

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

**Diagnostic difficulties and new therapeutic options in acquired
haemophilia A and congenital factor XIII deficiency**

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Diagnostic difficulties and new therapeutic options in acquired haemophilia A and congenital factor XIII deficiency

Short thesis for the degree of doctor of philosophy (PhD) in clinical medicine.

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The Examination takes place at 11:00 a.m., on 17th November, 2020, in online format.

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The PhD Defense will be held at 2:30 p.m., on 17th November, 2020, in online format.

To participate the online defense, please send your registration email to karanyi.zsolt@med.unideb.hu. Deadline for registration is 11:00 a.m., 16 th November, 2020.

1. Introduction

Acquired haemophilia A (AHA) and congenital factor XIII (FXIII) deficiency are rare bleeding disorders with an estimated prevalence of 1-3 cases/million inhabitants. Even in centres specialized in rare bleeding disorders only a few new cases are encountered annually. Accurate data on the incidence and prevalence are not available due to the tendency for the diseases to be often underdiagnosed and misdiagnosed in real-world clinical practice. In acquired haemophilia A bleeding tendency usually presents in adulthood due to the development of an inhibitory autoantibody against the endogenous coagulation factor VIII (FVIII). The characteristic symptoms are the extensive subcutaneous, mucosal or deep soft tissue haematomas and prolonged postoperative haemorrhages. Bleeding episodes are severe in 30-50% of cases with a potentially life-threatening outcome. In congenital FXIII deficiency delayed umbilical cord bleeding is a typical early presentation. Later intracranial, intramuscular and joint bleeding, impaired wound healing or recurrent miscarriages can occur.

In both diseases the first bleeding episode is often encountered by physicians not experienced in rare bleeding disorders (surgeons, traumatologists, urologists, internists, oncologists etc.). It leads to diagnostic difficulties and extreme periods of delay. Recognizing FXIII deficiency is not a simple clinical task, because the routine haemostasis tests are normal despite the obvious bleeding tendency. In AHA, results of international surveys and our data demonstrate that the time-lag between the onset of bleeds and the accurate diagnosis can be as long as 1.5 months. Regarding the main causes of the significant delay in the diagnosis of AHA, our observation also correlates with the international experience: in non-haematological wards even in severe haemorrhages there is a tendency 1. to neglect the evaluation of routine haemostasis tests or to use only the prothrombin time (PT) and 2. to ignore the prolonged, incorrigible activated partial thromboplastin time (APTT). Thus, there is a general consensus on the importance of early referral of cases with unexplained bleeding symptoms to centres with appropriate laboratory facilities and experience in the diagnosis of rare bleeding disorders.

Beyond the diagnostic process, acute and long-term management of AHA and FXIII deficiency also requires the clinical experience, laboratory and therapeutic equipment of haemostasis centres. Professional care of congenital FXIII deficiency consists of the „on demand” application of a FXIII concentrate guided by regular laboratory control of plasma FXIII (pFXIII) activity and the monthly prophylaxis with a FXIII product to avoid intracranial bleed, if pFXIII activity is <1%. In AHA with high-titer inhibitor bypassing agents and the recombinant porcine FVIII (rpFVIII) concentrate are the adequate therapeutic options for acute bleeds. To eliminate the inhibitor, immunosuppression should be introduced as soon as possible. However, in general wards haemorrhages of unexplained origin are tend to be treated with fresh frozen plasma that is usually effective in FXIII deficiency, but inadequate for patients with acquired inhibitor. Furthermore, patients with AHA are frequently operated to find and stop the source of intractable bleeds. A surgical intervention without appropriate haemostatic cover is a life-threatening process in these cases.

In rare bleeding disorders, only a limited number of well-structured clinical studies are available. In AHA international guidelines are usually based on experts' opinion, metaanalyses, summaries of registry or single-centre data and case reports. There are only a few publications and no clear recommendation on such a clinically important problem as prophylactic factor replacement in acquired haemophilia A. However, the risk of recurrent bleed exists until the acquired inhibitor is detectable. The European database reported a 25% recurrence rate of bleed during a median period of 14 days after the resolution of the first haemorrhagic event. Thus, patients with persisting inhibitor may benefit from prophylaxis with activated prothrombin complex concentrate (APCC). Potential thromboembolic complications and cost are also factors to consider. Recently, Italian authors demonstrated a favourable outcome with short-term, low-dose APCC prophylaxis in small prospective-retrospective, non-randomized studies. The indications of prophylaxis were not clearly defined. No bleeding occurred in the prophylaxis group. In the nonprophylaxis group patients experienced subsequent haemorrhages after the first resolved bleeding event. No thromboembolic complication developed during the 2-3 weeks of APCC prophylaxis.

Evidently, more structured clinical data are available about the care of congenital FXIII deficiency. A strong association has been explored between pFXIII activity and the bleeding tendency. The pharmacokinetic parameters of plasma-derived and recombinant FXIII concentrates are also particularly described. So nowadays, adequate haemostasis can be achieved safely in clinical situations with bleeding risk by the laboratory-controlled administration of FXIII concentrates in centres or in close collaboration with centres specialized in rare bleeding disorders. However, a clinical trial on a novel FXIII concentrate for on-demand therapy is not easy to carry out due to the rarity of the disease and the high rate of prophylactic treatment among patients with adequate diagnosis. A novel recombinant FXIII concentrate (rFXIII) has been licensed for prophylaxis in congenital FXIII-A subunit deficiency. Safety and efficacy of rFXIII in acute bleeding have not been tested. Though, on the basis of the pharmacokinetic profile, rFXIII at prophylactic dose should be suitable for the management of acute bleeding events.

In the thesis I am going to review the results of a nearly 10-year-long clinical research in the field of acquired haemophilia A and congenital FXIII deficiency. At first, I am going to evaluate the length, causes and consequences of the diagnostic delay in acquired haemophilia A in Eastern Hungary. I am also presenting here the clinical practice and outcome of our centre on long-term APCC prophylaxis for patients with acquired inhibitor to FVIII and severe bleeding tendency. Finally, I will demonstrate the efficacy of rFXIII-A₂ concentrate in the therapy of an acute bleeding episode in congenital FXIII-A deficiency through the presentation of a case report and pharmacokinetic parameters. This work is the summary of our recently published data.

2. Review of literature

2.1.Acquired haemophilia A

2.1.1.Clinical characteristics

Acquired haemophilia A (AHA) is a rare but potentially life-threatening bleeding disorder caused by neutralizing autoantibodies (inhibitors) against coagulation factor VIII (FVIII). Around half of the cases are idiopathic, while the other half are associated with malignancies, peripartum period, autoimmune disorders like rheumatoid arthritis or infections.

The estimated incidence of AHA is 1.2-1.48 cases/million inhabitants/year. Patients are often elderly, with a median age at diagnosis of 64-78 years. There is also a small peak of young women presenting with FVIII inhibitors associated with pregnancy or autoimmune disorders. Pediatric cases are exceptional with a reported incidence of 0.045 case/million children/year.

Typically, adult patients without any previous bleeding tendency present with a sudden onset of haemorrhage. The characteristic symptoms are subcutaneous, mucosal, deep soft tissue haematomas (muscle, retroperitoneal, intracranial) and prolonged postoperative haemorrhages. The bleeding phenotype in AHA is variable ranging from lethal bleeds to mild or no bleeds at all. Close observation is warranted even in patients with no or mild bleeds at presentation, because a fatal bleeding event can develop at any time while the inhibitor persists. The laboratory diagnosis is based on the prolonged, incorrigible activated partial thromboplastin time (APTT) with normal prothrombin (PT) and thrombin time (TT), low FVIII activity (<50%) and the quantification of the inhibitor (≥ 0.6 Bethesda Unit, BU) by the Bethesda assay. Due to the complex kinetics of type II inhibitors, Bethesda assay can provide only a rough estimate on inhibitor potency. Therefore, the titre of the acquired inhibitor and the residual FVIII activity do not correlate with the severity of bleeding tendency and fail to predict the response to bypassing agents.

The therapeutic aims in AHA are the control of acute bleeds and the eradication of the inhibitor. Due to the rarity of AHA and the severe clinical condition of patients at presentation, comparative clinical studies are hardly available on the management of haemorrhages and on inhibitor eradication. Published guidelines are often based on experts' opinion, metaanalyses and reports from tertiary centres. Referral of a patient with a newly diagnosed AHA to the regional haemostasis centre is often highly recommended to provide the best possible care in factor support, laboratory background, inhibitor eradication and the collective experience on rare bleeding disorders.

Consensus guidelines suggest that haemostatic treatment should be initiated in clinically relevant bleeding episodes, irrespective of the inhibitor titre or residual FVIII activity. Bypassing agents (BPA) or the recombinant porcine factor VIII (rpFVIII) concentrate are the recommended first-line treatment options of acute bleeds. Though parallel-group, comparative studies are missing, the available results of single agent studies demonstrate no difference in the efficacy between recombinant activated factor VII (rFVIIa, NovoSeven, NovoNordisk), activated prothrombin complex concentrate (APCC, Factor Eight Inhibitor Bypassing Activity, FEIBA, Takeda) and recombinant porcine factor VIII (rpFVIII,

susoctocog alfa, Obizur, Takeda). All three agents can resolve over 80-85% of the haemorrhagic events as first-line choices. The decision between the available agents and the dosage are based on the site and severity of bleeds, patient's age, previous response, factor availability and laboratory facilities. If a BPA or rpFVIII can not achieve the control of haemorrhage in 12-24 hours, therapy should be switched to the alternative agent. However, no validated laboratory test can predict or monitor the haemostatic efficacy of BPAs, so we should rely on the close clinical follow-up to ensure adequate bleed control and to avoid thromboembolic complications. On the other hand, after the initial dose of rpFVIII, plasma FVIII activity is determined every 2-3 hours to guide the subsequent injections (dose and dosing interval). In the European registry thromboembolic complications were reported with similar incidence of 2.9% for rFVIIa and 4.8% for APCC. In the pivotal trial of rpFVIII, no patient developed thromboembolic event.

Inhibitor eradication should be started right after the acquired inhibitor has been detected, because the risk of fatal haemorrhage persists until the autoantibody is present. The eradication approaches are based on immunosuppression due to the autoimmune origin of AHA. The optimal sequence of immunosuppressive protocols is not clear. However, the treating physician should be aware of the patient's general condition and comorbidities before the introduction of an immunocompromising therapy. Some new findings suggest that presenting inhibitor titre and residual FVIII activity have predictive value for the choice of immunosuppressive regimen. In the latest guideline steroid monotherapy (prednisolon of 1 mg/kg/day orally) is recommended as first-line treatment for patients with inhibitor titre of ≤ 20 BU and FVIII activity of $\geq 1\%$, while a combination of steroid and cyclophosphamide (1-2 mg/kg/day orally) or rituximab (375 mg/m²/week intravenously) was suggested if the presenting inhibitor titre is >20 BU and FVIII activity is $<1\%$. The expected rate of remission is around 60-80% with the first-line eradication therapy during a period of 5-8 weeks. In case of a refractory disease or relapse the previously unapplied first-line protocols or other regimens (cyclosporine, azathioprine) can be used. Budapest protocol (methylprednisolon, cyclophosphamide, FVIII concentrate), an anti-plasmacell protocol (vincristine, oradexon, FVIII concentrate) and CydRi (cyclophosphamide, oradexon, FVIII concentrate) are eradication approaches designed and widely used with high remission rates above 80% in Hungarian centres. As a relapse of an inhibitor can occur even after years, long-term follow up and haemostatic assessment before any invasive procedure by a trained haematologist is mandatory for the patients previously treated with AHA.

2.1.2. Diagnostic delay

A patient with acquired haemophilia A requires highly special care in view of acute bleeds and inhibitor eradication. If started in time, bypassing agents can stop over 85% of haemorrhages even the severe or life-threatening events; and proper immunosuppression can eliminate the autoantibody in more than 60% of cases. So early recognition of the acquired inhibitor is of major importance. It is an impedimental circumstance that, patients with first haemorrhages are often admitted to operative departments, internal or pediatric wards where

rare bleeding disorders are not in the frontline of the diagnostic algorithm. So referral of the cases to haematological or haemostaseological departments usually occurs with substantial delay. The importance of the issue is underlined by many publications - including our work - on the length of period elapsed from the onset of bleed till the establishment of the accurate diagnosis: in EACH2 the reported delay was median 3 days (interquartile, IQR 0-12), that of a Spanish centre was median 19 days (IQR 2-180), the Chinese National Registry reported a median elapsed time of 30 days (IQR 15-76), while in Eastern Hungary we found a median 1.5 months delay (3.0 days-9.0 months).

2.1.3. APCC prophylaxis

In the literature there is ample evidence on recurrent bleeds if the inhibitor persists. The European Acquired Haemophilia Registry (EACH2) reported a recurrence rate of 25.3% in a median period of 14 days, the same figures of the Chinese national registry were 66% and a median of 5 days. Mortality rate of recurrent bleed is around 10% according to French results. As no association exists between the haemostatic parameters and bleeding tendency in AHA, the individual probability of bleeding recurrence is unpredictable. Consequently, while the inhibitor is present one should count on the continuous threat for recurrent haemorrhages that can be difficult to control. Thus, prophylaxis with APCC may be beneficial for patients with persisting acquired inhibitor.

In congenital haemophilia with alloantibody inhibitor an international consensus guideline makes a clear suggestion to long-term APCC prophylaxis as secondary prophylaxis for patients suffering from a serious bleeding tendency. The occurrence of a single life-threatening bleed or repeated haemorrhages impairing the quality of life are defined as serious bleeding tendency. APCC prophylaxis can be introduced to patients in whom immune tolerance induction (ITI) has failed, who have been unsuitable for or declined ITI. The recommended starting dose is 50 U/kg APCC intravenously administered three times per week. If the clinical response is suboptimal, the dose of APCC should be increased to 85 U/kg at first, then the frequency can be switched to alternate days. If the dose adjustment of preventive APCC infusions fails to stimulate an optimal clinical response, prophylaxis should be terminated. The assessment of clinical response and inhibitor titre is scheduled for every 12 weeks. The authors emphasized that regular APCC infusions should be dosed with caution when body mass index (BMI) is above 30 kg/m² or the patient has an increased risk of arterial or venous thrombosis.

In acquired haemophilia A, no high level evidence or clear recommendations exist on APCC prophylaxis. In 2001, Grünewald et al reported a case of a 60-year-old male who suffered extensive intramuscular haematomas as presenting symptoms of AHA and received prophylactic APCC (dosage not specified by the author) for 45 days after the resolution of an acute bleed. In 2012, Kang et al published a case report about a 26-year-old woman who was treated with APCC at a dose of 5 U/kg twice a day for 14 extra days after the resolution of a large intramuscular haematoma. For both patients short-term APCC prophylaxis proved to be effective, but the indications for initiation and discontinuation were not particularly described.

In 2015, a small prospective study of 18 patients demonstrated a favourable outcome with short-term, daily-administered APCC infusion. Here the patient selection was arbitrary and finally seven patients received APCC injection daily for 2-3 weeks at a dose reduced by around 75% of the therapeutic dose. Recently, the analysis of the data of FAIR registry (FEIBA in the Acquired Haemophilia Italian Registry) revealed promising results with low-dose, short-term APCC prophylaxis from a prospective-retrospective study. APCC prophylaxis was introduced in 15 patients of 56 at the discretion of the treating physician, but inclusion and withdrawal criteria were not well-defined.

2.2. Congenital FXIII deficiency

2.2.1. Clinical characteristics

Congenital factor XIII (FXIII) deficiency is a rare and severe, autosomal recessive bleeding diathesis that can also be associated with impaired wound healing and recurrent miscarriages. Its estimated prevalence in developed countries is 1 cases/1-3million inhabitants, but a higher prevalence can be expected in societies where consanguinous marriages are usual.

Plasma FXIII (pFXIII) has a tetrameric structure (FXIII-A₂B₂) with a half-life of 8-12 days. Its catalytic A subunit (FXIII-A) is a protransglutaminase, while B subunit (FXIII-B) serves as an inhibitor/protective protein for FXIII-A. Cellular FXIII (cFXIII) exists as FXIII-A₂ homodimer in platelets, monocytes and macrophages. Activated pFXIII-A₂ promotes clot stability by cross-linking fibrin chains and incorporates antifibrinolytic proteins into the clot. In plasma all FXIII-A₂ is complexed with FXIII-B₂, only 1% exists in a non-complexed form. FXIII-B₂ is in excess: approximately 50% circulates in free form.

In FXIII deficiency delayed umbilical cord bleeding is a typical early presentation. Intramuscular haematomas, haemarthroses, subcutaneous and mucosal bleeds also develop spontaneously or after minor trauma. A life-threatening complication is intracranial haemorrhage that occurs far more frequently (30%) in congenital FXIII deficiency than in any other inherited bleeding diathesis.

Routine haemostasis tests are normal in FXIII deficiency. If the suspicion arises, the current guideline recommends a quantitative assay to determine FXIII activity as first-line screening test, then the assessment of FXIII-A₂B₂, FXIII-A₂ and FXIII-B₂ antigen levels for the classification. Genotyping is not mandatory to confirm the diagnosis, but it is important for prenatal diagnosis and is of research interest. Autoantibodies to FXIII can be detected by mixing studies and binding assays.

The therapy of congenital FXIII deficiency is based on factor substitution with the intention of on demand or prophylactic treatment. Sources of FXIII can be fresh frozen plasma, a plasma-derived FXIII concentrate (Fibrogammin®, CSL Behring GmBH) and a novel recombinant FXIII (rFXIII) concentrate (catridecacog, NovoThirteen®, NovoNordisk, Denmark). In clinical situations like pregnancy, planned surgical interventions or unexpected haemorrhages, the close monitoring of pFXIII activity is required parallel with factor replacement. During surgical procedures pFXIII level should be kept above 50% for the operation and above 20% for the wound healing. During early pregnancy trough level of 10-

20% is recommended, but for labour trough level of 30-50% is desired. For patients with pFXIII activity <1%, life-long prophylactic factor supplementation is mandatory to avoid intracranial haemorrhage. The aim of monthly administered FXIII support is to keep pFXIII activity above 5%.

2.2.2.Recombinant FXIII concentrate

Recombinant FXIII concentrate is manufactured in yeast and contains no human or mammalian products, so it can be considered safe for life-long factor replacement in the view of blood-transmitted agents. The product contains the A subunit of the tetramer (rFXIII-A₂) that forms a stable complex (FXIII-rA₂B₂) with the endogenous FXIII-B₂. In phase 1 studies half-life of rFXIII proved to be 6-14 days both in healthy volunteers and in FXIII-A deficient patients, but only 8.9 hours in FXIII-B subunit deficiency. In different studies median dose response was 1.77-2.4% increase of FXIII activity for every unit of rFXIII per kilogram administered. The pharmacokinetic parameters of rFXIII strongly resemble to those of native FXIII that has been extensively used for the prophylactic and on demand therapy with excellent clinical outcomes. Finally, rFXIII-A₂ of 35 U/kg intravenous dose was tested in phase 3 studies. It proved to be efficacious and safe for bleeding prophylaxis of patients with congenital FXIII-A subunit deficiency. The trough level of pFXIII persisted above 10% even on the 28th postinfusion day in >95% of patients. Pharmacokinetic profile supports the hypothesis that rFXIII would also be suitable for on demand therapy. However, rFXIII has not been tested in acute bleeds. Currently it is licensed for monthly prophylaxis in an intravenous dose of 35 U/kg in severe FXIII-A subunit deficiency.

3. Aims

3.1.Acquired haemophilia A

3.1.1.Diagnostic delay

To characterize the diagnostic delay of AHA in the Eastern Hungarian region by analysing the elapsed time, main causes and consequences.

3.1.2.APCC prophylaxis

To summarize the clinical practice and outcome with APCC prophylaxis introduced in our centre for patients with AHA.

3.2.Recombinant FXIII concentrate in a major bleed

To demonstrate the capability of rFXIII-A₂ (dose of 35 U/kg) to provide efficient haemostatic support for the therapy of a major bleed and for a minor surgical procedure in a male with severe, congenital FXIII-A subunit deficiency.

4. Patients and methods

4.1. Acquired haemophilia A

4.1.1. Diagnostic delay

4.1.1.1. Patients

The medical history of 11 patients with AHA was retrospectively analysed. The patients were referred to our centre from different county or smaller local hospitals from Eastern Hungary over the period of 2002-2011. All patients were consulted because of a sudden onset of bleeding symptoms without any clear sources or causes. The final diagnosis of AHA was established in our centre based on the clinical symptoms in cases with previously normal haemostasis, prolonged APTT which could not be corrected in mixing studies, low activity of FVIII and the presence of an inhibitor quantified by the Bethesda assay. The patients' clinical data were obtained from medical charts and laboratory records.

4.1.1.2. Characterization of the diagnostic delay

Diagnostic delay was defined as the period elapsed between the first bleeding episode and the accurate diagnosis. I separately evaluated the time that passed from the start of the bleeding symptoms and the measurement of APTT. The period needed for the correct interpretation of the prolonged APTT was calculated, as well. I also assessed the causes of the delay and summarized the non-invasive and invasive anti-haemorrhagic interventions that were performed before the accurate diagnosis of AHA.

4.1.2. APCC prophylaxis

4.1.2.1. Patients

In our practice APCC prophylaxis has been used in patients with AHA since 2002. Nineteen patients were diagnosed and treated with acquired inhibitor to FVIII in our ward between 2002 and 2016. Eleven of them received APCC prophylaxis, eight patients were treated exclusively on demand.

There was no difference in the clinical care between the prophylaxis and nonprophylaxis groups. Bleeding episodes were managed with either aPCC or rFVIIa. The decision between the two agents and the dosage were dependent on the site and severity of bleeds, patient's age, previous response and factor availability. Inhibitor eradication was performed according to the current guideline. However, eradication protocols including FVIII concentrate were not applied simultaneously with regular APCC injections. Cytotoxic agents and rituximab were administered concomitantly with the preventive APCC infusions.

Patients were under close clinical and laboratory control: history taking and physical examination were performed and complete blood count, routine clotting tests, FVIII activity and inhibitor titre were determined regularly. Clotting tests and FVIII activity were measured by routine reagents on BCS-XP coagulometer (Siemens Healthineers, Erlangen, Germany) and the titre of FVIII inhibitor by the Nijmegen-modified Bethesda assay on the same coagulometer. The visits were scheduled at baseline, then weekly, later every 2 weeks or in case of a new bleeding episode for the nonprophylaxis group. Patients receiving APCC

prophylaxis had weekly follow-up visits. When complete remission was reached, follow-up visits were organized every 2 months for a year, then every 6 months and at any time in case of an unexpected bleed or before an invasive procedure. Complete remission was considered when bleeding tendency stopped, the inhibitor stayed undetectable and FVIII activity normalized, even after the cessation of immunosuppression. Relapse means the reappearance of the inhibitor after a successful eradication attempt. If the autoantibody was successfully eliminated after the first or second relapse, we speak about second or third complete remission. Complete remission was regarded long-term, when it lasted at least for months after the cessation of immunosuppression.

4.1.2.2. Criteria for the initiation of APCC prophylaxis

In our centre, bleeding tendency is the main clinical factor considered by the treating physicians before indicating the preventive use of APCC in AHA. Patients with serious bleeding tendency and detectable inhibitor are candidates for APCC prophylaxis. Bleeding tendency is regarded serious if either a single episode of life-threatening haemorrhage occurs or recurrent, severe bleeding events develop. Bleeding tendency is mild, if no haemorrhage or nonsevere bleeding events occur or if a single episode of severe haemorrhage does not recur. Severity of an individual bleeding event is defined according to the categories used in EACH2. Severe bleeding episode is defined as a life-threatening, limb-threatening or organ-threatening episode or haemorrhage of the central nervous system; a bleed with a haemoglobin level below 80 g/l or a drop of more than 20 g/l; a bleed with red blood cell transfusion requirement of more than 2 units in 24 hours. All other bleeding events are regarded as nonsevere.

4.1.2.3. Dosing schedule of APCC prophylaxis

Prophylactic APCC was administered at a dose of 30-60 U/kg intravenously on two or three nonconsecutive days a week. The preventive dose was the lowest effective therapeutic dose having been used for acute therapy in the individual patient.

4.1.2.4. Termination of APCC prophylaxis

Prophylaxis with APCC is intended to be withdrawn when the inhibitor disappears. In case of a persisting inhibitor APCC prophylaxis is to be terminated when a breakthrough bleed develops or an eradication protocol including FVIII concentrate is to be initiated.

4.1.2.5. Data collection and statistical analysis

I retrospectively analysed the clinical courses of 19 patients treated with AHA over the period of 2002-2016 in our centre. The source of medical information was the MedSolution computer system and the hand-written records (e.g. drug charts, medical records). The indications, dosing, efficacy and safety of APCC prophylaxis were evaluated in details.

Descriptive statistics included case number, mean, median and interquartiles (IQR). For comparing the baseline characteristics of the prophylaxis and nonprophylaxis groups, unpaired *t* test was used for parameters with normal distribution and Wilcoxon rank-sum test

was used for parameters with nonnormal distribution. *P* values equal or below 0.05 were considered statistically significant. Statistical analyses were performed by R Project software version 3.0.2. (R Foundation for Statistical Computing, Vienna, Austria).

The limitation of the study arises from the retrospective way of data collection and the low number of patients, so the presented results have weak statistical power and serve only for informative purposes.

4.1.2.6. Informed consent

Informed consent was obtained from all patients before the initiation of APCC prophylaxis. The institutional Ethics Committee approved the retrospective data collection, the registration number of the approval is 4743-2017.

4.2. Recombinant FXIII concentrate in a major bleed

4.2.1. The patient

A 19-year-old man was referred to our haemostasis laboratory with a severe bleeding phenotype without clear diagnosis. His bleeding tendency manifested in infancy with a prolonged haemorrhage of the umbilical stump. Later he suffered a subdural haematoma, recurrent subcutaneous bleeds, intramuscular haematomas of both thighs and haemarthroses in the knees. Due to the poor condition of his teeth, severe gingivitis developed and caused repeated haemorrhages from the gum. He has had up to five major and two-three minor bleeding events annually. There was no family history of bleeding diathesis. Consanguineous marriages were denied in the family. Despite extensive evaluation for coagulation defects, no definitive bleeding disorder could be confirmed since his childhood. Only a mild form of platelet secretion defect was suspected. The bleeding episodes were treated successfully with fresh frozen plasma at a dose of 4.2-13.3 ml/kg that always stopped the haemorrhage.

In the Clinical Research Centre, University of Debrecen, complex laboratory reevaluation of the case was carried out with a new diagnostic line towards FXIII deficiency due to the impressively characteristic clinical course. And the accurate diagnosis was established after 19 years of uncertainty. The FXIII activity proved to be <1.0% in plasma and <2.0% in platelets. In plasma the FXIII-A₂B₂ and FXIII-A₂ antigens were undetectable. FXIII-A₂ antigen was undetectable in platelets, as well. Plasma level of total FXIII-B antigen was 33.5% of average normal. Neutralizing autoantibody against FXIII was excluded by mixing studies. According to the latest classification, the patient was suffering from severe congenital FXIII-A deficiency, type I.

The diagnosis was confirmed by genetic analysis. The patient proved to be compound heterozygote carrying two different known mutations in the FXIII-A gene. The heterozygous mutation c.1149G>C, p.Arg382Ser is located in exon 9 and causes the instability of FXIII-A. The mutation c.1201C>T, p.Gln400X is also found in heterozygous form in exon 9 and leads to the formation of a premature stop codon. Due to the autosomal recessive trait, available first-degree family members were also screened for the mutation. As

the mother died of breast cancer many years ago, blood samples were collected from his brother and father. No FXIII genetic abnormality was detected in the brother, but his father carried the mutation c.1149 G>C, p.Arg382Ser in heterozygous form.

Considering the established diagnosis of severe FXIII-A deficiency, life-long preventive factor supplementation was recommended with recombinant FXIII (rFXIII) concentrate for the patient due to his young age and negative viral tests toward human immunodeficiency, hepatitis B and C viruses. He was planned to receive rFXIII intravenously at a dose of 35 U/kg (2500 U NovoThirteen®) at four-week intervals.

On the week before the appointment for the first prophylactic rFXIII infusion, the patient was admitted to the clinic due to the swelling of the right thigh accompanied by unbearable pain and restriction in mobility. The symptoms began three days before hospitalization after having had performed hard physical activity. No traumatic event occurred. The pain and swelling were deteriorating despite local ice-compress and rest. Physical examination revealed a huge tough resistance in the upper third part of the right thigh leading to an eight cm surplus in the circumference compared with the proximal left thigh (62 cm vs. 54 cm). Arterial blood supply of the right leg was intact. Hemoglobin level did not fall out of the normal range at admission and over the observation period. Computed tomography (CT) described a huge intramuscular haematoma in the proximal and mediodorsal region of the right thigh with diameters of 7.7, 8.3 and 19.0 cm. Because of limitations in moving of the right lower extremity and a slight pain in the right lumbar region the possibility of retroperitoneal or pelvic haemorrhages arose, but the CT excluded both of them. The haemorrhage was categorized as a major bleed according to the definition of the European Network of Rare Bleeding Disorders due to the accompanying restriction in mobility and the consequent need of hospitalization. We decided to use rFXIII (at a dose approved for prophylaxis) for the treatment of the intramuscular haematoma. After obtaining the patient's informed consent, rFXIII of 35 U/kg was administered intravenously. We continued close clinical follow-up and local therapy with ice-compress and rest.

4.2.2. The assessment of pharmacokinetics and immunogenicity

The activity and antigen levels of plasma FXIII (antigens of FXIII-A₂B₂, FXIII-A₂, free and total FXIII-B₂) were monitored frequently during the first week following the administration of rFXIII. Blood sample was collected for baseline parameters right before rFXIII injection, then in the postinfusion 1st and 6th hours, 3rd and 6th days for follow-up. After the patient's mobility had been restored, FXIII was measured once a week during the next three weeks.

Plasma samples anticoagulated by trisodium citrate were used for the coagulation assays. An ammonia release method with plasma blank subtraction was used for the determination of FXIII activity using REA-Chrom FXIII kit (REANAL, Budapest, Hungary). Highly sensitive enzyme-linked immunosorbent assays (ELISA) developed in our laboratory were applied for quantifying FXIII-A₂B₂, FXIII-A and FXIII-B antigens. Screening for neutralizing anti-FXIII antibody was performed by mixing studies.

5. Results

5.1. Acquired haemophilia A

5.1.1. Demographic and clinical results

In the Thrombosis and Haemostasis Centre 19 patients (14 women, 5 men) were diagnosed and treated with acquired haemophilia A over the period of 2002-2016. Mean age of the whole cohort was 59.6 years (range 28-91) at diagnosis. Eleven patients were older than 60 years, and 13 of 19 patients suffered from at least one cardiovascular comorbidity like atrial fibrillation, advanced atherosclerosis, hypertension and diabetes mellitus. At presentation residual FVIII activity was <1% in each patients, while median inhibitor titre was 20.5 BU (range 1.2-272.0). In 10 of 19 cases, no related condition could be detected. In the remaining patients, malignancy (3 urothelial cancer, 1 prostate cancer) and pregnancy (4 cases) were the most common background disorders. In a 47-year-old woman an autoimmune condition (IgG4-related disease) provoked the development of an inhibitor to FVIII.

Bleeding tendency was characterized as serious for 11 of 19 patients and mild for 8 of 19 patients following the definitions previously described. Bypassing agents (APCC, rFVIIa) were applied for the therapy of acute bleeds according to the dosing instructions of consensus recommendations. APCC successfully stopped bleeds in 80.9% of the treated events, while this rate was 82.0% for rFVIIa. No fatal bleeding or thromboembolic complications occurred.

Inhibitor eradication was started as soon as the diagnosis of AHA was established in 12 of 19 patients. A postoperative condition or a delay in referring the patient to our centre were the causes of postponing immunosuppression in six cases. In the nonprophylaxis group an 84-year-old male could not receive any eradication therapy due to recurrent infections and frail condition. In our centre, steroid with or without cyclophosphamide, rituximab-based regimens and immunosuppressive protocols combined with FVIII concentrate were the most frequently used eradication approaches. The median follow-up period of the 19 patients was 34 months (IQR 15-62). Meanwhile the total rate of complete remission was 83% (15/18) for patients having received immunosuppression in one or more lines, and finally remission always persisted for months or years. During the same period mortality rate proved to be 21%: infection was the cause of death in 3 cases (2 of them related to immunosuppression) and 1 patient died of the underlying malignancy.

5.1.2. Characterization of the diagnostic delay

In the period of 2002-2011 eleven patients (7 women, 4 men) were referred to our centre due to the sudden onset of abnormal bleeding tendency without a clear origin. The referring institutions were county and local hospitals from Eastern Hungary. The bleeding symptoms consisted of subcutaneous, muscular, mucosal, deep soft tissue and prolonged postoperative haemorrhages with variable severity. In the Clinical Research Centre thorough laboratory evaluation proved the presence of acquired inhibitor to FVIII in the background of all cases. As the period elapsed from the first bleed to the accurate diagnosis seemed unreasonably long in our clinical practice, the retrospective analysis of the patients' medical history was carried

out to summarize the time, causes and consequences of real-life diagnostic delay in acquired haemophilia A in our region.

In our cohort, a median period of 1.5 months (range 3.0 days-9.0 months) was required to establish the diagnosis of AHA after the onset of bleeding symptoms. In four cases 4.0-9.0 months were needed to have the adequate diagnosis despite an obvious bleeding tendency. In primary care and also in some secondary care centres the routine coagulation tests were either not checked or only the prothrombin time was measured before invasive procedures or in case of abnormal bleeding symptoms. A median period of 4.3 weeks (range 0.0 day - 6.0 months) passed between the first bleeding events and the first determination of APTT. In four cases no importance was attributed to the prolonged APTT and its significance remained unnoticed. The median period between the measurement of a prolonged APTT and the recognition of acquired haemophilia A was 1.1 weeks (range 0.0 day – 9.0 months).

Before the recognition of AHA invasive procedures were attempted to stop haemorrhages in a few cases. The palliative resection of a urinary bladder tumour failed to cease the bleed due to the lack of accurate haemostatic support. The surgical exploration of an extensive subcutaneous haematoma led to an intractable bleed and hypovolaemic shock. A 54-year-old woman was operated on for ureter tumour with an unexplained prolongation of APTT to 64 sec that was ignored preoperatively. She suffered severe postoperative haemorrhage and a life-threatening hypovolaemic shock developed. Only the prompt diagnosis of acquired haemophilia suggested by the consulting haematologist and thus the immediate use of bypassing agents made the resolution of bleed possible. These patients required more than 20 units of red blood cell transfusion per person to cover the excessive blood loss related to the invasive procedures. Another common practice is the administration of fresh frozen plasma for intractable haemorrhages, but it is inadequate while the inhibitor persists. Our patients received fresh frozen plasma in a range of 0.0-15.0 units before the accurate diagnosis was made.

Clinical data of the patients with a delay below or above a month were analysed separately and compared with each other only for informative purposes. In five cases the diagnostic delay was less than a month: two patients had severe, three patients had nonsevere initial bleeds; the median inhibitor titre was 5.5 BU (3.2-30) at presentation; one patient died of infection related to immunosuppression. In six cases the diagnostic delay exceeded one month: three patients had severe, three patients had nonsevere initial bleeds; the median inhibitor titre was 50.8 BU (1.2-272) at presentation; two patients died of infection, one patient of malignancy. From these data no strict conclusion can be drawn, but in general we can unfold the fact that presenting haemorrhage had not an impact on the interval from first bleed to accurate diagnosis in our cohort.

5.1.3. APCC prophylaxis

5.1.3.1. Demographic and clinical characteristics of the prophylaxis and nonprophylaxis groups

No statistically significant difference was found in age (mean 62.3 vs. 55.9 years in the prophylaxis vs. nonprophylaxis groups, *P* value 0.5173) and inhibitor titre (median 34.0 vs. 6.5 BU in the prophylaxis vs. nonprophylaxis groups, *P* value 0.09008) at presentation between the two groups. Descriptive data on female-male ratio and the rate of cardiovascular comorbidities revealed no remarkable difference between the groups, though statistical analysis could not be performed. Underlying conditions were distributed equally between the groups. It is mentionable that all the malignancy associated cases presented severe bleeding tendency and required APCC prophylaxis. There was no significant difference between the two arms in the time elapsed from the diagnosis to the initiation of eradication therapy (median 1 vs. 1 day in the prophylaxis vs. nonprophylaxis groups, *P* value 0.5367) or to the elimination of the inhibitor (median 26 vs. 20 weeks in the prophylaxis vs. nonprophylaxis groups, *P* value 0.2889). The overall remission rate was 10/11 in the prophylaxis group and 6/8 in the nonprophylaxis group. In the prophylaxis group, 6 out of 11 cases required rescue eradication attempts. In the nonprophylaxis group, 5 out of 7 patients developed remission for first-line immunosuppression. To describe the severity of bleeding tendency in an objective manner, factor consumption for acute therapy was calculated and compared between the two groups. In the prophylaxis arm, consumption of APCC and rFVIIa was summarized for the period before the initiation of APCC prophylaxis. In the nonprophylaxis arm, consumption of APCC and rFVIIa was calculated for the whole clinical course. In the acute setting, APCC consumption was significantly higher for patients who received prophylaxis beyond the resolution of haemorrhage (median 1336 vs. 217 U/kg in the prophylaxis vs. nonprophylaxis groups, *P* value 0.0431). Although the difference in the amount of rFVIIa was not statistically significant between the two groups, numerical data demonstrated clearly a higher consumption of rFVIIa for patients who received APCC prophylaxis after the cessation of acute bleeding (median 902 vs. 312 µg/kg in the prophylaxis vs. nonprophylaxis groups, *P* value 0.3120). Among the nonprophylaxis cases, two patients presented with such mild bleeding phenotype that they did not require any bypassing agent.

5.1.3.2. Clinical outcome in the prophylaxis and nonprophylaxis groups

Eleven patients with severe bleeding tendency received APCC at a dose of 30-60 U/kg intravenously on two or three nonconsecutive days a week. The prophylactic dose was the lowest effective therapeutic dose used for acute therapy in the individual patient. Nine cases of the eleven with severe bleeding tendency did not experience any bleeding, but bruises while on APCC prophylaxis. In seven cases the preventive use of APCC was terminated due to the successful elimination of the acquired inhibitor. In two patients onset of breakthrough haemorrhages was the cause of the withdrawal of prophylaxis. Namely, a 28-year-old female developed nonsevere haematuria refractory to APCC and rFVIIa without any obvious abnormality in the urinary tract. Nonsevere haematuria resolved parallel with the fall of the

inhibitor titre after the start of eradication therapy. A 65-year-old male patient with inoperable urinary bladder tumour presented recurrent, severe haematuria resistant to APCC prophylaxis due to tumour progression. The severe haematuria ceased after performing palliative surgical resection of the tumour under haemostatic cover with APCC. In two patients prophylaxis was stopped when an eradication protocol including FVIII concentrate was introduced. Median duration of prophylaxis with APCC was 16 weeks (IQR 9-34). Two patients demonstrated fast disappearance of the autoantibody and required prophylaxis only for three weeks after having suffered a life-threatening bleed. Nine patients received regular APCC infusions beyond two months and six of them beyond four months. The extra factor consumption related to prophylaxis was calculated a median 1620 U/kg APCC per patient (IQR 1125-4110). No thromboembolic complication, allergic reaction, anamnestic response or other adverse event occurred in the prophylactic period.

In eight of the nineteen cases, bleeding phenotype was regarded mild and did not make it necessary to introduce the regular use of APCC. In the nonprophylaxis group, no severe bleeding complication could be reported while the inhibitor existed, only bruises developed.

5.2. Recombinant FXIII concentrate in a major bleed

5.2.1. Clinical outcome

By the next day of rFXIII infusion (35 U/kg), the pain, swelling and stiffness of the right thigh decreased spectacularly. The improvement of the patient's condition was carrying on through the following days and he was fully mobilized on the fourth postinfusion day. No additional fresh frozen plasma or red blood cell transfusion was required during his treatment. On the sixth postinfusion day he developed a sudden toothache caused by acute pulpitis. As the actual FXIII activity was 32%, the extraction of the painful tooth was performed without any extra factor supplementation. A second dental manipulation was scheduled on the day of the first prophylactic factor infusion and was carried out with FXIII activity of 68%. No bleeding occurred.

Regular prophylaxis began in the next month according to the recommended scheme: 35 U/kg rFXIII administered every four weeks. Currently the patient has been on monthly prophylaxis with rFXIII for six years and during this period no bleeding episode except bruises occurred despite he has been working as a stoneman.

5.2.2. Pharmacokinetic profile during the therapy of a major bleed

Preinfusion pharmacokinetic parameters were in correspondance with the known diagnosis. After injecting a single dose of rFXIII of 35 U/kg, pFXIII activity increased and then persisted on 70% within the first six postinfusion hours. Results on the 3rd, 6th, 13th, 21st, 24th and 28th postdose days demonstrated a gradual decline in pFXIII activity, though it still remained above 10% for three weeks. Trough level of pFXIII was 6% on the 28th day. Similar tendency was found in the levels of FXIII-A₂B₂ and FXIII-A₂. Half-life of rFXIII was calculated to be

around 6 days. Dose-normalized response was assessed to be 2% increase in pFXIII activity for 1 U/kg rFXIII administered.

Interestingly, there was an increase in total FXIII-B₂ antigen over the postinfusion three days. The tendency was then reversed, but the level of total FXIII-B did not fall below the baseline during the four week follow-up period. Simultaneously, there was an apparent decrease in free FXIII-B₂ antigen level from predose to 1 h postdose and then returned to baseline by 24 hours and persisted on this concentration during the next four weeks. These findings suggest that rFXIII-A₂ has regulatory effect on FXIII-B subunit: it can cause the release of FXIII-B to the circulation elevating total FXIII-B level and concurrently can bind to FXIII-B leading to the fall of free FXIII-B in plasma.

Presence of an inhibitor against rFXIII has been strictly monitored. No neutralizing anti-rFXIII-A₂ alloantibody was detected by mixing studies either in the acute phase or during 6 years of prophylaxis.

6. Discussion

6.1. Acquired haemophilia A

6.1.1. Diagnostic delay

Prompt recognition of acquired haemophilia A is critical due to the usually frail condition of the patients at presentation and the urgent need of special care during acute bleeds and the need of inhibitor eradication. The first registry addressing the issue of diagnostic delay was EACH2 reporting a median period of 3 days (IQR 0-12) between the onset of bleed and the establishment of adequate diagnosis of AHA. Fifty-two of 501 patients in the European registry suffered a delay of over a month. In the final analysis of EACH2, delay in diagnosis had no impact on overall survival.

From the everyday practice it was undoubtedly clear for us that quite a long period is passing from the onset of the first haemorrhage to the referral of cases with unexplained bleeding tendency to a specialized department. Therefore a research was carried out to characterize the diagnostic delay of acquired haemophilia A (length, causes, consequences) in Eastern Hungary. The source of data collection was the medical courses of 11 patients with acquired inhibitor who were treated in our regional haemostasis centre between 2002-2011. In our experience, patients with bleeding symptoms were usually admitted to operative and general wards, where the laboratory evaluation of clotting system was often inadequate. The lack of measuring the routine coagulation tests (4 of 11 patients) and the exclusive determination of the PT (2 of 11 patients) before an invasive procedure or in case of bleeding symptoms were common phenomena in the different hospitals. A median period of 4.3 weeks (range 0.0 day-6.0 months) passed between the initial bleeding episode and the first measurement of APTT. The second main problem was the misinterpretation of the prolonged, incorrigible APTT (4 of 11 patients). A median of 1.1 weeks (range 0.0 day-9.0 months) was found to elapse between the first measurement of an abnormal APTT and the recognition of AHA. All the patients debuted with bleeding symptoms. At the onset of the initial bleed, none

of them received anticoagulant or antiplatelet therapy as a confusing factor in the differential diagnostic process. In summary median 1.5 months (3.0 days-9.0 months) was calculated as diagnostic delay in our centre. In four cases the period passing without exact diagnosis was above four months. As a consequence of diagnostic obscurity surgical interventions were employed to cease bleeds in four cases, but it turn to be ineffective in one case and life-threatening in two patients due to the lack of adequate haemostatic cover. Another consequence was the use of large amount of fresh frozen plasma that is an ineffective product for acute bleed in the presence of an acquired inhibitor, also means an unnecessary volume load for patients with cardiovascular comorbidities and carries the risk of transmitting blood-borne agents.

In Spanish and Chinese reports – related later – median periods of 19 days (IQR 2-180) and 30 days (IQR 15-76) were determined as elapsing time related to the diagnostic uncertainty. The Spanish authors also made the detailed assessment of the diagnostic procedure in AHA: clotting times were not measured in 10 of 28 cases despite abnormal bleeding symptoms, coagulation tests were restricted to PT in 4 of 28 cases, prolonged APTT was neglected in 10 of 28 cases. Intramuscular haematomas of the lower extremities were misdiagnosed as deep vein thrombosis in eight patients, three of them received low-molecular weight heparin. Anticoagulation led to the further enlargement of the existing haematomas and in one case compartment syndrome developed. Seventeen patients used anticoagulant or antiplatelet agent at the time of the initial bleed. The Spanish study also emphasized the fact that most of the patients were encountered on operative or general wards with the initiating symptoms.

Neither ours nor the other surveys found association between the length of diagnostic delay and the severity of presenting haemorrhages, residual FVIII activity or inhibitor titre. In international cohorts diagnostic delay had no negative impact on survival data. Due to the low number of patients no statistical analysis could be performed on our data towards factors affecting survival. Mortality rate was 21% (3 patients died of infection and one of malignancy) and no fatal bleeding occurred among our patients.

From the international and Hungarian results the conclusion can be drawn that rare bleeding disorders are not in the frontline of diagnostic algorithm in routine clinical practice of non-haematological departments. However, it would be important to universalize the attitude that a bleeding patient should have complete clotting tests (APTT, TT, PT, fibrinogen) and complete blood count; a patient with an unexplained bleed or abnormal coagulation time without a clear reason should be referred with as little delay as possible to centres where appropriate laboratory diagnostic tests and therapeutic options are available.

6.1.2. APCC prophylaxis

The continuous risk of bleeding complication makes prophylaxis with APCC reasonable in patients with acquired haemophilia A and persisting inhibitor. Today no high level evidence or any guidance exists on the indications, optimal schedule and potential benefit of APCC prophylaxis in AHA. The feasibility of a prospective randomized study is an issue due to the

low prevalence of the disease. There are a lot of clinical factors to consider before the introduction of APCC prophylaxis: regular factor support may prevent bleeds, but also may create a prothrombotic condition in adult patients usually burdened by cardiovascular or thromboembolic risk factors. In our opinion only a selected group of patients with serious bleeding tendency may benefit from the preventive administration of APCC while the inhibitor is present.

In the past decade, our centre gathered experience in the field of prophylaxis with APCC for patients with acquired haemophilia A. Here I presented a retrospective case series of 19 patients with AHA to outline the indications and clinical outcome of preventive APCC administration in our practice. In every case, clinical bleeding tendency guided our decision on the initiation of APCC prophylaxis. For the assessment of individual haemorrhagic tendency we followed the same criteria those were previously defined in congenital haemophilia complicated with an alloantibody. APCC prophylaxis was introduced after the resolution of the first life-threatening bleeding event or in case of recurrent severe haemorrhages. If bleeding tendency was judged serious, APCC prophylaxis was suggested, independently of the inhibitor titre. Patients with mild bleeding tendency were treated on demand. As the candidates for prophylaxis were heavily burdened by thrombotic risk factors, the dosage of regular APCC infusion was minimized and immunosuppression combined with a FVIII concentrate was never coadministered with preventive APCC. The early observations proved that the twice-weekly, low-dose prophylaxis was less effective than the thrice-weekly regimen. So preventive APCC is usually administered three times a week in our centre. Twice-a-week regimen is preferred only in cases of poor venous access, high cardiovascular risk or patient's choice. The preventive dose of APCC was equal with the lowest effective therapeutic dose for the individual patients in the range of 30-60 U/kg. The APCC prophylaxis was intended to be continued until the inhibitor persists. Patients were under close clinical and laboratory control while on prophylaxis. With the statistical comparison of the prophylaxis and nonprophylaxis groups, no significant difference could be found in the view of presenting ages, background disorders or circumstances of inhibitor eradication. Only consumption of bypassing agents in the acute setting proved to be higher in the prophylaxis group, objectively demonstrating the severity of the members' bleeding tendency. In the prophylaxis group no breakthrough bleeding occurred in 9 of 11 cases, meaning that the regular use of APCC could prevent rebleeding in 81.8% of patients with clinically serious bleeding tendency. We experienced no arterial or venous thrombotic complication related to preventive APCC administration, despite its long median duration of 16 weeks (IQR 9-34). Eight patients of the nonprophylaxis group did not present any severe haemorrhage.

The number of available publications is quite low regarding the APCC prophylaxis for patients suffering from acquired haemophilia A. In 2015 a prospective, nonrandomized study of 18 patients was released about short-term APCC prophylaxis started after the resolution of the first bleeding episode. In the study the regular administration of APCC was indicated for seven patients irrespective of the bleeding phenotype. The prophylactic dose was calculated as a dose reduction with 50-75% of the individual therapeutic dose. Prophylaxis

was stopped when inhibitor titre decreased by more than 50% of the baseline level. Substantial decline of the autoantibody was considered as an indicator of good response to immunosuppression and the reduction of bleeding risk. Haemostasis parameters were controlled every five days. No bleeding occurred in the prophylaxis group. In the nonprophylaxis group, six patients of the eleven experienced subsequent bleed. No thromboembolic complication developed during the 2-3 weeks of APCC prophylaxis.

In 2019 the results of a prospective-retrospective study of 12 Italian centres were also published on short-term prophylaxis with APCC. In the prophylaxis group, APCC was continued beyond the first resolved episode in 15 of 56 patients at a lower dosage with a frequency of every 12-72 hours. Since it was a registry, no special protocol or clear indication were provided for the patients' prophylactic management. The length of prophylaxis was 20.5 ± 17.6 days. The criteria of prophylaxis discontinuation were not described, either. Bleeding relapses resulted significantly higher in the patients without preventive treatment with APCC.

In our experience, we found APCC prophylaxis effective and well tolerated in AHA, if clinically indicated. I would like to emphasize the 82% efficacy rate in preventing bleeding events among serious bleeders and the lack of subsequent severe haemorrhages among patients initially judged as mild bleeders. I am aware of the limitations of the positive results and the need for well structured studies to determine the role and place of APCC prophylaxis in the clinical care of acquired haemophilia A.

6.2. Recombinant FXIII concentrate in a major bleed

Here I demonstrated the first promising results on the capability of rFXIII-A₂ concentrate for the therapy of a major bleed in FXIII-A deficiency. In the reported case rFXIII-A₂ of 35 U/kg was administered for the management of an intramuscular haematoma causing immobility of a young man with severe FXIII-A deficiency. The rFXIII also provided adequate haemostatic cover for a dental surgical procedure on the 6th postinfusion day. Beyond close clinical observation, evaluation of pharmacokinetics was carried out. The haemostatic parameters guaranteed the clinical recovery of the patient and correlated with the experts' recommendation on factor levels in major bleeding and surgical intervention in FXIII deficiency. The measured laboratory results corresponded with data previously reported on rFXIII and plasma-derived FXIII products.

A few years later, reported data of MentorTM2 trial offered further confirmation on rFXIII being suitable for the management of bleeds and for providing haemostatic cover for minor surgeries. MentorTM2 is basically the extension trial of the pivotal study on testing the long-term safety and efficacy of monthly prophylaxis with rFXIII in FXIII-A deficient patients, but elective surgeries and breakthrough bleeds were permitted to be supported by rFXIII-A₂. This is the largest clinical trial performed to date in congenital FXIII deficiency. However, sixty patients could be enrolled. Twelve minor (mostly dental) surgical interventions were carried out within 21 days of the last scheduled rFXIII-A₂ dose without the need of additional factor substitution. Only one trauma-induced muscular bleed occurred while

on rFXIII prophylaxis. As the haemorrhage developed 24 days after the last rFXIII-A₂ injection, a single, repeated rFXIII-A₂ dose of 35 U/kg was administered. The haemostatic response was rated excellent.

Currently the available data on the use of rFXIII in acute and surgical setting is restricted to our work and the limited results of MentorTM2 trial. Though the initial data are favourable on rFXIII for on demand therapy, but further clinical evidence is required to find the optimal dosing scheme.

7. Summary

Here I present new clinical aspects of rare coagulopathies in the field of diagnostic difficulties and therapeutic approaches. Diagnostic delay in acquired haemophilia A is a major issue. I evaluated not only the period elapsing from the first bleed to the correct diagnosis, but also the causes and consequences of the delay. I found a period of median 1.5 months from the onset of bleeds to the correct diagnosis. Analysing the main causes of the delay I found that the routine haemostasis laboratory tests had not been checked or only the prothrombin time had been used exclusively to evaluate haemostasis and the prolonged activated partial thromboplastin time had gone unnoticed for a long time despite the obvious bleeding tendency. The period of delay was much longer in the Hungarian cohort, but the main causes were found to be the same in the Hungarian, pan-European and Spanish cohorts. The results underline the importance of early referral of patients with unexplained bleeding symptoms to regional haemostasis centres where the appropriate diagnostic and therapeutic facilities are available for the special management of these cases.

In our experience long-term APCC prophylaxis proved to be effective and safe for patients with acquired haemophilia and severe bleeding tendency until the inhibitor persisted. Considering the high thrombotic risk, the lowest effective therapeutic dose was recommended for prophylaxis on two or three days per week, with close laboratory control (weekly determination of inhibitor level). In two Italian studies short-term APCC prophylaxis was preferred with low-dose factor concentrate administered every 12-72 hours. The indications of prophylaxis were not very clearly demonstrated. The termination of regular factor support was scheduled if the inhibitor fall below the 50% of the baseline level. Results of both the Italian and our studies are promising, but further trials are needed to outline the clear indications and dosing schedules of APCC prophylaxis in acquired haemophilia A.

In the case report from our Centre, recombinant FXIII concentrate in a dose of 35 U/kg is suitable not only for the prophylaxis of bleeds, but also for the treatment of an acute bleeding episode and for haemostatic cover of minor surgical procedures. Beyond the demonstrated case, only a few reports are available on rFXIII as a therapeutic option for acute bleeds or surgery. The positive outcomes of these reports could stimulate the design of further well-structured studies in the field.

8. Major scientific results

1. Here I presented the first detailed assessment of the diagnostic delay in acquired haemophilia A in Hungary. The period of the delay was exceptionally long if compared with international data. The main causes were the frequent ignorance of testing the routine clotting times or the negligence of the unexplainable, prolonged APTT in preoperative situations or in cases of an obvious bleeding events.

2. Here I demonstrated the first clinical experience on long-term APCC prophylaxis for patients with acquired haemophilia A. Uniquely, the serious bleeding tendency was the main selection criteria for the initiation of APCC-prophylaxis that was intended to continue till the inhibitor persisted. The dosing schedule was low-dose APCC on two or three nonconsecutive days a week. In our practice APCC-prophylaxis proved to be safe and effective.

3. Here I presented the first clinical evidence on the efficacy of the recombinant FXIII-A₂ concentrate to achieve haemostasis for the therapy of a major bleed and for a minor surgical procedure in a patient with severe congenital FXIII-A deficiency. The applied dose was the same approved for monthly prophylaxis (35 U/kg).



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