SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Novel pieces of information on the structure and deficiency of coagulation factor XIII and on its changes in patients with end-stage renal disease

by Boglárka Hurják

Supervisor: László Muszbek, MD, PhD, DSc, emeritus professor,
member of the Hungarian Academy of Sciences

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF KÁLMÁN LAKI

DEBRECEN, 2023

NOVEL PIECES OF INFORMATION ON THE STRUCTURE AND DEFICIENCY OF COAGULATION FACTOR XIII AND ON ITS CHANGES IN PATIENTS WITH END-STAGE RENAL DISEASE

By **Boglárka Hurják**, Clinical Laboratory Scientist

Supervisor: László Muszbek,
MD, PhD, DSc, emeritus professor, member of the Hungarian Academy of Sciences

Doctoral School of Kálmán Laki, University of Debrecen

Head of the **Defense Committee**:	Pál Soltész MD, PhD, DSc
Reviewers:	György Pfliegler MD, PhD
	Előd Nagy MD, PhD
Members of the Defense Committee:	Mariann Harangi MD, PhD, DSc
	Klára Vezendi MD, PhD

The PhD Defense takes place at the Lecture Hall of Building "A", Department of Internal Medicine, Faculty of Medicine, University of Debrecen, the 20th of November 2023, 1:00 PM.

1. Introduction and Literature Review

1.1. Structure and function of factor XIII

Coagulation factor XIII (FXIII) is a transglutaminase (TG) proenzyme essential for maintaining hemostasis by stabilizing the fibrin clot and protecting it against fibrinolytic degradation. FXIII circulates in the blood as a heterotetramer (FXIII-A_2B_2) consisting of two potentially active FXIII-A and two inhibitory/protective FXIII-B subunits.

In plasma, FXIII-B is in excess over FXIII-A; about 50% of FXIII-B_2 exists in free non-complexed form. The cleavage of FXIII-A by thrombin and the Ca^{2+} induced dissociation of the two subunits transform it into an active transglutaminase that cross-links fibrin chains and $\alpha_2$plasmin inhibitor to fibrin. FXIII-A deficiency causes serious bleeding diathesis and deficient patients require regular substitution therapy.

The non-enzymatic FXIII-B subunit prolongs the lifespan of FXIII-A in the circulation and prevents its spontaneous activation. FXIII-B is a ~80 kDa glycoprotein that contains approximately 8.5% carbohydrate on two N-glycosylation sites (Asn142 and Asn525) in the third and ninth sushi domains.

Neither the structure nor the functional role of these glycans has been studied in detail as yet, in spite of the fact that the importance of such modifications has been confirmed by numerous studies in immunology, oncology, hematology, etc. Several members of the blood coagulation cascade are glycoproteins, e.g factors II, VII, VIII, and IX (FII, FVII, FVIII, and FIX) suggesting the importance of attached carbohydrates. It has been demonstrated that the stability and macromolecular interactions of FVIII changed by deglycosylation. It is also important to note that glycosylation can aid in promoting protein solubility. Furthermore, in vivo efficacy of several therapeutically relevant recombinant clotting factors increased by their natural glycosylation.

1.2. Factor XIII deficiency

The severe bleeding diathesis of patients with inherited FXIII-A deficiency clearly indicates the importance of FXIII in maintaining hemostasis. In the general population FXIII-A deficiency is among the rarest inherited coagulation disorders (one in two million), but in countries with a high frequency of consanguineous marriages, particularly if it is combined with a founder mutation, the frequency is much higher.

Autoantibodies formed against either of the FXIII subunits also result in severe, frequently life-threatening hemorrhagic complications with a mortality rate around 25%. It is also a rare condition, in a most recent review 48 well-established published cases, 47 with anti-FXIII-A and 1 with anti-FXIII-B antibody, were collected.

The autoantibody might be related to autoimmune disease; however, particularly in elderly patients it is frequently idiopathic. The autoantibody might interfere with the activation of FXIII, might inhibit the TG activity of FXIIIa and by forming immune-complex with the protein might accelerate its clearance from the circulation. A classification of anti-FXIII antibodies based on the above criteria has been proposed.

The classical method used for the diagnosis and for measuring the inhibitory strength of anti-FXIII-A autoantibodies is based on Bethesda-Nijmegen assay. We proposed to supplement this assay and the characterization of autoantibodies by the determination of the patient's immunoglobulin G (IgG) concentration required for 50% inhibition of FXIII activation/activity and by the determination of the binding affinity between FXIII-A and the patient's IgG.

1.3. Changes of the fibrinogen and factor XIII levels in ESRD patient’s

End-stage renal disease (ESRD) is the final outcome of a variety of chronic kidney diseases, which requires hemodiafiltration (HDF)/hemodialysis (HD) treatment or kidney transplantation for survival. HD removes low molecular weight uremic toxins simply by diffusion, while HDF, although it uses the same type of dialyzer with the same pore size

as HD, employs convective transport resulting in the excretion of small to mid-size toxic proteins, as well.

In general, ESRD is considered to be associated with risk of bleeding partly due to impaired platelet function. However, paradoxically, thrombotic manifestations involving both the arterial and the venous side are also rather common in this patient group. Such thrombotic complications include ischemic stroke, myocardial infarction, peripheral artery occlusion, deep vein thrombosis, pulmonary embolism and vascular access thrombosis. The coincidence of hemorrhagic diathesis and thrombotic tendency suggests a complex imbalance in the clotting system, platelet function and changes in the fibrinolytic system may also contribute to the hemostatic alterations.

Individual variations in the conditions and treatment of ESRD patients might contribute to the variability of clinical hemostatic symptoms. General functional tests and determination of various hemostatic parameters have been both used to characterize the hemostatic imbalance of ESRD patients and explore the pathomechanism leading to altered hemostasis. Concerning the clotting system investigations mainly targeted its activation leading to increased thrombin generation. In ESRD patients undergoing regular hemodialysis markers of thrombogenesis including thrombin-antithrombin complex, prothrombin fragment 1.2, D-dimer, fibrinopeptide A, and factor VIII became elevated, suggesting intravascular activation of the coagulation cascade.

In the present study we were interested how the constituents of the final stage of clotting cascade are influenced by ESRD and its treatment modality.

Fibrinogen and factor XIII (FXIII) are the key player in the formation of fibrin clot and its stabilization. FXIII is of tetrameric structure consisting of two potentially active A subunits (FXIII-A) and two protective/inhibitory B subunits (FXIII-B). Thrombin removes an activation peptide of 37 amino acids from the N-terminus of FXIII-A and in the presence of Ca^{2+} the truncated FXIII-A assumes an enzymatically active configuration. Active FXIII (FXIIIa) is a transglutaminase that cross-links fibrin α-, and γ-chains by isopeptide bonds and α_2PI, the main fibrinolysis inhibitor, to fibrin α-chains. This way it makes fibrin resistant to the shear stress of circulating blood and to proteolytic breakdown by the fibrinolytic system. Sporadic results on changes of fibrinogen, FXIII and α_2PI in ESRD have been reported; however, comprehensive analysis of these constituents in ESRD is still missing.

2. Objectives

1. The aim of our study was to comprehensively characterize the N-glycan structure of FXIII-B and achieve N-glycan removal under non-denaturing conditions for downstream experiments to understand the biological relevance associated with its glycosylation. In this study the effect of deglycosylation on the dimeric structure of FXIII-B and on its plasma clearance was investigated.

2. In the present study, a patient with anti-FXIII-A demonstrating unusual laboratory and clinical features was investigated. The results allowed us to point out difficulties in the diagnostic process and to test the recommended novel approach to the antibody characterization with the aim of introducing these techniques into laboratory practice. The described unusual clinical complication could draw clinicians' attention for such a possibility.

3. The aim of the present study was to explore novel aspects concerning components important in fibrin formation and stabilization of the fibrin clot in ESRD patients.

    a. We determined fibrinogen and FXIII antigen concentrations and FXIII activities in ESRD patients and compared the effect of HDF and HD treatments.

    b. It was also investigated if inflammation, tested by C-reactive protein (CRP) measurement, influenced these parameters, and if fibrinogen concentration was associated with other investigated parameters.

    c. While in the first part of the study, the long-term combined effects of ESRD and its treatment by HDF or HD were investigated, in the second part the short-term effect of an actual HDF or HD treatment was studied. In the latter case, changes of fibrinogen and FXIII levels were monitored during the four-hour HDF or HD treatments. To take the decrease in plasma water during the dialysis treatments and its effect on protein concentrations into account, the above parameters corrected for albumin concentration were also calculated.

3. Materials and methods

3.1. N-glycosylation of blood coagulation factor XIII subunit B and its functional consequence

3.1.1. PNGase F digestion under native conditions

Eighty micrograms of FXIII-B was dissolved in 50 μL of 20 mmol/L $NaHCO_3$ (pH 7.0) followed by the addition of 8.1 mU PNGase F enzyme in 10 μL, ie, 10 times higher than that regularly used for denatured proteins. This reaction mixture was transferred to a 10 kDa spin-filter (VWR, Radnor, PA) and incubated overnight at 37°C. Then, 100 μL HPLC grade water (Millipore, Darmstadt, Germany) was added to the reaction mixture and the released N-glycans were centrifuged through spin-filters at 11 270 × *g* for 10 minutes, followed by drying in a SpeedVac system (Thermo Scientific) prior to fluorophore labeling. The remaining pellet was dissolved in 50 μL 20 mmol/L pH 7.0 $NaHCO_3$ buffer and re-digested with 1 μL (0.81 mU) PNGase F enzyme by incubating overnight at 37°C. Then, 100 μL HPLC grade water was added to the reaction mixture and the released N-glycans were centrifuged through the spin-filter at 11 270 × *g* for 10 minutes, followed by drying in a SpeedVac system prior to fluorophore labeling.

3.1.2. PNGase F digestion under denaturing conditions

After digestions of the native protein, as described in Section 3.1, the remaining pellet was denatured and digested again with PNGase F. The pellet was dissolved in 10 μL of high-performance liquid chromatography (HPLC) grade water and 1 μL denaturing buffer (400 mmol/L DTT, 5% SDS) was added to the mixture on the 10 kDa filter. After incubation at 65°C for 10 minutes 100 μL HPLC grade water was added and the filter was centrifuged at 11 270 × *g* for 10 minutes to remove any remaining denaturing buffer. FXIII-B was digested in situ on the filter by the addition of 49 μL 20 mmol/L $NaHCO_3$ buffer (pH 7.0) and 1 μL (0.81 mU) PNGase F. The reaction mixture was incubated at

37°C overnight. Then, 100 μL HPLC grade water was added to the reaction mixture and the released N-glycans were centrifuged through the spin-filters at 11 270 × *g* for 10 minutes. The samples were dried in SpeedVac. The liberated N-glycans were then APTS labeled as described below. Results with FXIII-B sample treated with PNGase F under the denaturing protocol were also used for comparison to establish the effectiveness of the digestion of non-denatured FXIII-B.

3.1.3. Fluorophore labeling

Six microliter 20 mmol/L APTS in 15% acetic acid and 2 μL 1 mol/L $NaCNBH_3$ (in THF) was added to the dried glycan samples and incubated at 37°C overnight. The labeled samples were magnetic bead purified (SCIEX Fast Glycan Sample Preparation and Analysis kit) following the instruction manual of the kit and immediately analyzed by capillary electrophoresis with laser induced fluorescence detection (CE-LIF) analysis or stored at −20°C for later work.

3.1.4. Exoglycosidase array based carbohydrate sequencing

Four microliter HPLC grade water and 1 μL 50 mmol/L CH_3COONH_4 buffer (pH 7.5) were added to 5 μL of APTS labeled N-glycans. This mixture was digested at 37°C overnight with an array of α(2-3,6,8,9) sialidase A, α(1-2,3,4,6) fucosidase, β(1-4,6)-galactosidase, and β(1-2,3,4,6)-N-acetylhexosaminidase. The digested samples were dried in SpeedVac then analyzed by CE-LIF.

3.1.5. Capillary electrophoresis with laser induced fluorescence (CE-LIF) detection

A P/ACE MDQ System (SCIEX) was used to perform all capillary electrophoresis analyses. The separations were monitored by LIF detection using a 488 nm Ar-ion laser with a 520 nm emission filter. Fifty cm effective length (60 cm total) 50 μm ID bare fused silica capillaries were employed with the N-CHO separation gel buffer (both from SCIEX) for the analysis. The samples were injected at 1 psi for 5 seconds and the separation was accomplished in reversed polarity mode by applying 30 kV. The 32 Karat software (SCIEX) was used for data acquisition and processing.

3.1.6. Molecular weight determination by gel filtration

Native and deglycosylated FXIII-B were analyzed by gel filtration using ÄKTA chomatography system (Amersham Biosciences, Uppsala, Sweden). Size-exclusion chromatography was carried out on a HiPrep™ 16/60 Sephacryl®S-300 HR column (GE-Healthcare, Chicago, IL). Elution was performed at room temperature in Tris-buffered saline (50 mmol/L Tris, 150 mmol/L NaCl, pH 7.4) at a flow rate of 0.5 mL/ min. Elution of proteins was monitored at 214 nm. Thyroglobulin, ferritin, aldolase, conalbumin, and ovalbumin were used as standards (GEHealthcare, Chicago, IL) for the calibration curve.

3.1.7. Generation of F13B knock-out mouse line and genotyping strategy

CRISPR/Cas9 technology was used to knock-out the F13B gene by introducing inframe stop codons into its second exon. Translation of the modified gene starts from the translation initiation site in the first exon, but it is halted after incorporating 29 amino acids out of the total 669. Modification is based on a double stranded break by the Cas9 enzyme (Integrated DNA Technologies; IDT, Coralville, IA) directed by cr/tracr RNAs (IDT), and homology directed repair for which the template with the desired mutation was provided on a single stranded oligodeoxyucleotide (ssODN) template (IDT). The

target specific sequence for the crRNA was: ATCCTTCCATTTTCCACGGT. Cas9 protein (30 ng/μL), cr/tracrRNAs (1-1 pmol/μL), and ssODN (15 ng/μL) were microinjected into the pronuclei of fertilized eggs of C57Bl/6NTac mice. Sequence of the ssODN template (modified bases are in bold and italics): CCTCTCAGGAGAACTCTATGCAGAAGAGAAACAGTGTGATTTTCCT***TAGTGA*** GGAAAATGGAAGGATTGCCCAATATTATTATACGTTTAAAAGCTTTT.

A polymerase chain reaction (PCR) was used for genotyping. First from founder mice, the target region was PCR amplified by F13B specific general forward (TGCAAACTGAAAGATCTGCCG) and reverse (TGTAGCACCTTGGGTTTGGAG) primers, and the sequence was verified. Sequencing was repeated in F1 generation. Wild type and knock out (KO) specific forward primers (AACAGTGTGATTTTCCTACCGTG and AACAGTGTGATTTTCCTTAGTG, respectively) together with the general reverse primer (described above) were used to identify the mice carrying the genetic modification.

3.1.8. Clearance of deglycosylated FXIII-B

The study was approved by the Animal Care Committee of the University of Debrecen. 100 μL 300 μg/mL native non-delycosylated and deglycosylated FXIII-B were injected into the tail vein of seven and six FXIII-B knock out mice, respectively. There were two males in each group. The mice were 8 to 12 weeks old. The body mass of the animals receiving non-deglycosylated and glycosylated FXIII-B was 22.3 ± 2.6 and 22.2 ± 2.3 g, respectively. One hour, 48 hours, and 120 hours later 150 μL blood was drawn from the retro-orbital sinus in heparinized capillary under isofurane anesthesia. Plasma samples were obtained by centrifugation at 1300 g, for 15 minutes. The collected plasma samples were stored at −20°C until the measurement of FXIII-B by enzyme-linked immunosorbent assay (ELISA).

3.1.9. Determination of FXIII-B concentration

The concentration of FXIII-B was determined from plasma samples collected from KO mice using a one-step ELISA technique previously developed in our institute. This was performed by adding biotinylated anti-FXIII-B capture and horseradish peroxidase (HRPO)-labeled anti-FXIII-B detection monoclonal antibodies to plasma samples diluted with 0.15 mol/L (pH 7.2) phosphate buffer containing 0.5 mol/L NaCl, 0.05% Tween 20 and 5 g/l bovine serum albumin (BSA) on streptavidin-covered microplates. The two FXIII-B antibodies were directed against different epitopes and bound equally to free and complexed FXIII-B. The mixture was incubated for 1 h and then, after washing 3 x 300 ul with phosphate buffer, tetramethylbenzidine (TMB, 200 µl, 0.1 mg/L) and phosphate buffer containing 0.006% H_2O_2 (0.05 mol/L, pH 5) were added to the microplate wells. After 30 min, the reaction was stopped with H_2SO_4 (2 mol/L, 50 µL) and the absorbance was measured at 450 nm on an iEMS Reader MF microplate reader (Labsystem, Budapest, Hungary).

3.2. Autoimmune factor XIII deficiency with unusual laboratory and clinical phenotype

3.2.1. Previous case history

Blood samples we obtained were from a 67-year-old French woman with a history of hypertension, morbid obesity (weight 114 kg) and knee osteoarthritis. No bleeding complications occurred during a previous appendectomy, 3 caesarean sections and a recent cataract surgery. There was no history of spontaneous bleeding.

The patient was briefly hospitalized in November 2017 due to a cortisone injection in the knee. During hospitalization, following a minor trauma, a large muscular hematoma developed on both thighs (220x97mm on the right side and 195x55mm on the left side). Despite 8 red blood cell transfusions, his hemoglobin concentration remained low.

Afterward she was admitted to hospital for 45 days during which period the correct diagnosis could not be established.

In January 2018, he came for a medical consultation at the haemophilia centre of Dijon University Hospital. Extended haemostasis tests confirmed reduced FXIII levels in the patient. A 17% FXIII activity was measured using a Berichrome assay (Dade, Behring Marburg, Germany). Mutational gene analysis of FXIII (bidirectional sequencing of exons and the boundary intron regions) was performed, but no mutation was detected. A 1.51 BU antibody titer was detected by the Bethesda Nijmegen assay. No other clotting factor deficiency was revealed. No evidence of other autoimmune disease, infectious disease or neoplasia was found.

In May 2018, a computed tomography scan was performed for abdominal pain, during which a large hematoma was identified in the right diaphragm. FXIII substitution was started with 5000 IU (44 UI/kg) Fibrogammin® (CSL Behring, Marburg, Germany) and repeated 6 hours later. 17-20% FXIII activities were measured by the Berichrome assay in the patient's plasma samples taken 30 min, 1 and 2 h after FXIII infusion. Interventional radiology was used to perform embolization to stop bleeding. In addition, the patient received 1 g tranexamic acid three times daily and two red blood cell transfusions.

3.2.2. Protein preparations

Purified FXIII-A_2B_2 used in the experiments was prepared from human plasma. Recombinant FXIII-A_2 (rFXIII-A_2) was a kind gift from Dr. E Olsen (Novo Nordisk, Måløv, Denmark). The patient's and normal IgG were isolated by affinity chromatography, using HiTrap Protein G HP column (Amersham, Uppsala, Sweden).

3.2.3. Plasma FXIII activity and antigen determinations

Patient samples in 0.109 M trisodium citrate were collected and centrifugated to obtain the plasma in Dijon, France. The samples were stored at -80°C until the transportation to Debrecen, Hungary.

Plasma FXIII activity was determined both in Dijon and in Debrecen with modified ammonia release assay using Berichrom® FXIII assay kit (Marburg, Germany) and Technochrom® FXIII assay kit (Vienna, Austria) respectively. FXIII-A_2B_2 complex, FXIII-A and FXIII-B antigen levels were determined by ELISA.

3.2.4. Binding assay of the patient's IgG to FXIII

The binding affinity of the patient's IgG to FXIII-A_2 was measured by surface plasmon resonance (SPR) using Biacore 3000 instrument (GE Healthcare, Little Chalfont, UK). The patient's IgG was immobilized to flow cell 2 (FC2) of CMD sensor chip (XanTec, Düsseldorf, Germany). Control flow cell 1 (FC1) was covered by normal IgG. Various concentrations of rFXIII-A_2 in 10 mM HEPES, 150 nM NaCl, 3mM EDTA, 0.005 % (v/v) surfactant (pH 7.4) were used as analytes.

The association rate constant (ka), the dissociation rate constant (kd) the equilibrium dissociation (Kd) and affinity constant (Ka) were calculated by BIAevaluation software (GE Healthcare).

3.2.5. Inhibiton of FXIII activation/activity

Anti-FXIII inhibitor activity was evaluated by Nijmegen modification of the Bethesda assay. The 50% inhibitory concentration (IC_{50}) of the patient's IgG was determined as follows: 8.3 μg/mL FXIII-A_2B_2 was incubated with various concentrations of normal and patient's IgG in 50 mM HEPES, 100 mM NaCl, pH 7.4 for 60 min at 37°C. Then FXIII was activated with 20 U/ml human thrombin and 10 mM $CaCl_2$ and FXIII activity was measured with ammonia release assay.

Determination of the effect of patient's IgG on the proteolytic cleavage of the FXIII-A by thrombin was performed by western blotting. In this case 8.3 μg/mL FXIII-A_2B_2 was incubated with 300 μg/mL patient's or normal IgG in HEPES-buffered saline (pH 7.4) at 37°C for 60 min. Then FXIII was activated with 5 U/ml human thrombin and 10 mM $CaCl_2$. After various time of incubation aliquots were removed for the western

blot analysis. Sheep polyclonal anti-FXIII-A IgG antibody (Affinity Biologicals, Ancaster, Canada) with Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) was used for detection.

We also tested the effect of patient's IgG on Ca^{2+} induced activation. 8.3 μg/mL thrombin cleaved FXIII was incubated with 300 μg/mL patient or normal IgG at 37°C for 60 min, then activated with 10 mM $CaCl_2$ and the transglutaminase activity was measured.

Finally, we investigated the effect of patient's IgG on thrombin and Ca^{2+} activated FXIII (FXIII-A*$_2$). FXIII was activated with 20 U/mL thrombin and 10 mM $CaCl_2$ and then preactivated FXIII (FXIIIa) was incubated with 300 μg/ml patient's or normal IgG for 60 min at 37°C. After the incubation the FXIII activity was determined.

3.3. Fibrinogen and factor XIII levels in patients with End-Stage Renal Disease undergoing hemodiafiltration or hemodialysis treatment

3.3.1. Patients and Blood Sampling

Thirty ESRD patients (15 females and 15 males) on chronic dialysis treatment were enrolled for the study. Their age was in the range of 18–70 years (median: 57 years, interquartile range: 41.5–64.5 years). Patients with diabetes mellitus, malignant disease and hematological or hemostasis disorders were excluded. At the time of recruiting, the ESRD patients were on the HDF treatment, the preferred modality in our dialysis center, for at least twelve months (median period of HDF treatment: 54 months, interquartile range: 33–108 months).

During the investigations there was a modality change, for two weeks the patients were switched to HD treatment, after which the HDF treatment was reinstalled. The dialysis sessions were provided three times a week and one session lasted for effective 240 min for both treatment modalities. Before, one and four hours after the actual HDF or HD treatment peripheral blood samples were drawn from the efferent line port into vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing 0.109 mol/L

sodium citrate. Plasma from citrated blood was separated by centrifugation (1500 x g, 20 min, 4°C), aliquots of plasma samples were stored at -70°C until further analysis.

The study protocol fully complied with the Declaration of Helsinki and it was approved by the Ethics Committee of the University of Debrecen. Informed consent was obtained from the participating patients.

3.3.2. Laboratory Measurements

FXIII activity was determined by the ammonia release assay with blank correction using TECHNOCHROM® reagent kit (Technoclone, Vienna, Austria) on Modular EVO P800 analyzer (Roche/Hitachi, Mannheim, Germany). The complex FXIII antigen (FXIII-A2B2) concentration was measured by ELISA.

Plasma fibrinogen concentration was measured by the Clauss method using the Labexpert LX fibrinogen assay kit (Labexpert Ltd., Debrecen, Hungary).

High sensitivity CRP (HS) reagent from Diagnosticum Ltd. (Budapest, Hungary) was used for the measuring CRP concentration.

Serum albumin concentration was measured by the bromocresol purple absorption method on Roche/Hitachi cobas c system.

3.3.3. Statistical analysis

Statistical analysis of the results was performed by the Statistical Package for Social Science (SPSS, 22.0, Chicago, IL, USA) and by GraphPad Prism version 5.0 (GraphPad Software, Inc., San Diego, CA, USA). Distribution of the data was evaluated by the Kolmogorov Smirnov test. GraphPad Prism software was used for the construction of the figures. In case of nonparametric distribution, the results measured after HDF treatment and following the two-week HD treatment were compared by related-samples Wilcoxon signed rank test, while in case of parametric distributions related sample t-test was used.

Differences in tested parameters measured before, one and four hours after the initiation of individual HDF and HD treatment were compared by Friedman test (nonparametric distribution) or by repeated measure ANOVA test (parametric distribution). The association between various parameters were analyzed by Spearman rank correlation.

4. Results

4.1. N-glycosylation of blood coagulation factor XIII subunit B and its functional consequence

To identify the N-glycan structures on FXIII-B, first comprehensive asparagine linked carbohydrate analysis of the denatured protein was performed. The denatured FXIII-B subunit was treated with PNGase F, the liberated sugars were labeled with APTS, and analyzed by CE-LIF after magnetic bead mediated sample purification. The denaturing step was necessary to completely unfold the glycoprotein, and therefore to ensure full access of the endoglycosidase enzyme to cut off the N-linked sugar structures.

In addition, exoglycosidase digestion of FXIII-B was performed to verify the database suggested structures. The APTS labeled N-glycan pool released from denatured FXIII-B after treating it with an array of exoglycosidase mixtures containing sialidase, sialidase + β-galactosidase, sialidase + β-galactosidase + fucosidase and sialidase + β-galactosidase + fucosidase +hexosaminidase.

As a result of this exoglycosidase enzyme array based carbohydrate sequencing, the following structures were identified: a trisialo (A3G(4)3S(6,6,6)3), a bisialo (A2G(4)2S(6,6)2), a disialo bisecting (A2BG(4)2S(6,6)2), a disialo core fucosylated (F(6)A2G(4)2S(6,6)2), a disialo core fucosylated bisecting (F(6)A2BG(4)2S(6,6)), a monosialylated bisecting (2A2[3]BG(4)1S(6)1), a monosialylated (A2G(4)2S(6)1) and two monosialo core fucosylated structures (F(6)A2[6]G(4)2S(6)1), F(6)A2[3]G(4)2S(6)1. Abbreviated glycan structural names followed the nomenclature proposed by Harvey et al.

Because we were also interested in the deglycosylation efficiency for the native form of FXIII-B, it was treated with PNGase F without denaturation, using a two-step protocol starting with an enzyme concentration 10-fold higher than regularly used for the digestion of denatured proteins. This step was followed by digestion using the regular PNGase F concentration as described in Materials and Methods. Repeated PNGase F digestion of the native protein using two different enzyme concentrations resulted in successful deglycosylation, which was confirmed by the lack of remaining protein-linked carbohydrate structures. In this experiment the non-denatured protein that remained after this double digestion protocol was denatured and subjected to an additional PNGase F treatment. The first endoglycosidase PNGase F digestion in non-denaturing conditions removed about 70% of carbohydrates. The second native condition PNGase F treatment apparently removed the residual sugar structures. Denaturation and another PNGase F digestion of the remaining pellet did not result in any detectable glycans proving complete deglycosylation of the native protein.

We are aware of only a single attempt to deglycosylate FXIII-B; however, in this case the extent of deglycosylation, the composition of released carbohydrates, and the completeness of deglycosylation were not investigated. Complete deglycosylation of native FXIII-B, as described in this study, provides a tool for exploring the functional/structural role of the N-glycan structure linked to the protein.

Here we first studied if deglycosylation influences the dimeric structure of FXIII-B. The sites responsible for forming FXIII-B dimer are on the fourth and ninth sushi domains and considering the closeness of glycan moieties to these sites one may presume that they might influence the dimerization of FXIII-B. The molecular weight of deglycosylated FXIII-B was somewhat less than that of the native one; however, it was expected because the carbohydrate part is removed from the molecule. The closeness of the determined molecular weight of the two species indicates that the dimeric structure of FXIII-B was preserved after deglycosylation.

The effect of deglycosylation on the lifespan of FXIII-B in the circulation was investigated in FXIII-B knock-out mice. The species difference, i.e., the injection of human FXIII-B into mice, very likely influenced, and probably accelerated, the rate of elimination. The elimination rate of native human FXIII-B_2 in FXIII-B KO mice was faster than expected, which might be due to faster elimination of a protein from non-

identical species. However, the robust difference in the elimination rate of native and deglycosylated human FXIII-B_2 suggests that the glycan moiety prolongs its lifespan in circulation.

4.2. Autoimmun factor XIII deficiency with unusual laboratory and clinical

A 67-year-old female, during an intended brief hospitalization for cortisone injection in her osteoarthritic knees, was accidentally hurt and huge hematomas developed at the posterior side of both thighs. No previous history of spontaneous bleeding and post-surgical hemorrhagic complication were recorded. Despite eight transfusions of red blood cells hemoglobin concentration remained low. She was hospitalized for 45 days with several misdiagnoses. After this period, medical consultation at the Hemophilia Care Center, University Hospital of Dijon suspected FXIII deficiency and 17% FXIII activity was measured using the Berichrom assay (Dade Behring) without blank compensation.

As such an extent of FXIII deficiency does not explain the severity of bleeding, FXIII activity measurement was repeated by the ammonia release assay without and with blank compensation (Technochrom FXIII assay: Technoclone, Vienna, Austria). Correction for blank revealed that the real FXIII activity was below the limit of detection. Such undetectable FXIII activity was confirmed by the complete lack of fibrin cross-linking in the clot of the patient plasma. No mutation was found in the *FXIIIA1* gene by bidirectional sequencing of exons and flanking intronic regions.

The presence of inhibitory anti-FXIII autoantibody was revealed by mixing study and 74 Bethesda unit (BU) was measured by Bethesda-Nijmegen assay. No FXIII-A_2B_2 and FXIII-A_2 antigen were detected in the patient's plasma by enzyme-linked immunosorbent assay (ELISA), while FXIII-B antigen was in the reference interval. However, the fact that a considerable amount of FXIII-A was detected in the plasma by western blotting suggested that the autoantibody interfered with the binding of monoclonal anti-FXIII-A antibodies used in the ELISAs.

Most recently, further techniques were proposed for the more precise characterization of anti-factor autoantibodies in general, and anti-FXIII autoantibodies in

particular. In our case the affinity of the autoantibody to recombinant FXIII-A_2 was determined by surface plasmon resonance (SPR) using Biacore 3000 instrument. As expected the antibody showed high affinity toward FXIII-A_2 with a KD of $2.77 \pm 0.66 \times 10{-}9$ mol/L.

We also determined 50% inhibitory concentration (IC_{50}) of the patient's IgG, which, in our opinion, is a more accurate measure of the autoantibody's inhibitory power than the Bethesda unit. Fifty percent inhibition was achieved at 74 ± 8.6 μg/mL patient's IgG concentration, while normal IgG, even in the highest concentration, had no effect on FXIII activity.

The autoantibody might interfere with the cleavage of FXIII-A by thrombin, with the Ca^{2+} induced structural changes and with the transglutaminase activity of FXIIIa. It may also exert a combined effect. To properly classify the inhibitory effect of the autoantibody we tested these possibilities separately.

The effect of the patient's IgG on the proteolytic cleavage of the FXIII-A by thrombin was studied by western blotting. Comparison of the time course of FXIII-A truncation in the presence of normal and patient's IgG suggests that the removal of the activation peptide by thrombin was not prevented by the antibody.

We also tested the combined effect of patient's IgG on Ca^{2+} induced FXIII activation and on the activity of FXIIIa. In this set-up the patient's IgG exerted a close to total (92.3%) inhibition.

Finally, we investigated the effect of patient's IgG on fully activated FXIII. In this case the inhibition of FXIIIa by the patient's IgG was moderate: 44% of the transglutaminase activity was inhibited by 300 μg/mL IgG suggesting that the inhibition of transglutaminase activity only partially contributed to the combined inhibition of Ca^{2+} induced activation and FXIIIa activity. According to the proposed classification the neutralizing anti-FXIII-A autoantibody is of combined type (type IV).

The patient received tranexamic acid and corticoids throughout the observed period. She suffered major bleeding complications at two occasions, first right diaphragma pillar hematoma, then left diaphragma pillar hematoma together with a large rectus abdominis muscle hematoma. Supplementation with plasma derived FXIII concentrate (Fibrogammin®, CSL Behring) could not control the bleedings and only

minor temporary elevation of FXIII activity was observed. The bleedings were finally stopped by embolization using an interventional radiological technique.

After the first bleeding episode, an eradication strategy was initiated, which included Rituximab, immunoglobulin, and later cyclophosphamide or bortezomid. The eradication considerably decreased the inhibitor titer, but the inhibitor was never eliminated; its lowest titers were in the range of 0.9-4.0 BU and FXIII activity remained below the limit of detection. Unexpectedly, 4 months after the last hemorrhagic episode, she presented left femoral deep vein thrombosis complicated by pulmonary embolism. A filter was placed in the inferior vena cava due to contraindication of anticoagulant treatment. Evolution was favorable with a good recanalization.

4.3. Fibrinogen and factor XIII levels in patients with End-Stage Renal Disease undergoing hemodiafiltration or hemodialysis treatment

In 56.6% of ESRD patients being on HDF treatment fibrinogen concentration was above the reference interval (1.5–4.0 g/L), mean fibrinogen concentration was 4.21 ± 0.82 g/L. Modality switching from HDF to HD treatment did not influence the fibrinogen concentration significantly. In 50.0% of HD-treated patients it remained above the reference interval, mean: 4.23 ± 0.87 g/L. It is to be noted that even fibrinogen values that felt in the reference interval concentrated predominantly in the upper half of the interval.

As fibrinogen is an acute phase protein and ESRD patients frequently suffer from inflammatory conditions, the concentration of CRP, an excellent marker of inflammatory reactions, was also measured and its correlation with fibrinogen concentration was evaluated.

In 56.6% of the patients on HDF treatment, the CRP level was above the upper limit of reference interval (females: 4.6 mg/L, males: 5.2 mg/L). The median CRP plasma concentration of ESRD patients was 5.95 mg/L, with interquartile range (IQR): 4.02–15.15 mg/L and there were a few outliers. The CRP level of four HDF treated patients was above 50 mg/L, due to acute inflammatory conditions.

In spite of the few individual changes during the two-week period of HD treatment, the CRP levels, in general, did not change significantly. In 53.0% of the patients it remained above the upper limit of reference interval (median: 4.75 mg/L, IQR: 2.52–14.33 mg/L).

Fibrinogen concentration was an exponential function of CRP level. The high coeffcients of determination (r^2: 0.647 and 0.671 in case of HDF and HD treated patients, respectively) demonstrate significant correlation ($p < 0.001$) between the inflammatory condition and fibrinogen levels. Neither the length of HDF treatment period (r^2: 0.018, $p = 0.590$) or the age of patients (r^2: 0.035, $p = 0.267$) correlated significantly with the fibrinogen levels There was no significant difference between females and males in the pre-dialysis fibrinogen and CRP concentrations.

In the following experiments, we investigated whether the concentration of FXIII which are essential for maintaining normal hemostasis and are involved in the pathomechanism of thrombotic diseases, changes in ESRD.

FXIII was measured both by functional test and antigen assay. Similar results were obtained by the two measurement techniques as it was demonstrated by the coefficients of determination both in the HDF (r^2: 0.876, $p < 0.001$) and in the HD (r^2: 0.916, $p < 0.001$) group.

In the HDF group the mean FXIII activity was 127.1 ± 27.3% and the mean FXIII antigen concentration was 25.9 ± 5.6 mg/L. Two weeks of hemodialysis treatment did not change significantly the FXIII values (FXIII activity: 134.0 ± 29.8%, FXIII antigen: 27.1 ± 6.7 mg/L). The means in all cases were in the upper tertile of the reference interval.

In the HDF group 27% of individual FXIII activity values and 33% of FXIII antigen values exceeded the upper limit of the reference interval. In the HD group 43% (activity) and 40% (antigen) were above the reference interval. These results indicate that in ESRD patients, as compared to healthy individuals, FXIII concentration becomes elevated.

Both FXIII activity (r^2: 0.132, $p = 0.061$) and antigen (r^2: 0.147, $p = 0.070$) showed weak nonsignificant correlation with the patients' age. The length of HDF treatment did not correlate with FXIII levels (r^2: 0.004, $p = 0.769$ for FXIII activity and r^2: 0.003, $p = 0.272$ for FXIII antigen). Neither fibrinogen nor CRP concentration influenced FXIII activity or antigen levels significantly in HDF treated patients (r^2: 0.001 and <0.001 for

fibrinogen, r^2: 0.007 and 0.004 for CRP). There was no gender-specific difference in the FXIII activity/concentration of HDF- or HD treated patients.

The above results demonstrate the long-term effect of HDF and HD treatments on three key terminal phase components of the hemostasis system. A further interesting question was whether these parameters change during the four-hour individual HDF or HD treatment. As the relation of plasma water and plasma proteins significantly changes during the four-hour treatments, such changes were monitored by the determination of albumin concentrations and results of hemostasis parameters corrected for albumin concentration were also calculated.

As expected, albumin concentration gradually elevated during the 4-hour HDF and HD treatments. In HDF treated patients, pre-dialysis albumin concentration (median: 37 g/L, IQR: 35–40 g/L) became significantly elevated after 4-hour HDF treatment (median: 42 g/L, IQR: 38–44 g/L, $p < 0.001$). In the HD group a similar elevation was observed (pre-dialysis median albumin concentration: 39 g/L, IQR: 36–41 g/L; post-dialysis median albumin concentration: 44 g/L, IQR: 40–48 g/L, $p < 0.001$).

Similarly, plasma concentration of fibrinogen also became significantly elevated during the four-hour treatments. When corrected for albumin concentration the differences between the fibrinogen levels measured before and after the 4-h treatment disappeared, i.e., the measured elevations were due to the relative decrease of plasma water and not to the elevated amount of fibrinogen in the plasma.

In case of FXIII the situation was different. FXIII activity noncorrected for albumin also showed marked elevation during both HDF and HD treatments, however, a statistically significant elevation still remained after correction. The change of FXIII antigen levels was identical to the changes in FXIII activity.

5. Discussion

In summary, our experiments with the FXIII-B glycoprotein have led to the complete characterization of the N-glycan profile of FXIII and the development of a protocol that allowed the complete deglycosylation of the protein with the PNGase F enzyme. The protein deglycosylated in this way can be used in further biochemical

experiments that will help to understand the biological significance of FXIII-B N-glycosylation.

Only one communication is known in which deglycosylation of FXIII-B was attempted, but in this case neither the extent of deglycosylation, nor the composition of the carbohydrate components released, nor the success of deglycosylation was investigated. The complete deglycosylation of native FXIII-B, as described in this study, offers an opportunity to elucidate the functional and structural role of N-glycans associated with the protein.

We first investigated whether deglycosylation affects the structure of the FXIII-B dimer. The sites responsible for the formation of FXIII-B dimers are located at sushi domains 4 and 9 and given the proximity of glycan structures to these sites, one of our hypotheses was that they might affect FXIII-B dimerization. The molecular weight of the deglycosylated FXIII-B was slightly less than the native form, a consequence of the removal of carbohydrate components from the protein. However, the close proximity of the determined molecular weights of deglycosylated and native FXIII-B indicates that the dimeric structure is retained after deglycosylation.

The effect of deglycosylation on the lifespan of FXIII-B in the circulation was investigated in FXIII-B knock-out mice. The species difference, i.e. injection of human FXIII-B into mice, most likely affected and accelerated the rate of elimination. No data are available on the half-life of free human FXIII-B in plasma. In FXIII-A deficient patients, the half-life of FXIII-A_2B_2 complex as well as recombinant FXIII-A_2 (which is coupled in the circulation to the FXIII-B subunit of these patients) varied between 6.2 and 16 days. However, the half-life of FXIII-B_2 in the complex can differ significantly from the circulating time of the non-complexed protein. The rate of elimination of native human FXIII-B_2 in FXIII-B KO mice was faster than expected, which may be due to the fact that the proteins administered were not of the same species. However, the strong difference between the elimination rates of native and deglycosylated FXIII-B_2 suggests that the glycan structure prolongs the lifespan of FXIII-B_2 in the circulation.

A further aim of my PhD thesis was to describe in detail the history and correct diagnosis of a patient with acquired FXIII deficiency caused by an autoantibody against FXIII-A, thus enriching the literature on the pathological significance of antibodies against FXIII.

The severe haemorrhagic symptoms of patients with hereditary FXIII-A deficiency illustrate the importance of FXIII in maintaining normal haemostasis. In the general population, FXIII-A deficiency is one of the rarest inherited coagulation disorders (one in two million). Haemorrhagic predisposition caused by autoantibodies against FXIII is also a rare condition, a comprehensive study in 2018 summarised 48 previously reported well-defined cases, identifying autoantibodies against FXIII-A in 47 patients and against FXIII-B in 1 patient. The appearance of autoantibodies may be associated with autoimmune pathologies, but idiopathic occurrence is particularly common in elderly patients. The autoantibody formed may interfere with FXIII activation, inhibit FXIIIa TG activity and, by forming an immune complex with the protein, accelerate its clearance from the circulation.

Thrombotic complications are considered rare in patients with autoantibodies against FXIII, only a few cases have been described and some of them have been attributed to venous compression due to massive haematoma. Similar to our case, two other cases of patients who developed pulmonary embolism have been described.

The diagnosis and clinical management of acquired FXIII deficiency due to autoantibodies against FXIII-A is quite challenging. The case we present demonstrates that unexpected variations in the evaluation of laboratory results can significantly complicate the correct diagnosis and unpredictable clinical events can complicate the clinical course.

In the case we have described, the following conclusions can be drawn:

1. the determination of FXIII activity without blind correction can be significantly misleading in judging the severity of factor deficiency.
2. the presence of an anti-FXIII-A autoantibody in the patient may interfere with the antibody used in the immunoassay, resulting in a high underestimation of FXIII-A_2 and FXIII-A_2B_2 antigen levels.
3. in addition to the Nijmegen-Bethesda assay, the determination of the IC_{50} and dissociation constant is also useful for proper characterisation of the autoantibody.
4. identification of the mechanism by which the autoantibody interferes with FXIII activation/activity is necessary for proper classification.
5. an autoantibody against FXIII-A with inhibitory activity does not protect the patient from thromboembolic complications.

In ESRD patients, the risk of developing both thrombotic and haemorrhagic pathologies is significant. Alterations in the coagulation system may contribute to the pathogenesis of these lesions. Therefore, the study of coagulation and fibrinolytic factors may help to reveal the reasons underlying this paradoxical situation. An interesting question is whether elevated FXIII levels in ESRD patients contribute to atherothrombotic or thromboembolic pathologies. This hypothesis has been investigated in a few studies, but no general conclusion could be drawn.

Our study in ESRD patients on hemodialysis had a dual objective. Firstly, to investigate the long-term effects of hemodialysis treatment on the activity and concentration of components that play a key role in the final phase of coagulation and, in this respect, to compare the two hemodialysis treatments, HDF and HD. Secondly, to study the short-term effects of these treatments, i.e. to monitor individual changes during the four-hour dialysis treatments.

Fibrinogen, a precursor protein of fibrin, which, in addition to its key role in haemostasis, also plays a major role in the wound healing process. Elevated fibrinogen concentration is an independent risk factor for atherothrombotic diseases such as haemorrhagic stroke and ischaemic heart disease. Some studies in ESRD patients receiving chronic HD treatment have reported elevated fibrinogen concentrations in blood samples taken before dialysis treatment, although in some of them the elevation was not statistically significant. Similar to the results of previously reported studies, our measurements showed elevated fibrinogen levels in pre-dialysis samples. In 56.6% of patients on HDF treatment and 50.0% of patients on HD treatment, plasma fibrinogen concentrations were above the reference range. No significant difference was found between the effect of HD and HDF. For both treatment types, pre-dialysis fibrinogen and CRP levels were strongly correlated and high coefficients of determination suggested that at least partly the elevated synthesis of acute phase proteins was responsible for the elevated fibrinogen levels. Other variables tested, such as length of dialysis treatment, age and sex, did not affect the fibrinogen concentration measured before dialysis. Elevated values before treatment are presumably determined by prolonged effects on fibrinogen synthesis; the acute effect of a single dialysis treatment is likely to be influenced by other rapidly acting factors. We observed a gradual increase in fibrinogen concentrations during both HDF and HD treatments. We hypothesized that these

increases were due to the concentration of patients' plasma during dialysis treatment, and this hypothesis was supported by the fact that fibrinogen levels no longer showed a gradual increase during the treatments after correction for albumin values in each sample.

The effect of haemodialysis treatment on FXIII levels in ESRD patients has only been investigated in a few studies. Kolb et al. measured normal FXIII activity and FXIII-A antigen levels in samples taken before dialysis treatment, but two other studies reported significantly elevated FXIII-A concentrations.

In our case, FXIII activity and FXIII-A_2B_2 complex concentration were determined in parallel. In the HDF group, 27% of the individual FXIII activity values and 33% of the FXIII antigen values exceeded the upper limit of the reference range. In the HD group, 43% of activity values and 40% of antigen values fell above the reference range. The results show that a significant proportion of ESRD patients have elevated FXIII concentrations compared to healthy individuals. During 4 h HDF and HD treatments, FXIII activity showed a significant increase. In contrast to fibrinogen levels, FXIII levels remained elevated after correction for albumin concentration, although the level of significance decreased somewhat. These results suggest that the concentration of plasma in patients during the treatments does not explain the elevated FXIII levels and further studies are needed to elucidate the mechanism that leads to elevated FXIII levels in ESRD patients during hemodialysis treatment.

6. Summary

The protective/inhibitory B subunits of coagulation factor XIII (FXIII-B) is a ~80 kDa glycoprotein containing two N-glycosylation sites. Neither the structure nor the functional role of the glycans on FXIII-B has been explored. We attemted to reveal the glycan structures linked to FXIII-B, to design a method for deglycosylating the native protein, to find out if deglycosylation influences the dimeric structure of FXIII-B and its clearance from the circulation. Asparagine-linked carbohydrates were released from human FXIIII-B by PNGase F digestion. The released N-linked oligosaccharides were fluorophore labeled and analyzed by capillary electrophoresis. Structural identification utilized glycan database search and exoglycosidase digestion based sequencing. The structure of deglycosylated FXIII-B was investigated by gel filtration. The clearance of deglycosylated and native FXIII-B from plasma was compared in FXIII-B knock out mice. PNGase F completely removed N-glycans from the denatured protein. Deglycosylation of the native protein was achieved by repeated digestion at elevated PNGase F concentration. The total N-glycan profile of FXIII-B featured nine individual structures; three were fucosylated and each structure contained at least one sialic acid. Deglycosylation did not change the native dimeric structure of FXIII-B, but accelerated its clearance from the circulation of FXIII-B knock out mice. Characterization of the glycan moieties attached to FXIII-B is reported for the first time. Complete deglycosylation of the native protein was achieved by a deglycosylation workflow. The associated glycan structure is not required for FXIII-B dimer formation, but it very likely prolongs the half-life of FXIII-B in the plasma.

Hemorrhagic diathesis due to anti-factor XIII (FXIII) autoantibody is a rare but severe disorder. Challenges of the diagnosis and treatment is demonstrated by the case of a 67-year-old female without previous bleeding history, who suffered a huge muscular hematoma. Without blank subtraction 18% plasma FXIII activity was measured; however, after correction for blank the activity was below the limit of detection and the lack of fibrin cross-linking in the patient's plasma confirmed the latter result. FXIII-A_2 antigen was not detectable by enzyme-linked immunosorbent assay (ELISA); however, it was well detected by western blotting. The autoantibody showed high affinity toward

FXIII-A_2. Its considerable inhibitory activity was demonstrated by high titer in Bethesda units and the low immunoglobulin G concentration required for inhibition. The main biochemical effect was the inhibition of Ca^{2+}-induced FXIII-A activation. Eradication therapy was only partially successful. Four months after the last hemorrhagic event the patient suffered deep vein thrombosis complicated by pulmonary embolism.

Hemostasis disorder in patients with end-stage renal disease (ESRD) is frequently associated with bleeding diathesis but it may also manifest in thrombotic complications. Analysis of individual coagulation and fibrinolytic factors may shed light on the background of this paradox situation. Here we explored components essential for fibrin formation/stabilization in ESRD patients being on maintenance hemodiafiltration (HDF) or hemodialysis (HD). Pre-dialysis fibrinogen, factor XIII (FXIII) antigen concentrations and FXIII activity were elevated. The inflammatory status, as characterized by C-reactive protein (CRP) was a key determinant of elevated fibrinogen concentration. During a 4-hour course of HDF or HD, fibrinogen concentration and FXIII levels gradually elevated. When compensated for the change in plasma water, i.e., normalized for plasma albumin concentration, only FXIII elevation remained significant. There was no difference between HDF and HD treatments. Elevated fibrinogen and FXIII levels in ESRD patients might contribute to the increased thrombosis risk.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/181/2023.PL
Subject: PhD Publication List

Candidate: Boglárka Hurják
Doctoral School: Kálmán Laki Doctoral School

List of publications related to the dissertation

1. Bovet, J., **Hurják, B.**, Maistre, E. D., Katona, É., Pénzes-Daku, K., Muszbek, L.: Autoimmune Factor XIII Deficiency With Unusual Laboratory and Clinical Phenotype.
   J. Thromb. Haemost. 18 (6), 1330-1334, 2020.
   DOI: http://dx.doi.org/10.1111/jth.14811
   IF: 5.824
2. **Hurják, B.**, Kovács, Z., Döncző, B., Katona, É., Haramura, G., Erdélyi, F., Shemirani, A. H., Sadeghi, F., Muszbek, L., Guttman, A.: N-glycosylation of blood coagulation factor XIII subunit B and its functional consequence.
   J. Thromb. Haemost. 18 (6), 1302-1309, 2020.
   DOI: http://dx.doi.org/10.1111/jth.14792
   IF: 5.824
3. Pénzes-Daku, K., **Hurják, B.**, Katona, É., Becs, G., Balla, J., Muszbek, L.: Terminal Phase Components of the Clotting Cascade in Patients with End-Stage Renal Disease Undergoing Hemodiafiltration or Hemodialysis Treatment.
   Int. J. Mol. Sci. 21, 1-15, 2020.
   DOI: http://dx.doi.org/10.3390/ijms21228426
   IF: 5.924

Total IF of journals (all publications): 17,572
Total IF of journals (publications related to the dissertation): 17,572

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

30 May, 2023