Received: 11 December 2018 | Accepted: 14 December 2018

DOI: 10.1002/rth2.12185

COMMENTARY

rpth research & practice in thrombosis & haemostasis

Will the 'true' factor level make itself known? Measuring factor therapy for treatment of hemophilia in the era of enhanced half-life products

Peter V. Jenkins PhD[1,2]

[1]Department of Haematology, University Hospital of Wales, Cardiff, Wales, UK

[2]Department of Medicine, Cardiff University, Cardiff, Wales, UK

This is a commentary on *Persson et al* [2018]: https://doi.org/10.1002/rth2.12167

Correspondence

Peter V. Jenkins, Haemostasis & Thrombosis, Department of Haematology, University Hospital of Wales, and Department of Medicine, Cardiff University, Heath Park, Cardiff, Wales, UK.

Email: vince.jenkins@wales.nhs.uk

Factor (F)VIII N8-GP, turoctocog alfa pegol, is a 40-kDa glycopegylated version of the B domain truncated recombinant FVIII Novoeight (N8; Novo Nordisk). Variability in the predicted and measured factor level of N8-GP between one-stage clotting assay (OSA) and chromogenic factor VIII assay, and between various OSA depending on activated partial thromboplastin time (aPTT) reagents, notably those with silica-based activators has been observed.[1,2] In *Research and Practice in Thrombosis and Haemostasis*, Persson and colleagues investigated the mechanisms underlying these differences.[3] By measuring FXIa generated on contact activation, measuring activation of N8-GP by thrombin and measuring FXa generation in the presence of various OSA aPTT reagents, the authors showed that the variable results are, at least in part, the result of the differing times of contact activation within each assay. Longer incubation times for contact activation in the OSA resulted in relatively higher levels of FXIa accumulation. This, together with an overall slower rate of activation of N8-GP by endogenous thrombin formed in the OSA, resulted in underestimation of the N8-GP level. Interestingly, shortening the contact activation time diminished the underestimation in reagents associated with a discrepancy, whereas prolonging the time resulted in underestimation in OSA previously not associated with a discrepancy. The underestimation was not the result of silica based activators per se, but a variability in assay conditions. The study highlights the problems involved in ensuring accurate measurement of enhanced half-life (EHL) product treatment where even modest differences in assays reaction conditions can give rise to apparently discrepant assay results.

EHL replacement factor concentrates have increased the available treatment options for hemophilia A and hemophilia B. This new generation of concentrates promises effective treatment by increasing the treatment half-life while conserving the coagulant ability of FVIII or FIX. Prolonging the period between treatment infusions reduces the burden of treatment and will improve the quality of life for many patients. However, due to the relative novelty of the treatments, close monitoring, including laboratory monitoring, of EHL treatment is recommended for each patient.[4] This may appear to be relatively straightforward, but it is increasingly apparent that EHL products may not behave in factor assays in an identical manner to the corresponding native plasma factor.

Discrepancies between assays for recombinant factor products has been previously recognized, however, EHL factor concentrates are specifically designed to behave both differently and identically to the corresponding parent plasma factor. EHL factors are modified by attachment of moieties to the protein by chemical or recombinant means, to alter the life cycle of the protein. The modification either shields the protein from clearance receptors (PEGylation) or induces alternative clearance and recycling mechanisms (immunoglobulin FC fusion and albumin fusion). However, once activated, FVIIIa cofactor or FIXa enzyme supports coagulation near identically to the corresponding activated plasma factor. Given the nature of the designed modifications, it is perhaps then less surprising that the modified factors may behave differently to native plasma derived factor VIII or IX in one-stage and/or chromogenic factor assays.

In Europe, the chromogenic assay is required by the European Medicines Agency to assign FVIII concentrate potency, whereas FIX potency is assigned using by OSA, both using calibrants metrologically traceable to WHO plasma-derived concentrate standards. However, most clinical laboratories use the clotting OSA assay in both diagnosis of hemophilia and monitoring of treatment with factor concentrate, as it is relatively simple, cost-effective, and easy to automate.

The FVIII chromogenic assay is solely a measure of the cofactor ability of activated FVIII. The pro-cofactor is activated using excess exogenous thrombin; and in the presence of added FIXa, phospholipid converts substrate FX to FXa, this in turn is detected by conversion of a specific chromogenic substrate. The FVIII OSA, however, reflects both the activation of FVIII by endogenous thrombin generated produced within the reaction and the cofactor ability of the activated FVIII, as measured by a coagulation endpoint. Due to the different nature of the assays, modifications that modestly affect thrombin cleavage and activation can be predicted to give rise to a discrepancy between recovered OSA and chromogenic assay values. This has been demonstrated for a single-chain FVIII variant modified with a FC fusion molecule, where the OSA gave activity values 40% of that obtained by chromogenic assay. This was shown to be due to delayed thrombin activation of the single-chain variant and release from von Willebrand Factor as compared to two-chain FVIII.[5] A similar molecule, sequence modified single-chain FVIII EHL concentrate Afstyla (CSL Behring, King of Prussia, PA, USA) but with no added modification has similarly reduced FVIII levels by all OSA at approximately 55%-60% of the chromogenic assay, however, the underlying mechanism causing the difference in assays for this molecule is, as yet, undescribed.[6]

Discrepancies between predicted and recovered levels for certain EHL concentrates are seen even within the OSA. Although the principle of the OSA is the same irrespective of reagent and platform, assay reagents and calibrants vary between different manufacturers and these differences can give rise to differing performance in measuring EHL concentrate level. Contact activators used in different aPTT reagents include kaolin, micronized silica, and ellagic acid. The source and proportions of phospholipids also vary between aPTT reagents, from synthesized mixtures to plant-derived and also animal tissue sources. The modifications associated with certain EHL products affect the performance of particular OSAs depending on the aPTT reagents. For example, the pegylated FVIII product BAY 94-9027 (Bayer Healthcare, Berkeley, CA, USA) is underestimated by certain silica based OSA. The 60-kDa PEG moiety prolongs silica-based clotting time but is not affected by ellagic acid–based aPTT reagents.[7] In contrast, certain silica-based FIX OSA greatly overestimate the FIX level of the N-glycopegylated FIX product, N9-GP. Rosen and colleagues elegantly showed that this was due to N9-GP adsorption to the silica particles and subsequent enhanced activation of N9-GP, even in the absence of Ca^{2+} ions. This enhanced activation resulted in a shortening of clotting times and gross overestimation of N9-GP levels.[8] To further complicate matters, the effects are not seen in all silica-based aPTT reagents. Indeed, in this study of N8-GP Persson and colleagues show that although N8-GP has a similar 40-kDa covalently linked glycopegylated moiety as N9-GP, there was no additional adsorption of N8-GP to silica particles compared to the unmodified FVIII product, and it was solely the length of contact activation together with the level of thrombin cleavage that affected the relative FVIII recovery levels.[3] Furthermore, OSA discrepancies are not limited to pegylated EHL products. Studies using a variety of OSA reagents showed that when measuring the recombinant FIX Fc fusion protein Alprolix (Bioverativ Therapeutics Inc, Waltham, MA, USA), kaolin based activators underestimated recovered levels by ~50%.[9]

Clearly, the varying performance of certain assays with particular EHL concentrates presents challenges for clinical teams and laboratories in the monitoring of individual patient therapy. Ongoing developments in hemophilia treatment such as factor mimetics and gene therapy will also present further challenges; however, a number of valuable steps and safeguards can be taken in order to minimize the possible snags. Good communication between clinicians and laboratory is essential to ensure that appropriate assays are used for treatment monitoring. Clinicians prescribing EHL treatment should be aware of the assays and reagents used in their laboratory for monitoring patient treatment, and their suitability for measurement of the prescribed products. Similarly, laboratory scientists and testing laboratories performing factor assays should be aware of the behavior of their assay reagents with specific EHL products. This requires both an awareness of published clinical studies and product characteristic information, and local verification of the suitability of their chosen assays for use with specific EHL products. Importantly, the laboratory should be informed of the individual patient's specific concentrate treatment when factor levels for monitoring treatment are required.

Safeguards need not be limited to the clinical and laboratory team. Patient awareness of their treatment can also help ensure appropriate monitoring, especially when being performed by other centers or laboratories to their usual treatment center.

The aim of measuring treatment levels is to monitor the behavior of the prescribed EHL dose in a patient and ensure appropriate treatment. The prescribed dose is based on, and calculated using, the labelled potency. The “true” value of an EHL product therefore is best estimated by the same method that was used to assign potency. The exact reagents and conditions used by manufacturers in potency assignment are rarely detailed. As described above, FVIII product potency in Europe is assigned using a chromogenic assay, though this is not necessarily the situation in the USA. Although the preferred method for monitoring FVIII treatment is the OSA, knowledge of the method used to assign potency, including details of reagents would be valuable. This is especially the case for FIX EHL products where both potency and monitoring are generally, though not exclusively, performed by OSA.

Assay discrepancies present problems for clinical teams and laboratories, nonetheless, by further investigating underlying assays differences in EHL concentrate measurement, Persson and colleagues not only give insight to those mechanisms, but also further illuminate the biological science of the assays themselves.

RELATIONSHIP DISCLOSURE

Dr. Jenkins reports personal fees as an advisory board member from Novo Nordisk and Roche outside the submitted work.

ORCID

Peter V. Jenkins https://orcid.org/0000-0001-5301-464X

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