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Approximation of emicizumab plasma levels in emergency situations. A practical approach

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Abstract

Introduction: A dedicated emicizumab assay based on the modified one-stage factor VIII (FVIII) assay (mOSA) is mainly available in haemophilia treatment centres (HTC). A method to estimate emicizumab plasma levels based on a widely available assay would be desirable, especially for emergency situations.

Aim: A method for emicizumab plasma level approximation (ELA) using a routine FVIII activity measurement with standard one-stage assay (sOSA) was developed and evaluated.

Method: Within this pilot study, 59 samples from patients with severe haemophilia A with (n = 8) and without (n = 8) inhibitors under emicizumab treatment were analysed using sOSA following a manual 1:8 sample pre-test dilution with saline. The sOSA was determined in two different laboratories, using two different analyser platforms each.

Results: The results demonstrated an excellent correlation of approximated emicizumab plasma levels (ELA) with the emicizumab plasma concentration determined with mOSA ($r > .9$; $p < .05$). The ELA showed a sensitivity of 93.3% and a specificity of 89.6% to predict a pre-defined cut-off-value of ≤30 µg/ml for the discrimination between subtherapeutic and therapeutic emicizumab plasma levels.

Conclusion: Approximation of emicizumab levels by standard one-stage FVIII assay discriminates between subtherapeutic and therapeutic emicizumab levels and might facilitate clinical decision-making in emergency situations, such as bleeding, trauma or urgent surgery in case that dedicated emicizumab assays are not available.

KEYWORDS

bispecific antibody, blood coagulation tests, emergency, emicizumab, Haemophilia A, one-stage assay, urgent surgery

1 | INTRODUCTION

Emicizumab provides prophylaxis against spontaneous bleeds in patients with severe haemophilia A (HA) independently of anti-factor VIII (FVIII) inhibitor status.[1,2] Emicizumab mimics haemostatic activity of FVIIIa in the intrinsic tenase complex, but is not neutralised by anti-FVIII antibodies.[3] The functional and structural characteristics of emicizumab lead to strong interferences with coagulation monitoring as these tests are optimised for evaluation of plasma FVIII activity.[4,5] In the presence of emicizumab, all coagulation assays based on an intrinsic pathway activation step and chromogenic FVIII assays based on human substrates will give misleading results. Subtherapeutic emicizumab plasma levels of

2.5–10 μg/ml normalise the aPTT for most of the commercially available aPTT reagents. Therefore, normalised aPTT results do not indicate emicizumab levels within its therapeutic range. In samples containing emicizumab, the aPTT-based standard FVIII one-stage assay (sOSA) reports FVIII levels that are well above the reference range, even at subtherapeutic concentrations of the drug.[4–6] Chromogenic FVIII assays using human FIXa and FX (CSA_h) substrates provide a concentration-dependent effect of emicizumab on FVIII activity readout throughout the clinically applied dose range for emicizumab.[7] A dedicated assay was introduced for the quantification of emicizumab levels, which is based on sOSA and uses calibrators and controls containing emicizumab. This so-called modified one-stage assay (mOSA) is considered as the generally accepted standard method for determining emicizumab plasma levels.[7] While emicizumab has predictable efficacy based on body weight adjusted dosage regimen and does not require routine monitoring, the opportunity to determine emicizumab concentration is desirable in various situations. These include trauma, urgent surgery, bleeding events and situations of unclear patient compliance. As mentioned, two specialised methods are available for the detection of emicizumab levels. However, these methods are not widely available in centres other than haemophilia treatment centres (HTCs). In emergency situations, acutely ill patients are mostly allocated to the nearest appropriate hospital, which often will not have an attached HTC.

One can expect that in such centres currently three options exist for the management of emicizumab treated patients after trauma or breakthrough bleeds: a) management of patients without control of emicizumab level by any laboratory guidance: in this case, after treatment of acute bleeding situations with factor concentrates, possibly unnecessary up-titration of emicizumab might be performed, b) performance of aPTT and / or FVIII activity measurement using the sOSA levels, which can provide misleading results in respect to the emicizumab concentration in the patient or c) performance of specialised emicizumab assays in haemophilia laboratories, which can be logistically challenging, expensive (when eg samples are transported to specialised centres over longer distances) and in particular provide results with a large delay.

We present an alternative procedure for an approximation of emicizumab activity based on the widely available sOSA methodology.

2 | MATERIALS AND METHODS

2.1 | Subjects

This laboratory pilot study was conducted in accordance with the recommendations of the University's Ethical Committee and with the Declaration of Helsinki. Following the provision of written informed consent to use anonymised left-over samples of citrate platelet-poor plasmas from routine coagulation analysis, 16 subjects ≥18 years of age with severe HA with (n = 8) and without (n = 8) inhibitory antibodies were enrolled. We obtained 59 samples covering different time points during the loading and maintenance dose of emicizumab treatment, with (n = 35) and without (n = 24) anti-FVIII antibodies. None of the subjects were under concomitant use of FVIII- or rFVIIa concentrate at the time of sample acquisition. Samples were collected in 3.2% trisodium citrate blood collection tubes. The blood was centrifuged at 2500 g/15°C for 15 minutes and was stored at minus 80°C until batch analysis.

2.2 | Assays

In our study, we applied two commercially available assays: the mOSA as the standard assay for determination of emicizumab plasma levels [μg/ml] and the standard one-stage FVIII activity assay [%] for the approximation of emicizumab plasma levels (Figure 1). The assay methodologies are described below.

2.3 | Emicizumab plasma level measurement with the modified one-stage assay (mOSA)

Emicizumab plasma levels were measured for all patient samples with the mOSA following the manufacturer's protocol for the Werfen analyser (ACL TOP 600 CTS, Instrumentation Laboratory Company) and reagents (SynthASil, SS; HemosIL).[7,8] The assay was calibrated against emicizumab using commercially available and certified emicizumab calibrator and controls (r^2 Diagnostics, South Bend, Indiana, United States calibrator and controls). The results were reported as μg/ml emicizumab plasma concentration. According to the manufacturer's validated protocol the mOSA test is performed with a higher automated sample dilution (1:80; final dilution 1:320) compared to the standard one-stage FVIII assay (eg sOSA, ACL TOP/SS; 1:10; final dilution 1:60). This higher sample dilution allows for the determination of emicizumab in a broad, clinically relevant measuring range (between 10 and 100 μg/ml). The mOSA is considered currently as an accepted standard for emicizumab plasma concentration measurement.[9]

2.4 | Standard one-stage FVIII assay (sOSA)

Standard one-stage FVIII assays were performed based on the manufacturers' protocols on the ACL TOP and Siemens BCS analysers (Siemens Healthcare Diagnostics Inc.) with respective reagents and FVIII deficient plasmas (aPTT reagents: SynthASil, SS; HemosIL and Pathromtin SL, PSL). The sOSAs are fully automated and in routine use, final dilutions of the patients' plasma samples are 1:60 for sOSA with SS reagent and 1:40 for sOSA with PSL reagent. Results were quantified by reference to plasma calibrators, either Siemens Standard Human Plasma (#10446238) or Werfen HemosIl (#0020003700) calibrator as appropriate.

2.5 | Standard one-stage assay for approximation of emicizumab plasma levels (ELA)

Adamkewicz et al[7] demonstrated that 1 μg/ml emicizumab spiked into FVIII deficient plasma showed 8%–12% surrogate FVIII activity in the

Coagulation laboratory	Coagulation laboratory	Haemophilia treatment centre
Routine assay	Absence of mOSA#	Routine assay
sOSA	1:8 pre-diluted (saline) patient's plasma **sOSA**	**mOSA**
Calibrated against normal plasma	Calibrated against normal plasma	Calibrated against emicizumab calibrators
FVIII activity [%]	ELA [%] Emicizumab plasma level approximation	Emicizumab plasma level [µg/ml]

FIGURE 1 In Germany, for instance, approximately 90 laboratories offer sOSA. Most of these laboratories are associated or cooperate with hospitals. There are far fewer HTCs with specialised coagulation laboratories, providing the full laboratory diagnostic set for patients treated with emicizumab. Figure 1 gives an overview of the different assays, calibrators, and their reported test results. The emicizumab plasma level approximation with sOSA (ELA) might be useful in emergency situations when dedicated emicizumab assays are not available. Abbreviations: ELA [%] emicizumab plasma level approximation (=1:8 pre-diluted (saline) plasma samples analysed with sOSA)), HTC haemophilia treatment centre, mOSA modified one-stage assay, sOSA standard FVIII one-stage assay. # in the absence of emicizumab calibrators

sOSA. Therefore, we hypothesised that an approximation of emicizumab plasma levels (ELA) could be possible with a comparable assessment as describe for the mOSA; but without a calibration against emicizumab.[7] In preliminary experiments, we observed that 1:8 diluted plasma samples provided a FVIII activity, which resembled the emicizumab level in µg/ml of the undiluted sample. Following these considerations, the samples were 1:8 manually pre-diluted with unbuffered saline and then analysed using standard one-stage FVIII activity assays (sOSAs) as described above. The reported results of the sOSAs would be (1) strictly speaking, 1:8 diluted FVIII activities [%] but (2) since the plasma sample does not contain FVIII, (3) it seemed to be more correct to choose an arbitrary name "ELA [%]".

To show feasibility and reproducibility, 10 out of 59 samples were measured in two different laboratories using the same reagents and analyser systems. Patient samples were shipped on dried ice.

2.6 | Clinical interpretation of emicizumab plasma levels (mOSA)

In the clinical study programme for emicizumab, the investigated dosage regimes resulted in maintenance plasma levels ranging between ~30 and 80 µg/ml plasma concentration in the.[2,7,10,11] Therefore, the emicizumab plasma levels and the ELAs will be stratified as subtherapeutic when ≤30 µg/ml and as therapeutic when >30 µg/ml.

2.7 | Quality control of mOSA and sOSA

For this pilot study, all results were measured with certified and standardised assays and analyser systems. The laboratories participate in internal and external quality control programmes. Intra- and inter-day percent coefficient of variance (CV) for sOSAs, and the mOSA are below 10%.

2.8 | Statistical analysis

Statistical analysis was performed using GraphPad software and MS Excel (Microsoft Corporation). Descriptive statistics were performed and presented as median and interquartile range (IQR). Where applicable, normality testing was carried out using the Kolmogorow-Smirnow goodness of fit test. Normal distributed data were analysed using students t-test for unpaired data with different variances (analysis between inhibitor and non-inhibitor patients). A p-value of <.05 was considered statistically significant. Pearson's correlation coefficient (r) was calculated to show the strength of the linear relationship between the ELA and the corresponding emicizumab plasma levels for each analysed batch and aPTT reagent. For agreement analysis of the mOSA and the ELA, the Cohens coefficient, (κ) has been used.[11] No further formal hypothesis testing has been performed in this pilot study.

3 | RESULTS

3.1 | Emicizumab plasma levels results with mOSA

The results cover the whole range of measurement (min. 0|max. 94 µg/ml). The median is at 27 µg/ml (IQR 38; Q_1 5|Q_3 53 µg/ml) meaning that 50% of data points approximately encompass subtherapeutic and 50% therapeutic emicizumab levels.

3.2 | Emicizumab level approximation (ELA)—comparison to mOSA

The results of sOSA measurement after 1:8 unbuffered saline pre-dilution of the plasma samples were expressed as emicizumab plasma level approximation (ELA [%]). The ELA results obtained by sOSA-SS and sOSA-PSL ranged between 0 and 103% (median 32%; IQR 44; Q_1 17|Q_3 61) and 0%–71% (median 22%; IQR 32; Q_1 10|Q_3 42), respectively. A strong and significant linear association was shown with the Pearson's correlation coefficient for each aPTT reagent (SynthASil®, Pathromtin®) and batch (Figure 2A). No significant difference in the ELA results has been observed between the inhibitor and non-inhibitor group. We observed an up to 1.25-fold variability of ELA results between aPTT reagents and after batch change of the SynthASil-reagent an up to 2-fold variability. It was hypothesised that the approximate value could be of use for decision-making at a categorical level, for example subtherapeutic or therapeutic emicizumab level. Therefore, data were stratified depending on the value to the subtherapeutic or therapeutic range for further analysis. The sensitivity and specificity calculation for the cut-off point of 30 µg/ml (95% CI 25.4|34.5 µg/ml) was 93.3% and 89.6%, respectively. For agreement analysis of the ELA and the mOSA, the Cohens coefficient was calculated and demonstrated a substantial agreement of the results (Cohen coefficient κ of 0.8) (Table 1). 10 samples were analysed in two different laboratories with the same analyser platforms and reagents. The results confirmed that ELAs were able to discriminate between subtherapeutic and therapeutic emicizumab plasma levels (Figure 2B).

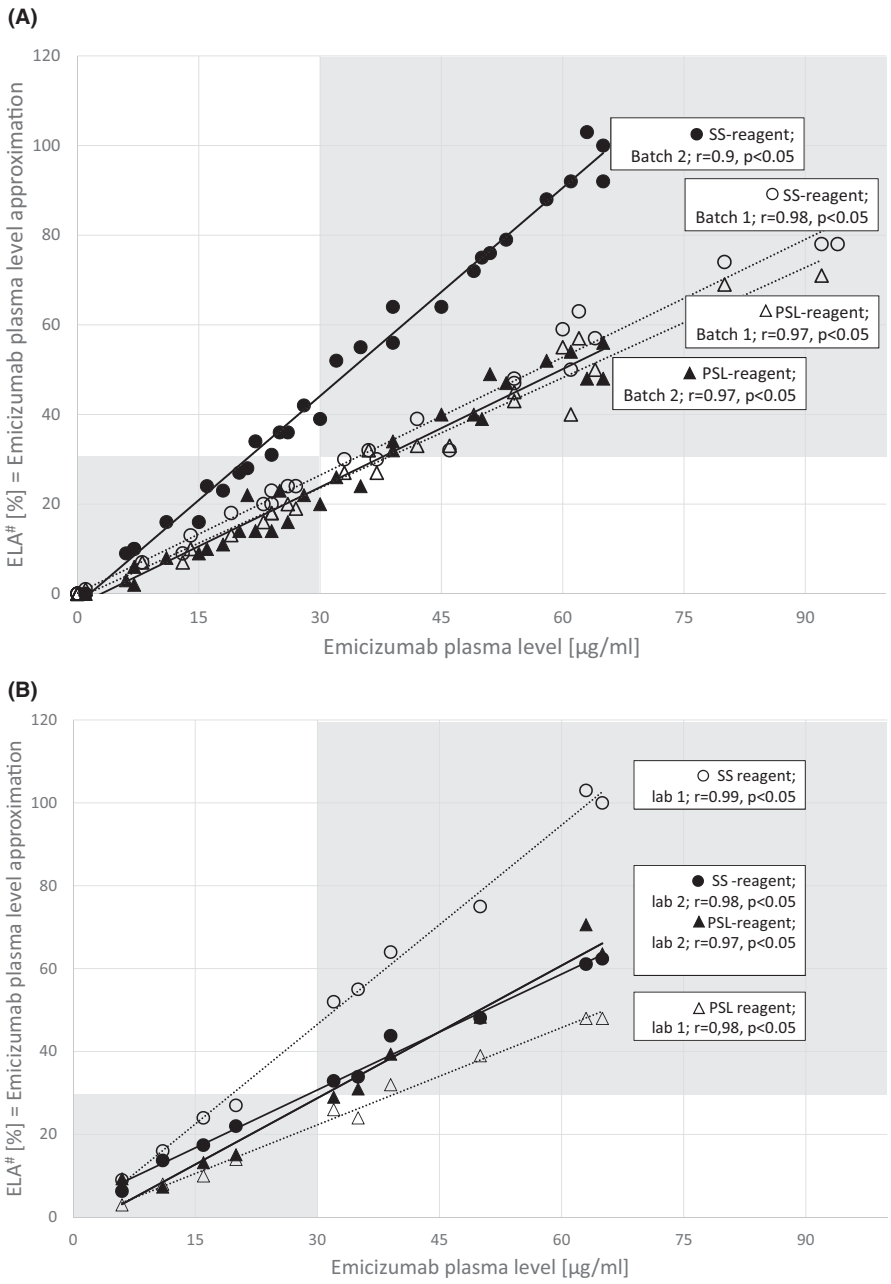

FIGURE 2 (A) Figure 2a shows emicizumab plasma levels [µg/ml] versus ELA [%] = emicizumab level approximation for two different aPTT reagents: SS reagent (circles) and PSL reagent (triangles), measured in two batches (batch 1 open circle/triangle, batch 2 closed circle/triangle). After lot-change for SS reagent and FVIII deficient plasma, we observed systematically lower results for batch 2. For the PSL reagent, the results of batch 1 and 2 show minor shifts between batches. Grey areas indicate (l) subtherapeutic (r) therapeutic emicizumab plasma concentrations. Abbreviations: ELA, emicizumab plasma level approximation; PSL, Pathromtin®; SS, SynthASil®; #ELA results obtained with sOSA using manually 1:8 pre-diluted (saline) patient plasma sample. (B) ELAs (n = 10; inhibitor sample n = 7, non-inhibitor samples n = 3) determined in two different laboratories with the same coagulation analysers and reagents showed comparable correlations with mOSA emicizumab plasma levels and were able to differentiate between sub- and therapeutic emicizumab plasma levels. Grey areas indicate (l) subtherapeutic (r) therapeutic emicizumab plasma concentrations. Abbreviations: ELA, emicizumab plasma level approximation; PSL, Pathromtin®; SS, SynthASil®; #ELA results obtained with sOSA using 1:8 manually pre-diluted (saline) patient plasma sample

4 | DISCUSSION

Emicizumab as a subcutaneous haemophilia A (HA) medication with a long half-life and predictable pharmacokinetics, is applied in body weight adjusted dosing and no routine monitoring is required.[1,2,12,13] A specialised laboratory assay for determination of the emicizumab concentration is commercially available and is widely used in haemophilia treatment centres (HTCs). Emicizumab leads to increased quality of life and mobility of HA patients, and therefore, patients can be expected to expose themselves to more risky situations and to reside in larger distance from their haemophilia centres. In emergency situations, like trauma, breakthrough bleeds or in case of emergency surgery most patients will be admitted in non-specialised hospitals for medical treatment. In this setting, a specialised assay for emicizumab monitoring will often not be available. However, for the clinical decision-making the information whether the emicizumab level is therapeutic or subtherapeutic might still be beneficial.[14,15] Clinical studies have shown that most patients under emicizumab maintenance therapy have emicizumab plasma levels of 30–80 µg/ml.[1,2,12,13] Within this concentration range, no difference of efficacy of the treatment was observed. This means that while the determination of a subtherapeutic or treatment level of emicizumab can have therapeutic consequences, the exact quantification of the plasma concentration of emicizumab will mostly not be required in this emergency setting.

Here, we demonstrated a method for emicizumab plasma level approximation using the widely available standard one-stage FVIII activity assay methodology. The emicizumab plasma levels (ELAs) were approximated by diluting patients' plasma samples 1:8 in unbuffered saline and then determining FVIII levels using standard methodologies. As no FVIII was present in the samples, the results were expressed as the ELA.

Our study demonstrated an excellent correlation of ELA with the emicizumab plasma concentration. Using a cut-off value of ≤30 µg/ml subtherapeutic from therapeutic levels were discriminated with a sensitivity and specificity of 93.3 and 89.6%, respectively. The agreement between ELA and emicizumab concentration resulted in a Cohen coefficient κ of 0.8; demonstrating a substantial agreement of results. Stronger variations of ELA were seen at emicizumab levels above 30 µg/ml, which are however of little clinical relevance.

The ELA is not a substitute to the modified one-stage assay (mOSA) or the chromogenic FVIII determination using human substrates (CSA_h). Therefore, some limitations should be noted: Experimental data using two different aPTT reagents, have been presented which indicate the utility of such an assay. Now clinical experiences with this strategy are required to fully evaluate the benefits and limitations of this strategy. In addition, further studies are desirable to confirm performance of this assay when different aPTT reagents and batches are applied. It must be ––expected that a high FVIII activity in the patient sample, will lead to false-high ELA level. However, this is also true for the mOSA which—as the ELA—is also based on the one-stage FVIII assay.[16,17] Obviously, the CSA_h, using human substrates, is strongly influenced by the presence of FVIII in the sample. This limitation of the ELA should therefore be taken into account, when emicizumab treated patients received FVIII concentrates in addition, for example in the case of bleeding complications or surgery. One strategy would be to determine the ELA before the FVIII is administered. Due to its long half-life, the emicizumab level should then be stable during the clinical course of the patient. If the level of the FVIII given shall be quantified, this could be performed using a chromogenic FVIII assay using bovine compounds (in case such assay is available in the institution).

In addition, as shown in our investigations, there can be variations of the ELA based on the used reagents and instrumentation applied for the sOSA. We observed an up to 1.25-fold variability of ELA results between aPTT reagents and after batch change of the SynthASil-reagent an up to 2-fold variability. However, for the discrimination between subtherapeutic and therapeutic concentrations these variabilities had little effect. Therefore, despite the presented assay and batch influence

TABLE 1 The agreement between ELA and emicizumab concentration resulted in a Cohen coefficient κ of 0.8; demonstrating a substantial agreement of results

		ELA# [%] Emicizumab plasma level approximation		Total	Relative frequency
		0-≤30	>30	Σ	[%]
mOSA Emicizumab plasma level intervals [µg/ml]	0-≤30	**56**	6	62	52,5%
	>30	6	**50**	56	47,5%
Total	Σ	62	56	118	
Relative frequency	[%]	52,5%	47,5%		

Observed agreements; p_0 [%]	**89,8%**
Agreements expected by chance; p_e [%]	**50,1%**
Cohens κ	**0,80**

#ELA results obtained using sOSA using 1:8 manually pre-diluted (saline) patient plasma sample.

the ELA concept would still provide valuable orientation in the absence of dedicated emicizumab assays. Still, further evaluations would be desirable in subsequent studies to determine FVIII assays more or less suitable for the ELA determination. Adamkewicz et al. reported a quite uniform dose-response of emicizumab using 13 different aPTT reagents. [7] Therefore, it can be expected that the ELA methodology should allow to be adapted to a large number of aPTT reagents in the market.

Drugs or diseases which can interfere with the sOSA, such as direct oral anticoagulants and lupus anticoagulants, can also be expected to influence ELA and the mOSA, however potentially to a lesser extend due to the high sample dilution applied in these two methodologies.

Our pilot study presents a practical approach for the approximation of emicizumab plasma levels with a reasonable accuracy. In emergency situations, the ELA might beneficially contribute to the following aspects of clinical decision-making, when specific assays for emicizumab are not available:

The aPTT is oversensitive to emicizumab and normalises even at very low emicizumab levels (2,5 µg/ml).[7,18,19] Thus, the aPTT can be in its reference range up to six months after the last emicizumab injection and is therefore an inadequate indicator for its haemostatic activity or the patient's therapy compliance. In contrast, ELA could help identify missing patient compliance, the development of an anti-drug antibody and a subtherapeutic emicizumab dose due to haemodilution following trauma-induced bleeding. The consequence of the detection of a subtherapeutic ELA in an emicizumab patient may include education of the patient, with the investigation of a potential missing patient compliance, subsequent analyses in respect to a potential inhibitor formation to emicizumab or simply the application of an additional emicizumab dose.

From a laboratory perspective the presented methodology is simple enough to be reliably communicated to non-specialised laboratory staff with a high likelihood of success. In this respect, we avoided variations making the ELA more complicated or expensive, such as diluting the patient sample in FVIII deficient plasma as this could interfere with the intended application of the ELA method in emergency situations. However, one should be aware of potential risks such as the reporting of a false FVIII activity in the patients' medical file. This risk is not unique to the ELA method, because also aPTT, thrombelastography or FVIII results in the normal range could be misinterpreted by the treating physicians.

5 | CONCLUSION

The approximation of emicizumab plasma level (ELA) is easy to perform using a standard one-stage FVIII activity assay (sOSA) without substance-specific calibration, following manual pre-dilution of the plasma sample with saline. We demonstrated that the ELA allows for the discrimination between subtherapeutic and therapeutic emicizumab levels. Eventually, in emergency situations when a dedicated emicizumab assay is not available, the ELA can give valuable information on the patient's haemostatic situation and support clinical decision-making.

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IP and MS designed the research study. IP, GC, CP and AG performed the research and analysed the data. IP wrote the manuscript. MS, CP, AG and SG reviewed the manuscript. The research was conducted without external funding.

CONFLICT OF INTERESTS

IP has received honoraria or grants from BMS, Bayer Healthcare, LFB, Novo Nordisk, Roche, Shire, and Sobi for participating in advisory boards, speaker bureaus, and/or research and reports fees from Sobi, LFB, Roche, Shire, and grants from Novo Nordisk, outside the submitted work. GC reports no conflicts of interest. CP has received speaker honoraria from BMS, Pfizer, Roche, Shire and CSL Behring. CP served as medical advisor for CSL Behring, Bayer Healthcare, Roche, Chugai, Shire, Novo Nordisk and Pfizer during the last three years, and received research grants from Bayer Healthcare, Shire and LeoPhrama outside the submitted work. AG has received honoraria from Siemens, Stago, Bayer, Pfizer, Shire/Takeda and Novo Nordisk. AS has received research grants from Sobi, Novo Nordisk, Bayer Healthcare, Takeda, Diagnostica Stago and Pfizer outside the submitted work. SG declares no conflicts of interest. MS has received grants from Shire and Pfizer and personal fees from Roche, Sobi, LFB, Bayer, Shire, Pfizer, Uniqure, Novo Nordisk and CSL Behring and reports grants from Shire, Pfizer, personal fees from Roche, Sobi, LFB, Bayer, Shire, Pfizer, Uniqure, Novo Nordisk, CSL Behring, outside the submitted work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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