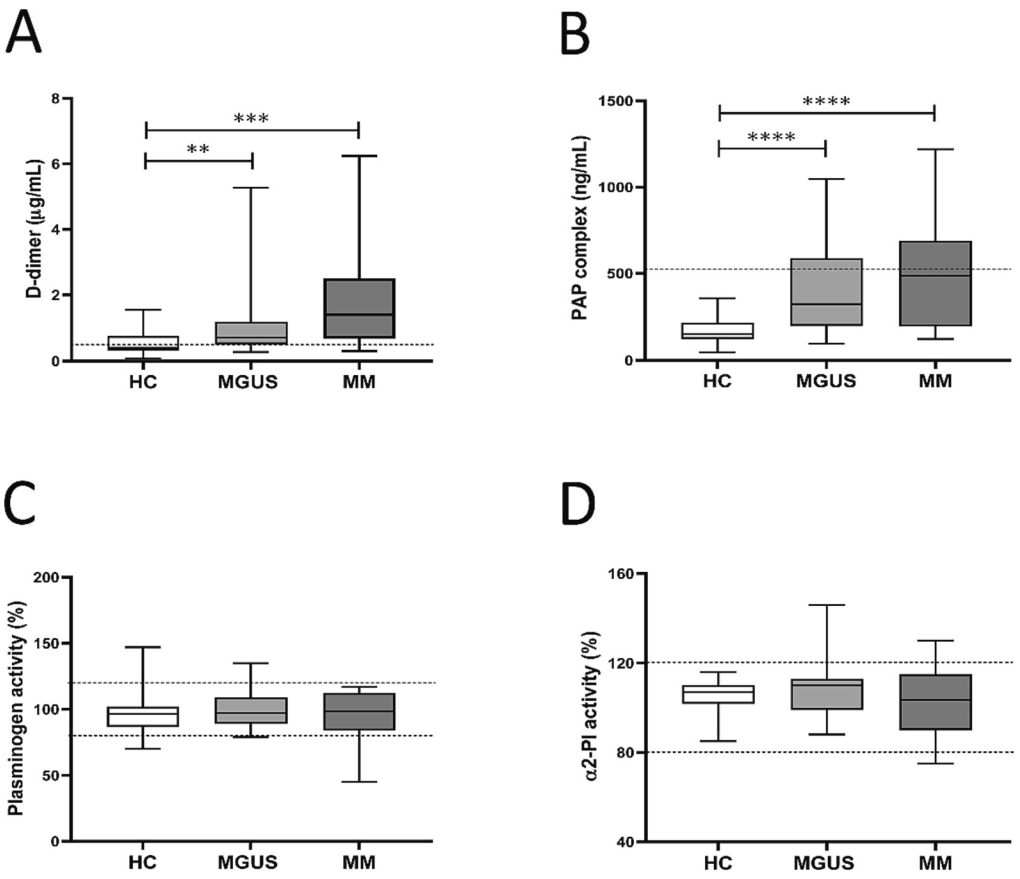

Fig. 3. Box plots of D-dimer (A), plasmin-α2-antiplasmin complex (B), plasminogen activity (C), and α2-plasmin inhibitor activity (D) in healthy individuals (white boxes), MGUS (light grey boxes) and MM (dark grey boxes) patients. The lower and upper box boundaries represent the 25th and 75th percentiles, respectively, horizontal solid lines represent the median, and whiskers indicate range. Horizontal dashed lines indicate reference ranges or limits of reference (plasminogen and α2-PI activities: 80–120 %; D-dimer: <0.5 µg/mL; PAP complex: <514 ng/mL). HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; PAP complex, plasmin-α2-*antiplasmin complex*; α2-PI activity, α2-plasmin inhibitor activity. **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, Kruskal-Wallis with Dunn's post-hoc test (A, B).

3.3. Baseline hemostasis tests, pro-thrombotic and fibrinolytic markers in the study population

To further clarify the pathomechanism behind the decreased FXIII levels detected in MM patients, markers of hypercoagulation and enhanced fibrinolysis were assessed. Fibrinogen levels were similar between all groups (Fig. 2A). Quantitative FM was significantly elevated in patients with MM [median: 4.5 (IQR: 3.3–6.1) µg/mL, $p = 0.0009$] and MGUS [median: 3.7 (IQR: 2.7–5.0) µg/mL, $p = 0.0246$] compared to controls [median: 2.5 (IQR: 2.3–3.1) µg/mL] (Fig. 2B). TAT complex levels were significantly higher in myeloma patients than in controls [6.6 (4.8–10.0) µg/L vs. 3.3 (2.8–5.1) µg/L, $p = 0.0004$] (Fig. 2C), suggestive of hypercoagulation and increased TG in MM patients.

Table 2
Comparison of ClotPro TPA-assay parameters among the study groups.

Parameters	HC ($n = 30$)	MGUS ($n = 33$)	MM ($n = 17$)	p-Value
TPA-assay				
CT (s)	49.0 (43.8–57.0)	50.0 (46.3–57.5)	56.0 (47.0–65.0)	0.0397 *0.0369
MCF (mm)	31.7 ± 6.4	33.8 ± 9.2	39.2 ± 9.0	0.0141 *0.0106
LT (s)	230.0 (211.0–246.0)	247.0 (217.5–289.0)	247.0 (219.5–358.0)	0.0357 *0.0427

Mean ± SD or median (interquartile range) are shown. Comparison between *HC vs. MM. HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; TPA, tissue plasminogen activator; CT, clotting time; MCF, maximum clot firmness; LT, lysis time.

The median D-dimer levels in MM [1.4 (0.7–2.5) μg/mL, $p = 0.0002$] and MGUS [0.7 (0.5–1.2) μg/mL, $p = 0.0095$] patients were higher than in control subjects [0.4 (0.3–0.8) μg/mL] (Fig. 3A). This increase was associated with a significant elevation in PAP complex levels in patients [485.2 (196.3–689.0) ng/mL, $p < 0.0001$ for MM and 320.9 (199.9–588.0) ng/mL, $p < 0.0001$ for MGUS] compared to controls [153.0 (124.2–217.8) ng/mL] (Fig. 3B), suggestive of enhanced fibrinolysis in both patient groups. There were no differences in plasminogen activity (Fig. 3C) and α2-PI activity (Fig. 3D) between the patients and control groups.

3.4. Global assays and correlation studies with FXIII

To find out whether the decrease in FXIII levels in MM patients affects the balance of fibrinolysis, global assays were performed, and lysis parameters were correlated with FXIII levels. Results of the Clotpro TPA-assay measurements are shown in Table 2. The clotting time (CT) parameter of the assay was significantly prolonged, while maximum clot firmness (MCF) was higher in myeloma patients compared to healthy controls. The lysis time (LT) parameter was also significantly prolonged in the cohort of MM patients compared to controls (Table 2). We found no significant differences in PG assay parameters and CLA test parameters between patients and controls (Supplementary Tables 1 & 2).

FXIII activity and FXIII-A_2B_2 levels showed a significant positive correlation with ClotPro TPA lysis time and peak plasmin in healthy control individuals. Such associations were abolished in patients with MM or MGUS (Table 3). Similarly, α2-PI activity and PAP complex positively correlated in healthy controls but not in the patient groups (Table 3).

4. Discussion

This study is, as far as we are aware, the first comprehensive evaluation of FXIII levels in patients with MM, in comparison with MGUS patients and healthy age- and sex-matched controls. Surprisingly, in a large fraction (38 %) of MM patients, FXIII levels were found to be below the lower limit of reference already at the time of diagnosis, and FXIII levels were significantly lower in this group of patients as compared to healthy controls and MGUS patients. The pathomechanism associated with decreased FXIII levels in this cohort of MM patients was a non-immune mechanism. Median FXIII-B subunit levels were at the upper limit of reference in all groups, which, according to the literature is expected based on the age of the cohort [37]. As total FXIII-B levels were comparable across groups, the lower FXIII-A_2B_2 antigen and activity levels observed in MM patients are most probably due to increased consumption of the FXIII-A active subunit due to excessive activation of coagulation. Evidence of increased fibrinolytic turnover, including elevated D-dimer and PAP-complex levels was found in MM patients, which, together with the increased free/total FXIII-B ratio is indicative of enhanced activation of FXIII. As D-dimers are derived from cross-linked fibrin by FXIIIa, most likely FXIII is consumed in this process of ongoing activation of coagulation and fibrinolysis, while the ratio of free FXIII-B subunits/total FXIII-B increases. Similar phenomenon has been described in a number of clinical scenarios, including in COVID-19 [46] and in critically ill patients [47]. Similarly to our findings, the presence of hyperfibrinolysis and acquired FXIII deficiency has been demonstrated in newly diagnosed pediatric hematological malignancies [48].

It is difficult to estimate the clinical consequences of such decrease in FXIII levels. According to the global assays of fibrinolysis performed in this cohort, plasmin generation and clot lysis was unaltered in MM and MGUS patients. In the ClotPro TPA-assay, significantly increased clot firmness and longer clot lysis time was found in MM patients, but this was not associated with FXIII levels. In fact, as opposed to healthy controls, levels of individual fibrinolysis proteins (e.g., FXIII, α2-PI) did not correlate with results of global fibrinolytic assays in MM and MGUS patients, suggesting that additional key players can be accounted for the disturbed balance of fibrinolysis. These results also suggest that the loss of FXIII in MM patients is a secondary result of ongoing fibrinolysis, and not the primary cause.

Altered fibrinolysis may be a significant but often unnoticed cause of a bleeding phenotype in a patient. Secondary or 'reactive' hyperfibrinolysis occurs when fibrinolytic activation increases in response to hypercoagulability, in which case the clinical phenotype might be even more complex [49]. Interestingly, we detected marked hypercoagulable changes and reactive fibrinolysis (increased fibrin monomer, D-dimer and PAP complex) in both MM and MGUS patient groups. In our previous study [12], we reported elevated levels of FVIII and VWF, as well as increased TG in patients with MM and MGUS. Elevated FM and TAT complex levels have been reported in newly diagnosed MM patients [50], but such alterations of hemostasis balance in MGUS patients have not been reported as yet. In this cohort, FXIII-A_2B_2 was below the reference range in already two MGUS patients (6 %) at presentation. The relevance of such findings in MGUS patients is to be determined. As MGUS is considered a cancerous, yet indolent pathology, in which most often only a close follow-up without specific treatment is suggested, it is somewhat unexpected to see a disturbed hemostasis and fibrinolysis balance at presentation. Our results indicate that during the follow-up and the management of MGUS patients, the possibility of such alterations indicating increased TG and altered fibrinolysis must be considered by physicians. As some studies have shown that both bleeding and thrombotic complications might be more frequent in MGUS patients as compared to healthy individuals [51], awareness of the underlying etiology is important. Consistent with previous studies [52], increased D-dimer was found in 88 % of myeloma patients as compared to 37 % of control subjects, but notably, elevated D-dimer levels were found in as high as 76 % of the MGUS cases in this cohort. This finding, in line with a previous study, calls attention to the distinct coagulation and fibrinolysis profile already present in MGUS [53].

The clinical prognosis of the scenario with increased TG, ongoing fibrinolysis and low FXIII is difficult to estimate. Viscoelastometry is one of the few techniques for studying *coagulation* and fibrinolysis in whole blood, allowing the contribution of cells, particularly platelets, to be investigated. According to some reports, it may help towards determining the overall balance of hemostasis and fibrinolysis in certain

Table 3
Correlations between FXIII activity, FXIII-A_2B_2, α2-plasmin inhibitor activity, plasmin-α2-antiplasmin complex and global assays in patients and controls.

	FXIII activity (%)			FXIII-A_2B_2 (mg/L)			α2-PI activity (%)			PAP complex (ng/mL)		
	HC	MGUS	MM	HC	MGUS	MM	HC	MGUS	MM	HC	MGUS	MM
ClotPro TPA lysis time	$r = 0.5136$ (0.1286–0.7640) **$p = 0.0122$**	$r = 0.2147$ (−0.2167–0.5759) $p = 0.3253$	$r = 0.3282$ (−0.2720–0.7445) $p = 0.2736$	$r = 0.4591$ (0.1113–0.7067) **$p = 0.0122$**	$r = 0.1570$ (−0.1969–0.4747) $p = 0.3828$	$r = 0.4183$ (−0.1136–0.7638) $p = 0.1077$	$r = 0.3737$ (0.0154–0.6469) **$p = 0.0420$**	$r = 0.0961$ (−0.2654–0.4341) $p = 0.5945$	$r = 0.3747$ (−0.1643–0.7414) $p = 0.1520$	$r = 0.5050$ (0.1589–0.7406) **$p = 0.0052$**	$r = 0.0205$ (−0.3345–0.3705) $p = 0.9096$	$r = -0.1502$ (−0.6113–0.3870) $p = 0.5760$
Peak plasmin	$r = 0.4595$ (0.0583–0.7329) **$p = 0.0274$**	$r = -0.1319$ (−0.5160–0.2964) $p = 0.5486$	$r = 0.3645$ (−0.2334–0.7624) $p = 0.2208$	$r = 0.3445$ (−0.0251–0.6313) $p = 0.0672$	$r = 0.1655$ (−0.1885–0.4815) $p = 0.3572$	$r = 0.3366$ (−0.1909–0.7133) $p = 0.2023$	$r = 0.3685$ (0.0094–0.6433) **$p = 0.0451$**	$r = -0.2589$ (−0.5603–0.1031) $p = 0.1457$	$r = -0.0722$ (−0.5483–0.4392) $p = 0.7903$	$r = 0.4900$ (0.1394–0.7314) **$p = 0.0070$**	$r = 0.1858$ (−0.1785–0.5053) $p = 0.3005$	$r = 0.3317$ (−0.1963–0.7106) $p = 0.2095$

α2-PI: α2-plasmin inhibitor, FXIII: factor XIII, TPA, tissue plasminogen activator; HC, healthy control; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma, PAP complex: plasmin-α2-antiplasmin complex. The 95 % confidence intervals are provided in brackets.

clinical conditions, and may be predictive of future hemostasis events, particularly bleeding [54,55]. Trelinski et al. [56] demonstrated that at the time of diagnosis, patients with myeloma show changes in rotational thromboelastometry (ROTEM) parameters that may indicate a prothrombotic state. Thromboelastographic parameters were also assessed in MM and MGUS patients, but no differences were found between patients and controls [53]. In our study, for the first time, we compared the parameters of ClotPro TPA-test, a similar technique that measures the viscoelastic properties of whole blood, among MM and MGUS patients and healthy controls. Herein, MCF was significantly higher in myeloma patients than in healthy controls, indicating a prothrombotic state. The lysis time using this assay was significantly prolonged in myeloma patients. Studies have shown that plasma fibrin clot structure and function are altered in myeloma patients at the time of diagnosis, with clot formation occurring more slowly and with decreased lysability. This mechanism has been linked to a higher TG potential during clot formation, which modifies coagulant properties of fibrinogen and possibly other proteins involved in fibrin formation and/or degradation, resulting in the formation of more compact clots composed of thin fibers [19]. Purified myeloma IgG has also been shown to inhibit fibrin polymerisation, resulting in clots with thin fibrin fibers that are more difficult to lyse [20], as it was found in our cohort using the ClotPro TPA test. In our study, similarly to a previous report [20], despite the impaired lysis efficiency, an increased D-dimer and PAP complex was found, indicating a secondary or reactive, disturbed balance of fibrinolysis. Interestingly, parameters of plasma-based fibrinolytic assays (PG, CLA) did not show significant differences between groups in our cohort, which might be explained by the different sensitivity of these tests to alterations in the fibrinolytic system.

As the methods used in this study are generally not part of routine diagnostic work-ups in MM and MGUS patients, it is important to highlight that the above listed hemostasis alterations are already evident at presentation in a considerable fraction of patients. Screening tests of hemostasis and fibrinogen levels, the most commonly used hemostasis laboratory assays did not differ significantly from controls in this tested cohort, and it must be kept in mind that these measurements provide only partial picture of the balance of hemostasis. Our data raises the potential need for awareness during the initial laboratory testing in MM and MGUS. Without the direct measurement of FXIII levels and markers of hypercoagulation and fibrinolysis (e.g. TAT levels, D-dimer, PAP levels), cases with low FXIII and disturbed fibrinolysis balance will be unnoticed. A more comprehensive laboratory approach at the time of diagnosis may help to identify patients at risk of bleeding or hypercoagulation during or after provoking factors (e.g. invasive procedures, etc.) and may influence treatment strategies.

This study's limitations include the small sample size resulting from the extensive use of exclusion criteria during recruitment, a factor that must be considered when interpreting our findings. For the same reason, we were unable to determine which stage of myeloma diagnosis was associated with decreased FXIII levels and ongoing fibrinolysis. To determine which MM and MGUS patients will develop a bleeding or thrombotic complication in the future was beyond the scope of our work. As the main aim of the study was to uncover how monoclonal paraproteinemias affect the balance of hemostasis and fibrinolysis in newly diagnosed patients, the lack of assessing cases longitudinally is a limitation of our study, as the lack of longitudinal laboratory assays to determine how fibrinolysis markers change over the course of disease is another limitation. The strength of our study, however, lies in its novel study design, which employs a comprehensive panel of specific FXIII assays to determine the possibility of acquired FXIII deficiency, together with fibrinolysis tests including global assays. The current study contributes to a relatively under-researched area, with potential clinical relevance in the management of patients with MM and MGUS and provides direction for future research.

5. Conclusions

Findings of this study reveal low FXIII in newly diagnosed, untreated MM patients as compared to MGUS and healthy controls. The cause of decreased FXIII levels in MM patients was found to be consumption as a result of hypercoagulation and ongoing fibrinolysis. Interestingly, signs of procoagulant hemostasis balance and reactive fibrinolysis were detected in MGUS patients as well, and in a small fraction of MGUS patients FXIII levels were below the reference range. These results indicate that despite the benign nature of MGUS, hemostasis alterations are already evident at presentation, raising the need for awareness during monitoring. A longitudinal study is needed to determine whether acquired FXIII deficiency in MM patients can result in pathological bleeding tendencies, and whether patients with low levels of FXIII and clinical symptoms may benefit from FXIII replacement therapy.

Ethical approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Debrecen (approval number: DE RKEB/IKEB 5906-2021). All participants provided written, informed consent voluntarily.

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CRediT authorship contribution statement

J.K., Z.B., and H.G. contributed to the conception and design of the study; L.R., and L.V. recruited the patients and collected clinical data and blood samples; L.L. and H.G. recruited the healthy controls and collected clinical data and blood samples; H.G., R.O.K., E.K., I.B.D., and L.L. performed the experiments; H.G., Z.B., D.H., E.K., R.O.K., and J.K. analyzed and interpreted the data; H.G., Z.B., and E.K. prepared the figures; H.G., J.K., and Z.B. drafted the original manuscript; Z.B. reviewed and edited the manuscript; J.K. supervised the research. All authors revised and approved the final version of the manuscript for submission.

Declaration of competing interest

The authors declare no competing interests.

Data availability

The data presented in this study are available on request from the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2023.12.004.

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