AUS DEM LEHRSTUHL
FÜR INNERE MEDIZIN III
Prof. Dr. med. Wolfgang Herr
DER FAKULTÄT FÜR MEDIZIN
DER UNIVERSITÄT REGENSBURG

FACTOR XIII DEFICIENCY: EVALUATION OF THE INCIDENCE, CLINICAL SIGNIFICANCE AND MANAGEMENT AT THE UNIVERSITY HOSPITAL REGENSBURG

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

der
Fakultät für Medizin
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vorgelegt von
Didzis Gailis

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Tag der mündlichen Prüfung: 10.06.2022

Content

List of abbreviations

A: A-subunit of factor XIII

aPTT: activated partial thromboplastin time

ARDS: acute respiratory distress syndrome

AT: antithrombin

A2B2: inactive plasma factor XIII

A2*: calcium activated factor XIII

A'2B2: inactive intermediate factor XIII after thrombin cleavage

B: B-subunit of factor XIII

Ca2+: calcium

CLSI: The Clinical and Laboratory Standards Institute

Cryo: cryoprecipitate

D: D Domain

dl: deciliter

E: E Domain

ECMO: extracorporeal membrane oxygenation

e.g.: for example

ELSO: Extracorporeal Life Support Organisation

fg: femtogram

FFP: fresh frozen plasma

FII: factor II

FV: factor V

FVa: activated factor V

FVIIa: activated factor VII

FVIII: factor VIII

FVIIIa: activated factor VIII

FIX: factor IX

FIXa: activated factor IX

FX: factor X

FXa: activated factor X

FXI: factor XI

FXIa: activated factor XI

FXIIa: activated factor XII

FXIII: factor XIII

FXIIIa: activated factor XIII

FXIII-A: A-subunit of factor XIII

FXIII-A2: two A-subunits of factor XIII

FXIII-A2-B2: assembly of 2 A- and B-subunits of factor XIII

FXIII-B: B-subunit of factor XIII

FXIII-B2: two B-subunits of factor XIII

FXIII': inactive plasma factor XIII

g: grams

GP Ib Receptor: glycoprotein Ib receptor

GvHD: graft-versus-host disease

h: hours

HIT: heparin induced thrombocytopenia

ICU: intensive care unit

i. e.: namely

INR: International Normalized Ratio

ISTH-SSC BAT: The Scientific and Standardization Committee of International Society on Thrombosis and Haemostasis: Bleeding Assessment Tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders

ITP: immune thrombocytopenic purpura

IU: international units

kg: kilogram

l: litre

LVAD: left ventricular assist device

M: men

mg: milligram

min: minutes

ml: milliliter

MPN: myeloproliferative neoplasm

n: amount

NID: no information documented

nm: nanometer

nl: nanoliter

No.: number

PEG: percutaneous endoscopic gastrostomy

Pre-PMF: prefibrotic primary myelofibrosis

PPSB: prothrombin complex concentrate

PT: prothrombin time

RCo: ristocetin cofactor

OAC: oral anticoagulant drugs

S: active site cysteine

SD: standard deviation

sec: seconds

TF: tissue factor

TFPI: tissue factor pathway inhibitor

UFH: unfractionated heparin

V.: vein

vaECMO: veno-arterial ECMO

vvECMO: veno-venous ECMO

VP shunt: ventriculoperitoneal shunt

vWD: von Willebrand disease

vWF: von Willebrand factor

vWM: von Willebrand multimers

W: women

µg: microgram

1 INTRODUCTION

1.1 Overall structure and biology of FXIII

FXIII, also known as a fibrin-stabilising factor, circulates in plasma as a tetrameric molecule comprised of two potentially active catalytic FXIII-A2 and two carrier/inhibitory non-catalytic FXIII-B2.[1-3] Additionally, 50% of the total fibrin-stabilising activity in blood is found in platelets where FXIII is present as a dimeric molecule consisting only of FXIII-A2.[4, 5, 6]

FXIII is an inactive precursor of a transglutaminase enzyme (i.e., a protransglutaminase), and is one of nine members of a family of transglutaminase proteins with diverse biological functions. FXIII is best recognised for its critical role in blood clot stability and wound healing.[7]

FXIII-A2 are mainly produced by cells originating in the bone marrow, namely macrophages and megakaryocytes.[8] A recent study by Wölpl et al. suggested that plasma FXIII-A2 are predominantly produced by resident tissue macrophages in the heart.[9, 7] Whereas the FXIII-B2 are synthesised in the liver.[8] Once released into the plasma, the FXIII-A2 and FXIII-B2 assemble rapidly to form the non-covalent heterotetramer FXIII-A2-B2.[10] Assembly of the FXIII-A2-B2 complex is essential, as the FXIII-B2 subunits are required for the stabilisation of FXIII-A2 in plasma.[11, 7]

In contrast to plasma FXIII-A2-B2, a cellular form of FXIII exists only as a homodimer of two catalytic subunits (FXIII-A2). Cellular FXIII-A2 is found in a wide variety of cells, including megakaryocytes, macrophages, leukocytes, and osteoblasts.[7] In particular, FXIII-A2 is present in high concentrations in platelets.[7,12]

1.2 Coagulation cascade and the functions of FXIII

As a fibrin-stabilising factor, FXIII not only plays a critical role in plasma coagulation, ensuring both clot stabilisation and crosslinking of fibrin polymers, but is also involved in many essential non-haemostatic functions, including maintenance of pregnancy, physiological vascular permeability, cartilage and bone mineralization, and survival after myocardial infarction.[13]

FXIII is activated during the final stages of blood coagulation. Plasma coagulation is initiated by exposure to a triggering molecule in the blood. This process may occur during a blood vessel injury, when extravascular TF, which is expressed in the subendothelial tissue, is exposed to

plasma procoagulants and binds with FVIIa to form the TF/FVIIa complex (the extrinsic pathway). Under pathological conditions, when blood contacts an artificial surface, a change in the conformation of FXII occurs, resulting in the formation of small amounts of FXIIa (the intrinsic/contact pathway).[14] In either pathway, a series of regulated proteolytic reactions result in the activation of FIX to FIXa, FX to FXa, and prothrombin to thrombin (the common pathway).

Thrombin has several important functions in the coagulation cascade. First, thrombin activates platelets by cleaving protease-activated receptors. Second, thrombin activates coagulation co-factors (i.e., FVIII to FVIIIa and FV to FVa). Subsequently, these reactions produce positive feedback loops to amplify the clotting response even further. Importantly, thrombin also mediates the cleavage and removal of N-terminal peptides from fibrinogen Aα- and Bβ- chains, which results in the production of insoluble fibrin monomers. These fibrin monomers assemble into fibrin protofibrils, and then into fibrin fibres, which form a web-like network that stabilises the components of a clot.[7,15-17] A schematic illustration of primary and secondary haemostasis is shown in Figure 1.

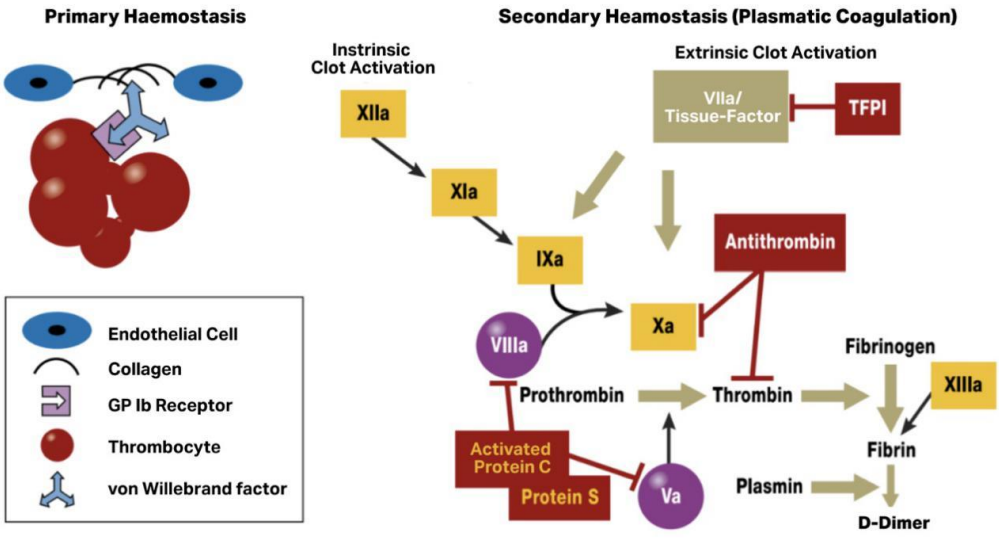

Figure 1: (Luxembourg B, Krause M, Lindhoff-Last E. Basiswissen Gerinnungslabor, Deutsches Ärzteblatt. Jg. 104, Heft 21|25. Mai 2007) Overview of the coagulation cascade. Diagram of the multistep intrinsic and extrinsic pathway. Both pathways combine into the common pathway leading to activation of FX and then subsequent thrombin-fibrin activation and finally formation of the fibrin clot. FXIII as a fibrin stabilising factor ensures clot stabilisation by crosslinking of fibrin polymers (TFPI=tissue factor pathway inhibitor; GP Ib Receptor=glycoprotein Ib receptor).

Moreover, thrombin also activates plasma FXIII-A2-B2, a process that is coordinated with the onset of fibrin formation.[7] Thrombin mediates the cleavage of the FXIII-A2, a necessary step in the activation of the plasma tetramer and dimeric platelet FXIII.[18] Fibrin polymers are an important cofactor in the generation of FXIIIa.[19-24] A complex between thrombin, fibrin polymers, and plasma FXIII accelerates cleavage of the FXIII-A2, which has important implications for haemostasis.[6]

Upon thrombin-mediated activation of FXIII, N-terminal, 37-amino acid activation peptides are cleaved and removed from the FXIII-A2. After activation, the peptides are released, and calcium induces a conformational change in FXIII that promotes the release of the FXIII-B2, which leads to the full activation of FXIII-A2 (FXIIIa). Dissociation of the FXIII-B2 from the FXIII-A2 is necessary to expose the active site cysteine in the plasma FXIII-A2. Because plasma FXIII-A2-B2 circulates in the blood bound to fibrinogen, and the formation of fibrin accelerates FXIII activation, the activation of plasma FXIII-A2-B2 is very efficient and immediately localises activated FXIII at the site of the newly-forming clot.[21]

Unlike other coagulation enzymes that proteolytically cleave their substrates, FXIIIa induces the formation of new covalent bonds. FXIIIa cross-links the fibrin γ- and α- chains, generating γ-γ dimers and high molecular weight fibrin species, composed of both γ- and α-chains.[7] The schematic activation of FXIII is depicted in Figure 2. Fibrinogen polypeptide cross-linking is depicted the Figure 3.

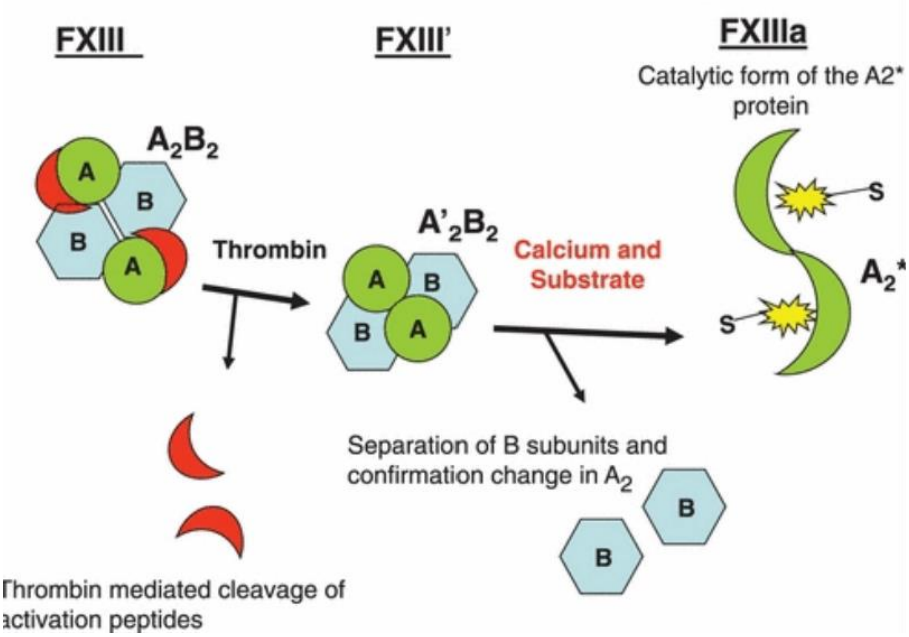

Figure 2: (Hsieh L, Nugent L. Factor XIII deficiency. Haemophilia, Volume: 14, Issue: 6, Pages: 1190-1200, First published: 30 October 2008, DOI: (10.1111/j.1365-2516.2008.01857.x) Activation of FXIII by thrombin and calcium is a two-step process. Thrombin cleaves an arginine-lysine bond in the FXIII-A and calcium causes dissociation of the FXIII-B, exposing the active site on the FXIII-A (FXIIIa) (A=FXIII A-subunit; B= FXIII B-subunit; A_2B_2, FXIII'=inactive plasma FXIII; A'_2B_2=inactive intermediate FXIII after thrombin cleavage, A_2*=calcium activated FXIII; S: active site cysteine).

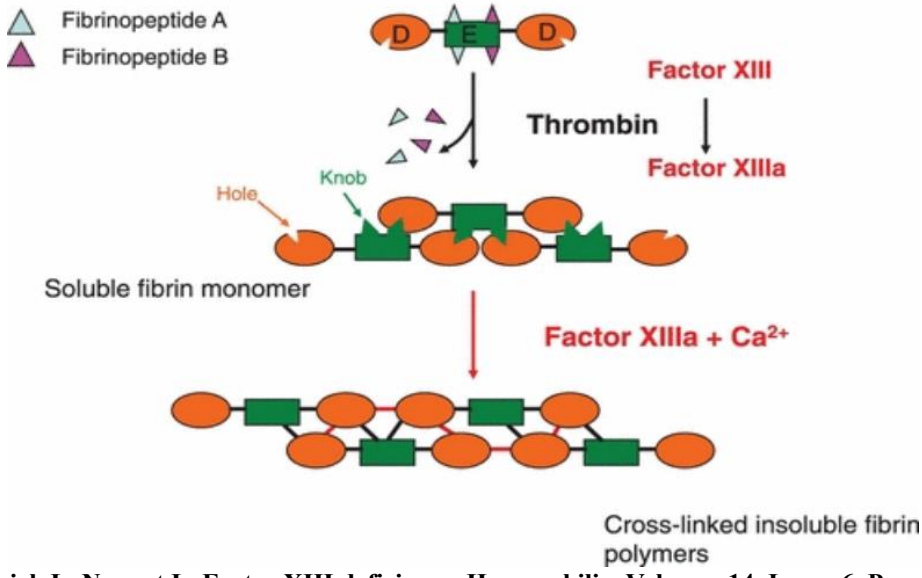

Figure 3: (Hsieh L, Nugent L. Factor XIII deficiency. Haemophilia, Volume: 14, Issue: 6, Pages: 1190-1200, First published: 30 October 2008, DOI: (10.1111/j.1365-2516.2008.01857.x)) The conversion of soluble fibrinogen to insoluble fibrin. Initially thrombin mediates the cleavage of fibrinopeptides A and B from the α- and β-chains of fibrinogen; the residual molecule is called fibrin monomer. Further the loose fibrin clot develops as fibrin monomers spontaneously polymerize. During the last step FXIII ensures the formation of a firm insoluble fibrin gel by cross-linking of the polymer (Ca2+=calcium; D=D Domain; E=E Domain).

In a review by Wolberg et al., it was noted that fibrin cross-linking has minor effects on the network structure of fibrin clots, whereas the effects on the biophysical properties of the individual fibrin fibres and stability are considerable.[13,25] First, FXIIIa cross-linking protects the clot from premature dissolution by fibrinolytic enzymes. This effect is primarily mediated by the ability of FXIII to cross-link α2-antiplasmin to the α-chain of fibrin.[26] Second, FXIIIa cross-linking increases the stiffness of the individual fibrin fibres and, consequently, the entire fibrin network. In the absence of crosslinking of the α-chains in fibrin, the clot shows reduced stability and resistance to proteolytic enzymes. The cross-linking of both fibronectin and collagen (types I, II, III, and V) to fibrin suggests that these reactions could stabilise the extracellular matrix that forms at the sites of tissue injury.[27-29] [6] Third, studies have shown that plasma FXIII promotes red blood cell retention in clots during platelet-mediated clot contraction by cross-linking the contractile proteins actin and myosin in platelets.[30,31]

1.3 Laboratory diagnosis of FXIII deficiency and the assessment of FXIII activity and antigen levels

In individuals with FXIII deficiencies, standard laboratory clotting tests typically show that PT, aPTT, fibrinogen level, platelet counts, and bleeding time are normal, which creates a diagnostic challenge for the laboratory diagnosis of FXIII deficiencies. Reliable diagnostic methods for the determination of plasma FXIII activity and antigen levels have been recently developed. In vitro assays to confirm diagnosis of FXIII deficiencies include the following: clot solubility test, FXIII activity assay, FXIII antigen assay, inhibitor assay, and molecular diagnosis.

Reference intervals for FXIII activity (69–143%) and for the FXIII-A2-B2 antigen (14–28 mg/l; 67–133%) were established according to CLSI guidelines.[32-34] No reference interval has currently been established for FXIII-B antigen and platelet FXIII. Based on the analysis of FXIII-A in the platelet lysate of 41 individuals, 60±10 fg FXIII-A antigen per platelet was calculated.[34]

1.4 Inherited FXIII deficiency

Congenital FXIII deficiency is one of the rarest inherited bleeding disorders, with a frequency of one case per 2-3 million individuals in the general European and North American population.[35] The disease is transmitted as an autosomal recessive trait and there is often a history of consanguinity within certain families of FXIII-deficient individuals.[36,37]

Inherited FXIII deficiency can be the result of a defect in FXIII-B (formerly known as type I

deficiency), or a defect in FXIII-A (formerly known as type II deficiency), and a combined deficiency is due to defects in both FXIII-A and B subunits.[36,38]

A deficiency of FXIII results in 'delayed haemorrhage' after trauma. While primary haemostasis in individuals with these characteristics is normal, delayed haemorrhage is predominantly caused by premature lysis of haemostatic clots.[39] Recently, patients with this deficiency have been diagnosed early and treated appropriately, so that severe bleeding complications associated with this deficiency are rare.[39]

In around 80 % of the patients with FXIII deficiency bleeding symptoms typically appear soon after birth, with a bleeding from the umbilical stump as the most common and characteristic symptom of the inherited FXIII deficiency.[36] Bleeding from the umbilical stump usually occurs a few days after birth, when the cord has been separated, and can be serious and life-threatening in homozygous FXIII deficiency.[40-42]

The most life-threatening bleeding complication in affected individuals is intracranial haemorrhage, which may be the first indication of FXIII deficiency. Intracranial haemorrhage may occur in up to one-third of patients without prophylactic FXIII replacement therapy, more than in any other congenital bleeding disorder. Intracranial haemorrhage was documented in 80% of patients with inherited FXIII deficiency who died due to haemorrhage.[40,43]

The registry of patients with inherited FXIII deficiency shows that the most common bleeding symptoms reported in 2007 were subcutaneous bleeds (57%), delayed umbilical cord bleeding (56%), muscle haematoma (49%), haemorrhage after surgery (40%), haemarthrosis (36%), intracerebral bleeding (34%), and abnormal wound healing (29%), which were probably caused by fibrinolytic abnormalities and altered vascular permeability, as well as impaired angiogenesis.[44]

Recurrent miscarriages and pregnancy losses are a common complication in women with FXIII deficiency. As Eshghi et al. noted in a review, FXIII is not required for ovulation, fertilisation or implantation of the fertilised egg; however, it is essential for proper anchoring of the cytotrophoblasts after they pass into the endometrium.[36] In addition, typical primary haemostatic defects such as menorrhagia and intra-abdominal bleeding may also occur in women with FXIII deficiency.

Recently, clinicians have become aware of heterozygous FXIII deficiency (FXIII activity of approximately 30–60%), which differs markedly in terms of symptoms from severe inherited FXIII deficiency.[39] Patients with this form of deficiency do not normally bleed spontaneously. Nevertheless, patients with heterozygous FXIII deficiency may experience delayed haemorrhage after surgeries (e.g., tonsillectomy), dental extraction or any form of physical trauma. In addition, heterozygous FXIII deficiency may lead to unsuccessful in-vitro fertilisation attempts in affected.[45]

1.5 Acquired FXIII deficiency

Acquired FXIII deficiency is of a multifactorial origin and is characterised by a broad clinical heterogeneity. Both clinically silent cases and those with severe wound healing disorders or fatal haemorrhages have been described. As recently as the 1960s, the first survey of nearly 100 different forms of acquired FXIII deficiency was published.[46]

A secondary deficit in FXIII is in most of the cases a non-specific comorbidity in association with various internal diseases that could be attributed to decreased hepatic synthesis of the FXIII-B (e.g., hepatitis, acute liver failure) and/or increased utilisation due to leukaemia, inflammatory bowel disorder, sepsis, major surgery, trauma, pulmonary embolism, stroke, Henoch Schoenlein purpura, liver cirrhosis and DIC.[38] Depending on the severity of the underlying disease, up to 40% of patients may show decreased levels of FXIII of less than 30%.[39,47]

Perioperative blood loss, haemodilution, and increased utilisation can lead to a reduction of FXIII activity of approximately 30–70%.[39,48,49] Especially in the case of extensive surgical interventions, abdominal or heart surgery can lead to the increased utilisation of FXIII, resulting in severe bleeding complications, intra- or postoperatively, and prolonged wound healing. In particular, extracorporeal circulation seems to be associated with the development of an acquired FXIII deficiency. This assumption is supported by studies on patients in whom a heart–lung machine was employed intraoperatively. [50]

A study by Lorenz et al. showed that treatment of patients with small bowel bleeding due to Henoch-Schoenlein purpura or large bowel bleeding in ulcerative colitis with plasma-derived FXIII concentrate can be effective in controlling bleeding.[51] Furthermore, the development of

autoantibodies against the FXIII-A (primarily) or FXIII-B (very rare) can also lead to acquired FXIII deficiency.[52] One third of these cases had been diagnosed with systemic lupus erythematosus.[53]

1.6 FXIII deficiency and haemorrhagic diatheses during ECMO therapy

Data from the ELSO registry has confirmed that bleeding is generally a major concern for patients during ECMO therapy.[54] More than 30% of ECMO patients experience treatment-related bleeding, including intracranial haemorrhage, with overall incidence ranging from 5% up to 19%, and outcomes that are predominantly fatal.[55] [56,57] Data from Australia and New Zealand have also confirmed that 71% of deaths in these patients were due to haemorrhage.[58]

In addition, identification of factors leading to haemorrhage during ECMO therapy with standard tests is often unsuccessful, as current standard coagulation tests do not typically reveal FXIII deficiency.

The exact mechanisms underlying the association of ECMO therapy and haemorrhagic diatheses are under active investigation and still need to be clarified. The most commonly proposed reasons for the reduction of FXIII activity in the context of ECMO therapy includes the pump itself, as well as the positive and negative pressures generated in the system, which can mechanically damage blood cells and platelets, in particular. Such a damage leads to impairment or loss of function or even lysis of the cells.[59,60] Moreover, blood contact with the artificial surfaces of the ECMO equipment induces a systematic inflammatory syndrome and diffuse activation of the coagulation system with consequent worsening of plasma coagulation.[61] In addition, as noted by Kalbhenn et al., the development of acquired vWD and the loss of high molecular weight vWM during ECMO therapy has been recently demonstrated.[62]

Based on published reports for adult and paediatric cohorts following cardiac surgery, perioperative blood loss, dilutional coagulopathy, and increased utilisation could lead to a post-operative decrease in FXIII ranging from 30–70%, as a secondary response to haemodilution.[63] As Fahlbush et al. described in a study on the influence of FXIII activity on postoperative transfusion in congenital cardiac surgery, one of the principal causes of severe FXIII deficiency, and the elevated risk of postoperative bleeding in the intensive care setting, is dilutional coagulopathy.[63] In another study, Kalbhenn et al. found FXIII deficiency in 88% of all patients

treated with vvECMO therapy.[64] That the reduction in FXIII activity could possibly be a sign of an active or impending haemorrhage was highlighted in a study by Theusinger et al., which found that during a period of 50 ± 16 min, FXIII concentration decreased by 20 ± 5% in patients in the emergency department.[65]

1.7 Treatment of FXIII deficiency

FFP, stored plasma, plasma-derived concentrate and Cryo are adequate sources of FXIII, and have all been used successfully in the treatment of FXIII deficiency.[66] Although FFP and Cryo are good sources of FXIII (1 and 3 IU/ml of coagulation FXIII respectively), they are no longer recommended for the treatment of FXIII deficiency due to the risk of transmission of blood-borne viruses. Moreover, the recent development and approval of pasteurised FXIII concentrates provides increased safety and higher titres of FXIII (about 240 IU/vial).[38,40,67] The first commercially available human FXIII concentrate was derived from placenta (Fibrogammin HS®), which was later initially replaced by plasma-extracted FXIII concentrates [Fibrogammin P® (CSL Behring, Marburg, Germany)], followed by a recombinant FXIII concentrate, (Novo Nordisk, Bagsvaerd, Denmark), which is currently not approved by Germany's Federal Institute for Drugs and Medical Devices.

A limitation of recombinant FXIII concentrate, compared to plasma-derived FXIII, is that it lacks the FXIII-B and will most likely be ineffective in patients who are FXIII-B-deficient.[39] A small number of randomised crossover studies have reported no differences in pharmacokinetics or tolerability between FXIII prepared from either human plasma or placenta or recombinant FXIII.[39,68]

In patients with acute bleeding episodes, the recommended treatment is administration of 15–30 IU/kg of FXIII concentrate or a bolus of 1250 IU until the bleeding has stopped.[36] A dosage of 1 IU of FXIII concentrate per kg increases the activity of FXIII in plasma by 1–2%.

Relative contraindications for the use of FXIII concentrate include recent thromboses or pulmonary embolisms.[69]

1.7.1 Therapeutic options for inherited FXIII deficiency

Due to the long half-life of plasma FXIII (9–14 days), and the observation in clinical practice

that even low levels of FXIII (> 3–10%) are usually sufficient to prevent spontaneous bleeding, prophylaxis is the current management strategy of choice. Life-long prophylactic therapy is recommended with 20–30 IU/kg per month for mild and moderate levels of bleeding, while severe bleeding might require increased dosages up to 50 IU/kg of body weight and administration at more frequent intervals.[37,39]

Affected individuals who have sustained a trauma or will be undergoing surgery require more intensive replacement therapy with FXIII concentrates. For major surgical procedures the plasma concentration of FXIII should be maintained above 5% until wound healing is complete. To achieve this level of concentration, administration of 20–30 IU/kg of FXIII per day is recommended. For minor interventions, the administration of 10–20 IU/kg FXIII per day for two till three days is recommended.[37]

1.7.2 Therapeutic options for acquired FXIII deficiency

Studies on acquired FXIII deficiency and the optimal monitoring and treatment goals in the case of reduced FXIII activity are few in number. Moreover, no uniformly acceptable threshold for the administration of FXIII has yet been established and discussions of treatment strategies have been controversial. Most treatment strategies focus on the immediate treatment of acute bleeding using Cryo, FFP, plasma-derived FXIII concentrate or recombinant FVIIa.

According to the recommendations of guidelines for haemotherapy, the administration of the FXIII concentrate may be justified in cases of extensive and uncontrolled bleeding events, after other coagulation disorders have been excluded, even without laboratory confirmation of FXIII deficiency.[70] Guidelines from the European Society of Anaesthesiology for the management of severe perioperative bleeding suggest that in cases of ongoing or diffuse bleeding and low clot strength despite adequate serum fibrinogen concentrations, it is likely that FXIII activity is critically reduced; therefore, in cases of significant FXIII deficiency (i.e., < 60% activity), the administration of FXIII concentrate can be considered. However, even in regard to these scenarios, the guidelines emphasise that more data are needed on the effects of FXIII concentrate on bleeding and transfusion requirements. Furthermore, the guidelines from the European Society of Anaesthesiology for the management of severe perioperative bleeding suggests maintaining FXIII levels above 50–60% when severe perioperative bleeding occurs.[70] Further prospective randomised trials are essential to develop specific strategies for managing severe bleeding in patients with acquired FXIII deficiency in surgical and intensive care settings,

particularly in the context of ECMO therapy, in order to develop efficient algorithms for the management and treatment of bleeding complications in ECMO patients.

1.8 The aim of the current research

In contrast to inherited FXIII deficiency, the clinical relevance of acquired FXIII deficiency is unclear and currently very few studies had investigated optimal monitoring and treatment targets in patients with acquired FXIII deficiency. In addition, the uniformly accepted indication for initiating prophylactic measures in acquired FXIII deficiency is not clearly defined in the current medical literature and remains to be clarified.

The aim of the present study was to characterise the incidence, diagnostic methods, and FXIII replacement therapy in patients with FXIII deficiency at the University Hospital Regensburg and to evaluate the clinical relevance of FXIII deficiency.

In 2017, FXIII activity assays were performed once per week. In 2019, a new laboratory order for FXIII activity analysis was introduced, increasing the availability of this analysis on a daily basis. Another aim of this study was to investigate whether any differences in the administration of plasma-derived FXIII concentrates were observed in 2019 compared with 2017, as a result of the introduction of the new laboratory order for FXIII activity analysis.

This research was conducted at the University Hospital Regensburg. The University Hospital Regensburg is a maximum medical care provider in the eastern Bavarian region with a total of 839 beds. On average, 37,338 patients are treated annually in an inpatient/day-care setting, and 159,771 patients, in the outpatient setting (https://www.ukr.de/ueber-uns/Daten_und_Fakten/index.php, status: 20.07.2020).

2 MATERIALS AND METHODS

2.1 Data collection

This study was conducted as a retrospective, evidence-based investigation and encompasses a single-center series at the University Hospital Regensburg. The research was carried out in accordance with the Declaration of Helsinki. The local Clinical Ethics Committee at the University Hospital Regensburg provided a waiver of approval for the study (Kennzeichen 19-1330-104).

During the period from 01.01 to 31.12.2017 all performed FXIII activity assays (%) in the coagulation laboratory of the University Hospital Regensburg were included. Quantitative assays (functional activity assays) were carried out using commercially available reagents for the ammonia release assay method (Berichrom FXIII Siemens, Marburg, Germany).

To assess the incidence, diagnostic methods, and therapeutic options for FXIII deficiency at Regensburg University Hospital and to evaluate the clinical relevance of FXIII deficiency, medical and clinical reports and laboratory data of patients with severely reduced FXIII (< 41%) were reviewed. Based on the medical reports and the laboratory findings, the following parameters were identified and documented: age, sex, department in which the patient was admitted, blood count, thromboplastin time (Quick value), INR, aPTT, fibrinogen and AT. The acquisition of retrospective data was performed using the laboratory information system Lauris (version 15.09.29.9 Swisslab GmbH, Berlin, Germany) and SAP (Krankenhausinformationssystem) software solutions. Furthermore, diagnoses related to bleeding complications, clinically relevant documented bleeding events, the amount of administered blood transfusions (FFP, platelet concentrates, and red blood cells concentrates) during the period of hospitalisation, and therapy with ECMO were also included. Because the interpretation of FXIII activity values may be distorted in cases of anticoagulation with argatroban, the type of anticoagulation administered was also documented.

In addition, the author examined the medical and clinical reports and laboratory findings of six patients diagnosed with moderately reduced FXIII activity (41–70%) during routine coagulation diagnostics at the coagulation outpatient clinic at the University Hospital Regensburg to assess whether there was a documented predisposition to bleeding events in the medical history, clinical presentation, or laboratory findings.

2.2 FXIII activity assays

The ammonia release assay is the most commonly used and convenient method because of the short time required to perform it.[71] In the ammonia-release assays FXIII is activated by thrombin and calcium and the resulting FXIIIa cross-links a small molecular weight amine substrate to a glutamine containing oligopeptide.[72] Ammonia released in the transgluataminase reaction is spectrophotometrically monitored by glutaminase dehydrogenase-mediated NAD(P)H-dependent indicator reaction at 340 nm.[34,73,74] Commercially available ammonia release assays are: Berichrom FXIII (Siemens, Marburg, Germany), REA-chrom FXIII (Reanal-ker, Budapest, Hungry) and TECHNOCHROM FXIII (Technoclone, Vienna, Austria) tests. The ammonia release assays are rapid, one-step with good reproducibility and can be adapted to automatic coagulation assays.[72]

The range of plasma FXIII activity in the population is very broad, ranging from 60-250% of the normal plasma level. FXIII levels are also influenced by non-genetic variables such as age and gender.[32] Reference intervals for FXIII activity over 70% (>70%) were described in this study as the normal range of activity, reference intervals between 70% and 41% as moderately reduced FXIII activity and the reference intervals less than 41% (<41%) as severely reduced FXIII activity. With all the performed FXIII activity levels during this period were attained additional data of specific time of requested FXIII assays, patients age, gender, and the specific department, which requested this assay.

2.3 FXIII replacement therapy

With the assistance of the Institute of Pharmacy of University Hospital Regensburg the data of all the dispensed FXIII concentrates Fibrogammin® at the University Hospital Regensburg during 2017 were collected to investigate whether there was a correlation between the FXIII activity levels and the administration of the FXIII concentrate and in which clinical situations this replacement therapy was administrated. Therefore, the medical reports and laboratory data of patients who received replacement therapy with FXIII concentrate but had moderately reduced or normal FXIII activity levels were also investigated. As mentioned above, age, gender, department, in which the patient was admitted, laboratory findings, specifically, blood cell counts and extended haemostaseological diagnostics, diagnoses related to the bleeding complications, clinically relevant documented bleeding events, number of blood transfusions

administered, therapy with ECMO and the anticoagulation were also documented.

In order to compare the obtained data from 2017 with the newly introduced laboratory order in which FXIII activity assays were made available on a daily basis, the author selected all performed FXIII activity assays (%) in the coagulation laboratory of the University Hospital Regensburg in 2019 and compared them with the obtained data from 2017. With all FXIII activity values performed from 01.01.2019 to 31.12.2019, additional data were collected on the exact timing of the requested FXIII assays, the age and gender of the patients, and the department that requested the assays. Furthermore, with the support of the Institute of Pharmacy of University Hospital Regensburg, the data of all FXIII concentrates dispensed at the University Hospital Regensburg in 2019 were collected and compared to the data from 2017.

2.4 Statistical analysis

For statistical analysis, Microsoft Excel for Windows 20.00 program was used (Microsoft Excel Inc., IBM, Somers, New York, USA). All the data used to evaluate were summarized in tables and graphs.

3 RESULTS

3.1 FXIII activity assays and FXIII replacement therapy in 2017

3.1.1 FXIII activity assays

In the period from 01.01 to 31.12.2017, a total of 592 FXIII activity assays (ammonia release assay method, Berichrom FXIII [Siemens, Marburg, Germany]) were performed on 458 different patients in the coagulation laboratory at the University Hospital Regensburg. Overall, 255 (55.7%) of the patients studied were women and 203 (44.3%) were men. Mean age was 39.0 years (range 2-91 years). FXIII activity tests were most frequently performed in the age group between 1 and 20 years. The percentage distribution of FXIII activity tests across age groups is shown in Figure 4.

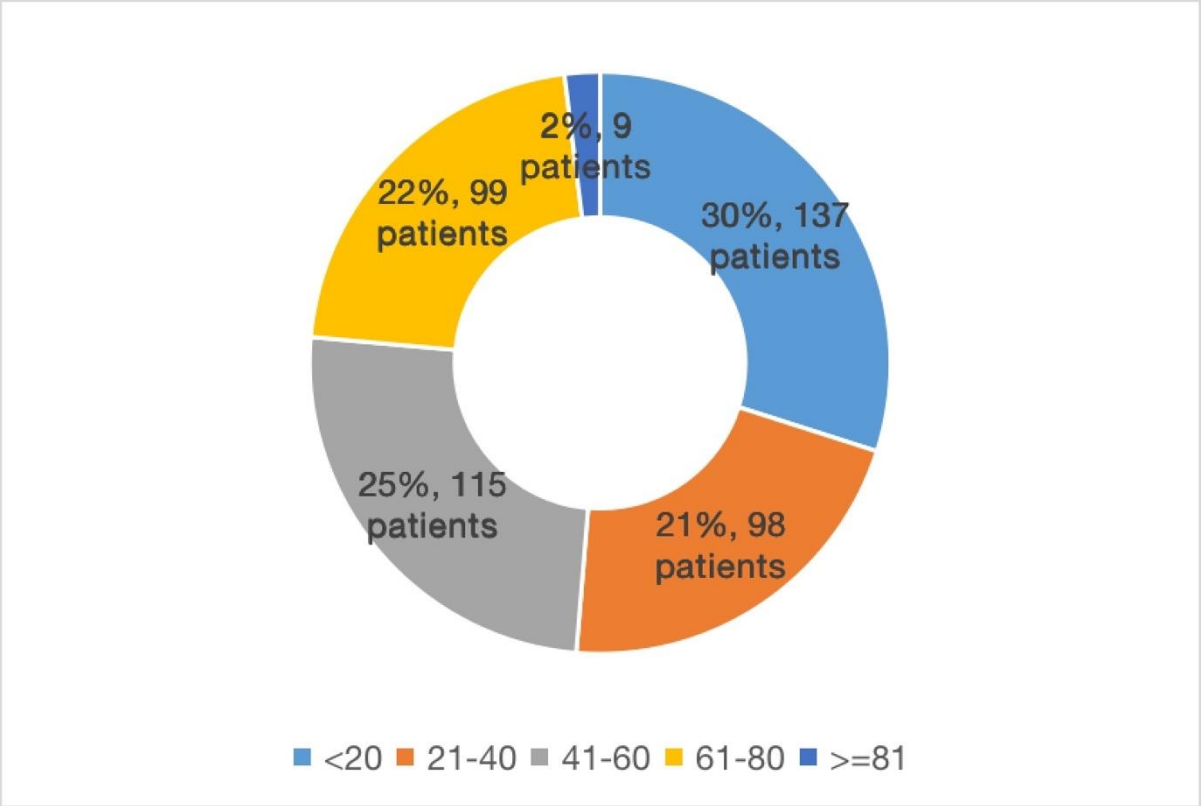

Figure 4: The percentage distribution of FXIII activity tests across age groups.

Severe FXIII deficiency (0-40%) was identified in 46 tests (31 patients), moderate deficiency (41-70%) in 126 tests (91 patients), and normal FXIII activity (>70%) in 420 tests (380 patients). The distribution of FXIII activity values is shown in Figure 5.

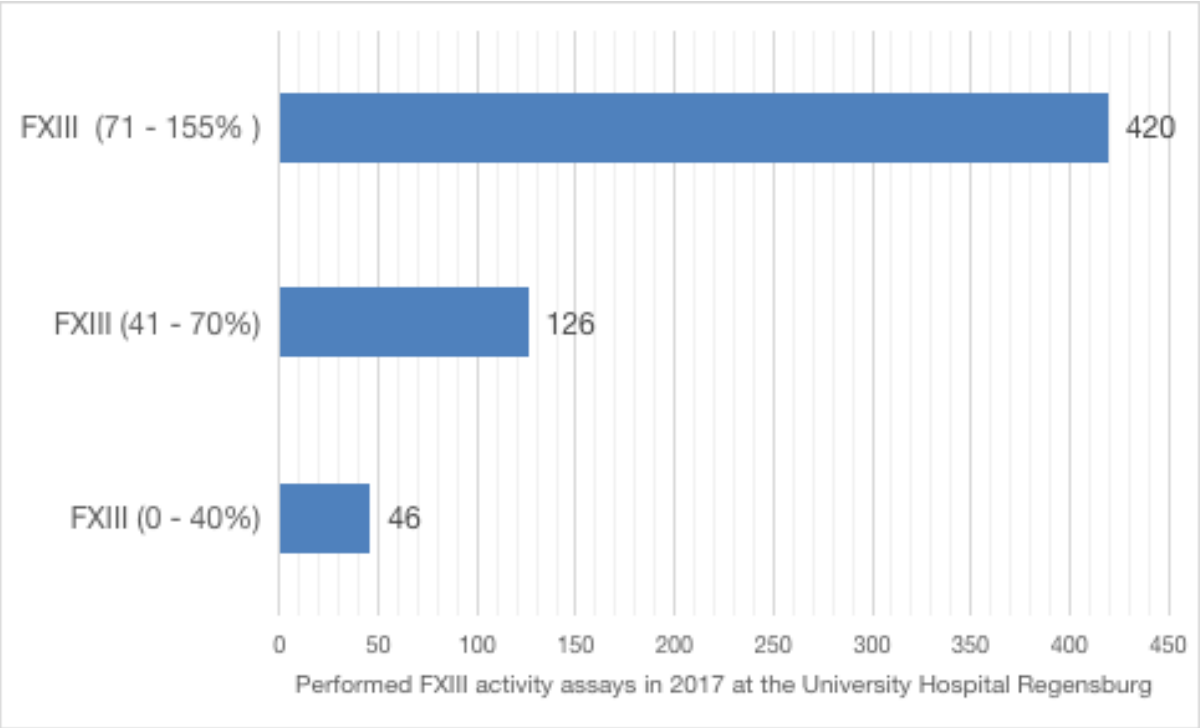

Figure 5: The distribution of FXIII activity values.

Overall, 91.3% of the 46 FXIII activity assays in patients with severe FXIII deficiency were requested by the various ICUs across several departments at the University Hospital Regensburg, with most requests from the internal medical ICU (ward 93, cardiology), with a total of 30 tests, followed by the cardio-thoracic surgical ICU (ward 97) (n=11). Another five tests were requested from the stem cell transplantation ward (ward 21) (n=2), the paediatric clinic (ICU and paediatric outpatient clinic) (n=2) and the cardio-thoracic surgical intermediate care (ward 60) (n=1). The percentage distribution of FXIII tests for severely decreased FXIII activity across departments is shown in Figure 6.

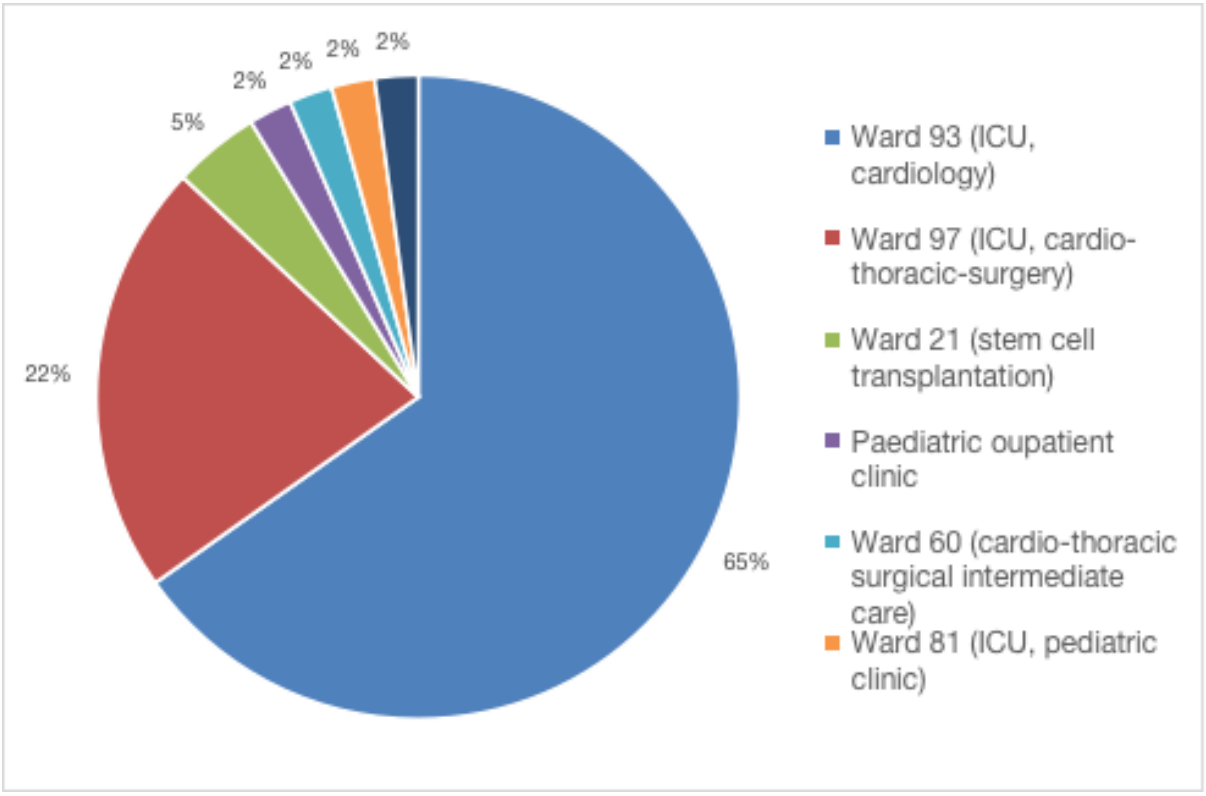

Figure 6: The percentages distribution of FXIII activity assays with severely decreased activity across departments.

In patients with moderate FXIII deficiency (41-70%), most FXIII activity assays (80.2%) were requested by various ICUs. Most requests came from the internal medical ICU (ward 93, cardiology) with 53 tests, the cardio-thoracic surgical ICU (ward 97) (n=22), and the operative ICU (ward 90) (n=19). In addition, 5.6% (n=7) of FXIII activity tests for moderate deficiency were requested by the coagulation outpatient clinic, and 3.9% (n=5) by the stem cell transplantation ward (ward 21). The percentage distribution of FXIII activity tests in patients with moderately reduced activity across departments is shown in Figure 7.

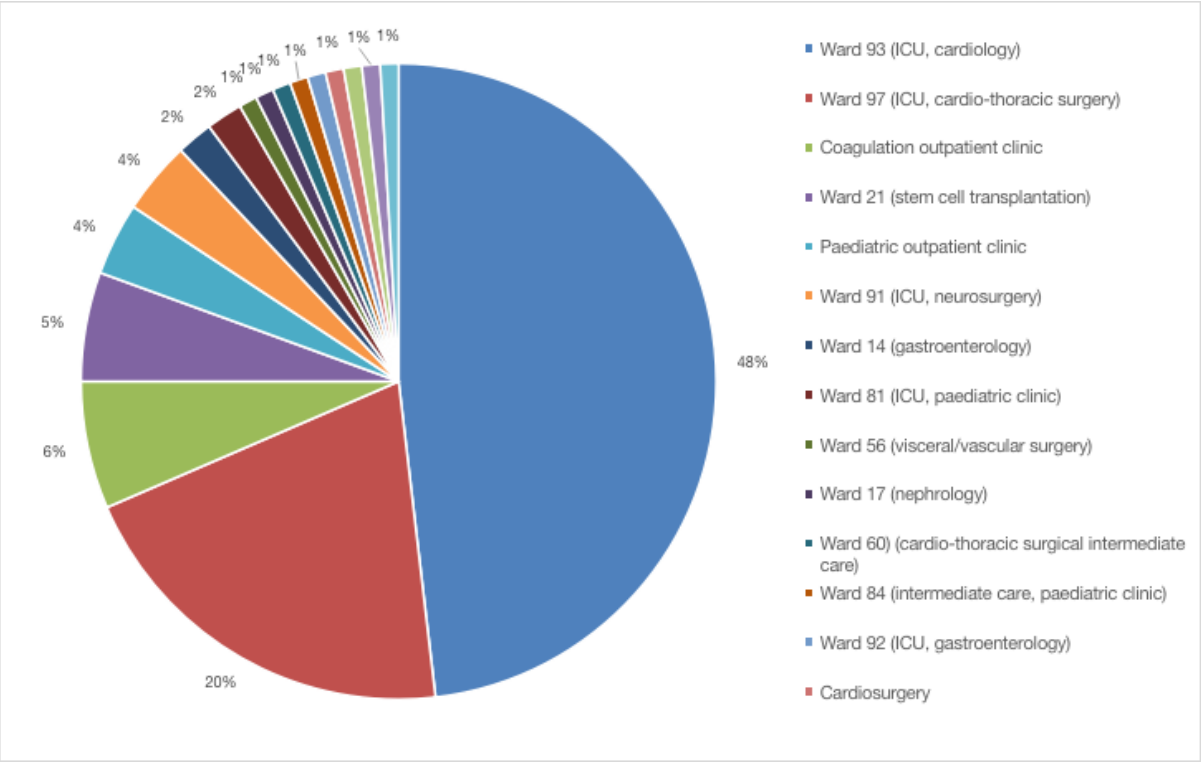

Figure 7: The percentage distribution of FXIII activity assays with moderately reduced activity across departments.

Normal FXIII activity was diagnosed in 95.9% (n=166) of the total 173 FXIII activity tests performed in the coagulation outpatient clinic in 2017 as the part of routine coagulation diagnostics. Moderate FXIII deficiency was detected in 4.1% (n=7). None of the patients with severely reduced FXIII activity were diagnosed in the coagulation outpatient clinic in 2017. The distribution of FXIII activity values in FXIII tests in the coagulation outpatient clinic is shown in Figure 8.

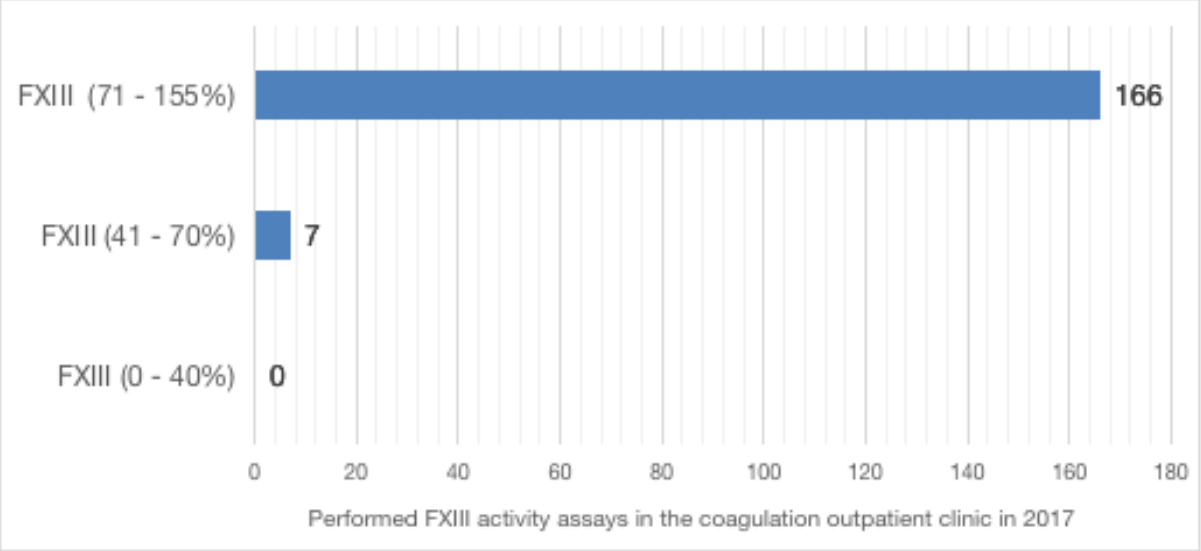

Figure 8: The distribution of FXIII activity values in FXIII assays in the coagulation outpatient clinic.

The highest number of FXIII activity tests in 2017 was requested by the coagulation outpatient clinic: 173 tests, representing 29.2% of all FXIII activity tests performed that year. Overall, 36.8% (n=218) of the tests performed were requested by various ICUs, with most requests coming from the internal medical ICUs (ward 93/92, cardiology/gastroenterology), with 102 requests and the cardio-thoracic surgical ICU (ward 97), with 43 requests. Overall, 20.1% (n=119) of the tests performed were requested by the various departments of the paediatric clinic (ICU and paediatric outpatient clinic). General wards (internal medicine/surgery) requested 5.6 % (n=33) of the FXIII activity assays performed. Overall, 3.9% (n=23) of the assays performed were requested by various intermediate care units: stem cell transplantation (ward 21) (n=15), cardio-thoracic surgery (ward 60) (n=5), and paediatric intermediate care (ward 84) (n=3). Individual requests for evaluation of FXIII activity were documented from dermatology, otorhinolaryngology, radiation therapy, and the emergency department. The distribution of requests for FXIII activity assays across departments is depicted in Figure 9.

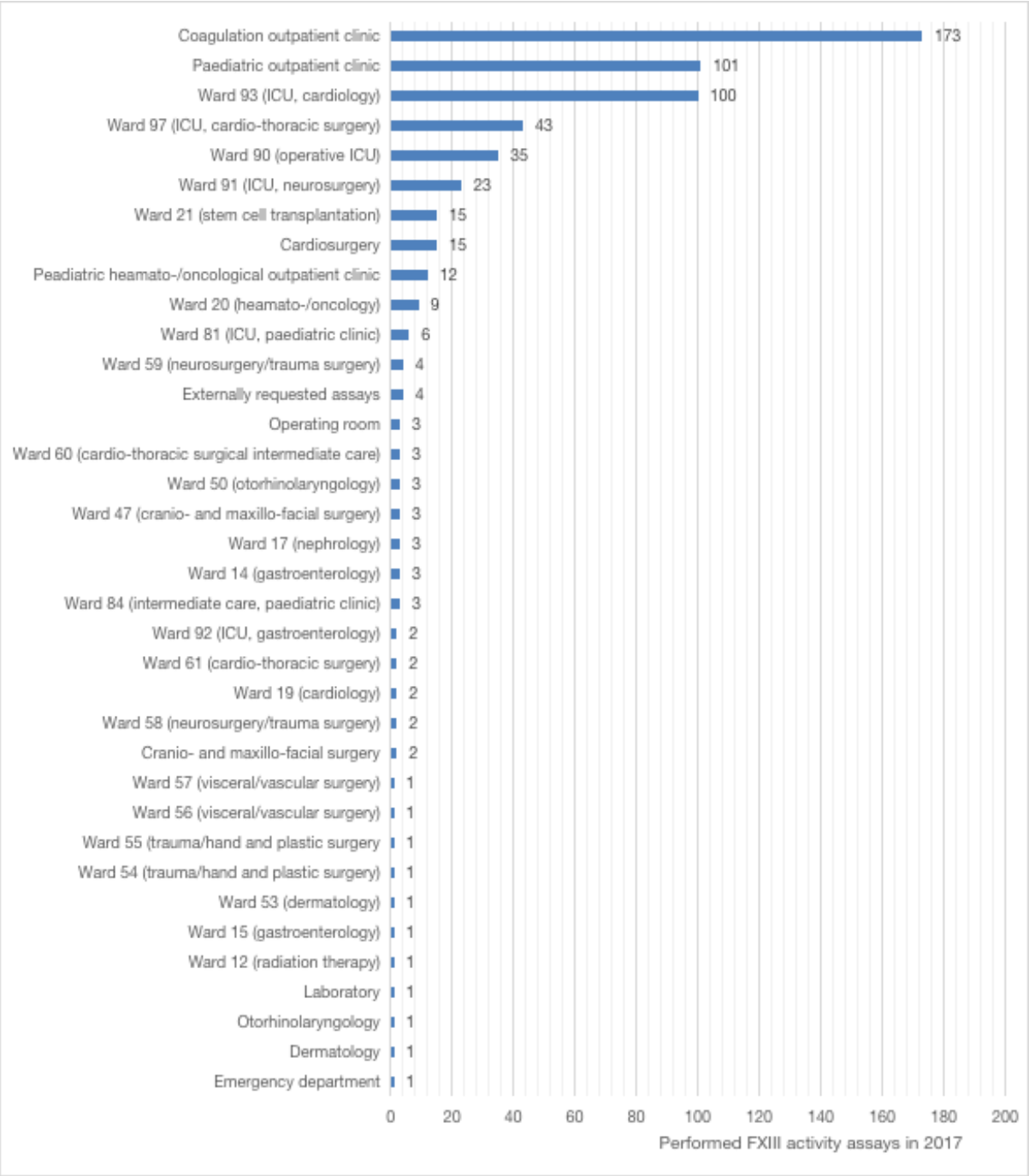

Figure 9: The distribution of requests for FXIII activity assays across various departments at the University Hospital Regensburg in 2017.

3.1.2 Characteristics of patients with severe FXIII deficiency

Severe FXIII deficiency was diagnosed in 31 patients in the coagulation laboratory in 2017. Among these, 7 (22.5%) patients were women and 24 (77.4%) were men. The median age was 48 years, with the youngest patient 4 years and the oldest 71 years of age. An average of 1.5 tests were performed to determine FXIII activity, with a maximum of 4 tests in a single patient. The laboratory findings in these patients are shown in Table 1.

3.1.2.1 Laboratory findings

	Haemoglobin	**Platelets**	**Quick**	**INR**	**PTT**	**Fibrinogen**	**Antithrombin**
Reference range	11.2 - 15.7	182.0 – 369.0	> 70	0.85–1.15	25.9 - 36.6	210 - 400	79.4 - 111.5
Units	g/dl	/nl	%	-	sec	mg/dl	%
n	41	40	40	40	40	38	38
Average value	9.0	108.6	66.7	1.5	51.1	355.5	78.8
Mean value	8.8	95.0	68.5	1.3	49.3	255.4	76.7
Maximal value	13.6	250.0	100.0	6.0	120.0	1080.0	126.0
Minimal value	7.4	13.0	6.0	1.0	27.5	40.0	23.9
Standard deviation	1.4	60.5	22.2	1.0	19.8	238.9	23.1

Table 1: Laboratory findings in patients with severe FXIII deficiency (n=amount).

3.1.2.2 Clinical characteristics of bleeding events

The most frequent bleeding complications were associated with ECMO therapy; overall, bleeding from the puncture sites of ECMO cannulae was reported in 29% of all patients (n=9). The second most common bleeding complication was nasopharyngeal bleeding (22.6%) and haematomas (22.6%) at various sites, including right ventricle, left thigh, right cleft lip, retroperitoneal, and pericardial. Overall, 4 patients (12.9%) had both upper and lower gastrointestinal bleeding. Severe intrathoracic haemorrhage was found in 4 patients (12.9%) and included bleeding from chest tubes and tracheal tubes, endobronchially confirmed bleeding in the right lower lobe, haemoptysis, and bleeding after intubation. Bleeding-related events were not recorded in 8 patients (25.8% of cases). Interestingly, in 2 patients, a history of severe bleeding tendency was documented prior to hospital admission. The most common bleeding events are shown in Figure 10. A detailed description of all clinically documented bleeding events is provided in Table 2.

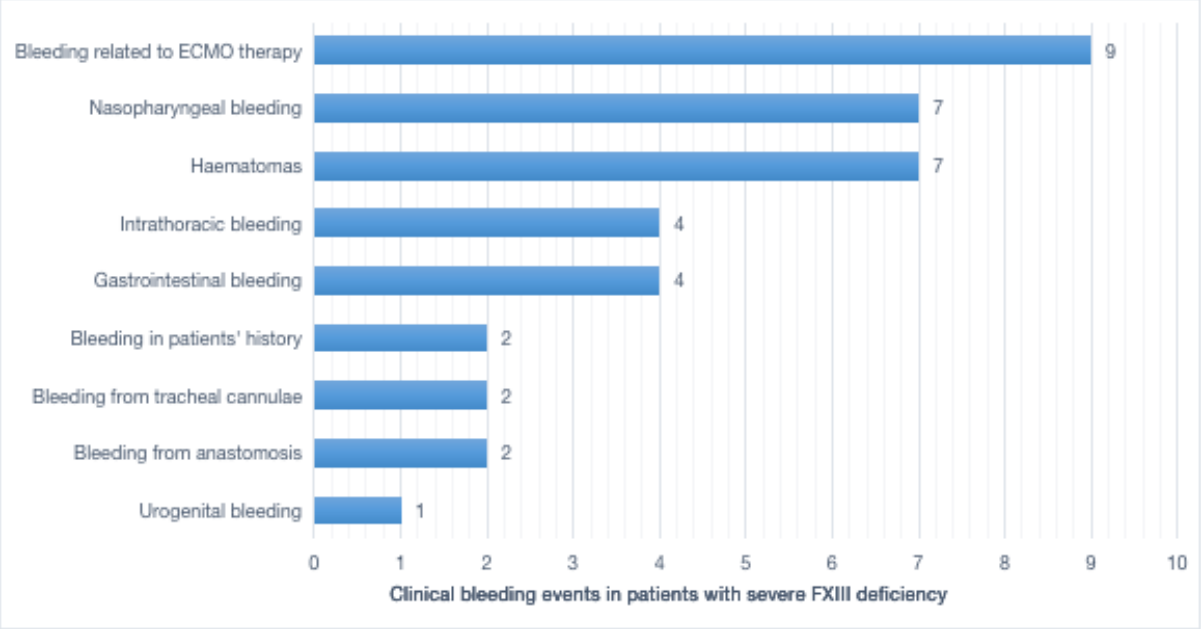

Figure 10: The most common bleeding events in patients with severe FXIII deficiency.

Patient No.	Age	Sex	Documented clinical bleeding events
1	43	M	Haemoglobin-relevant upper gastrointestinal bleeding by ulcus duodeni, bleeding from the tracheal cannula
2	34	M	Bleeding complications during previous surgical interventions
3	54	M	Nasopharyngeal bleeding under ECMO, diffuse mucosal bleeding
4	44	W	Bleeding from the puncture site
5	47	M	Bleeding from the puncture site under ECMO
6	24	W	Nasopharyngeal bleeding, suspected haematoma
7	33	M	Extensive bleeding from puncture sites of ECMO cannulae
8	69	M	NID
9	62	W	Recurrent bleeding from puncture sites of ECMO cannulae
10	57	M	Bleeding from the puncture site in the femoral artery, haemothorax on the left side
11	46	W	Bronchoscopy: blood in the right inferior lobe - endobronchially abundant bloody secretion, epistaxis
12	1	W	NID
13	38	M	Diffuse bleeding tendency, haemodynamically relevant, haematoma in front of the right ventricle, suspected bleeding from the anastomosis, diffuse bleeding from the chest drains, haemohorax on the right side
14	39	W	NID
15	48	W	NID
16	49	M	Discrete bleeding from puncture sites of ECMO cannulae
17	41	M	Oropharyngeal and nasopharyngeal bleeding
18	19	M	Discrete bleeding from the tracheotomy, haemoptysis
19	50	M	Haemorrhage in the left thigh, arterial bleeding from puncture sites of ECMO cannulae
20	68	M	NID
21	63	M	Nasopharyngeal bleeding, bleeding from the puncture sites of ECMO cannulae, gastrointestinal bleeding, haematoma in the right cleft lip.
22	19	M	Bleeding from all the puncture sites, after the intubation a lot of bloody,

			foamy secretion from the tube
23	69	M	NID
24	61	M	NID
25	56	M	Recurrent bleeding (retroperitoneal haematoma, haematoma in the area of leg, epistaxis, urogenital bleeding, unstoppable esophageal bleeding)
26	60	M	Pericardial haematoma, haemodynamically relevant, profuse thoracic bleeding, haemothorax on the right side, increased bleeding from the drains, pericardial space, recurrent intrathoracic bleeding
27	52	M	Bleeding in the area of the arterial puncture in the right groin
28	17	M	Injury at soccer games with impending compartment syndrome. Bleeding, epistaxis once a month, gum bleeding, 40 days postpartum in the hospital umbilical cord stump bleeding
29	48	M	Tarry stools, coffee-like reflux, gastrointestinal bleeding, renewed bleeding in the esophagogastroduodenoscopy - active arterial bleeding in the fundus
30	62	M	Slight tendency to haematomas and petechiae, a diffuse tendency to bleeding especially bleeding from the distal anastomosis, mucosal bleeding
31	64	M	NID

Table 2: Detailed description of all clinically documented bleeding events (M=man; NID=no information documented; W=women).

3.1.2.3 Administration of blood transfusions

Overall, 2 patients with severe FXIII deficiency did not receive the replacement therapy with FFP, platelet concentrates, or red cell concentrates during their inpatient stay. At the time the FXIII activity assay was performed, 30 patients (96.8%) had received replacement therapy with red cell concentrates, 18 patients (58.1%) with platelet concentrates, and 15 (48.4%) patients with FFP. On average, patients received 19 red cell concentrates (range 0-102), 7 platelet concentrates (range 0-125), and 44 FFP (range 0-339). The distribution of replacement therapy administered is shown in Table 3.

Patient No.	Age	Sex	Red blood concentrates	Platelets concentrates	FFP
1	43	M	2	1	-
2	34	M	-	1	-
3	54	M	3	-	-
4	44	W	2	-	-
5	47	M	-	-	-
6	24	W	5	-	6
7	33	M	6	1	-
8	69	M	2	-	-
9	62	W	18	-	6
10	57	M	13	1	-
11	46	W	16	-	12
12	1	W	2		2
13	38	M	102	20	66
14	39	W	4	1	-
15	48	W	2	1	36
16	49	M	3	1	-
17	41	M	16	13	97
18	19	M	9	-	339
19	50	M	7	1	4
20	68	M	-	-	-
21	63	M	12	-	18
22	19	M	1	-	-
23	69	M	27	3	3
24	61	M	19	11	-
25	56	M	88	8	3
26	60	M	64	22	21
27	52	M	6	2	-

28	17	M	-	-	20
29	48	M	34	18	-
30	62	M	27	25	40
31	64	M	25	8	-

Table 3: The distribution of administered replacement therapy (M=man; W=women).

3.1.2.4 Diagnoses related to bleeding complications

The most common bleeding-related diagnoses were associated with ECMO therapy. Overall, 40.8% of recorded bleeding/coagulation disorders were complications related to ECMO therapy, among which the most common diagnoses were ECMO-associated thrombocytopenia, hyperfibrinolysis, thrombosis, or local bleeding. The most common diagnosis documented in patients' medical reports was anaemia of various types, including normochromic normocytic anaemia, iron deficiency anaemia, and macrocytic hyperchromic anaemia. DIC was recorded in 5 patients (10.2%). HIT and pulmonary embolism were documented in 2 patients. AT deficiency, afibrinogenemia, seronegative autoimmune thrombocytopenia and FXIII deficiency were also reported. A total of 8 patients (25.8%) did not have a documented bleeding-related diagnosis. The most common diagnoses associated with bleeding/coagulation abnormalities are shown in Figure 11. A detailed description of all diagnoses associated with bleeding/coagulation abnormalities is shown in Table 4.

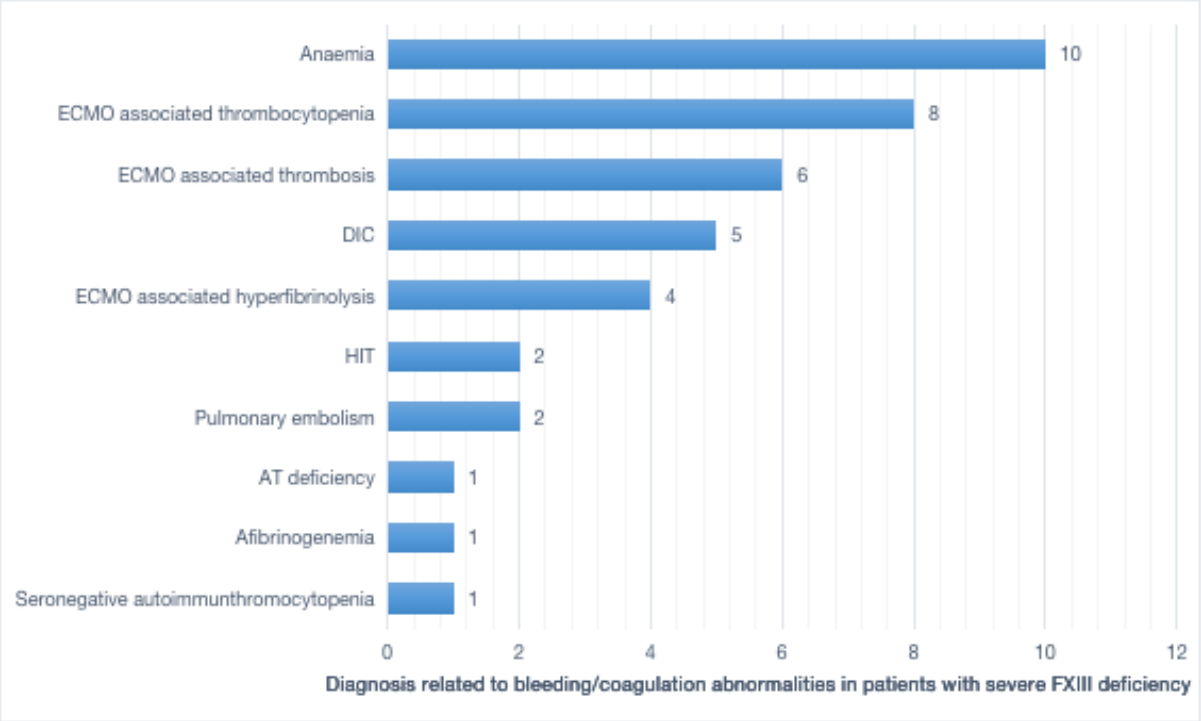

Figure 11: The most common diagnoses associated with bleeding/coagulation abnormalities in patients with severe FXIII deficiency.

Patient No.	Age	Sex	Diagnosis related to the bleeding/abnormalities complications
1	43	M	ECMO-associated thrombocytopenia, pulmonary embolism, thrombus
2	34	M	NID
3	54	M	DIC, macrocytic anaemia, ECMO initiated hyperfibrinolysis, thrombus in the right V. Jugularis. ECMO-associated thrombocytopenia
4	44	W	Thrombocytopenia, a.e. ECMO-associated coagulation abnormalities, hyperfibrinolysis
5	47	M	ECMO-associated thrombocytopenia, macrocytic anaemia, thrombosis in the right V. jugularis interna after ECMO therapy
6	24	W	Haemothorax, haemorrhagic shock, hyperfibrinolysis, iron deficiency anaemia, thrombosis in the right V. jugularis
7	33	M	DIC, ECMO-associated thrombocytopenia, iron deficiency anaemia
8	69	M	NID
9	62	W	ECMO and sepsis associated coagulation abnormalities, normochromic, normocytic anaemia, periarticular haematoma, haemorrhagic gastritis
10	57	M	HIT
11	46	W	Thrombosis in the right V. iliaca till V. cava inferior, thrombocytopenia, normochromic anaemia, left thigh haematoma
12	1	W	AT deficiency, anaemia
13	38	M	NID
14	39	W	NID
15	48	W	DIC
16	49	M	Retroperitoneal haemorrhage by forefoot ischemia, ECMO-associated thrombocytopenia, macrocytic hyperchromic anaemia
17	41	M	DIC
18	19	M	Inferior V. cava thrombosis after ECMO, thrombosis in the right V. jugularis after ECMO, HIT
19	50	M	Haemoglobin-relevant arterial bleeding in left groin after removal of ECMO cannulae, epistaxis, haemorrhage in the thyroid gland

20	68	M	NID
21	63	M	Hyperfibrinolysis under ECMO therapy, ECMO-associated thrombocytopenia, haemoglobin-relevant gastrointestinal bleeding
22	19	M	Thrombosis in V. cava und V. jugularis, pulmonary embolism in inferior lobe on the left side
23	69	M	Thrombosis in the LVAD system, large intracavitary thrombus in the heart, iron deficiency anaemia, hemodynamically relevant pericardial temponade
24	61	M	NID
25	56	M	FXIII deficiency, recurrent bleedings under anticoagulation with UFH by existing LVAD system
26	60	M	NID
27	52	M	Pancytopenia
28	17	M	Afibrinogenemia
29	48	M	NID
30	62	M	DIC, seronegative autoimmunthrombocytopenia, macrocytic anaemia
31	64	M	NID

Table 4: Detailed description characterization of all diagnoses associated the with the bleeding/coagulation abnormalities (M=men; NID=no information documented; W=women).

3.1.2.5 Anticoagulation

Overall, 51.6% of patients (n=16) with severe FXIII deficiency received anticoagulation therapy with intravenous heparin. In 1 patient, when HIT was suspected, anticoagulation with heparin was switched to argatroban. Overall, 19.4% of patients (n=6) received argatroban. In 2 patients, anticoagulation was switched from argatroban to heparin during their inpatient stay. In 3 cases, the patients were not given anticoagulation during their inpatient stay. In 3 patients, information about the anticoagulation prescribed was not intelligible in the medical reports.

3.1.2.6 ECMO therapy

Overall, 25 patients (80.7%) were diagnosed with severe FXIII deficiency in the course of the ECMO therapy during their stay in ICU. Among these, 24% of patients (n=6) were treated with vaECMO and 76% of patients (n=19) with vvECMO therapy.

3.1.3 Replacement therapy with FXIII

3.1.3.1 Severe FXIII deficiency

Overall, 5 patients (16.1%) of a total of 31 patients with documented severe FXIII deficiency received replacement therapy with Fibrogammin® in 2017. In most cases (3 out of 5), Fibrogammin® was prescribed for 1 patient in the cardio-thoracic surgical ICU (ward 97), 1 patient in the stem cell transplantation ward (ward 21), and 1 patient in the cardio-thoracic surgical intermediate care (ward 60). The distribution of patients who received Fibrogammin® across departments is shown in Figure 12.

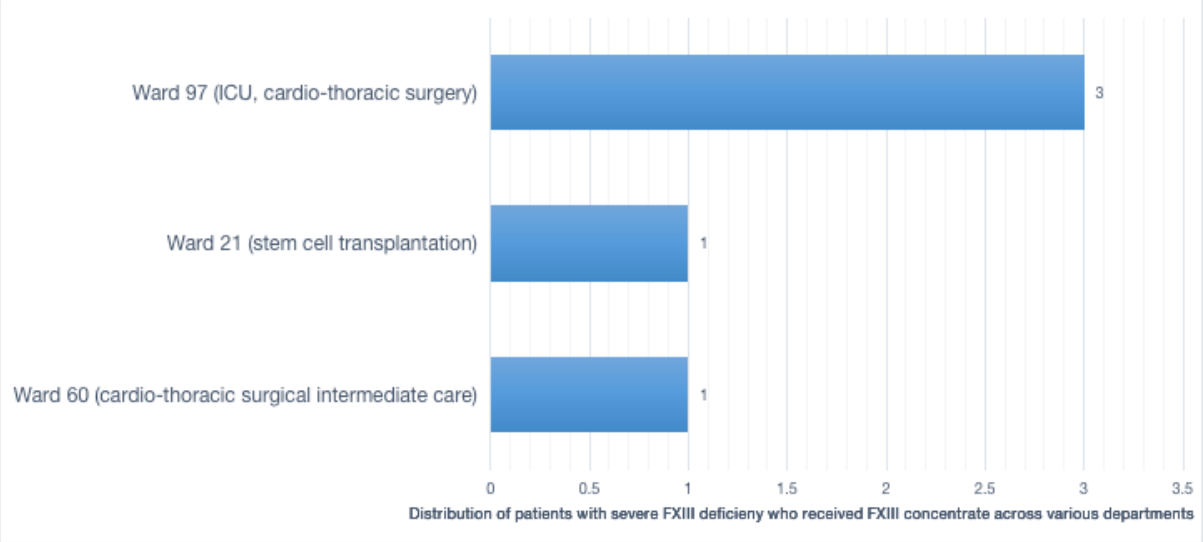

Figure 12: The distribution of patients with severe FXIII deficiency who received Fibrogammin® across various departments.

In 2017, 22,500 IU of Fibrogammin® were administered to patients with severe FXIII deficiency. Detailed data on the exact doses of Fibrogammin® were not recorded in the medical reports. Administration of Fibrogammin® occurred before (in 9 patients) or after (in 9 patients) determination of FXIII activity levels. None of the patients received Fibrogammin® replacement therapy on the same day that FXIII activity was determined. The percentage distribution of Fibrogammin® administration according to the time of determination of FXIII activity is shown in Figure 13.

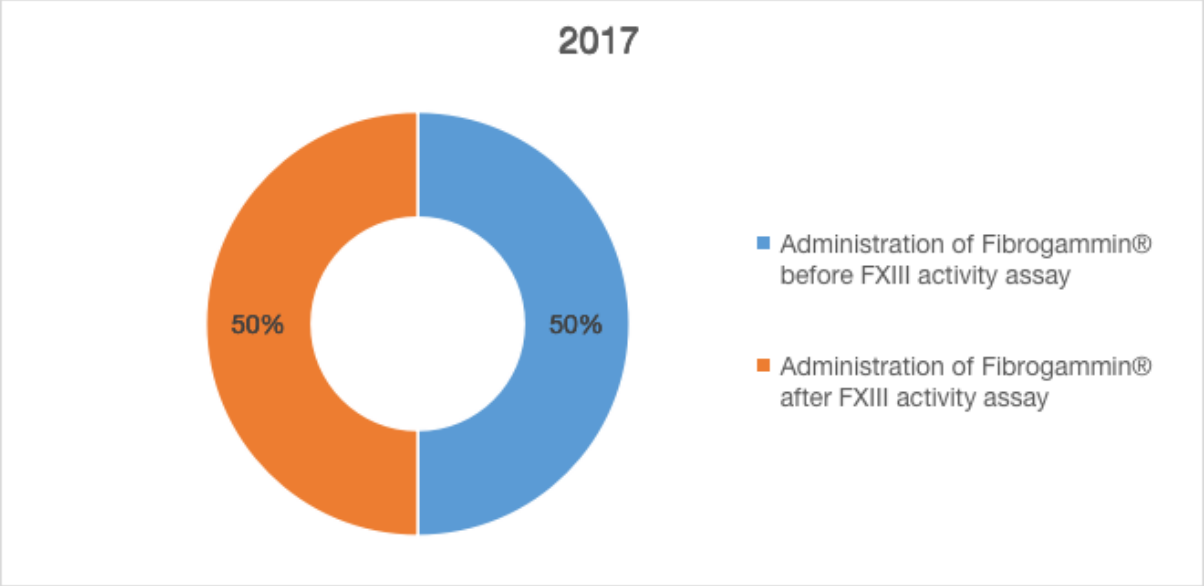

Figure 13: The percentage distribution of Fibrogammin® administration in patients with severe FXIII deficiency, according to the time of determination of FXIII activity.

3.1.3.2 Moderate FXIII deficiency

Overall, 20 patients (21.9%) with moderate FXIII activity received replacement therapy with Fibrogammin® in 2017. In most cases (n=9), Fibrogammin® was prescribed in patients in the cardio-thoracic surgical ICU (ward 97). 7 patients who received Fibrogammin® were in the operative ICU (ward 90), 2 patients in the neurosurgical ICU (ward 91), 1 patient in the stem cell transplantation ward (ward 21), and 1 patient in the cardio-thoracic surgical intermediate care (ward 60). The distribution of patients receiving Fibrogammin® across departments is shown in Figure 14.

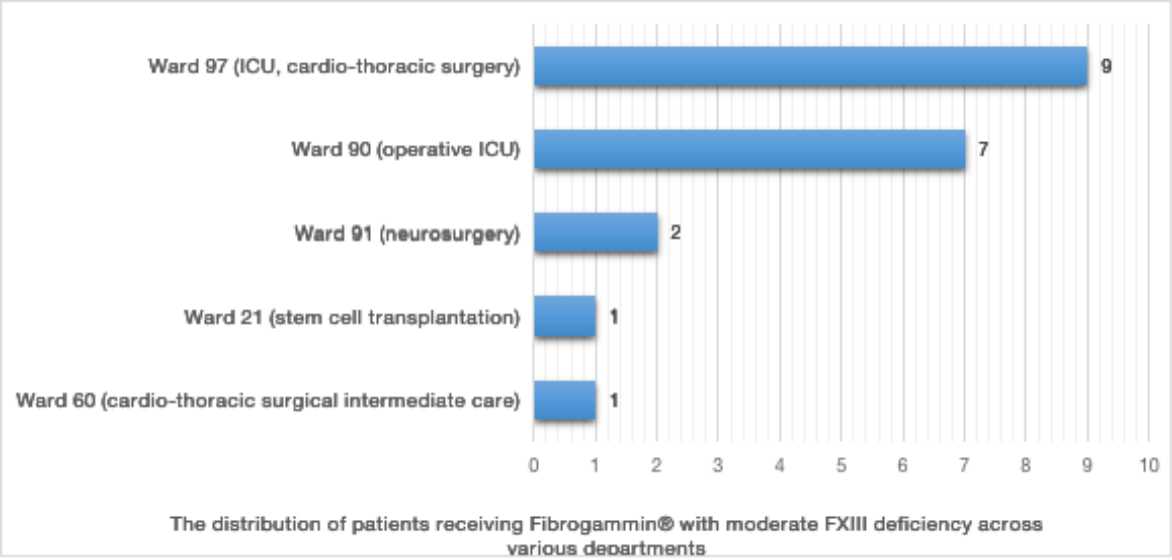

Figure 14: The distribution of patients receiving Fibrogammin® with moderate FXIII deficiency across various departments.

A total of 42,500 IU of Fibrogammin® were administered to patients with moderate FXIII deficiency in 2017. Detailed data on the exact doses of Fibrogammin® administered were not recorded in the medical reports. In most cases (n=14), patients received replacement therapy with Fibrogammin® after determination of FXIII activity. In 35% of cases (n=12), Fibrogammin® was administered on the day of FXIII activity determination. In 8 patients, Fibrogammin® was administered before the FXIII assay was performed. The percentage distribution of Fibrogammin® administration according to the time of determination of FXIII activity is shown in Figure 15.

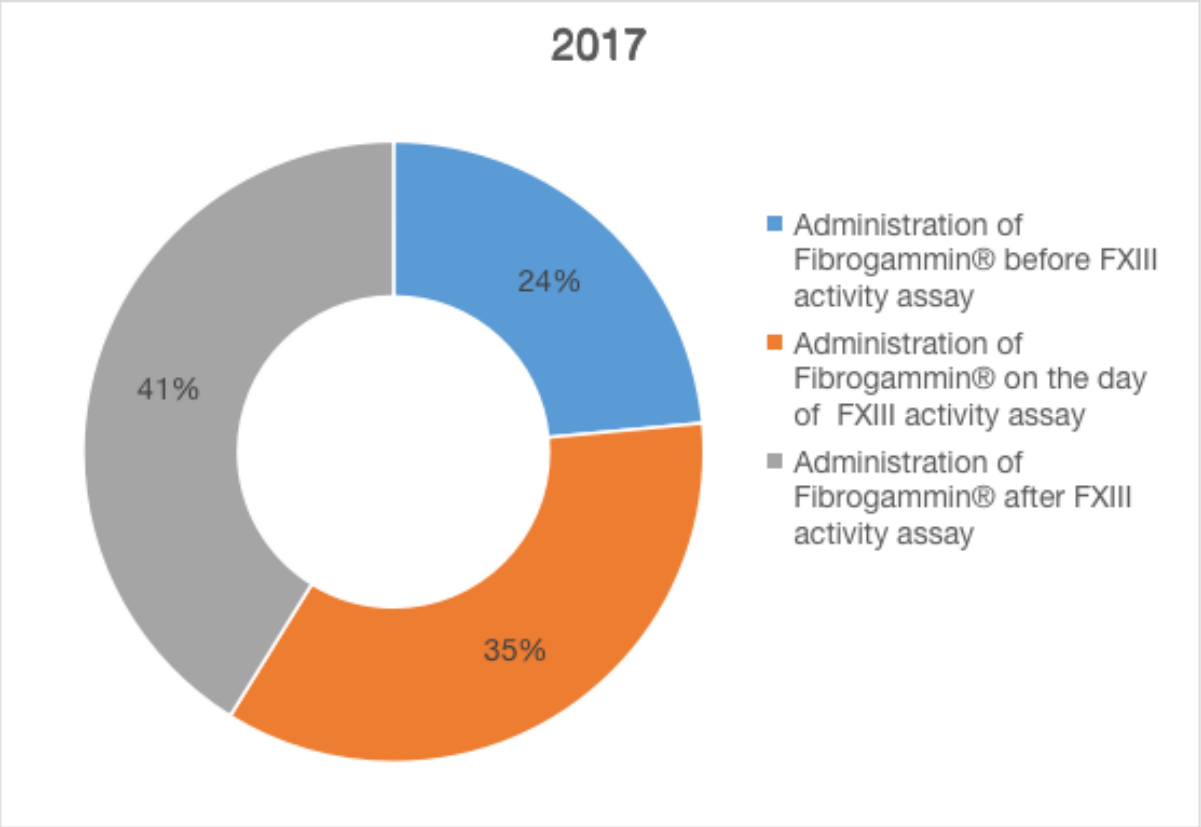

Figure 15: The percentage distribution of Fibrogammin® administration in patients with moderate FXIII deficiency according to the time of determination of FXIII activity.

3.1.3.3 Normal FXIII activity

Among a total of 380 patients with documented normal FXIII activity, 21 patients (5.5%) received replacement therapy with Fibrogammin® in 2017. Similar to cases of severe and moderate FXIII deficiency, Fibrogammin® therapy was prescribed most frequently in patients (n=14) in the cardio-thoracic surgical ICU (ward 97) and the operative ICU (ward 90). Similar to cases of severe and moderate FXIII deficiency, 2 patients from the stem cell transplantation

ward 21 and 1 patient from the cardio-thoracic surgical intermediate care (ward 60) received replacement therapy with Fibrogammin®. The distribution of patients receiving Fibrogammin® across various departments is shown in Figure 16.

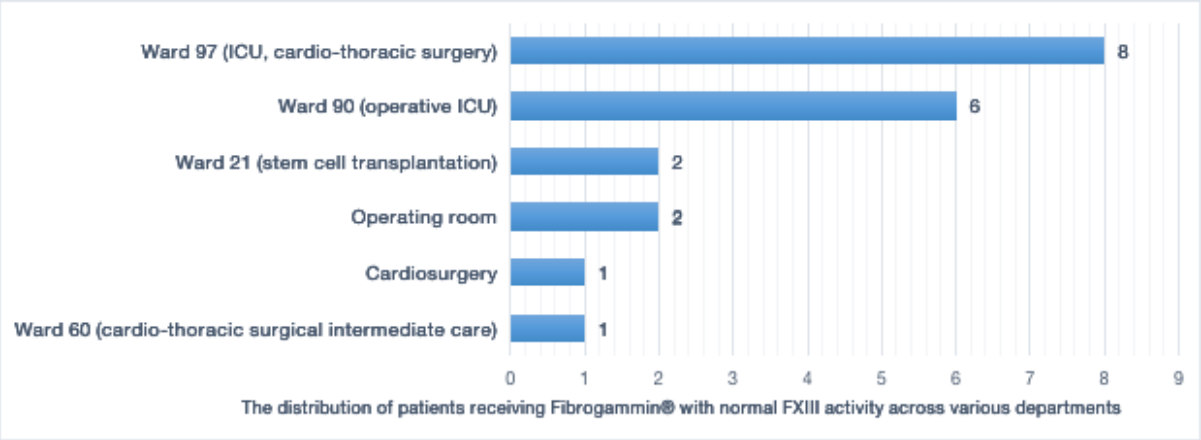

Figure 16: The distribution of patients receiving Fibrogammin® with normal FXIII activity across various departments.

A total of 88,750 IU of Fibrogammin® were administered to patients with normal FXIII activity in 2017. Detailed data on the exact doses of Fibrogammin® administered were not recorded in the medical reports. In more than 50% of cases (n=36), patients with normal FXIII activity received Fibrogammin® replacement therapy before FXIII activity was determined. Overall, 27 (38%) patients received Fibrogammin® after determination of FXIII activity, and 8 patients received Fibrogammin® on the day of FXIII determination. The percentage distribution of Fibrogammin® therapy, according to the time of determination of FXIII activity, is shown in Figure 17.

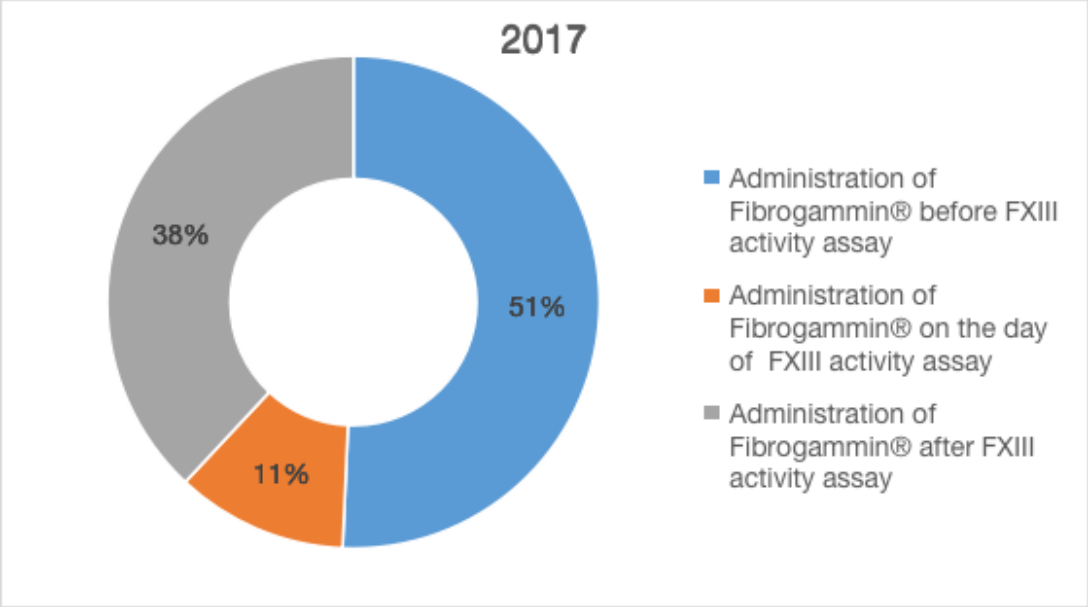

Figure 17: The percentage distribution of Fibrogammin® therapy in patients with normal FXIII activity, according to the time of determination of FXIII activity.

3.1.4 Characteristics of patients with moderate FXIII deficiency who received FXIII replacement therapy

Among 91 patients with documented moderate FXIII deficiency, 20 patients (22%) received replacement therapy with Fibrogammin® in 2017. Of these, 4 patients (20%) were women and 16 (80%) were men. Mean age was 55.8 years, with the youngest patient 20 years and the oldest 85 years of age. In patients receiving replacement therapy with Fibrogammin®, an average of 1.3 tests were performed to determine FXIII activity, with a maximum of 4 tests in a single patient. Overall, of 2 patients no medical reports were available, and data from 4 patients were already included in the group of severe FXIII deficiency, as more than one FXIII activity test was performed for these patients.

3.1.4.1 Clinical characteristics of bleeding events

By far, the most common bleeding complication was gastrointestinal bleeding (n=5) from a wide variety of causes, including upper gastrointestinal bleeding and bleeding after PEG placement with indicated interventional treatment in 2 patients. The second most common bleeding complication was combined bleeding from drainage systems and postoperative bleeding from surgical wounds. The third most common bleeding complication in patients treated with Fibrogrammin® were combined (2 cases each) intrathoracic bleeding, haematomas (retroperitoneal and inguinal), and intracerebral/subdural bleeding. In addition, postpartum haemorrhage after caesarean section was recorded in 1 patient. Only 2 patients who received Fibrogammin® had no documented bleeding-related events. The most common documented bleeding events in patients with moderate FXIII deficiency who received replacement therapy with Fibrogammin® are shown in Figure 18. Detailed description of clinically documented bleeding events are provided in Table 5.

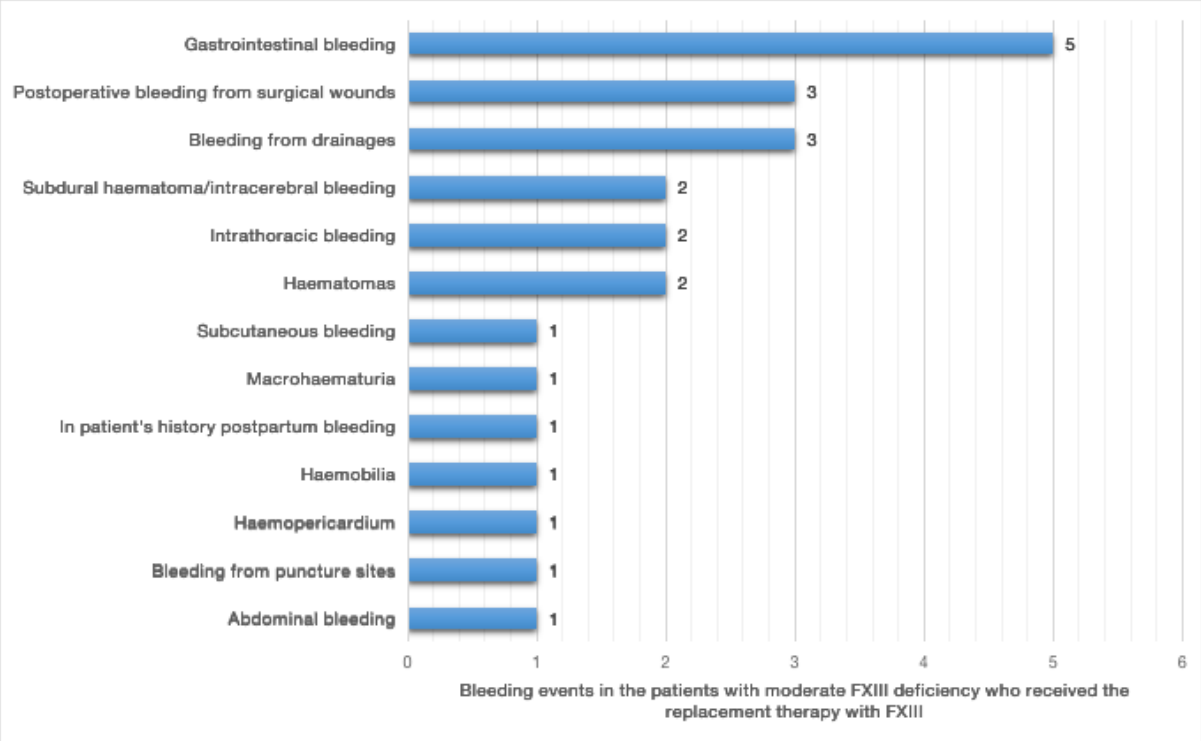

Figure 18: The most common documented bleeding events in patients with moderate FXIII deficiency who received Fibrogammin®.

Patient No.	Age	Sex	Documented clinical bleeding events
1	63	M	Gastric haemorrhage after PEG application, postoperatively increasing bleeding through the inserted drainage as well as over the laparoscopic wound, renewed gastroscopy - an endoluminale bleeding. Over the course renewed upper gastrointestinal bleeding with clipping (haemoglobin-relevant bloody stool)
2	18	M	NID
3	78	M	Haemoglobin-relevant macrohaematuria, persistent bleeding from meatus. Haemoglobin-relevant haemobilia under OAC
4	60	M	Diffuse bleeding tendency with formation of haemothorax and resulting in haematoma clearing
5	78	M	Positive haemoccult test
6	85	W	Perimesencephal subdural haematoma with ventrical invasion
7	52	M	Haemoglobin-relevant haematemesis with diffuse bleeding from diverse puncture sites with indicated substitution of blood concentrates
8	59	M	Retroperitoneal haematoma extended on the left, inguinal haematoma, haemopericardium, haemothorax bilateral, bleeding from ventricotomy, subcutaneous bleeding
9	51	M	Postoperative bleeding in the area of the medial cranial abdominal wound. Later again acute bleeding from the wound area with another surgical revision with haemostasis and renewed debridement
10	74	M	Intraoperatively extensive bleeding tendency with consecutive blood loss - after the operation a new discrete haemorrhage frontal in the puncture channel of the pressure probe. Intracerebral mass haemorrhage on the right side, internal haematocephalus
11	36	W	Increasing haemoglobin-relevant bleeding into the drainage system. An emergency surgical revision - large amounts of blood suctioned from the abdominal cavity, no active source of bleeding. On the day of admission again haemoglobin-drop. Postpartum bleeding after Ceasarean section according to patient’s history
12	60	M	NID

13	20	W	Upper gastrointestinal bleeding
14	70	M	Haemoglobin-relevant gastrointestinal-bleeding, positive haemoccult test

Table 5: Detailed description of the clinically documented bleeding events in patients with moderate FXIII deficiency who received Fibrogammin® (M=men; NID= no information documented; W=women).

3.1.4.2 Administration of blood transfusions

All patients with moderate FXIII deficiency who received replacement therapy with Fibrogammin® during their inpatient stay also received blood transfusions of either FFP, platelet concentrates, or red blood cell concentrates. At the time the FXIII activity assay was performed, all patients had received replacement therapy with red blood cell concentrates, 8 patients (57.1%) platelet concentrates, and 10 patients (71.4%) FFP. On average, patients with moderately reduced FXIII activity received 14.6 (range 2-35) red blood cell concentrates, 4.6 (range 0-12) platelet concentrates, and 14.7 (range 0-45) FFP concentrates. The distribution of replacement therapy administered to patients receiving Fibrogammin® is shown in Table 6.

Patient No.	Age	Sex	Red blood concentrates	Platelets concentrates	FFP
1	63	M	9	1	9
2	18	M	25	5	45
3	78	M	6	-	8
4	60	M	33	-	4
5	78	M	15	5	7
6	85	W	2	-	6
7	52	M	16	-	6
8	59	M	20	3	9
9	51	M	35	12	41
10	74	M	2	-	-
11	36	W	3	4	-
12	60	M	11	1	-
13	20	W	2	2	-
14	70	M	25	-	3

Table 6: The distribution of replacement therapy administered to patients with moderate FXIII activity receiving Fibrogammin® (M=men; W=women).

3.1.4.3 Diagnoses related to the bleeding complications

The most common bleeding-related diagnoses in this patient group were combined DIC and anaemia of various origins, including suspected haemolytic anaemia and aplastic anaemia, both

documented in 3 patients. Liver failure leading to impaired production of coagulation factors was recorded in 2 patients. Haemorrhagic shock, vWD type 2, portal V. thrombosis, and cholestasis due to biliary obstruction by the blood clot were also documented in the medical reports of patients with moderate FXIII deficiency who underwent replacement therapy. Bleeding/coagulation-related diagnoses were not documented in 3 patients with moderate FXIII deficiency who received Fibrogammin® therapy. The most common diagnoses associated with bleeding/coagulation abnormalities are shown in Figure 19. Details of all diagnoses associated with bleeding/coagulation abnormalities are provided in Table 7.

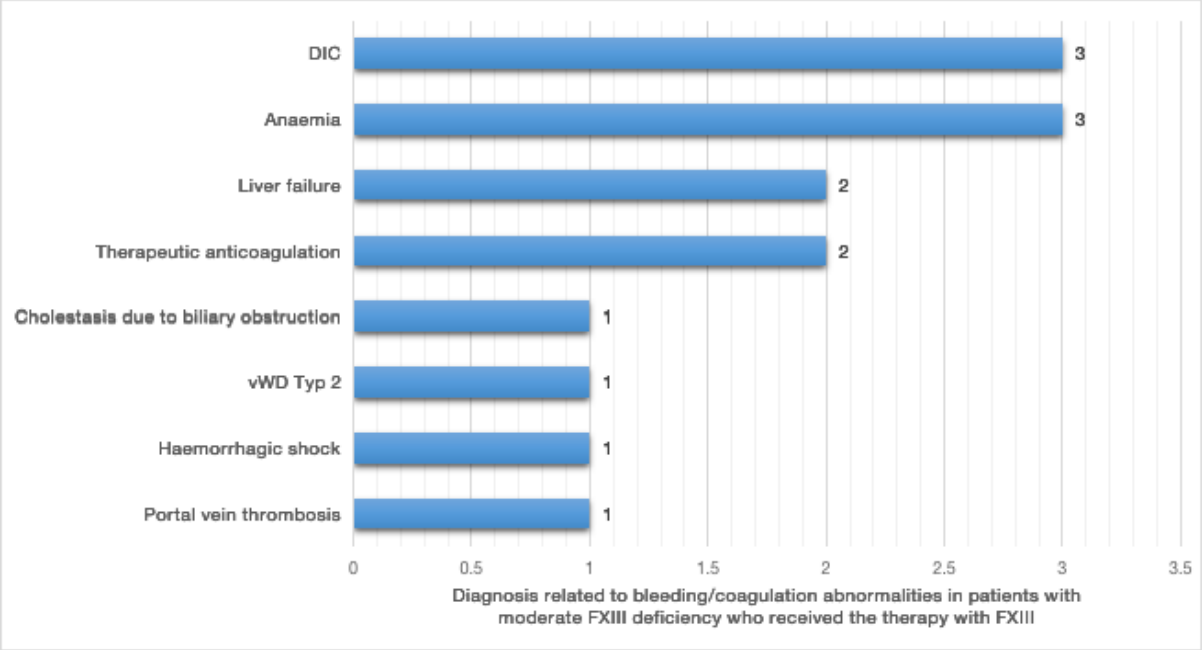

Figure 19: The most common diagnoses associated with bleeding/coagulation abnormalities in patients with moderate FXIII activity who underwent the replacement therapy with FXIII.

Patient No.	Age	Sex	Diagnosis related to bleeding/coagulation abnormalities
1	63	M	NID
2	18	M	DIC, liver failure
3	78	M	Cholestasis due to biliary obstruction due to blood clot, due to atrial fibrillation, permanent therapeutic anticoagulation
4	60	M	Portal V. thrombosis, anaemia
5	78	M	Suspected DIC, liver failure
6	85	W	FXIII deficiency
7	52	M	DIC
8	59	M	Suspected haemolysis
9	51	M	Haemorrhagic shock after postoperative bleeding
10	74	M	Therapeutic anticoagulation with enoxaparin
11	36	W	vWD Typ 2
12	60	M	NID
13	20	W	Severe aplastic anemia
14	70	M	NID

Table 7: Detailed description of all with the bleeding/coagulation abnormalities related diagnosis in patients with moderate FXIII activity who underwent the replacement therapy with FXIII (M=men; NID=no information documented; W=women).

3.1.4.4 Anticoagulation

Overall, 57.1% of patients (n=8) with moderate FXIII deficiency who received therapy with Fibrogammin® were anticoagulated with intravenous heparin. In 1 case, when HIT was suspected, anticoagulation with heparin was switched to argatroban. Overall, 1 patient received prophylactic anticoagulation with enoxaparin subcutaneously. In 2 patients, no anticoagulation was given during their inpatient stay. In 2 patients, information about specific anticoagulation was not clearly described in the medical reports.

3.1.4.5 ECMO therapy

Overall, 35.7% of patients (n=5) with moderate FXIII deficiency received ECMO therapy

during their stay in ICU. Among these, 3 patients were treated with vaECMO and 2 patients with vvECMO.

3.1.5 Characteristics of patients with normal FXIII activity who received FXIII replacement therapy

Among a total of 380 patients with documented normal FXIII activity, 21 patients (5.5%) received replacement therapy with Fibrogammin® in 2017. Of these 21 patients, 7 patients (33.3%) were women and 14 (66.7%) were men. Mean age was 55.8 years, with the youngest patient 20 years and the oldest 78 years of age. Among patients who received Fibrogammin®, an average of 1.5 tests were performed to determine FXIII activity, with a maximum of 4 tests in a single patient. Overall, 3 patients were also included in the group of patients with severe FXIII deficiency, as more than one FXIII activity assay was performed in these patients.

3.1.5.1 Clinical characteristics of bleeding events

By far, the most common documented bleeding complication was gastrointestinal bleeding (n=8), in most cases due to erosive gastritis, and in 1 case due to acute intestinal GvHD. The second most common bleeding complication was combined bleeding from drains and intrathoracic bleeding. Bleeding from chest drains, Robinson drains, and Redon drains was also recorded. Severe intrasurgical abdominal bleeding, with massive blood transfusion and replacement of clotting factors, including Fibrogammin®, and multiple surgical re-interventions, were recorded in 4 patients. Postoperative bleeding from surgical wounds was noted in 3 patients, including extensive bleeding from the negative pressure wound system. In 1 patient, the formation of a bifrontal subarachnoid haemorrhage, a subdural bifrontal haematoma, and the formation of an interhemispheric haematoma was documented. In another patient an intracerebral haemorrhage with incipient incarceration was recorded. Only 1 patient with normal FXIII activity who received Fibrogammin® did not have any with bleeding-related events. The most common clinically documented bleeding events are shown in Figure 20. Details of all clinically recorded bleeding events are provided in Table 8.

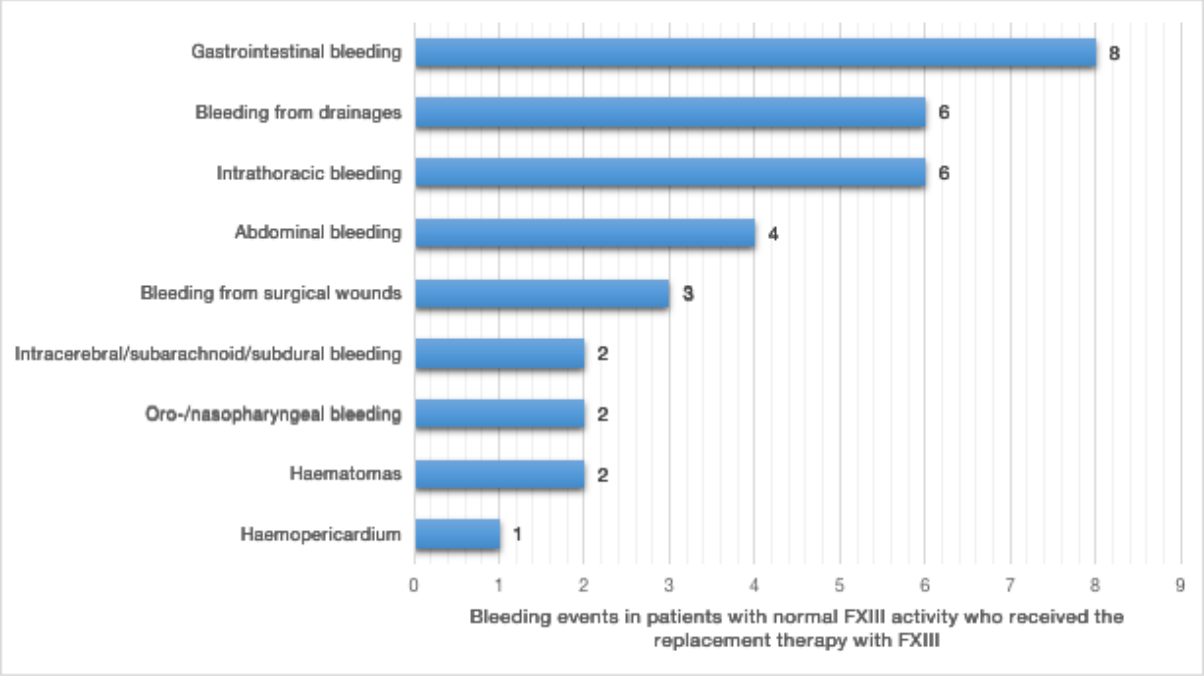

Figure 20: The most common documented bleeding events in patients with normal FXIII activity who received the replacement therapy with FXIII.

Patient No.	Age	Sex	Documented clinical bleeding events
1	65	M	Intraperitoneal haematoma, retroperitoneal haematoma, infected haematoma of the left proximal thigh, tarry stool, ulcer in the duodenal bulb covered by the fresh blood, haemothorax, negative pressure wound therapy promotes a lot of bloody secretion, drainages discrete bloody
2	57	W	Gastrointestinal bleeding with haematochezia
3	43	M	FXIII administration by diffuse bleeding tendency, diffuse bleeding from the operated area and mouth / nasal cavity, blood loss via the wound
4	75	W	Bleeding tendency. By severe traumatic brain injury - subarachnoid haemorrhage bifrontal and perimesenencephalic on the right side, subdural haematoma bihemispherical, haematoma in the interhemispheric gap, galeahaematoma occipital and bifrontal
5	78	M	Haemoptosis
6	71	W	Suspected bleeding from the ascending aorta at the aortic valve level with increasing haemopericardium, gastrointestinal bleeding, a diffuse bleeding in the area of the liver capsule
7	41	M	Thoracic haematoma, the thoracoscopic removal of haematoma
8	58	W	Septic eroding bleeding of the right hemisphere with midline shift, incipient incarceration
9	20	M	Suspected acute abdominal bleeding, surgical intervention indicated, macroscopically massive bleeding from several, most likely inflammatory, lacerations of the portal V. and aorta at the right renal artery. Intraoperatively: 8 red blood concentrates, 15 FFP, 3 platelet concentrates, renewed bleeding in this area, 2 further surgical revisions, further 5 red blood concentrates, 3 FFP. In the further clinical course recurrent abdominal bleeding with massive blood transfusions, which could not be controlled even through multiple packing attempts. 4 FFP, 1 platelet concentrates, 300 ml intraoperative blood salvage
10	71	W	Spontaneous haemothorax on the right side, recurrent bleeding in tracheostoma bleeding

11	55	M	1.5-2 l haematochesia by acute intestinal GvHD, gastrointestinal bleeding requiring blood re-transfusions
12	78	W	Patient fell under anticoagulation with phenprocoumon and large haematoma developed. In the patient's history protracted bleeding of the gums, mucous membranes, bleeding from inguinal arteries
13	54	M	Erosive bleeding with massive blood transfusions
14	29	M	Intraoperative extensive bleeding in the area of the abscess cavity of the spleen, transfusion of 25 red blood concentrates, 31 FFP, 6 platelet concentrates, 3350 ml intraoperative blood salvage, fibrinogen 15 g. Postoperatively the abdominal drainages promoted bloody secretion in the first 12 h, 7 red blood concentrates, FXIII, 8 FFP, 4 g fibrinogen, tranexamic acid
15	36	W	Haemoglobin-relevant bleeding from Redon-Drainage, post-operative bleeding from the operated area, active bleeding from an ulcer in the area of the Corpus gastricum, which was stopped by applying a clip and injecting fibrin
16	71	M	In the event of acute bleeding during the surgery, mass blood transfusions, bleeding from V. cava and from distal anastomosis over-sutured, this also over-sutured, abdominal tamponade with compresses, 9 red blood concentrates, 10 FFP, 7 g fibrinogen, 2 platelet concentrates, 5000 IU PPSB
17	70	W	Haemoglobin-relevant bleeding from the chest drains, the nasogastric tube promotes haematin, haemoccult-test positive (after surgery) yellowish/bloody secretion endotracheally
18	54	M	NID

Table 8: Detailed description of all the clinically documented bleeding events in patients with normal FXIII activity who received Fibrogammin ® (M=men; NID=no information documented; W=women).

3.1.5.2 Administration of blood transfusions

All patients with normal FXIII activity treated with Fibrogammin® during their inpatient stay also received replacement therapy with either FFP, platelet concentrates, or red blood cell concentrates. At the time the FXIII activity assay was performed, all patients had received replacement therapy with red blood concentrates, 16 patients (88.8%) received platelet concentrates, and 14 patients (77.8%) received FFP. On average, patients with normal FXIII

activity received 38.1 (range 1-115) red blood cell concentrates, 16 (range 0-34) platelet concentrates, and 39.3 (range 0-132) FFP. The distribution of replacement therapy administered is shown in Table 9.

Patient No.	Age	Sex	Red blood concentrates	Platelets concentrates	FFP
1	43	M	35	-	6
2	34	M	43	44	2
3	54	M	90	31	132
4	44	W	1	-	-
5	47	M	10	6	10
6	24	W	42	19	65
7	33	M	20	10	20
8	69	M	21	10	13
9	62	W	11	5	49
10	57	M	47	4	3
11	46	W	48	27	12
12	1	W	15	6	-
13	38	M	81	22	112
14	39	W	115	34	78
15	48	W	35	2	3
16	49	M	37	12	45
17	41	M	18	17	-
18	19	M	16	7	-

Table 9: The distribution of replacement therapy administered in patients with normal FXIII activity (M=men; W=women).

3.1.5.3 Diagnoses related to the bleeding complications

The most common bleeding-related diagnosis was thrombocytopenia (n=5) due to various origins, mostly toxic side effects of medications, postoperative coagulation abnormalities, or unknown origin. In 5 patients, thrombosis was documented in different areas: in the right internal jugular V., right femoral V., superficial cephalic and basilic V. and thoracic aorta.

Overall, 3 patients were found to have anaemia due to a wide variety of causes, including iron deficiency anaemia, anaemia associated with DIC, and anaemia associated with toxic side effects of medications. DIC was also documented in 3 patients with normal FXIII activity who had received replacement therapy. Pulmonary embolism, long-term anticoagulant therapy and postoperative healing problems were also documented in the medical reports of patients with normal FXIII activity. Bleeding/coagulation-related diagnoses were not recorded in 5 patients (27.8%) with normal FXIII activity who received Fibrogammin® therapy. The most common diagnoses associated with bleeding/coagulopathy in patients with normal FXIII activity who received Fibrogammin® replacement therapy are shown in Figure 21. Details of all diagnoses associated with bleeding/coagulation abnormalities are provided in Table 10.

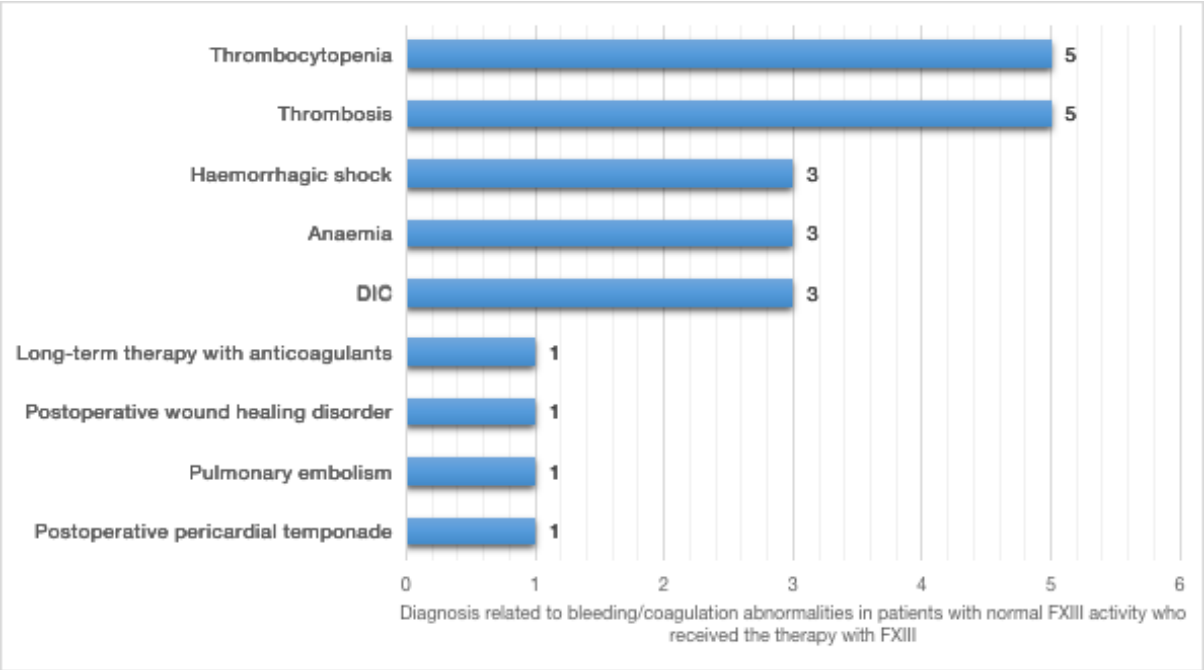

Figure 21: The most common diagnoses associated with bleeding/coagulation abnormalities in patients with normal FXIII deficiency who received Fibrogammin®.

Patient No.	Age	Sex	Diagnoses related to the bleeding/coagulation abnormalities
1	63	M	Thrombosis in the right internal jugular V.
2	18	M	NID
3	78	M	DIC
4	60	M	NID
5	78	M	Thrombocytopenia of unknown origin, pulmonary embolism with infarct pneumonia
6	85	W	DIC, splenic infarction, massive transfusions
7	52	M	NID
8	59	M	Iron deficiency anaemia, most likely within the septic erosion bleeding, pump thrombosis (LVAD Systems)
9	51	M	NID
10	74	M	Postoperative pericardial tamponade
11	36	W	NID
12	60	M	Thrombocytopenia, deep V. thrombosis in the right femoral V., pulmonary embolism
13	20	W	Haemorrhagic shock
14	70	M	Haemorrhagic shock, anaemia, most likely of medical-toxic origin, thrombocytopenia with splenomegaly and suspected ITP
15	36	W	Wound-healing disorder of the adductor muscles of the left thigh, postoperative coagulation abnormalities with thrombocytopenia and anaemia, requiring transfusions. Thrombosis of the superficial cephalic and basilic V., pulmonary embolism, deep V. thrombosis left lower leg
16	71	M	Haemorrhagic shock with haemorrhage from the V. cava and distal anastamosis of the aortobiliac prosthesis, partially thrombosed infrarenal abdominal aneurysm, mural thrombi in the thoracic aorta

Table 10: Detailed description of diagnoses associated with bleeding/coagulation abnormalities in patients with normal FXIII deficiency who received Fibrogammin® (M=men; NID=no information documented; W=women).

3.1.5.4 Anticoagulation

Overall, 72.2% of patients (n=13) with normal FXIII activity received anticoagulation therapy

with intravenous heparin. One patient was treated with argatroban. Another patient received prophylactic anticoagulation with enoxaparin subcutaneously. In 1 case, the patient did not receive anticoagulation therapy during their inpatient stay. In 2 patients, information about the specific anticoagulation therapy was not clearly provided in the medical reports

3.1.5.5 ECMO therapy

Overall, 27.8% of patients (n=5) with normal FXIII activity received ECMO therapy during their stay in ICU. Among patients who received ECMO therapy, 5 patients were treated with vaECMO and 1 patient with vvECMO.

3.1.6 Moderate FXIII deficiency in coagulation outpatient clinic

A total of 173 FXIII activity tests were performed in the coagulation outpatient clinic in 2017. Of these tests, only 6 patients (3.5%) were diagnosed with moderate FXIII deficiency and no patient was diagnosed with severe FXIII deficiency. Of these 6 patients, 4 were women and 2 were men. Mean age was 35.3 years, with the youngest patient 21 years and the oldest 64 years of age. Overall, a mean of 1.8 FXIII activity tests were performed.

Overall, 2 patients in the coagulation outpatient clinic were diagnosed with moderately reduced FXIII activity as a consequence of consumption of coagulation factors in the context of current bleeding events: in 1 patient due to extensive epistaxis (patient No. 1) and in another patient due to severe hypermenorrhoea (patient No. 2). In the first case (patient No. 1), the patient also suffered from documented severe haemophilia A with FVIII <1% with extensive bleeding events, such as intracerebral haemorrhage. Severe hypermenorrhoea (patient No. 2) was treated surgically with supracervical hysterectomy by continuous bleeding under conservative therapy with tranexamic acid. In 1 case (patient No. 5), physiologically decreased FXIII activity was diagnosed at 33 weeks of gestation, with no current clinical bleeding signs other than heavy menstrual bleeding.

In 1 case (patient No. 2), decreased FXIII activity was determined during the previously diagnosed liver failure, resulting in vitamin K deficiency and suspected coagulopathy without any clinical bleeding signs. In addition, the patient was diagnosed with myeloproliferative neoplasia with acquired vWD, which further impaired haemostasis. One case (patient No. 6) was diagnosed with moderate FXIII deficiency due to postoperatively increased bleeding after laparoscopic cholecystectomy, but without any other bleeding signs in the patient's history. In 1 patient moderately reduced FXIII activity was directly associated with lifelong bleeding tendencies, including bleeding after extraction of wisdom teeth and severe diffuse permanent bleeding after periodontal treatment. A recommendation was made for prophylactic therapy with tranexamic acid in future surgical procedures and control of FXIII in the coagulation outpatient clinic. Detailed description of all documented bleeding events and diagnosis associated with bleeding/coagulation abnormalities are provided in Table 11.

Patient No.	Age	Sex	Diagnoses related to the bleeding/coagulation abnormalities	Documented clinical bleeding events
1	21	M	Severe haemophilia A, FVIII <1%, FXIII deficiency	Recurrent epistaxis, status after intracerebral haemorrhage and recurrent bleeding with application of a VP shunt in hydrocephalus
2	64	W	MPN with dysplasia of erythropoiesis and megakaryopoesis and Pre-PMF. Acquired vWD. Vitamin K deficiency, suspected coagulopathy by hepatic insufficiency, folic acid deficiency	No signs of bleeding
3	37	W	Thrombosis of the left V. tibialis posterior after hysterectomy by hypermenorrhea, discrete FXIII deficiency	Persistent bleeding after abrasion by existing hypermenorrhea. Massive bleeding, resulting in assisted supracervical hysterectomy
4	29	M	Marginal FXIII activity	Bleeding after extraction of wisdom teeth. Status post heavy, diffuse persistent bleeding after periodontal treatment. Epistaxis. Gum bleeding during tooth brushing
5	25	W	No evidence of haemorrhagic diathesis, 33 weeks of gestation	After the birth of a child heavy, vaginal bleeding that lasted for about a month, hypermenorrhea (6 days)
6	36	W	No evidence of haemorrhagic diathesis	Increased bleeding tendency after laparoscopic cholecystectomy in the patient's history, hypermenorrhea

Table 11: The detailed description of all documented bleeding events and diagnoses associated with bleeding/coagulation abnormalities in patients with moderate FXIII deficiency in coagulation outpatient clinic (M=med; W=women).

3.2 FXIII activity assays and FXIII replacement therapy in 2019

3.2.1 FXIII activity assays

In the period from 01.01 to 31.12.2019, a total of 1,405 FXIII activity tests (ammonia release assay method, Berichrom FXIII [Siemens, Marburg, Germany]) were performed in 523 patients in the coagulation laboratory at the University Hospital Regensburg. Overall, 42.5 % of the FXIII activity assays performed were in women and 57.5% in men. The mean age was 45.5 years. Of the patients receiving FXIII activity tests, the mean age 50.5 years. FXIII activity assays were most frequently performed in the age group between 61 and 80 years. The percentage distribution of FXIII tests by the age group is shown in Figure 22.

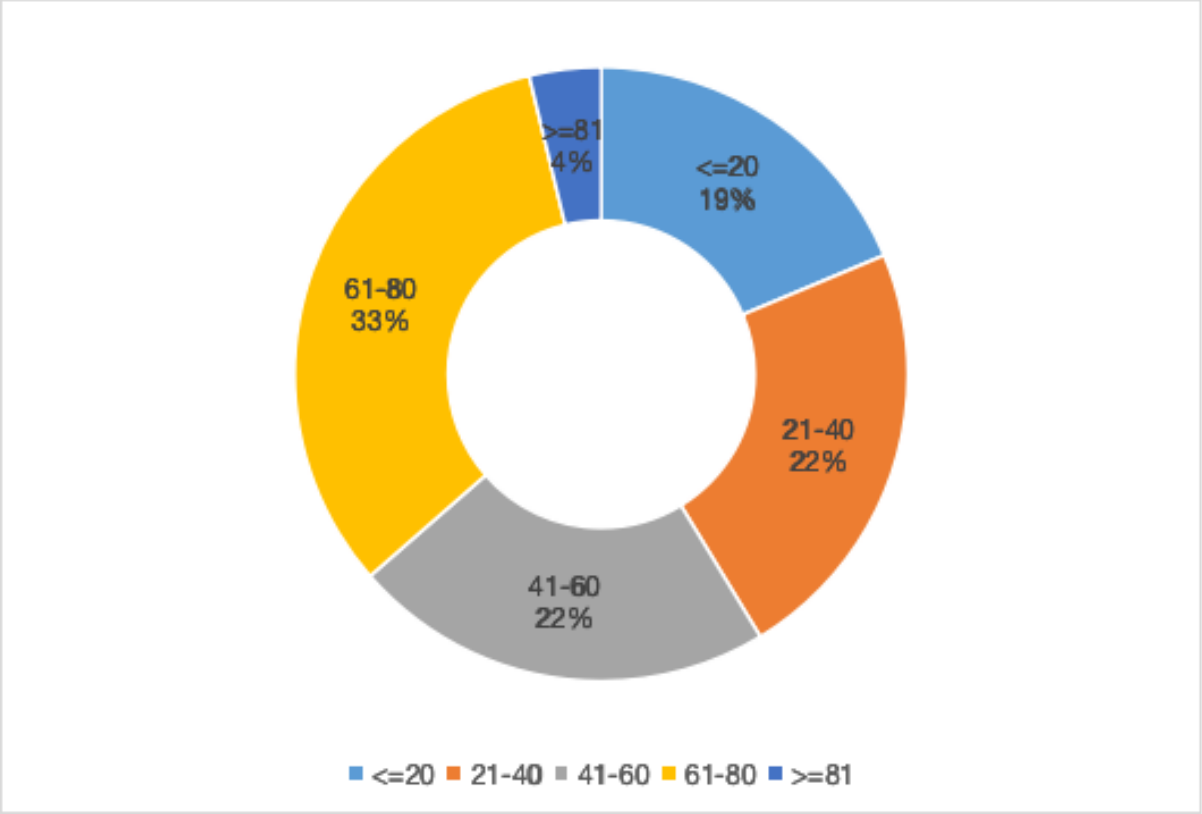

Figure 22: The percentage distribution of FXIII activity assays by the age groups.

Most FXIII activity assays were requested by a wide variety of ICUs, comprising 67.3% (n=946) of all 1,405 FXIII activity assays performed in 2019. Most requests came from the internal medical ICU (ward 93, cardiology) with 529 requests, the cardio-thoracic surgical ICU (ward 97) with 184 requests, and the operative ICU (ward 90) with 116 requests. Overall, 5.8% (n=82) of FXIII activity tests were requested by the intermediate care unit – stem cell transplantation ward 21. General wards (internal medicine/surgery) requested 12.5% (n=175)

of all the FXIII activity tests, with most requests (n=61) coming from the general haemato-/oncology ward 20. Overall, 178 FXIII activity assays were requested by the coagulation outpatient clinic in 2019. The distribution of requests for FXIII activity tests in 2019 is shown in Figure 23.

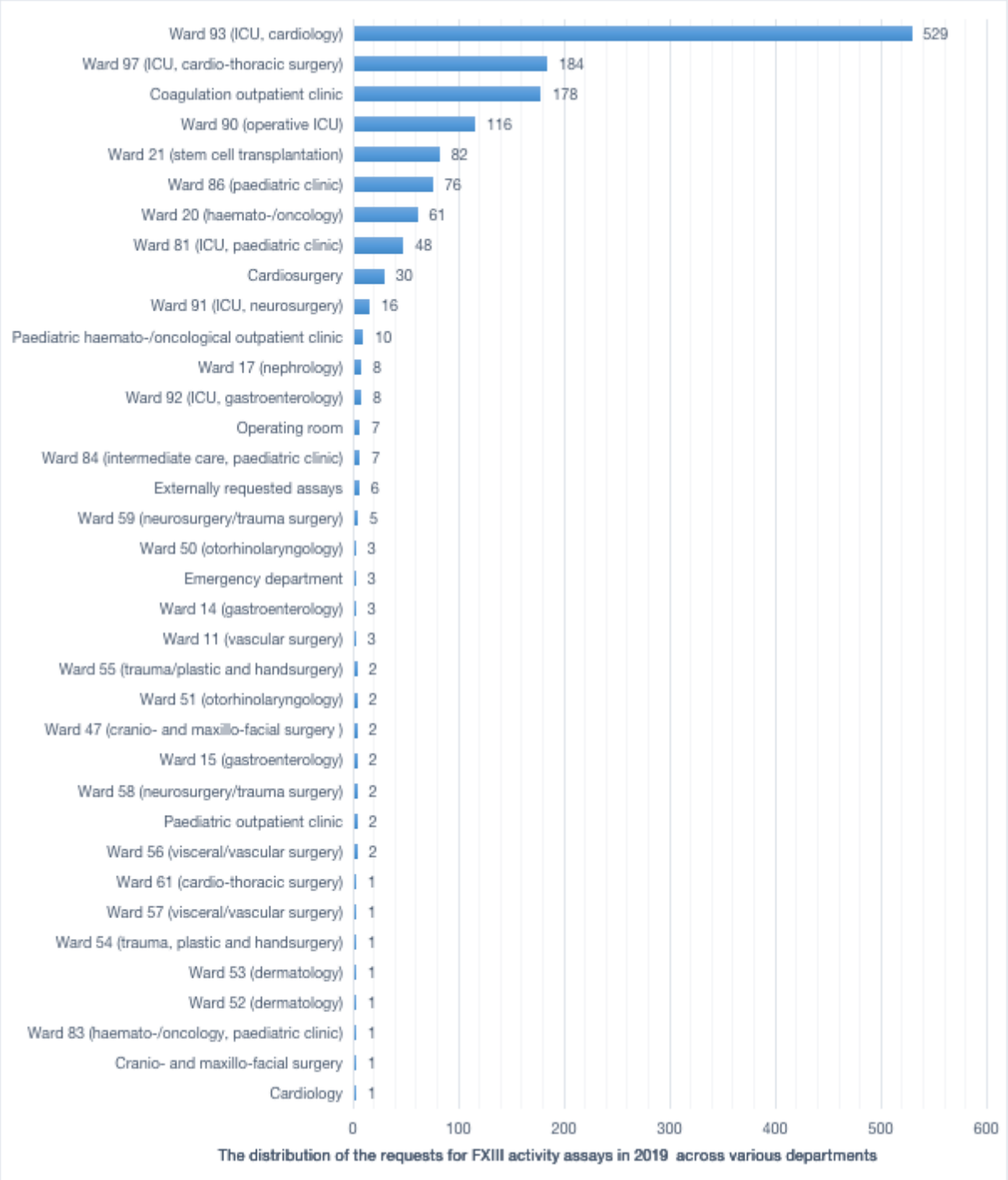

Figure 23: The distribution of the requests for FXIII activity assays across various departments at University Hospital Regensburg.

In 2019, a total of 178 FXIII activity assays were performed in the coagulation outpatient clinic. Normal FXIII activity was diagnosed in 86.7% (n=169) of all FXIII activity tests performed in the coagulation outpatient clinic as part of routine coagulation diagnostics. Moderate FXIII deficiency was diagnosed in 4.5% (n=8). One patient with severely reduced FXIII activity was diagnosed in the coagulation outpatient clinic in 2019. The distribution of FXIII activity levels in the tests performed in the outpatient coagulation clinic is shown in Figure 24.

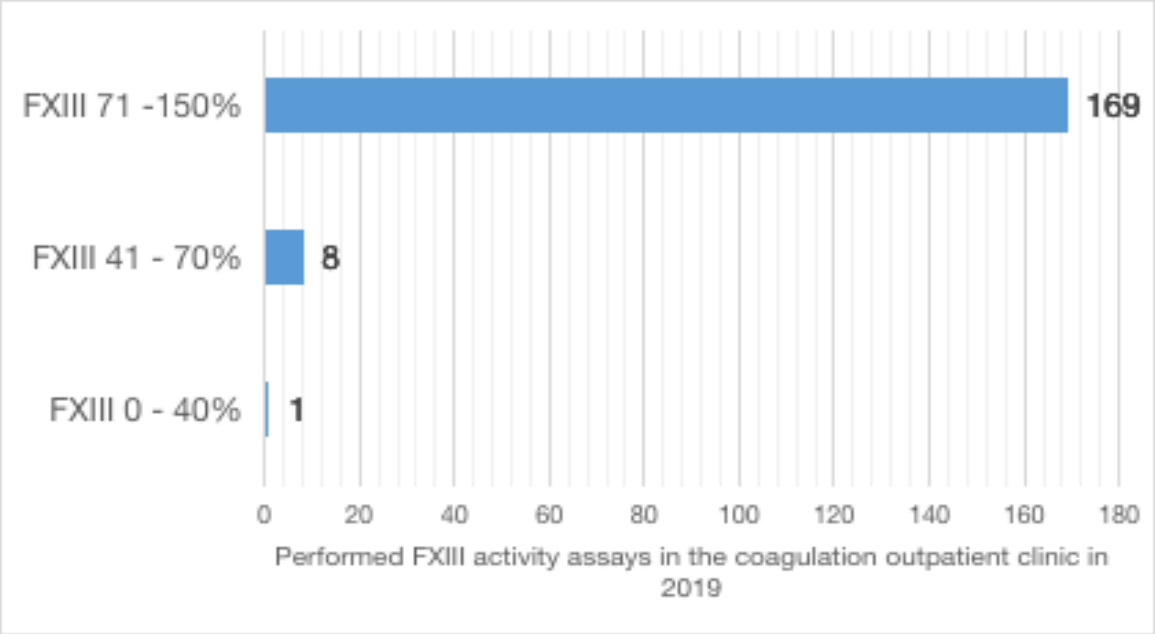

Figure 24: The distribution of FXIII activity levels in the assays performed in the coagulation outpatient clinic.

Severe FXIII deficiency (0-40%) was diagnosed in 404 tests (131 patients), moderate FXIII deficiency (41-70%) in 509 tests (172 patients), and normal FXIII activity (>70%) in 492 tests (375 patients). The distribution of FXIII activity values in the FXIII activity tests is shown in Figure 25.

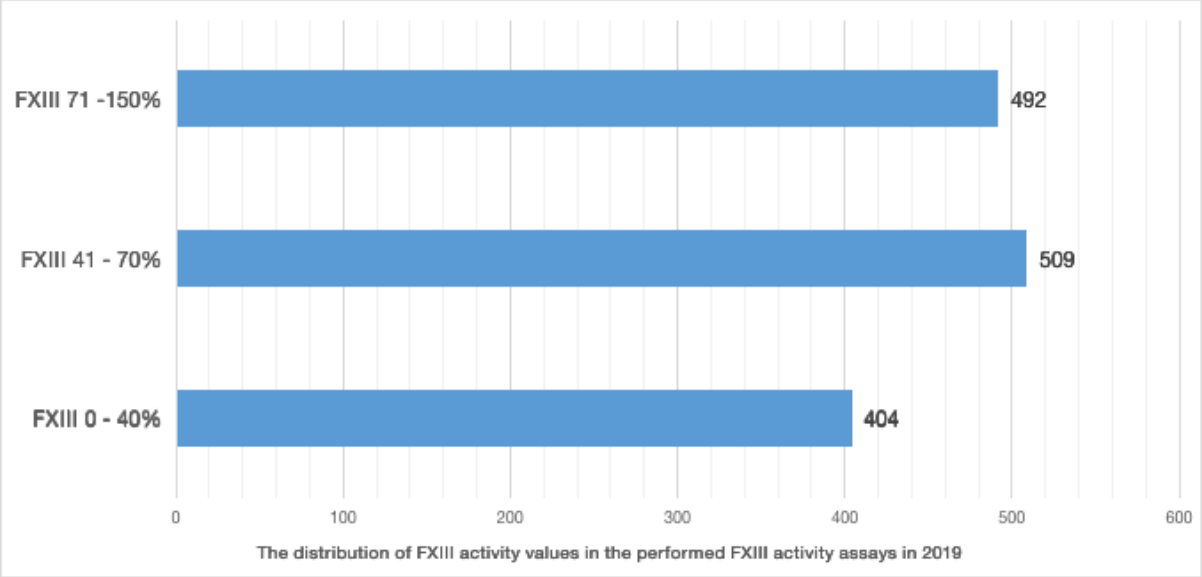

Figure 25: The distribution of FXIII activity values in the FXIII activity assays.

Overall, 88.6% of the 404 FXIII activity assays in patients with severe FXIII deficiency were requested by ICUs of several departments at the University Hospital Regensburg, with the majority of requests from the internal medical ICU (ward 93, cardiology), with a total of 235 tests, and the second most from the cardio-thoracic surgical ICU (ward 97) (n=68). Another 23 assays in patients with diagnosed severe FXIII deficiencies were requested from the stem cell transplantation ward 21, 9 from the haemato-/oncological ward 20, and 6 from the nephrology ward 17. The distribution of FXIII activity tests in patients with severe FXIII deficiency across departments in 2019 is shown in Figure 26.

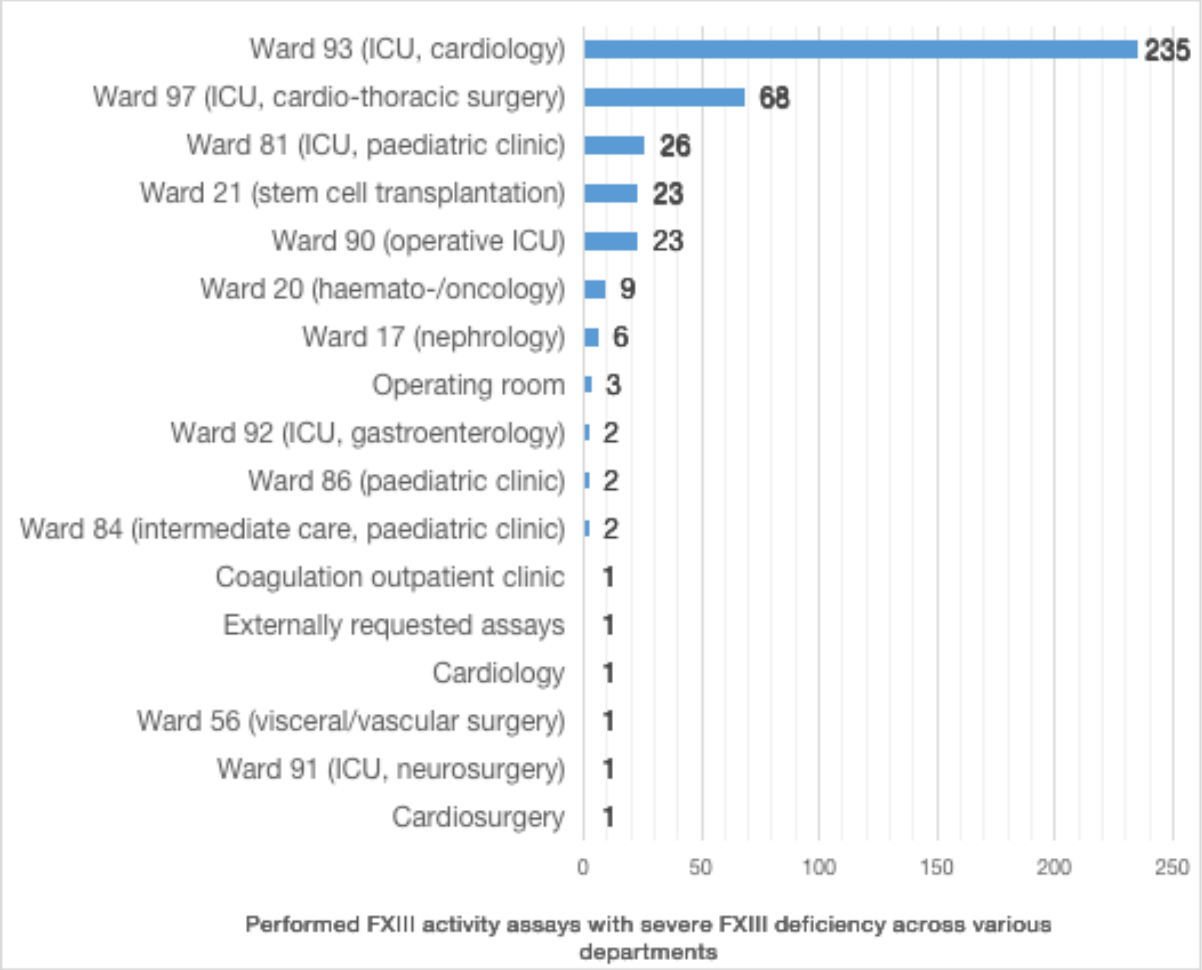

Figure 26: Performed FXIII activity assays with severe FXIII deficiency across departments at the University Hospital Regensburg.

Overall, 65.6% of cases (264 FXIII activity assays in 60 patients) diagnosed with severe FXIII deficiency received anticoagulation with argatroban in the intensive care setting. The mean argatroban concentration in these patients was 0.09 µg/ml (SD 0.27).

In patients with moderate FXIII deficiency, most FXIII activity tests (79.4%) were requested by the various ICUs at the University Hospital Regensburg. The majority of requests (n=228) came from the internal medical ICU (ward 93, cardiology), the cardio-thoracic surgical ICU (ward 97) (n=82), and the operative ICU (ward 90) (n=64). In addition, 14.7% of FXIII activity tests in patients with moderate deficiency were requested from the stem cell transplantation ward 21 (n=48) and the general haemato-/oncology ward 21 (n=27). The distribution of FXIII activity tests in 2019 in patients with moderate FXIII deficiency, across departments, is shown in Figure 27.

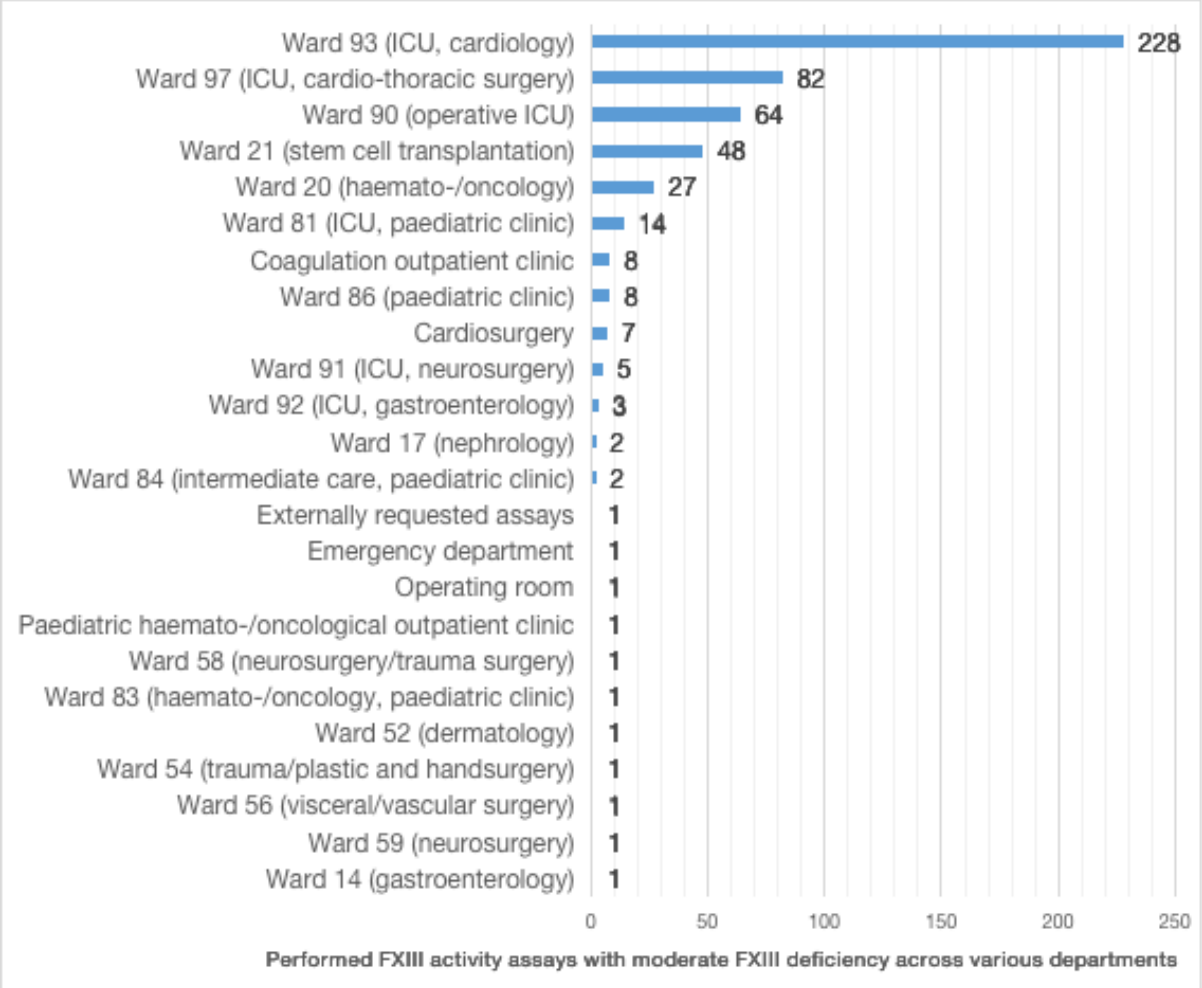

Figure 27: The distribution of FXIII activity tests in 2019 with moderate FXIII deficiency across departments.

In contrast to severe and moderate FXIII deficiency, the majority of patients with normal FXIII activity (n=169) were identified in the coagulation outpatient clinic and accounted for 36.6% of all tests performed in patients with normal FXIII activity. In almost as many cases (n=166), normal FXIII activity was diagnosed in ICUs, with 66 tests performed in the internal medical ICU (ward 93, cardiology), 34 tests in the cardio-thoracic surgical ICU (ward 97), and 23 tests in the operative ICU (ward 90). In addition, 22.6% of FXIII activity tests in patients without any FXIII deficiency were requested by general wards. The distribution of FXIII activity tests in patients with normal FXIII activity, across departments, in 2019 is shown in Figure 28.

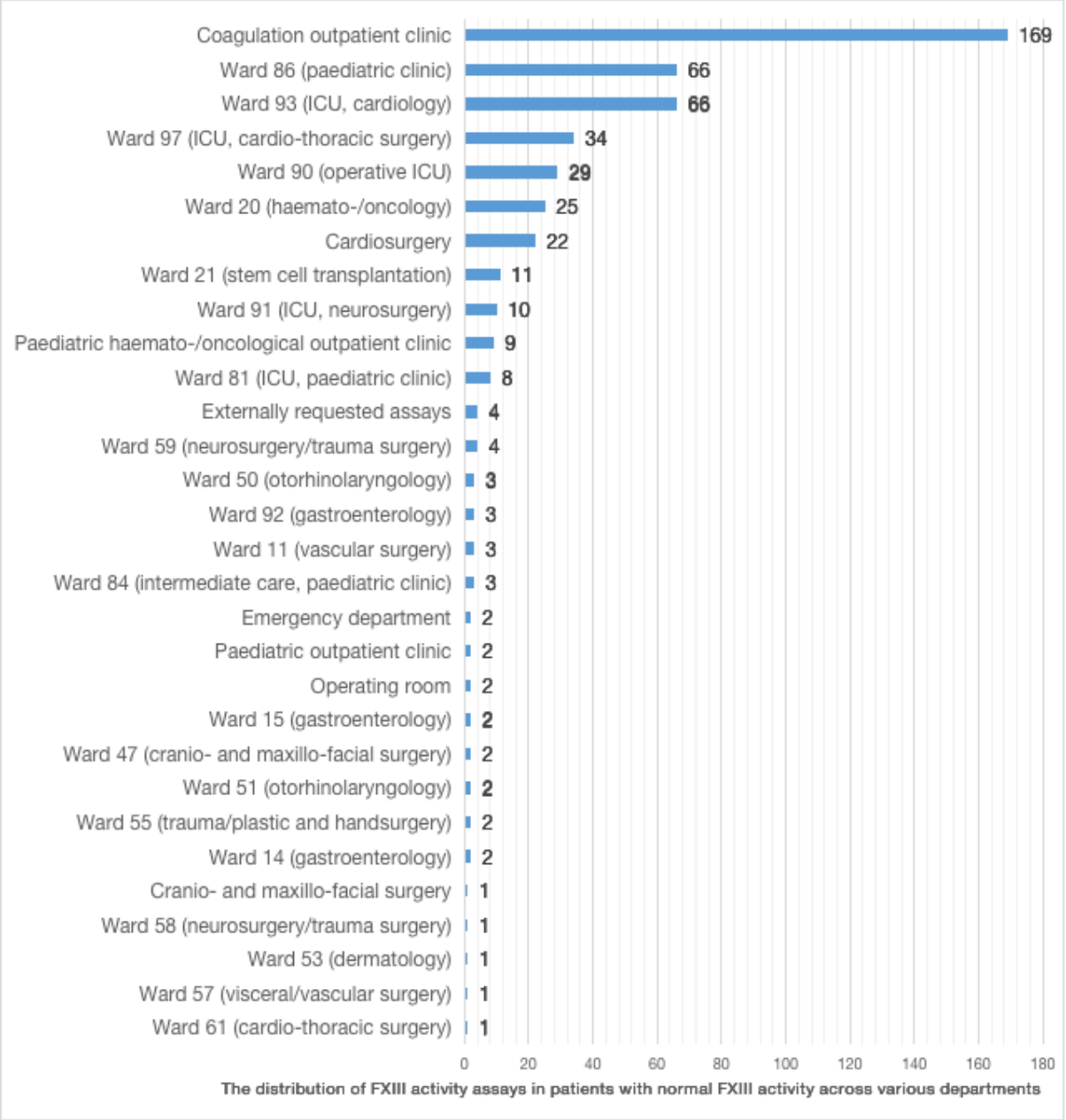

Figure 28: The distribution of FXIII activity assays in patients with normal FXIII activity across departments.

3.2.2 FXIII replacement therapy

3.2.2.1 Patients with severe FXIII deficiency

Overall, 39 patients (24.5%) of a total of 131 patients with severe FXIII deficiency received replacement therapy with Fibrogammin® in 2019. In most cases (13 in total), replacement therapy with Fibrogammin® was prescribed in patients in the cardio-thoracic surgical ICU (ward 97), followed by the internal medical ICU (ward 93, cardiology) and the operative ICU (ward 90). Replacement therapy with Fibrogammin® was administered to 3 patients from the general wards: 2 patients from general haemato-/oncology ward 20 and 1 patient from the nephrology ward 17. One patient received Fibrogammin® in the outpatient setting. The distribution of patients with severe FXIII deficiency who received Fibrogammin® among the various departments is shown in Figure 29.

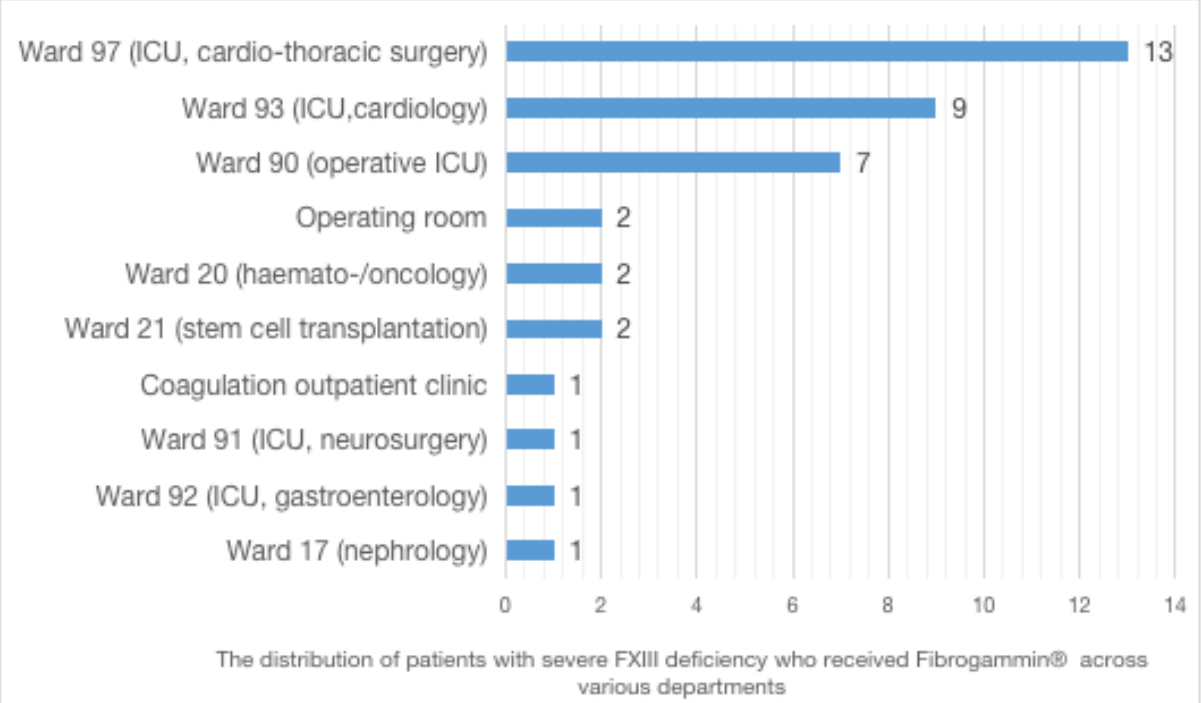

Figure 29: The distribution of patients with severe FXIII deficiency who received Fibrogammin® across the various departments.

A total of 155,000 IU of Fibrogammin® were administered to patients with severe FXIII deficiency in 2019. In most cases (n=58), Fibrogammin® was administered on the day of FXIII activity determination. In 40% of cases (n=31), Fibrogammin® was administered after FXIII determination was performed. In 16 cases, Fibrogammin® replacement therapy was administered before FXIII activity was determined. The percentage distribution of the administration of Fibrogammin®, according to the time of determination of FXIII activity is

shown in Figure 30.

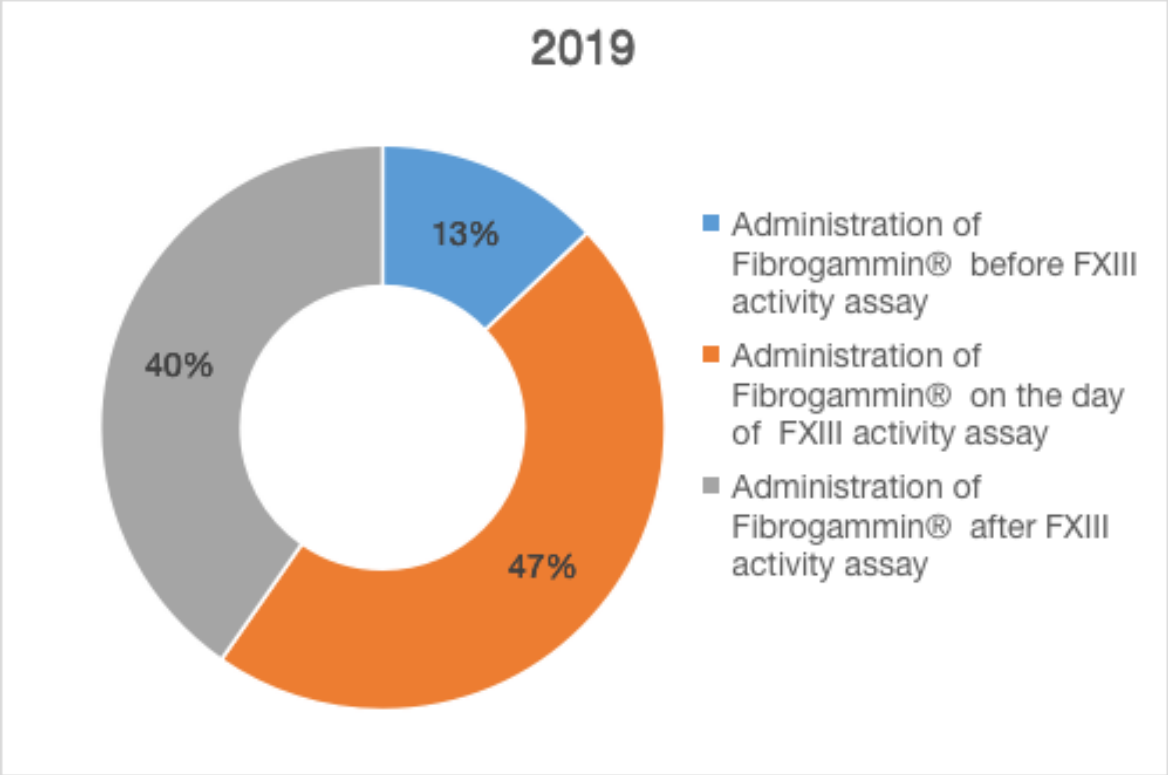

Figure 30: The percentage distribution of the administration of Fibrogammin® in patients with severely decreased FXII activity, according to the time of determination of FXIII activity.

3.2.2.2 Patients with moderate FXIII deficiency

Overall, 54 patients (31.4%) of a total of 509 patients with moderate FXIII deficiency received Fibrogammin® in 2019. In most of these cases (n=32, 77.8%), and similar to severe FXIII deficiency, replacement therapy with FXIII was prescribed primarily for patients in the various ICUs, with most patients (n=18) receiving Fibrogammin® therapy in the cardio-thoracic surgical ICU (ward 97), followed by the operative ICU (ward 90), with 14 patients, and in the internal medical ICU (ward 93, cardiology), with 6 patients. A total of 5 patients receiving Fibrogammin® therapy were from the stem cell transplantation ward 21and 45 patients were from the general haemato-/oncology ward 20. The distribution of patients with moderate FXIII deficiency who received replacement therapy with Fibrogammin® in 2019 across departments is shown in Figure 31.

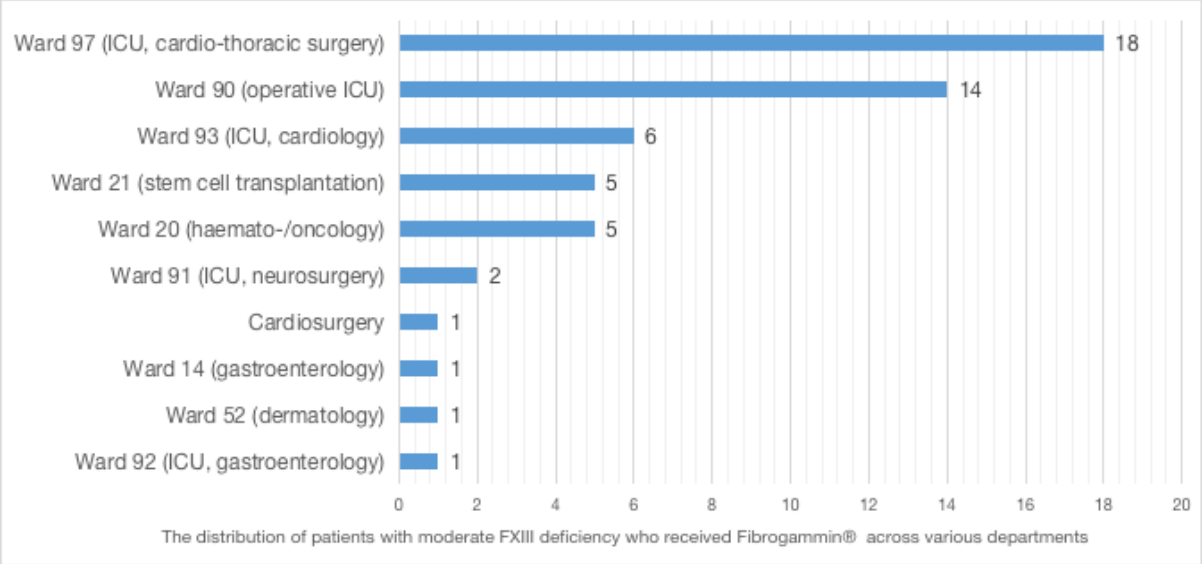

Figure 31: The distribution of patients with moderate FXIII deficiency who received Fibrogammin® across departments.

A total of 213,750 IU of Fibrogammin® were administered to patients with moderate FXIII deficiency in 2019. Similar to severe FXIII deficiency, Fibrogammin® was mostly administered on the day that the FXIII activity test was performed or shortly thereafter. In 44% of cases (n=77), patients with moderate FXIII deficiency received Fibrogammin® substitution therapy on the same day the FXIII activity test was performed. In almost as many cases (n=74), Fibrogammin® was administered after the FXIII activity assay. In 24 cases, Fibrogammin® substitution therapy was administered before the FXIII activity assay was performed. The percentage distribution of the administration of Fibrogammin®, according to the time of determination of FXIII activity, is shown in Figure 32.

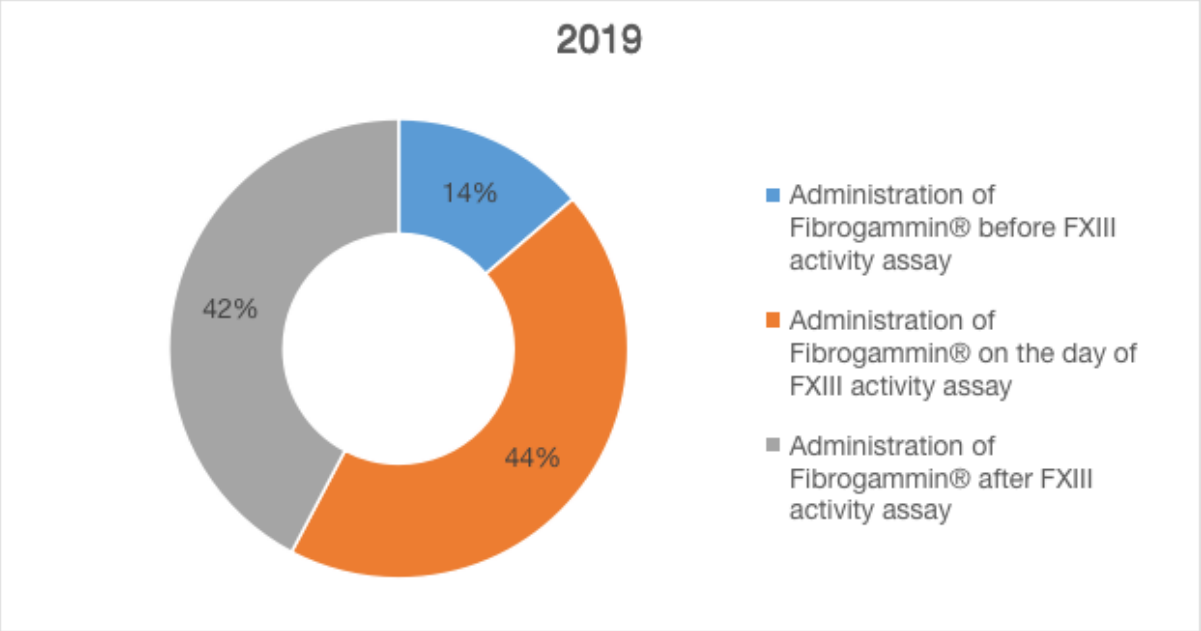

Figure 32: The percentage distribution of the administrations of Fibrogammin®, according to the time of determination of FXIII activity.

3.2.2.3 Patients with normal FXIII activity

Overall, 32 patients (8.5%) of a total of 492 patients with normal FXIII activity received Fibrogammin® in 2019. Similar to patients with severe and moderate FXIII deficiency, Fibrogammin® was prescribed most frequently (n=20, 87.0%) in patients in ICUs, with the majority (n=10) from the cardio-thoracic surgical ICU (ward 97), the operative ICU (ward 90), and the internal medical ICU (ward 93, cardiology). In addition, as in the case of severe and moderate FXIII deficiency, 2 patients from the general haemato/-oncology ward 20 and 1 patient from the stem cell transplantation ward 21 received replacement therapy with Fibrogammin®. The distribution of patients who received Fibrogammin® across departments is shown in Figure 33.

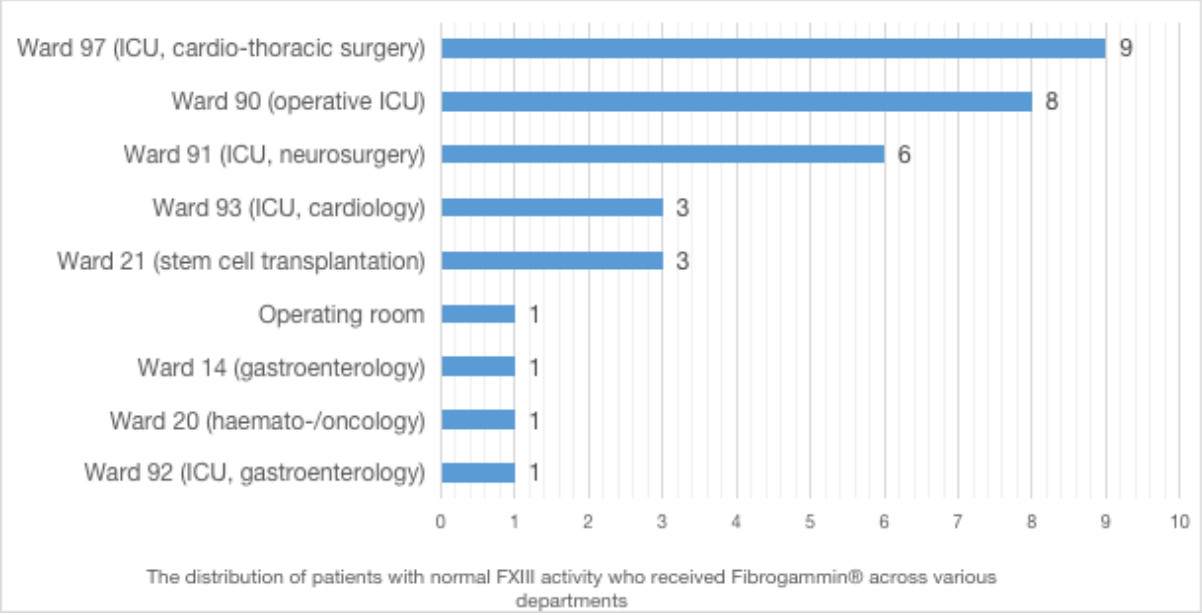

Figure 33: The distribution of patients with normal FXIII activity who received Fibrogammin® across various departments.

A total of 80,000 IU of Fibrogammin® were administered to patients with normal FXIII activity in 2019. Similar to severe or moderate FXIII deficiencies, patients with normal FXIII activity received Fibrogammin®, mostly on the day of FXIII activity determination. In 44% of cases (n=28), patients with normal FXIII activity received replacement therapy on the day of FXIII activity determination. In 30% of cases (n=19), Fibrogammin® was administered after the FXIII activity assay was performed. In 26% of cases (n=17), Fibrogammin® was administered before the FXIII activity assay. The percentage distribution of administration of Fibrogammin®, depending on the time of determination of FXIII activity is shown in Figure 34.

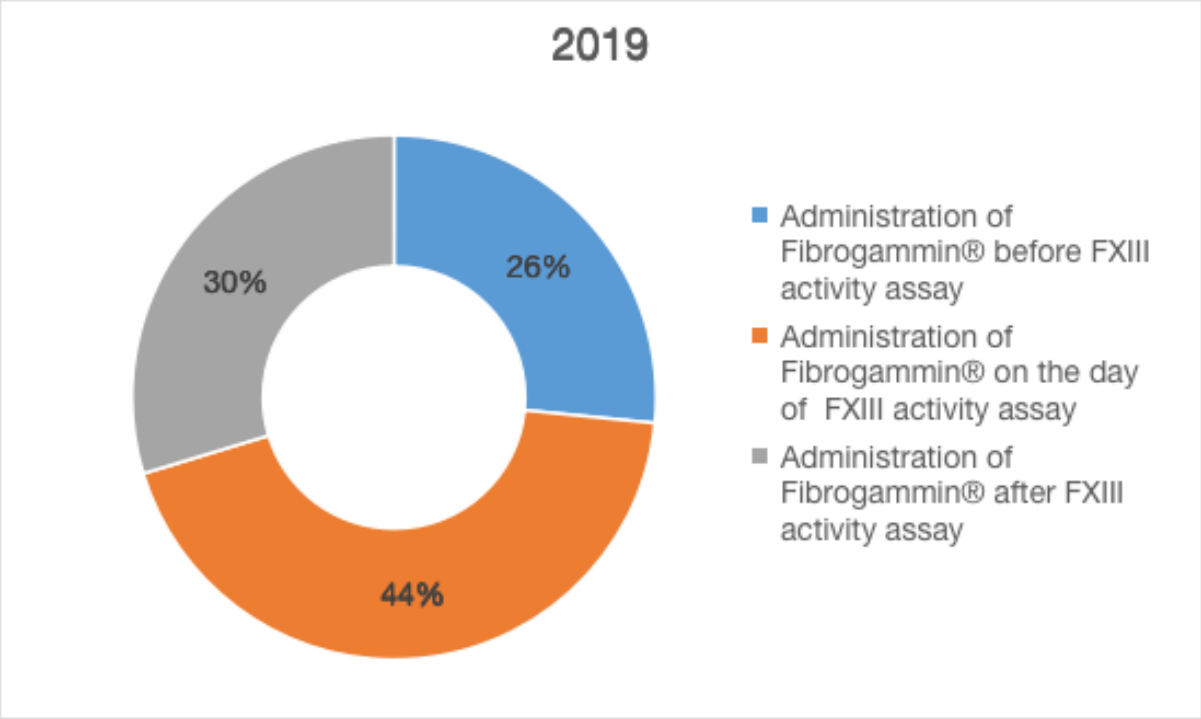

Figure 34: The percentage distribution of administrations of Fibrogammin® in patients with normal FXIII activity, according to the time of determination of FXIII activity.

3.3 Comparison between 2017 and 2019

In 2017, FXIII activity assays were performed once a week. In 2019, a new laboratory order for FXIII activity analysis was introduced, enabling assays to be performed on a daily basis.

The number of FXIII activity tests performed in 2019 at the University Hospital Regensburg increased by 57.4%, which means that an additional 813 FXIII activity tests were performed in 2019 compared to 2017. The number of patients for whom FXIII activity tests were performed also increased markedly by 12.4% in 2019.

Whereas in 2017 most FXIII activity tests were performed in the age group between 1 and 20 years (a total of 30% of all FXIII tests performed in 2017), in 2019 this pattern shifted to the age group between 60 and 81 years, with 33% of all FXIII tests performed. This finding is also reflected in the average age of patients for whom FXIII activity tests were performed. In 2017, the average age was 38.9 years, and in 2019, 45.5 years, a difference of over 6 years between 2017 and 2019.

The distribution of FXIII activity values in the tests performed changed between 2017 and 2019. Whereas in 2017, normal FXIII activity (71-150%) was diagnosed in a total of 71% of all FXIII tests performed, in 2019 the prevalence of normal FXIII activity had decreased to over 36%. In contrast, the prevalence of severely reduced FXIII activity (0-40%) increased markedly in 2019 to over 21% overall. For moderately reduced FXIII activity (41-70%), prevalence increased by 15% in 2019 and was the most commonly diagnosed FXIII activity level that year. A comparison between 2017 and 2019 of the prevalence of severe and moderate FXIII deficiency and normal FXIII activity is shown in Figures 35 and 36.

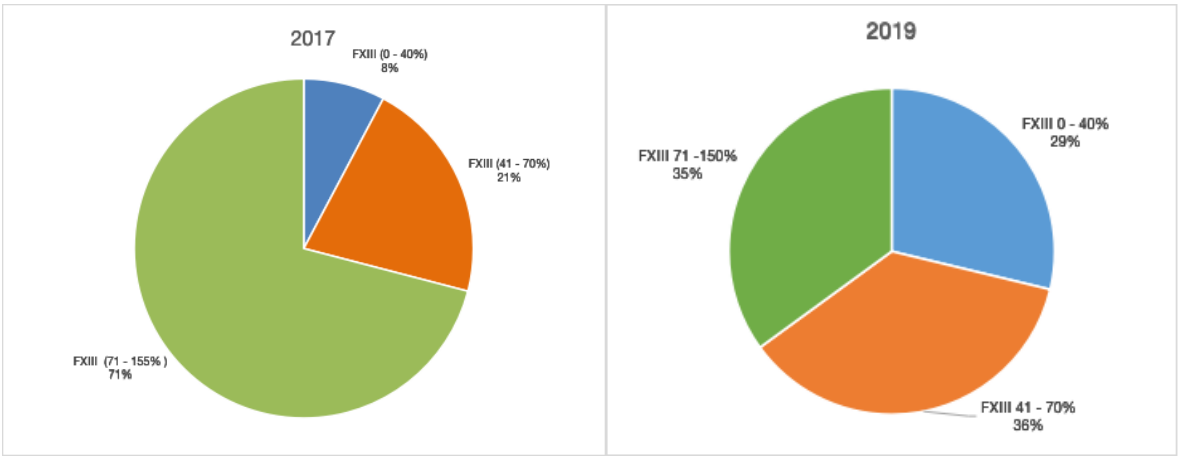

Figure 35 and Figure 36: Comparison of the prevalence of severe and moderate FXIII deficiency and normal FXIII in percentage between 2017 and 2019.

In 2017, most FXIII activity tests were performed in the outpatient setting, with a total of 286 FXIII activity tests, corresponding to 48.3% of all FXIII activity tests performed that year. In contrast, the number of FXIII activity tests performed in the outpatient setting decreased by 34.8% in 2019. In contrast to 2017, most FXIII activity tests in 2019 were performed in the various ICUs, which accounted for a total of 67.3% of all tests performed that year, 23.3% more than in 2017. In both years, most requests were made by the internal medical ICU (ward 93, cardiology), followed by the cardio-thoracic surgical ICU (ward 97), and followed by the operative ICU (ward 90). Whereas in 2017 a relatively large proportion of the FXIII activity tests performed (n=119, 20.1 %) were requested by various departments of the paediatric clinic (ICU, intermediate care and haemato-/oncological outpatient clinic), in 2019 the requests from the various departments of the paediatric clinic comprised only 4.8% of all FXIII activity tests performed. General wards (internal medicine/surgery) also saw a relevant increase in requested FXIII activity tests in 2019, with 18.5% of all FXIII activity tests performed, compared to 7.6% in 2017.

The total number of IUs of Fibrogammin® administered in 2019 increased by more than 65%, from 153,750 IU in 2017 to 448,750 IU in 2019. Table 12 shows the comparison between the general characteristics of FXIII activity tests and FXIII replacement therapy in 2017 and 2019.

		2017	2019
Performed FXIII tests		592 (458 patients)	1405 (523 patients)
Average age		38.9 years	45.5 years
The age group with most performed FXIII tests		1 – 20 years (30%)	61 – 80 years (33%)
FXIII tests in the intensive care setting		261 (44%)	946 (67.3%)
	Ward 93	100	529
	Ward 97	53	184
	Ward 90	32	116
FXIII tests in the outpatient setting		286 (48.3%)	190 (13.5%)
FXIII tests in the general wards		45 (7.6%)	260 (18.5%)
FXIII tests in the paediatric setting		119 (20.1%)	68 (4.8%)
Delivered Fibrogammin® in IE of patients with performed FXIII activity analysis		153,750 IU	448,750 IU

Table 12: Comparison between the general characteristics of FXIII activity assays and Fibrogammin® therapy in 2017 and 2019.

In both years, the highest prevalence of severe FXIII deficiency was documented in various ICUs, with a total of 91.3% in 2017 and 88.6% in 2019, with most assays performed in the internal medical ICU (ward 93, cardiology). In contrast to 2017, the prevalence of severe FXIII deficiency increased in 2019, not only in the intermediate care wards (from 2 to 23 cases in the stem cell transplantation ward), but in the general wards, with 9 recorded cases of severely decreased FXIII activity in the haemato-/oncology general ward 20 and 6 in the nephrology general ward 17. In 2019, there was one documented case of severe FXIII deficiency in the coagulation outpatient clinic.

In 2019, the use of Fibrogammin® therapy in patients with severe FXIII deficiency increased by 13.7% compared to 2017. The amount of Fibrogammin® dispensed to the various departments also increased significantly by more than 85.5% in 2019, from 22,500 IU in 2017 to 155,000 IU in 2019. In both years, the highest quantity of Fibrogammin® was dispensed to various ICUs, in 60% of cases in 2017 and in 84.6% of cases in 2019. In contrast to 2017, Fibrogammin® was also delivered to general wards (haemato-/oncology and nephrology) in 3

cases in 2019. In 2017, when FXIII activity determination was available only once a week, in none of the cases Fibrogammin® was administered on the day of FXIII activity determination. In 2019, Fibrogammin® was administered on the day of FXIII activity determination in 47% of the cases. In 2019, the administration of Fibrogammin® before the actual FXIII assay decreased to 37%. In addition, the use of argatroban as an anticoagulant in the intensive care setting increased for 23.2% in 2019. Table 13 shows the comparison between the characteristics of severe FXIII deficiency, FXIII replacement therapy, and anticoagulation with argatroban in 2017 and 2019.

			2017	**2019**
Activity levels of FXIII tests	**FXIII 0 – 40 %**		**46 (31 patients)**	**404 (131 patients)**
		Tests in ICUs	42 (91.3%)	358 (88.6%)
		Anticoagulation with argatrobran	7 patients (22.6%)	60 patients (45.8%)
		Patients treated with Fibrogammin ®	5 (16.1%)	39 (29.8%)
	Ward 97		3	13
	Ward 93		-	9
	Ward 90		-	7
		Delivered Fibrogammin ® in IE	22 500 IU	155 000 IU
	Administration of Fibrogammin® before FXIII analysis		50%	13%
	Administration of Fibrogammin® on the day of FXIII analysis		-	47%
	Administration of Fibrogammin® after FXIII analysis		50%	40%

Table 13: Comparison between characteristics of severe FXIII deficiency, FXIII replacement therapy and anticoagulation with argatroban in 2017 and 2019.

Moderate FXIII deficiency (41-70%) was also most commonly diagnosed in the intensive care

setting with a total of 80.2% assays in 2017 and 79.4% in 2019, with most tests in both years performed in the internal medical ICU (ward 93, cardiology).

Similar to severe FXIII deficiency, the frequency of Fibrogammin® therapy administered to patients with moderate FXIII deficiency increased by over 63% in 2019. The amount of Fibrogammin® dispensed also increased substantially by over 80.1% in 2019, from 42,500 IU in 2017 to 213,750 IU in 2019. In both years, the greatest amount of Fibrogammin® was prescribed by the various ICUs, in 90% of cases in 2017 and 77.8% in 2019. In both years, Fibrogammin® was prescribed in patients in the stem cell transplantation ward, to 1 patient in 2017 and a total of 5 patients with moderate FXIII deficiency in 2019. With regard to the administration pattern of Fibrogammin®, according to the timing of FXIII analysis in the patients with moderately reduced FXIII activity, no relevant difference between 2017 and 2019 was observed. Table 14 shows the overall comparison between the characteristics of moderate FXIII deficiency and FXIII replacement therapy in 2017 and 2019.

		2017	2019
Activity levels of FXIII tests	**FXIII 41 - 70%**	**126 (91 patients)**	**509 (172 patients)**
	Tests in ICUs	101 (80.2%)	405 (79.4 %)
	Patients treated with Fibrogammin ®	20 (21.9%)	54 (31.4%)
	Ward 97	9	18
	Ward 93	-	6
	Ward 90	7	14
	Delivered Fibrogammin ® in IE	42,500 IU	213,750 IU
	Administration of Fibrogammin® before FXIII analysis	24%	14%
	Administration of Fibrogammin® on the day of FXIII analysis	35%	44%
	Administration of Fibrogammin® after FXIII analysis	41%	42%

Table 14: Comparison between characteristics of moderate FXIII deficiency, FXIII replacement therapy in years 2017 and 2019.

Whereas in 2017 the highest incidence of normal FXIII activity was observed in the outpatient setting, in 2019 the incidence of normal FXIII activity was found to be 36% in both - the outpatient setting and the intensive care setting.

In contrast to severe and moderate FXIII deficiency, the amount of Fibrogammin® administered in 2019 decreased slightly in patients with normal FXIII activity: from 88,750 IU in 2017 to 80,000 IU in 2019. Similar to severe and moderate FXIII deficiency, the largest quantity of Fibrogammin® was prescribed in ICUs in both years. The ability to perform a FXIII activity assay at any time reduced the administration of Fibrogammin® by 8% in patients with normal FXIII activity, after the activity level was determined. In 2019, an overall increase of

33% in the administration of Fibrogammin® on the same day the FXIII activity assay was performed was observed. Table 15 shows the overall comparison between normal FXIII activity and FXIII replacement therapy in 2017 and 2019.

			2017	2019
Activity levels of FXIII tests	**FXIII 71– 150 %**		**420 (380 patients)**	**492 (375 patients)**
		Tests in ICUs	100 (23.8%)	178 (36.2%)
		Patients treated with Fibrogammin ®	21 (5.5%)	32 (8.5%)
	Ward 97		8	9
	Ward 93		-	3
	Ward 90		6	8
		Delivered Fibrogammin ® in IE	88,750 IU	80,000 IU
	Administration of Fibrogammin® before FXIII analysis		51%	26%
	Administration of Fibrogammin® on the day of FXIII analysis		11%	44%
	Administration of Fibrogammin® after FXIII analysis		38%	30%

Table 15: General comparison between characteristics of normal FXIII activity, FXIII replacement therapy in 2017 and 2019.

4 DISCUSSION

In contrast to inherited FXIII deficiency, the incidence of acquired FXIII deficiency is much higher and the clinical significance is not yet fully understood. There are currently very few studies on the incidence, optimal monitoring and treatment targets of acquired FXIII deficiency. There is no reliable evidence from prospective randomised trials showing a causal relationship between perioperative acquired FXIII deficiency and the occurrence of confirmed bleedings. In addition, a uniformly accepted indication for prophylactic or therapeutic administration of FXIII concentrates has not yet been clearly defined in the current medical literature. Therefore, the aim of this retrospective research study was to characterise the incidence, diagnostic methods and FXIII replacement therapy in patients with FXIII deficiency at the University Hospital Regensburg, and to evaluate the clinical relevance of FXIII deficiency. An additional aim was to investigate whether any differences in the FXIII replacement therapy were observed with the introduction of the new laboratory order, providing FXIII activity assays on a daily basis. This retrospective study was based on the analysis of performed FXIII tests in the coagulation laboratory of the University Hospital Regensburg as well as medical documents, laboratory values and files of patients with severe FXIII deficiency.

4.1 Incidence of FXIII deficiency

Currently no data on the incidence of acquired FXIII deficiency in the general population as well as in the outpatient or inpatient setting are available. Furthermore, no uniformly defined classification of acquired FXIII deficiency in terms of the activity level has been developed. Normally FXIII activity levels range approximately from 69 to 143%.[32,33] Congenital FXIII deficiency usually presents clinically with severe bleeding diatheses with plasma FXIII levels less than 1 %. However, as the prospective study of Gödje et al. showed, postoperative blood loss and transfusion requirements in patients after coronary surgery with extracorporeal circulation were significantly higher in patients with FXIII activity already less than 70%. [75] Therefore we proposed in this study a classification of FXIII deficiency into three groups according to the activity values - severe (0-40%), moderate (41-70%) FXIII deficiency and normal FXIII activity (71-150%).

Taking in account that at the University Hospital Regensburg altogether 37,338 patients are annually treated in inpatient/day-care setting and 159,771 patients, respectively, in the outpatient setting, the incidence of both - the severe and moderate FXIII deficiency in patients

in the inpatient and outpatient setting was 0.06 % in 2017 and 0.15% in 2019. The incidence was higher in both years in the inpatient setting: 0.03% in 2017 and 0.18% in 2019. Thus, during the course of these two years only 15 cases with moderate FXIII deficiency were diagnosed in the outpatient setting, with only one case of severe FXIII deficiency. This is the first study that implemented the incidence of acquired FXIII deficiency in both outpatient and inpatient settings.

4.2 Overview of FXIII assays performed in 2017 and 2019

In 2017, a total of 592 FXIII activity assays were performed on 458 different patients in the coagulation laboratory at the University Hospital Regensburg. Following the introduction of the new laboratory order for FXIII activity analysis, which made this analysis available on a daily basis, the number of FXIII activity tests performed increased by 58.9% in 2019, resulting in a total of 813 additional FXIII activity tests. Although the number of FXIII tests performed increased in 2019, the number of patients for whom this test was performed increased only by 12.4%. The sharp increase in the number of FXIII activity tests may be related to the newly introduced laboratory order, that allows physicians to determine FXIII activity levels on a daily basis in patients with clinically observed bleeding events and with otherwise normal laboratory findings in coagulation diagnostics, providing physicians with additional therapeutic options.

4.2.1 FXIII activity assays in the outpatient setting

The majority of FXIII activity tests were carried out in 2017 in the outpatient setting. A total of 286 FXIII activity tests were requested, representing 48.3% of all FXIII activity tests performed in 2017. Overall, 173 of these tests were performed in the coagulation outpatient clinic, and comprised 29.2% of all FXIII activity tests performed in 2017. Almost the same number of FXIII activity tests were also performed in the coagulation outpatient clinic in 2019, resulting in a total of 178 tests. This finding generally corresponds with the fact that the FXIII activity test is a standardised component of bleeding-diagnostics for all patients at the initial presentation in the coagulation outpatient clinic. The laboratory diagnostics additionally include the following parameters: INR, aPTT, fibrinogen, vWF antigen, vWF activity, FVIII, FIX, and platelet function testing (aggregometry according to Born), as well as determination of the blood group.

Although the FXIII activity assay is a standard component in bleeding diagnostics at the

coagulation outpatient clinic, the present study shows that moderate FXIII deficiency was diagnosed in only 6 patients in 2017 and in 8 patients in 2019, in an outpatient setting. In addition, no cases of severe FXIII deficiency were diagnosed in 2017, and only one case was diagnosed in 2019. Further examination of the medical reports of these 6 patients with moderately reduced FXIII activity from 2017 revealed that in more than 50% of the cases, acquired FXIII deficiency was diagnosed secondary to the utilisation of coagulation factors due to a current bleeding event or due to pre-existing hepatic insufficiency. In addition, one case was diagnosed with a physiologically reduced FXIII deficiency during pregnancy.

In view of these findings, the discontinuation of standard testing for FXIII activity in all patients in the coagulation outpatient clinic at the initial presentation should be discussed. One approach could include the implementation of further risk stratification, with the use of the ISTH-SSC BAT, for example, in which FXIII activity is more likely to be assessed in cases of a pre-existing and long-standing bleeding tendency, especially in the perioperative setting, by otherwise normal coagulation diagnostics or in the cases of diseases in which decreased FXIII activity is expected, such as hepatic insufficiency, haemato-oncologic disorders or inflammatory bowel diseases.[76]

4.2.2 FXIII activity assays in the inpatient setting

The majority of FXIII activity assessments in 2017 and 2019 were requested by a wide variety of ICUs. Overall, 44% of FXIII activity assays were performed in the intensive care setting in 2017, with significantly more requests in 2019, in which 67.3% of the performed FXIII assays were request by different ICUs. In both years, most requests were made by the internal medical ICU (ward 93, cardiology), followed by the cardio-thoracic surgical ICU (ward 97), and by the operative ICU (ward 90). An increase in the number of FXIII activity tests performed in the intensive care setting in 2019 could not be explained by the increased number of patients receiving ECMO therapy, as a total of 186 patients were treated with ECMO in 2019, which is 16 fewer than that in 2017, according to data from the ECMO technical support team at the University Hospital Regensburg.

The new standardised protocol for coagulation diagnostics, including the determination of FXIII activity, during the course of ECMO therapy, which was introduced in August 2017, could also explain the sharp increase in FXIII activity assays in critical care in 2019. FXIII

activity levels are determined before the initiation of ECMO therapy, on the first, fifth and tenth day, on the last day of ECMO, and on the first and second day following the termination of ECMO therapy. Along with FXIII, the ECMO protocol includes diagnostic assessments for vWF and FVIII, as well as the monitoring of argatroban and impedance-aggregometry.

4.3 Severe and moderate FXIII deficiency

In 2017 and 2019, the highest incidence of severe FXIII deficiency was found in the intensive care setting. Overall, 91.3% and 88.9% of all cases in 2017 and 2019 of severe FXIII deficiency were diagnosed in the various ICUs. The incidence of moderate FXIII deficiency was also the highest in the intensive care setting, with 80.2% in 2017 and 79.4%, respectively, in 2019, of all cases of moderate FXIII deficiency.

In addition, more than 80% of all cases of severe FXIII deficiency were diagnosed in association with ECMO therapy. Bleeding events were documented in 75.2% of patients with severe FXIII deficiency during the course of ECMO therapy. In most cases bleeding events were related to the ECMO therapy itself. Moreover, a total of 74.2% of patients with severe FXIII deficiency had diagnoses related to bleeding or other coagulopathies. The most common bleeding-related diagnoses were also mainly related to ECMO therapy, including thrombocytopenia, hyperfibrinolysis or local haemorrhages.

Due to the high prevalence of bleeding events and bleeding-related diagnoses in patients with severely reduced FXIII activity, over 93% of the patients in this group received blood transfusions. At the time the FXIII activity assay was performed, 87.1% of patients with severely reduced FXIII activity were receiving replacement therapy with red blood cell concentrates, 58.1% of patients were receiving platelet concentrates, and 48.4% of patients were treated with FFP.

Data from this present study corresponds with the current findings from relevant studies in the field. The register of the ELSO confirms that bleeding is generally the main complication during ECMO therapy.[54] A meta-analysis from 2013 noted that more than 30% of all the patients treated with ECMO suffer from bleeding, including intracranial bleeding with the incidence rates ranging from 5% up to 19%, and an average survival rate of 30%. [55] [56,57] [55,77]

The exact mechanisms of ECMO therapy leading to haemorrhagic diatheses are under active investigation and still need to be clarified. However, it has been observed that mechanical shear stress derived from the circuit and the blood flow damages blood cells, particularly platelets and leads to the uncoiling of vWF, resulting in thrombosis, impaired platelet function, and fibrinolysis.[78] In addition, artificial surfaces can induce a systematic inflammatory response syndrome and diffuse activation of the coagulation system with consecutive wastage of plasma coagulation factors.[61,64]

Also, our study shows that in patients with severe FXIII deficiency in intensive care setting, in particular in those patients during ECMO therapy, over 87.1% had documented thrombocytopenia. Because platelets contain FXIII-A, low platelet counts might account for the low FXIII levels observed.[79] However, a study by Zeerleder et al. found no correlation between platelets, FXIII-A, and FXIII cross-linking activity. In addition, no difference in FXIII levels in patients with and without thrombocytopenia was observed.[80]

As noted previously, there are limited data on acquired FXIII deficiency in ECMO patients, and the prevalence of acquired FXIII deficiency in these patients is unclear. A study by Kalbhenn et al. with a small sample of patients (n=20) reported that over 88 % of all patients undergoing vvECMO therapy showed a decrease in FXIII activity.[64] In a prospective study by Wettstein et al., 226 patients with unexplained intraoperative bleeding who underwent elective surgery were found to have significantly decreased FXIII activity compared with the control group without any bleeding complications.[81]

The direct mechanism underlying the reduction in FXIII activity in association with ECMO therapy remains to be elucidated. Acquired FXIII deficiency is rarely an isolated entity, but rather a constellation of multifactorial conditions, especially in the intensive care setting. Apart from the reasons mentioned above, also a perioperative blood loss, dilutional coagulopathy, and increased utilisation of clotting factors could lead to decreased FXIII activity between 30–70%, according to data in published reports of adult and paediatric cohorts.[63] Furthermore, Fahlbush et al. noted that haemostasis following surgery might be aggravated by hypothermia, acidosis and fibrinolysis, resulting in a worsening of patient's outcome.[63]

In a prospective study, Korte et al. showed that patients undergoing elective surgery who develop 'unexplained' intraoperative coagulopathies have significantly less FXIII at any point

in time than patients without such coagulopathies. The consequence was a significant loss of clot firmness that was associated with an increase in intraoperative blood loss.[82]

Interestingly, the study of Moerer et al. suggested that the activity of FXIII in patients undergoing vvECMO therapy is already considerably reduced even before the initiation of extracorporeal circulation. [83] This study evaluated the course of FXIII before, during and after vvECMO in ARDS patients. The activity of FXIII was analysed in 20 ECMO patients before and 6 h, one, three and seven days after the implantation of ECMO, as well as one and three days after the termination of ECMO therapy. It was shown that FXIII activity was already severely decreased to 37% before ECMO. FXIII activity was the only coagulation factor continuously declining during vvECMO, being significantly decreased on the third and seventh day compared to 6 h after the initiation of vvECMO. Three days after termination of vvECMO, platelet count and fibrinogen nearly doubled and FII, FV, FXI and FXIII showed spontaneous significant increases. Moreover, Moerer et al. concluded that incorporation of FXIII monitoring into the regular haemostaseologic routine during vvECMO therapy could be advisable.

4.4 Anticoagulation and the measurement of FXIII in the intensive care setting

UFH is the most commonly used systemic anticoagulant during ECMO therapy. In recent years, however, anticoagulation with direct thrombin inhibitors has gained acceptance. The main reason for this is that direct thrombin inhibitors do not cause an immune mediated thrombocytopenia, such as HIT. Therefore, direct thrombin inhibitors may provide a more predictable dosing regimen with less bleeding complications.[84] Argatroban is also preferably used as a standard anticoagulation in vvECMO in intensive care setting at the University Hospital Regensburg. The study of Fisser et al. showed that in patients without HIT on vvECMO, argatroban was non-inferior to UFH regarding bleeding and thrombosis with similarly distributed occurrence of technical complications. Interestingly, Frisser at el. observed in his study, that argatroban may have less impact on platelet decrease during ECMO, but this finding needs further investigations.[85]

As Tripodi et al. emphasised in his study, an important drawback in therapy and prophylaxis of venous thromboembolism with direct thrombin inhibitors is that FXIII may be underestimated when measured with chromogenic substrates in patients receiving the direct thrombin inhibitor. The is explained by the fact that FXIII is activated by thrombin.[86] Weigel et al. showed in his

study strong interferences of routine coagulations tests with increasing concentrations of the direct oral thrombin inhibitor dabigatran up to 0.48 µg/ml with a strong influence of FXIII activity levels, reducing the activity of FXIII over 70 % in the presence of dabigatran.[87]

Medical reports showed that 51.6% of patients with severe FXIII deficiency were anticoagulated with intravenous heparin in 2017. In one case, HIT was suspected and anticoagulation with heparin was switched to argatroban. 19.4% of patients were anticoagulated with argatroban. In addition, 2019 data showed that over 45% of patients with severely reduced FXIII activity were anticoagulated with argatroban. Therefore, certain degree of underestimation of FXIII activity in this study has to be discussed. Nonetheless, clinicians at the intensive care setting are informed of this matter and the measured 2019 argatroban levels (average value: 0.09 µg/ml) showed that relevant underestimation of FXIII activity is very unlikely, as the levels are very low.

4.5 FXIII replacement therapy

With the introduction of the new laboratory order, making FXIII activity assays available on a daily basis, a sharp increase in the use of plasma-derived FXIII concentrates of over 65% in 2019 was documented. The prevalence of the administered FXIII replacement therapy in patients with severe FXIII deficiency has increased over 13.7%, in patients with moderate FXIII deficiency over 9.5% and in patients with normal FXIII deficiency over 2.9%. Also, the delivered amount of Fibrogammin® during 2019 has considerably increased by 85.5% in patients with severe FXIII deficiency and by 80.1 % in patients with moderate FXIII deficiency, while in the group of patients with normal FXIII activity, the utilisation of Fibrogammin® decreased over 0.8% in 2019. Overall, the amount of administered Fibrogammin® rose up to 65.7%, namely from 153,750 IE in 2017 to 448,750 IE in 2019. Taking in account that 1250 IE plasma-derived FXIII concentrate Fibrogammin® costs approximately 490 euros, then altogether 219,887 euros were spent in 2019 for Fibrogammin®, which is 144,550 euros more than in 2017.

In both years the greatest amount of Fibrogammin® was delivered to various ICUs at the University Hospital Regensburg, in 2017 in 60% of the cases and in 2019 in 84.6% of the cases. In most of these cases Fibrogammin® was delivered to the cardio-thoracic surgical ICU (ward 97), the operative ICU (ward 90) and internal medical ICU (ward 93, cardiology). In contrast

to 2017, Fibrogammin® was also administered in 3 cases to the general wards (haemato-/oncology and nephrology) in 2019.

Few reports have been published on optimal management and treatment options for patients with FXIII deficiency. Furthermore, no specific, generally acceptable threshold for administration of FXIII concentrates has been established and its routine use has been the subject of controversy. To date, coagulation management, which in most cases does not include the assessment of FXIII activity, has primarily been based on clinical bleeding signs in support of a decision to initiate the administration of blood products, including the FXIII concentrate. This could be one of the approaches implemented at the University Hospital Regensburg and may explain why 32 patients (8.5%) in 2019 with normal FXIII activity levels and documented bleeding complications received FXIII replacement therapy. This observation also corresponds with further findings in the medical reports of patients who received FXIII replacement therapy in 2017. Documented bleeding complications occurred in 100% of patients with severely reduced FXIII activity and in 90% of patients with moderately reduced FXIII activity, who underwent replacement therapy with Fibrogammin®.

However, the sharp increase of over 65% in the use of the FXIII concentrate in 2019, especially in patients with severe and moderate FXIII deficiency, could also be explained by the introduction of the new standardised protocol for coagulation diagnostics, which includes the assessment of FXIII activity, during ECMO therapy. Assessment of FXIII activity before the initiation of ECMO therapy, as well as throughout the course of therapy, can provide clinicians with an important new tool for monitoring FXIII activity levels. Thus, replacement therapy with Fibrogammin® is based on actual FXIII activity levels, rather than on signs of clinical bleeding. This strategy could also reflect the association observed between the timing of performed FXIII activity assays and the administration of replacement therapy with Fibrogammin® 2019. Overall, in 87% of patients with severe FXIII deficiency and 86% of patients with moderate FXIII deficiency, the replacement therapy was initiated on the same day of the determination of FXIII activity or soon thereafter. Interestingly, approximately 75% of patients with normal FXIII activity who underwent FXIII replacement therapy, received Fibrogammin® even though the normal FXIII activity was confirmed priorly. This finding also raises the question of why, despite the introduction of the standardised protocol for coagulation diagnostics during ECMO therapy, only 39 patients (29.8%) with severe FXIII deficiency received the replacement therapy with Fibrogammin®. Therefore, further examination of

patients' medical records in 2019 would be useful in evaluating whether the introduction of the new laboratory order of FXIII activity assays, as well as the standardised protocol of coagulation diagnostics during ECMO therapy, have had an effect on decision-making with regard to the administration of Fibrogammin®.

In order to optimise coagulation diagnostics during ECMO therapy, and to test whether standardised screening for FXIII activity levels can reduce the risk of bleeding complications, a study by Kalbhenn et al. investigated the effect of using a coagulation protocol that included a standardised assessment and target-controlled replacement of coagulation factors among 19 patients in the control group and 20 patients in the intervention group. FXIII was determined by every patient twice per week and when clinical bleeding signs were present. Additionally, the following parameters were assessed: hemoglobulin every hour; platelets, INR; aPTT daily; and FVIII, vWF:antigen and vWF: RCo twice per week. Implementation of this protocol led to a reduction in the incidence and severity of spontaneous intracranial bleeding from 31% before implementation of the protocol to 10% after implementation.[64] Asam et al. reported 2 vvECMO patients with uncontrollable bleeding events and acquired FXIII deficiency, who were successfully treated with plasma-derived FXIII concentrates.[78] In a prospective randomised controlled study of patients undergoing elective surgery for gastrointestinal cancer, Korte et al. showed that replacement with 30 IU Fibrogammin® per kg increased clot strength and significantly reduced perioperative blood loss, as well as the transfusion rate of red blood cell concentrates.[88]

However, as noted by Kalbhenn et al., further prospective randomised controlled studies with a larger group of patients are needed to demonstrate statistical significance for the findings in their study, and to determine if patients would benefit from standardised screening for FXIII. Such an approach could also be implemented at University Hospital Regensburg to determine if patients benefit from the implemented ECMO protocol for coagulation diagnostics, and to establish a standard for the management of bleeding complications, particularly with regard to FXIII activity levels, in the intensive care setting.

In contrast to the results described by Kalbhenn et al., numerus studies did not demonstrate any significant clinical advantage of routine FXIII supplementation. A study by Fahlbusch et al. analysed whether pre- and post-operative FXIII levels were associated with the use of blood product supplementation and chest-drainage loss in the first 48 h following open-heart surgery

in infants with congenital heart defects necessitating cardiopulmonary bypass. No evidence of a significant correlation between FXIII activity and chest-drainage loss or any advantage of routine FXIII supplementation was observed in the study. Furthermore, results from the study were only able to show that a single post-operative administration of FXIII reduced effusions for the first 24 h after cardiac surgery, but not thereafter.[89] In another prospective randomised placebo-controlled study by Gödje et al. of 75 patients who underwent cardiac surgery, received either 0 IU (placebo), 1250 IU, or 2500 IU Fibrogammin after extracorporeal circulation. Postoperative blood loss and the transfusions requirement differed between the groups not significantly. However, postoperative blood loss and transfusion requirements in patients with FXIII activity > 70% were significantly lower compared to that in patients with FXIII activity < 70%.[75]

In 2017 and 2019, respectively, 8.7% and 6.6% of cases with documented severe FXIII deficiency were diagnosed in the stem cell transplantation ward 21. Around 60% of patients with acute leukemia present with acquired FXIII deficiency at the time of the initial diagnosis.[90] This deficiency usually normalises spontaneously within periods of haematological remission.[90] Both the causes and the clinical relevance of acquired FXIII deficiency in these patients remains unclear. In a randomised controlled study, Rasche et al. analysed whether replacement with plasma-derived FXIII concentrates influences the clinical course in patients with acute leukemia and acquired FXIII deficiency. Compared to the control group, no statistically significant differences in the frequency and severity of bleeding complications, transfusion requirements, and the number of remissions were observed.[91]

To date, there are no official recommendations for the monitoring as well as the FXIII replacement therapy during ECMO. As mentioned above, according to the recommendations of guidelines for haemotherapy, the administration of calibrated therapy with the FXIII concentrate in cases of extensive and uncontrolled bleeding events when other coagulation disorders have been excluded can be justified even without laboratory-confirmed evidence of an FXIII deficiency.[70] Guidelines from the European Society of Anaesthesiology for the management of severe perioperative bleeding suggest that in cases of ongoing or diffuse bleeding and low clot strength, despite adequate fibrinogen concentrations, it is likely that FXIII activity is critically low; therefore, in cases of significant FXIII deficiency (i.e., < 60% activity), the administration of FXIII concentrate can be considered. However, the guidelines also emphasise that more data are needed on the effects of FXIII concentrates on bleeding and

transfusion requirements. Furthermore, guidelines from the European Society of Anaesthesiology on the management of severe perioperative bleeding suggests maintaining FXIII levels above 50–60% during perioperative bleeding.[49,75,81,88] The guidelines highlight that low platelet counts, low plasma fibrinogen concentration, and FXIII deficiency are predictive of bleeding complications in intracranial surgery and major spinal surgery, particularly when these factors occur in combination.

Such an approach could be applicable for monitoring FXIII activity during ECMO therapy at University Hospital Regensburg. Carrying out FXIII activity assays in patients with concomitant low platelet counts or low plasma fibrinogen concentrations has the potential to avoid serious bleeding complications. Further prospective randomised trials are crucial for elucidating the exact mechanisms underlying acquired FXIII deficiency in the surgical and intensive care setting, especially in association with ECMO therapy, in order to establish efficient algorithms for the management and treatment of bleeding complications in ECMO patients.

In conclusion, with the introduction of the new laboratory order, which enables the determination of FXIII activity on a daily basis, an increase was observed not only in the number of tests performed, but in the use of plasma-derived FXIII concentrate in 2019. The increase in replacement therapy with Fibrogammin® was observed mainly in patients with severe or moderate FXIII deficiency, while a slight decrease in the utilisation of Fibrogammin® was observed in patients with normal FXIII activity. These results cannot be explained by the increasing number of patients receiving ECMO therapy, as the number of patients undergoing ECMO decreased in 2019. The sharp increase in utilisation of Fibrogammin® could be mostly explained by the newly introduced standardised protocol for coagulation diagnostics during ECMO therapy. To date, there are no uniform recommendations for the use of FXIII replacement therapy in the intensive care setting; therefore, we speculate that clinicians could initiate the replacement therapy with plasma-derived FXIII concentrate based on clinical findings. This is underscored by the fact that all patients treated with Fibrogammin® had relevant bleeding-related complications. Further studies are needed to investigate if the introduction of the standardised screening of FXIII activity levels during ECMO therapy with subsequent replacement of FXIII could reduce with bleeding complication in the intensive care setting.

4.6 Limitations of the study

This study has some limitations. First, the study was conducted as a retrospective, evidence-based investigation. Prospective randomised trials on the incidence, optimal monitoring, and treatment goals of acquired FXIII deficiency are essential to establish efficient algorithms for managing bleeding complications in the intensive care setting. Second, the author examined medical reports from patients with severe FXIII deficiency only from 2017. Further examination of the patients' medical records in 2019 would be useful to assess whether the introduction of the new laboratory order influenced decision-making with regard to the administration of plasma-derived FXIII concentrates. In addition, FXIII activity assays were only available once per week in 2017. Thus, evaluation of the association between FXIII activity tests and Fibrogammin® administration in 2017 was probably not quantifiable. Furthermore, with the assistance of the Institute of Pharmacy at University Hospital Regensburg the author was provided with information on when Fibrogammin® was delivered to the wards without any further details on the exact time when the patients received FXIII replacement therapy.

5 SUMMARY AND CONCLUSIONS

Our study shows, that with the introduction of the new laboratory order, which allows a daily assessment of FXIII activity, the number of FXIII tests and the use of plasma-derived FXIII concentrates at the University Hospital Regensburg increased. The highest incidence of severe and moderate FXIII deficiency at the University Hospital Regensburg was in the intensive care setting, particularly in association with ECMO therapy. Severe bleeding events with increased requirements for blood transfusions, which in most cases were related to the ECMO therapy itself, were documented in most patients with decreased FXIII activity levels. The increase in the use of FXIII replacement therapy was observed mainly in patients with severe or moderate FXIII deficiency, whereas Fibrogammin® utilisation decreased slightly in patients with normal FXIII activity compared to 2017. The increase of FXIII replacement therapy in 2019 may be explained by the newly introduced standardised protocol for coagulation diagnostics during ECMO therapy. As there are no uniform recommendations for FXIII replacement therapy in the intensive care setting, we speculate that clinicians might also have initiated FXIII replacement therapy on the basis of clinical findings. This presumption is underscored by the fact that all patients treated with Fibrogammin® had relevant bleeding-related complications, whereas only one third of patients with documented severed FXIII deficiency received Fibrogammin® replacement therapy. Further prospective randomised trials on the incidence, optimal monitoring, and treatment goals of acquired FXIII deficiency are crucial to establish efficient algorithms for managing bleeding complications.

6 ZUSAMMENFASSUNG DER DOKTORARBEIT AUF DEUTSCH

FXIII oder s.g. fibrinstabilisierender Faktor ist als letztes Glied in der Gerinnungskaskade zuständig für die Vernetzung und Stabilisierung des Fibrinthrombus und spielt dadurch eine wichtige Rolle in der Blutgerinnung. Darüber hinaus hat der FXIII eine Reihe bedeutsamer biologischer Funktionen wie etwa die Förderung der Wundheilung oder Erhalt der Schwangerschaft.

Der angeborene FXIII Mangel ist eine sehr seltene autosomal-rezessiv vererbte Gerinnungsstörung mit schwerer Blutungsneigung und ausgeprägter Wundheilungsstörungen. Im Gegensatz dazu ist die Inzidenz eines erworbenen FXIII Mangels wesentlich höher. So kann es z.B. bei großen operativen Eingriffen in der Allgemein- und Abdominalchirurgie sowie Herzthoraxchirurgie intra- und postoperativ zu einem Verbrauch von FXIII im Rahmen der Blutstillung und Wundheilung kommen. Ein erworbener FXIII Mangel tritt auch im Rahmen einer Verbrauchskoagulopathie bei akuten Leukämien oder schwerer Leberzirrhose auf.

Eine Verminderung der FXIII Aktivität wird nicht in der basalen Gerinnungsparametern abgebildet. Im Rahmen der Abklärung eines FXIII Mangels ist laborchemisch eine Messung der FXIII Aktivität notwendig. In Deutschlang steht mit Fibrogammin® ein plasmatisches FXIII Präparat im Rahmen einer Substitution zu Verfügung.

Im Gegensatz zum angeborenen FXIII Mangel ist die klinische Relevanz eines sekundären FXIII Mangels unklar und in der Literatur ist die Indikation zur Substitution eines FXIII Mangels nicht klar definiert.

Im Rahmen dieser medizinischen Doktorarbeit wurden die Inzidenz, die Diagnostik und die Behandlung des FXIII Mangels am Universitätsklinikum Regensburg analysiert und die klinische Relevanz des FXIII Mangels untersucht. Hierbei wurde in einem definierten Zeitraum (01.01. – 31.12.2017) ermittelt, wie viele FXIII Bestimmungen im Gerinnungslabor des Instituts für Klinische Chemie und Laboratoriumsmedizin angefordert wurden und in welchen klinischen Situationen eine FXIII Substitution erfolgte. Das retrospektive Forschungsvorhaben fußt dabei auf der Analyse von ärztlichen Dokumenten, Laborwerten und Akten von Patienten am Universitätsklinikum Regensburg, bei denen eine FXIII Bestimmung im oben angegebenen Zeitraum durchgeführt wurde.

Im Jahr 2017 wurden die FXIII Bestimmungen einmal pro Woche durchgeführt. Seit 2019 gibt es im Gerinnungslabor des Instituts für Klinische Chemie und Laboratoriumsmedizin eine neue Laborordnung für die FXIII Analyse, wodurch die Verfügbarkeit dieser Bestimmung auf täglicher Basis erhöht wurde. Ein weiteres Ziel dieser Studie war es zu untersuchen, ob Unterschiede bei der Verabreichung von FXIII Konzentraten durch die Einführung der neuen Laborordnung für die FXIII Analyse im Jahr 2019 gegenüber 2017 festgestellt wurden.

Zusammenfassend lässt sich feststellen, dass mit der Einführung der neuen Laborordnung, die Anzahl der FXIII-Bestimmungen sowie die Verwendung von plasmatischen FXIII-Präparaten am Universitätsklinikum Regensburg im Jahr 2019 deutlich gestiegen sind. Die höchste Inzidenz von schwerem und mittelschwerem FXIII Mangel am Universitätsklinikum Regensburg war im Bereich der Intensivmedizin zu verzeichnen, insbesondere im Zusammenhang mit der ECMO-Therapie. Bei den meisten Patienten mit verminderter FXIII-Aktivität wurden schwere Blutungsereignisse mit erhöhtem Bedarf an Bluttransfusionen dokumentiert, die in den meisten Fällen mit der ECMO-Therapie in Zusammenhang standen. Die Zunahme der Anwendung der FXIII-Präparaten wurde hauptsächlich bei den Patienten mit schwerem oder mittelschwerem FXIII Mangel beobachtet, während die Anwendung von Fibrogammin® bei den Patienten mit normaler FXIII-Aktivität im Vergleich zu 2017 leicht zurückging. Die Zunahme der FXIII-Ersatztherapie im Jahr 2019 lässt sich durch das neu eingeführte standardisierte Protokoll zur Gerinnungsdiagnostik während der ECMO-Therapie erklären. Da es bisher keine einheitliche Empfehlung für die FXIII-Substitutionstherapie gibt, spekulieren wir, dass Kliniker auch aufgrund von klinischen Befunden, eine Substitutionstherapie mit Fibrogammin® eingeleitet haben könnten. Diese Vermutung wird durch die Tatsache untermauert, dass alle mit Fibrogammin® behandelten Patienten relevante blutungsbedingte Komplikationen aufwiesen, während nur ein Drittel der Patienten mit nachgewiesenem ausgeprägtem FXIII-Mangel eine Fibrogammin®-Ersatztherapie erhielt. Weitere prospektive randomisierte Studien zur Inzidenz, optimalen Überwachung und zu den Behandlungszielen des erworbenen FXIII-Mangels sind von entscheidender Bedeutung, um effiziente Algorithmen für das Management von Blutungskomplikationen zu etablieren.

7 The Annex

7.1 List of Figures

7.2 List of Tables

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Acknowledgement

I would like to thank from the bottom of my heart to everybody who has supported me along the writing of my scientific theses.

I would like to thank Prof. Dr. Wolfgang Herr for the great opportunity to write my dissertation at the Klinik und Poliklinik IIII.

Above all, I want to thank PD Dr. med. Christina Hart for not only for supervising this dissertation but also for mentoring me throughout my residency and whom I truly look up to as an outstanding clinician.

At last, I want to thank my family, especially my mom, for her great love, always being there for me and motivating me.

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SPRACHEN

Lettisch	Muttersprache
Deutsch	fließend
Englisch	fließend
Russisch	fortgeschritten
Interessen	Reisen, Lesen, Oper, Kunstgeschichte

München, 23.02.2022