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Mimetics of active factor VIII (FVIIIa) and their impact on laboratory tests of haemostasis

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Summary

The introduction of long-acting, subcutaneously administered mimetics mimicking active factor VIII (FVIIIa) is undoubtedly a breakthrough in the prophylactic treatment of haemophilia A patients. The innovative nature and mode of action of FVIIIa mimetic molecules has significant impact on the current algorithms for laboratory monitoring of haemophilia treatment. Drug level monitoring for dose adjustment is not required; in clinical practice however, laboratory monitoring of haemostasis may be useful, if surgical intervention is required or bleeding occurs despite prophylaxis. In such cases, awareness of the impact of FVIIIa mimetic therapy on laboratory tests of haemostasis is a necessary condition for efficient utilization and interpretation of results.

Keywords: haemophilia A; haemostasis tests; factor VIII; FVIII mimetics; emicizumab; mim8

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Introduction

Haemophilia A (HA) is one of the most common congenital bleeding disorders. It is caused by the absence or impaired synthesis of plasma coagulation factor VIII (FVIII, Factor VIII). It is a genetically determined monogenic disease, inherited in a sex-linked recessive manner, which makes FVIII deficiency and the accompanying hemorrhagic diathesis manifest mainly in men, while women are carriers of a mutated variant of the gene encoding FVIII (F8, Xq28) [1]. The disease manifests as an excessive bleeding tendency and the severity of bleeding depends on the degree of FVIII deficiency. HA is diagnosed by measuring plasma FVIII activity. Depending on the degree of FVIII deficiency, there are 3 forms of HA:

— severe, when FVIII is less than one International Unit (IU)/dl;
— moderate, when FVIII is between 1–5 IU/dl;
— mild, when FVIII is > 5–50 IU/dl [1].

The severe form of Haemophilia A is a life-threatening bleeding disorder (FVIII < 1 IU/dl) which manifests with spontaneous bleeding, primarily into joints and muscles, typically beginning around 2–3 years of age [2]. The mainstay of treatment for severe HA is the use of deficient blood clotting factor concentrates (known as replacement therapy). Both the treatment of active bleeding and long-term prophylaxis of bleeding in this group of patients rely on intravenous injections of FVIII concentrate, the number of which can reach more than 150 per year if performed regularly. Moreover, the most serious complication of substitution

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Table 1. Bispecific antibodies mimicking FVIIIa used in hemophilia A, with or without inhibitors (adapted from [1])

FVIIIa mimetic	Route of administration	Drug dosage	Application/clinical research
Emicizumab	Subcutaneously	1 × per week, 1 × every 2 weeks or 1 × every 4 weeks	FDA and EMA approved; Adults, adolescents, and children with severe or moderate haemophilia A, with or without inhibitors
Mim8	Subcutaneously	1 × per week or 1 × per month	Phase III clinical trial: FRONTIER 4/5

FDA — Food and Drug Administration, EMA — European Medicines Agency

therapy (affecting about 20–40% of patients with severe and 3–13% with non-severe HA) is the occurrence of alloantibodies that neutralize FVIII activity (the so-called FVIII inhibitor), as a result of which prophylaxis and inhibition of bleeding in this group of patients do not lend themselves to FVIII substitution therapy [3–5]. In addition, the burden on the patient of having to administer FVIII concentrate 3–4 times a week can lead to variable clinician adherence and poorer treatment outcomes [6]. It should also be added that, as a consequence, suboptimal HA treatment can lead to an increased number of complications, such as recurrent joint bleeding, hemophilic arthropathy and reduced quality of life [6].

For the above reasons, the last few years have witnessed significant advances in innovative treatments for hemophilia, among which the development and introduction of molecules that mimic active FVIII, the so-called FVIIIa mimetics (FVIIIa mimetics), into the prophylactic treatment of HA is groundbreaking [1, 7] (Table 1). The development of such novel monoclonal antibodies has revolutionized the treatment of HA, expanding the therapeutic options for this hemorrhagic diathesis with so-called non-replacement therapy [7]. These drugs are characterized primarily by their long duration of action, lack of neutralization by the FVIII inhibitor and subcutaneous route of administration, which significantly improves the quality of life of HA patients and the effectiveness of prophylactic treatment [7].

The first FVIIIa mimetic introduced for the prophylactic treatment of bleeding in HA is emicizumab (Hemlibra®, Roche Switzerland) [3, 6]. This drug is a humanized, bispecific, monoclonal immunoglobulin G4 (IgG4) that, to restore hemostasis, replaces FVIIIa in the tenase complex by binding human coagulation factors IXa and X [8–11]. Emicizumab is an antibody that has no structural similarity to FVIII, making it non-neutralizable by alloantibodies (inhibitor) to FVIII [9, 12]. In November 2017, the U.S. Food and Drug Administration (FDA, Food and Drug Administration), followed by the European Medicines Agency (EMA, European Medicines Agency) in February 2018, approved the use of emicizumab in patients with HA complicated by FVIII inhibitor, and in subsequent years its use for prophylaxis in patients with severe and moderate HA without inhibitor, as it has been shown to significantly reduce bleeding rates in this group of patients as well [1, 3, 10, 13, 14]. Emicizumab was very quickly recognized as a drug signaling a new era of HA treatment, as in all phase III clinical trials, emicizumab prophylaxis led to a spectacular reduction in annualized bleeding rate (ABR, annualized bleeding rate), with a median ABR of 2.6 for all dosing regimens in patients in HAVEN trials 1 through 4, and more than 80% of participants had no bleeding after week 24 of therapy [11]. Emicizumab has a prolonged half-life in the bloodstream (4–5 weeks), which allows the drug to be dosed once every one, two or four weeks, compared to conventional replacement therapy [3, 8]. A second invaluable benefit of using Hemlibra® in HA patients is, unlike the use of standard FVIII concentrates, the subcutaneous route of administration and the associated increase in comfort and quality of life for this group of patients. For the above reasons, but primarily because of its effectiveness in inhibiting bleeding, the use of emicizumab is growing rapidly worldwide. The drug is currently approved for clinical use in many countries, with the understanding that in Poland, as of 06/01/2024. it can only be available for patients with severe HA complicated by an inhibitor and in exceptional cases in patients with severe hemophilia A without an inhibitor.

The search for an ideal HA treatment and the successful development of FVIIIa mimetic therapy using emicizumab has driven the development of further next-generation bispecific antibodies based on this concept of action and designed for subcutaneous prophylactic HA treatment with and

without inhibitors. In 2021, literature reported the development by Novo Nordisk of a molecule called denecimig (Mim8), which is a human next-generation bispecific antibody that, like emicizumab, mimics the action of FVIIIa by forming a complex with FX and FIXa on the phospholipid surface of activated platelets to accelerate FX activation [4, 7, 11, 15, 16]. Data from in vitro studies using human blood, as well as animal models of HA, indicate that Mim8 increases thrombin generation approximately 15-fold compared to the emicizumab analog [6, 15–17]. What differentiates Mim8 from emicizumab is the construction of a monovalent anti-FIX arm that more potently stimulates the proteolytic activity of FIX to activate FX [6, 15]. The drug is intended for subcutaneous prophylactic treatment of patients with HA with or without inhibitors, and its efficacy, safety, pharmacokinetics and pharmacodynamics are currently being evaluated in clinical trials. Current data from clinical trials showed that the half-life (t1/2) of Mim8 was approximately 30 days, confirming the possibility of dosing the drug weekly or monthly [1, 6].

Other next-generation bispecific FVIII mimetics in development include NXT007 (Chugai Pharmaceutical Co, Roche) and BS-027125 (Bioverativ, Sanofi), currently in preclinical evaluation [1, 3, 7, 11, 18, 19].

The so far published clinical data indicate that the treatment regimen with FVIIIa mimetics ensures the achievement of a stable and lasting response, making dose adjustment unnecessary. Adhering to the prescribed regimen ensures the attainment of the desired drug concentration and its persistence in the circulation, even for several months after the last dose injection [14]. Clinical situations in which experts currently agree that laboratory monitoring and haemostasis control can be useful include: surgeries and surgical procedures, breakthrough bleeding despite prophylaxis, bleeding following injuries, and verifying the effectiveness of ongoing treatment [10, 20, 21].

It should be emphasized that the innovative nature and mode of action of FVIIIa mimetic molecules bring significant implications for existing algorithms in the laboratory monitoring of haemophilia treatment. The half-life of a mimetic molecule, such as emicizumab, is approximately 4 to 5 weeks, which means that this therapy can impact laboratory test results for up to 6 months after administration [12, 22]. Awareness of the impact of the use of the aforementioned mimetic therapy on laboratory tests of hemostasis is a prerequisite for their efficient use and proper interpretation of their results. This article discusses the effect of FVIIIa mimetic drugs on individual hemostasis tests, with a particular focus on the monitoring of combined FVIII replacement therapy with FVIII concentrates.

Effects of FVIIIa mimetics on baseline and specialized tests of hemostasis

As mentioned, the mimetics in question are molecules that mimic activated FVIII and, unlike the FVIII protein, do not require thrombin-mediated feedback activation [12, 23, 24]. This feature of mimetic molecules significantly affects especially the routine test of hemostasis, which is the activated partial thromboplastin time (aPTT) and all tests derived from it (Table 2) [8, 12, 14–16, 20]. aPTT is indeed excessively shortened to varying degrees depending on the reagent used, usually below the normal reference range [8, 13, 14, 25]. Importantly, because FVIIIa mimetics are long-acting drugs, this shortening occurs after the first dose of the drug, even at very low concentrations, and may persist for several months after treatment is discontinued [12, 14, 22]. In addition, it is worth noting that the measurement of aPTT in a patient treated with FVIIIa mimetics is not suitable for monitoring this therapy, but its prolongation may indicate the patient's noncompliance with the drug regimen or the development of so-called Anti-Drug Antibody (ADA) [9, 13].

In the case of other routine haemostasis tests, a clinically insignificant impact of emicizumab and mim8 on prothrombin time (PT) measurements has been observed, depending on the reagent used. Associated determinations of factors II, V, VII, and X also remain without significant changes [8, 9, 14, 22, 27, 28]. Similarly, measurement of thrombin time (TT) and determination of fibrinogen concentration using the Clauss method remain without clinically significant impact from the action of the discussed drug [8, 27].

Specialized tests based on aPTT measurement, used, among other things, to examine the activity of factors in the intrinsic pathway of the coagulation system (VIII, IX, XI, and XII), show falsely elevated values [14, 20, 25]. This effect also applies to the measurement of protein C and S activity with the coagulation assay and the activated protein C resistance test (APCR), when performed based on aPTT measurement [8, 14, 22, 28, 29]. In all HA patients treated with emicizumab who require heparin therapy, a chromogenic anti-Xa test should be used to measure drug levels [8, 14].

Table 2. Effect of FVIIIa mimetics on the results of selected coagulation tests (adapted from [8, 9, 22, 26, 27])

Type of haemostasis laboratory test.	False result	Reliable result	Comment
Routine tests			
aPTT	√		False-shortened result (even < 20 s) Effect already seen at minimal drug concentration (> 5 μg/ml)
aPTT correction test	√		False negative result
PT		√	The result is normal or slightly prolonged
Fibrinogen (Clauss method)		√	Not affected
TT		√	Not affected
Specialized tests			
Determination of FVIII activity by a one-stage coagulation assay	√		False high result. Value from several hundred to even several thousand IU/dl [%]
Determination of FIX, FXI and FXII activity using a one-step coagulation test	√		False high result. Measurement based on aPTT
Determination of the activity of FII, FV, FVII, and FX by a one-stage coagulation assay		√	Not affected Measurement based on PT
Determination of inhibitor titer against human FVIII by the Bethesda assay with FVIII measurement using a coagulation method	√		False negative result Indicates lack of inhibitor
Determination of FVIII activity by a chromogenic assay using a reagent of human origin (FX, FIX)	√		False high result It serves as a "surrogate" for the drug's activity in a patient without FVIII treatment
Determination of FVIII activity by a chromogenic assay using a reagent of bovine origin (FX)		√	Not affected
Determination of inhibitor titer against human FVIII by the Bethesda assay with FVIII measurement using a chromogenic method with a reagent of bovine origin (FX)		√	Not affected
Determination using an immunological test (Elisa)		√	Not affected
Determination of anti-Xa using a chromogenic assay		√	Not affected

aPTT — activated partial thromboplastin time; PT — prothrombin time; TT — thrombin time; FVIII — factor VIII; FX — factor X

In addition, tests to detect FVIII inhibitors, i.e., a screening test based on aPTT measurement of a mixture of test plasma and normal plasma (the so-called "correction test") and the Bethesda test with measurement of FVIII with a one-step coagulation test show false-negative results [8, 14].

The impact of FVIIIa mimetics on the Measurement of FVIII in Plasma

Based on global experiences with the use of emicizumab in HA patients , it is known that long--term bleeding prophylaxis with this group of drugs

significantly reduces the risk of bleeding events. However, when bleeds identified as breakthrough bleeds, or those resulting from trauma, or the need for invasive surgery, or the deployment of ITI, need to be treated, the concomitant use of an FVIIIa mimetic and FVIII concentrate may be necessary, in which case the appropriate choice of tests to reliably monitor such therapy is important [8, 10, 14, 21]. It should be noted, however, that in a joined analysis of the HAVEN 1–4 study, breakthrough bleeds, both spontaneous and post-traumatic, were very rare during emicizumab prophylaxis [3].

In the diagnosis and monitoring of haemophilia A treatment, two main strategies are used to measure the activity of FVIII in plasma: the one-stage coagulation test (FVIII:C, FVIII coagulation activity) and the chromogenic test [4, 21, 25]. Currently, in the treatment of haemophilia A, there is a fairly wide range of plasma-derived or recombinant (rFVIII, recombinant Factor VIII) Factor VIII concentrates with standard (SHL, standard half-life) or extended half-life (EHL) [12, 30]. Discrepancies in the results of FVIII activity measured by the coagulation and chromogenic tests have been described in the literature for many years [4, 12, 25]. Initially, these discrepancies pertained to the classification of non-severe haemophilia A, and later emerged as a consequence of introducing structurally modified FVIII molecules (rFVIII, EHL) to the market, as well as gene therapy for haemophilia A [4, 14].

The impact of FVIIIa mimetics on FVIII coagulation tests

The most common test used worldwide for determining FVIII activity is the one-stage coagulation test based on aPTT measurement. This test is used to assess the coagulation activity of FVIII:C in the diagnosis, classification, and monitoring of replacement therapy for Haemophilia A. Due to the significant shortening of aPTT caused by Hemlibra® and Mim8, the FVIII:C activity in this test is falsely elevated [14]. The results of conducted studies suggest that the degree of overestimation of the FVIII:C result is to some extent dependent on the applied aPTT reagents, which differ from each other in the content of activator or phospholipids [4, 12]. For this reason, in patients using FVIIIa mimetics, the FVIII:C test is unreliable, and its performance is not recommended [8, 22, 25, 31].

It is worth noting that a certain modification of the FVIII:C test has found application in laboratory practice, involving the use of a specific calibrator for the drug. This has been validated for the emicizumab molecule, enabling quantitative determination of the drug concentration in the patient's plasma (see below) [12, 14].

Impact of FVIIIa mimetics on FVIII chromogenic assays

To monitor FVIII activity in HA patients, a chromogenic test is also used, but this test is usually less available and its use is limited to hemophilia treatment centers [14]. Studies conducted to date have shown that due to the false overestimation of FVIII in the coagulation test, the chromogenic test should be used to monitor FVIII replacement therapy in patients using FVIIIa mimetics (emicizumab, Mim8) [9, 10, 14, 15, 25]. Commercially available chromogenic assays differ in the source of the coagulation response activating agents: FIXa and FX, which can be of human (h, human) or bovine (b, bovine) origin [4]. Since FVIIIa mimetics bind only human FIXa and FX, chromogenic assays containing bovine-derived FIXa and FX do not present interference and aPTT shortening, as this bispecific molecule does not interact with them [4, 12, 21]. That's why, according to current recommendations, patients treated with emicizumab should have infused FVIII activity using a chromogenic test with reagents of bovine origin [e.g., Coamatic FVIII test (Chromogenix), Coatest SP (Chromogenix), or FVIII Chromogenic FVIII (Siemens)], Electrachrome FVIII (IL Werfen), TriniCHROM FVIII:C (Stago) [4, 8, 9, 14, 22, 25]. When using hybrid laboratory kits containing bFX and hFIX [e.g., Technochrome FVIII:C (Technoclone), Rossix FVIII (Rossix), BIOPHEN FVIII:C (Hyphen-Biomed)], initial validation and local verification of the reliability of FVIII assays is recommended [4, 8, 9, 15, 25].

It is important to note that chromogenic tests containing hFIXa and hFX are sensitive to the presence of FVIIIa mimetics in a concentration-dependent manner. However, they are not specific solely to the medication, as they also measure the presence of endogenous or infused FVIII in the tested plasma [10, 12, 14, 32].

Effect of FVIIIa mimetics on the determination of FVIII inhibitor (anti-hFVIII)

In any patient with HA complicated by an inhibitor, regular monitoring of FVIII inhibitor titer (concentration) is recommended as part of routine care [14]. This requirement also applies to the group of patients treated with prophylactic FVIIIa mimetics with a positive history of the inhibitor.

Determination of inhibitor titers (antibodies) to human FVIII (anti-hFVIII) in the laboratory is most often performed, as recommended, using the Bethesda test with Nijmegen modification (NBA, Nijmegen Bethesda Assay) [4, 25]. This test is a multi-step test that, among other things, includes measurement of residual FVIII activity performed routinely by a single-step coagulation method [8, 14]. Because it is dependent on the measurement of aPTT, the result of the FVIII inhibitor titer in this case is also unreliable, as it indicates the false absence or significantly reduced content of FVIII [12, 14, 20]. The initial laboratory procedure, in the form of heating the test plasma sample at 56°C before performing the Bethesda test, does not eliminate drug interference [4, 8, 12, 14]. Therefore, in order to determine the FVIII inhibitor titer in a patient receiving an FVIIIa mimetic, chromogenic measurement using bovine reagents should be used to assess residual FVIII activity in the Bethesda test [8–10, 25, 31]. According to expert recommendations, it is believed that appropriate tests for monitoring FVIII inhibitor should be available in every HA center, including a modification with chromogenic measurement of FVIII with bovine FXa [22, 25]. Importantly, in practice, to ensure consistency in FVIII inhibitor diagnosis, it is recommended that FVIII inhibitor titers be determined prior to initiation of therapy with Hemilibra® using the same chromogenic assay that will be used during emicizumab therapy [8, 9, 26].

Measurement of drug concentration in plasma

As mentioned above, routine determination of FVIIIa mimetic concentration for the purpose of dose adjustment is not considered necessary, but this test can be very useful in some situations (eg. when anti-drug antibodies are suspected) [3, 8, 9]. Moreover, currently experts recommend that tests that also monitor emicizumab concentration should be available in hemophilia treatment centers [14, 22, 25]. In addition, German recommendations published in 2020 specified the frequency of monitoring emicizumab concentrations 1 week after the last saturating dose, then every 3 months for a year, and successively every 6 to 12 months or in the absence of efficacy [22, 31]. Currently, most data are available in the literature for emicizumab, but it can be assumed that the principles of measuring the concentration of each FVIII mimetic will be performed in an analogous manner.

For the determination of FVIII mimetics, a modified assay based on the measurement of FVIII activity by single-step coagulation in diluted patient plasma against a drug-specific calibrator is most commonly used (mainly for emicizumab) [8, 9, 12, 14, 25, 31]. At the moment, a calibrator for emicizumab is commercially available, manufactured in a kit with assay controls (R2 Diagnostics, Enzyme Research Laboratories, https://r2diagnostics.com/), which contains 100 μg/mL of emicizumab and can be used on various analyzer platforms [8, 12, 13]. Studies conducted to date have used a portion of the drug, with an appropriately adjusted concentration, as reference material when determining Mim8 concentration [15]. When measuring emicizumab concentrations, a higher sample dilution was used compared to the one-step FVIII assay (pre-dilution of the sample in the analyzer 1:8), which allows measuring the range of emicizumab (10–100 μg) in the plasma sample [4, 12]. Emicizumab concentrations obtained with the modified one-step FVIII assay have been shown to correlate significantly with concentrations determined with the enzyme-linked immunosorbent assay (ELISA) previously used in HAVEN clinical trials. In addition, verification of the method with locally used reagents is recommended in each laboratory; studies have shown satisfactory reproducibility of the test despite the use of different measurement systems (APTT reagents, FVIII-deficient plasma and analyzers) [8, 13]. For the determination of drug concentration, it is also possible to use the chromogenic method to measure FVIII with reference to the calibrator described above [8, 12, 22, 24]. When interpreting the result of emicizumab concentration measurements, it should be taken into account that the possible concomitant use of FVIII concentrate and/or inhibitor bypass preparations may overestimate the drug concentration [20]. When it becomes necessary to determine the concentration of emicizumab in plasma in the presence of FVIII, thermal inactivation of the plasma sample may be considered [4].

Currently, for patients with suspected anti-emicizumab neutralizing antibodies, the World Federation of Hemophilia (WFH) recommends measuring emicizumab levels using a modified one-step assay that includes the aforementioned additional step of pre-diluting the test plasma and calibrating the assay using specific emicizumab calibrators [25]. Validated assays that directly measure the presence of anti-drug antibodies may also be used for this purpose, if available.

Table 3. Summary of the application of selected haemostasis tests in the presence of FVIIIa mimetic (modified from [8, 9, 12, 14, 25])

Purpose of the study	Laboratory test
Presence of the drug	Chromogenic test for measurement of FVIII with factors of human origin (hFIXa and hFX)
	Measurement of drug concentration using a modified coagulation test for FVIII calibrated against a specific calibrator
FVIII activity after infusion	Chromogenic test for measuring FVIII with bovine FXa (bFX) and bovine/human FIXa (bFIXa/hFIXa)
Inhibitor against FVIII	Bethesda assay with chromogenic measurement of FVIII using bovine FXa (bFX) and bovine/human FIXa (bFIXa/hFIXa)
Drug concentration	Modified coagulation assay for measuring FVIII calibrated against a specific calibrator
	Infusion of FVIII concentrate increases the result
ADA	No direct test
	Prolongation of aPTT may indicate the presence of ADA (Anti-Drug Antibody)
	Modified coagulation assay for measuring the drug concentration of FVIII

h — human; b — bovine, FIXa — activated factor IX; FX — factor X

Measurement of anti-drug antibodies (anti-emicizumab)

It is worth noting that with any drug therapy, there is a small risk of developing anti-drug antibodies (ADA). The suspicion of ADA formation arises when a patient's rate of breakthrough bleeding increases and aPTT prolongs. The use of emicizumab was associated with the development of ADA in 5.1% of patients (34/668) who participated in HAVEN clinical trials 1–4 [3]. In most of these patients, no significant change in plasma emicizumab levels or increase in bleeding incidence was observed, and antibodies neutralizing the drug were detected in < 1% of cases [3, 8]. Moreover, the appearance of ADA does not affect the efficacy of FVIII replacement therapy or inhibitor bypass therapy [3]. Since no commercially available laboratory kits for direct detection of ADA have been developed to date, determination of the drug concentration in the patient's blood is recommended when antibodies to emicizumab are suspected [8, 9, 14, 22].

A summary of the use of the laboratory tests discussed above for monitoring FVIIIa mimetics is summarized in Table 3.

Measurement of thrombin generation

To date, the role of global tests assessing hemostasis, including measurement of thrombin generation, in monitoring patients using FVIIIa mimetics has not been established [8, 9]. Despite the fact that the thrombin generation assay (TGA, thrombin generation assay) is not a routinely used test of hemostasis in clinical practice, mainly due to difficulties in interpretation, low reproducibility and low availability, its usefulness with regard to assessing the hemostatic potential of FVIIIa mimetics, especially emicizumab, has been reported more than once in the literature. Attempts to use this assay to assess thrombin generation in patient-derived or in vitro-prepared samples in a model with combined treatment of FVIII, rVIIa and aPCC appear to be particularly interesting [3, 24, 33]. The main idea of these studies is to analyze in vitro the effect of BPA therapy (by-passing agents therapy) in a patient treated with FVIIIa mimetics, prior to their administration to the patient. There are also attempts to use TGA for perioperative bleeding control to minimize the potential risk of thrombotic events [20].

Summary

The introduction of non-substitutive therapy with long-acting, subcutaneous FVIIIa mimetics into the treatment of hemophilia A represents a significant advancement in the treatment of this group of patients. The use of these modern molecules in HA patients and their novel mode of action require ongoing education of medical personnel, including laboratory personnel, for awareness of the impact of new-generation drugs on laboratory tests of hemostasis is a key component of the diagnostic process. The availability of appropriate laboratory tests and the continuous improvement of knowledge regarding new-generation drugs is

particularly important in hemophilia centers, as in the future mimetic therapy is likely to become the dominant paradigm in HA treatment. Monitoring these novel HA treatment options may therefore require ongoing adaptation of appropriate tests to provide clinicians with reliable results. In addition, it is not unlikely that FVIIIa mimetics will also find application in the prophylactic treatment of bleeding in von Willebrand's disease and acquired hemophilia A (an indication for emicizumab in clinical trials) [3, 7].

Conflict of interest

EO — participated in clinical trials and received remuneration for lectures given by following companies: Baxalta, Baxter, Shire, Takeda, Novo Nordisk, Roche, SOBI, Siemens, Werfen

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