Article:
Bowyer, A., Shepherd, M.F., Kitchen, S. et al. (2019) Measurement of extended half-life recombinant factor IX products in clinical practice. International Journal of Laboratory Hematology, 41 (2). e46-e49. ISSN: 1751-5521

https://doi.org/10.1111/ijlh.12953

Measurement of extended half-life recombinant factor IX products in clinical practice.

Bowyer AE, Shepherd MF, Kitchen S, Maclean RM, Makris M

Introduction/aim

The introduction of extended half-life (EHL) recombinant Factor IX (rFIX) products for the treatment of haemophilia B (HB) has improved patient quality of life [1] however the complexities of accurately measuring product recovery has created uncertainty within haemostasis laboratories.

Two EHL products; FIX albumin fusion protein, albutrepenonacog alfa, (rFIX FP, Idelvion, CSL Behring, King of Prussia, USA) and FIX Fc fusion, eftrenonacog alfa, (rFIX Fc, Alprolix, Bioverativ, Waltham, USA), have been in routine use in the UK since 2016. Following on from field study data involving artificially spiked plasma, both manufacturers advise against the use of certain APTT reagents in the laboratory measurement of their product by the one-stage APTT based assay (OSA) [2, 3]. The recommended potency assignment for replacement FIX products is by one-stage clotting assay (OSA) [4]. The potency for Alprolix was assigned by OSA using Actin APTT reagent [5]. To the best of our knowledge, the APTT reagent used for assignment of Idelvion potency is not in the public domain, however Pathromtin SL has been used in previous clinical trials of Idelvion [2]. Reports in the literature assessed EHL product recovery in assays of FIX activity (FIX:C) using *in vitro* spiked plasma and again, to the authors' knowledge, no reports of product recovery in patients have been published. Findings based on spiked samples should be confirmed with post infusion samples from patients in case of *in vivo* modifications or interactions that could affect assay results. The aim of this study was to assess recovery of FIX by OSA and chromogenic substrate assay (CSA) in HB patients treated with Idelvion or Alprolix.

Methods

FIX:C was measured by OSA and CSA in eighteen samples from three HB patients on prophylactic treatment with Idelvion and twenty-two samples from five HB patients

on prophylactic treatment with Alprolix. OSA were performed with Actin, Actin FS (AFS), Actin FSL and Pathromtin SL (PSL) APTT reagents and FIX deficient plasma (all Siemens, Marburg, Germany) on a Sysmex CS5100i analyser and APTT SP, SynthASil and SynthAFax APTT reagents and FIX deficient plasma (all Werfen, Barcelona, Spain) on an ACL TOP analyser. Specific calibration curves were performed for each reagent using Siemens standard human plasma or Werfen HemosIL calibrator plasma. Each sample was tested at 3 dilutions and results were obtained by double logarithmic plot then extrapolation of parallel line. CSA, at two sample dilutions, were performed on a Sysmex CS5100i with kits from Rossix (Mölndal, Sweden) and Hyphen Biomed (Neuville-sur-Oise, France) with calibration curves performed using Hyphen Biomed calibrator plasma.

Percentage difference was calculated per sample by (**a**-**b**/**b**) x 100 where **a** was the FIX:C using alternative APTT reagents or CSA and **b** was the FIX:C using either Pathromtin SL in Idelvion samples or Actin in Alprolix samples.

Results

Idelvion

The overall median OSA FIX:C for 18 samples from Idelvion-treated patients was 48 IU/dL with PSL, with other OSA ranging from 28 IU/dL(AFS) to 63 IU/dL (SynthAFax) and using CSA was 82 IU/dL with Rossix and 66 IU/dL with Hyphen (see table 1). At high levels, FIX:C ranged from 46 IU/dL (AFS) to 137 IU/dL (Rossix CSA) in the same sample; PSL was 78 IU/dL. At trough levels, FIX:C varied from 4 IU/dL (both CSA) to 9 IU/dL (Actin) in the same sample; PSL was 6 IU/dL. Percentage differences were calculated using PSL as the target result. For OSA the median percentage difference ranged from -3.5% with Actin to 42.5% with SynthAFax. For CSA the median percentage difference was 71.5% with Rossix and 41.1% with Hyphen (table 1 and figure 1).

Alprolix

The median OSA FIX:C for 22 samples from Alprolix-treated patients ranged from 33 IU/dL with PSL to 53 IU/dL with Actin. Median CSA was 49 IU/dL with Rossix and 34

IU/dL with Hyphen (see table 1). At high levels, the largest FIX:C variation in a single samples was 65 IU/dl with APTT SP and 94 IU/dl with Actin. A single patient had a IX:C of below 10 IU/dL with Actin and the range of results was 3 IU/dl (Rossix CSA) to 8 IU/dl (Actin, APTT SP and Synthasil) in this sample. Percentage differences were calculated using Actin as the target result. The median OSA percentage difference ranged from -22% with Synthasil to -27.4% with PSL. For CSA, the median percentage difference was -3.7%% with Rossix and -30.8% with Hyphen (table 1 and figure 1).

Discussion/ Conclusions

The laboratory monitoring of FIX products which have been modified by fusion to albumin or the Fc portion of IgG1 molecules or by linkage of polyethylene glycol, has become a challenging issue. The results of pharmaceutical company instigated field studies using plasma spiked with these EHL rFIX products have indicated that some commonly used APTT reagents would not be suitable for accurate measurement of these products [2, 3, 6]. A plethora of APTT reagents are in routine laboratory use worldwide and full details of the one-stage method used for potency assignment of the individual products are not always freely available.

The use of Actin FS or CK Prest APTT reagents to monitor Idelvion was reported to underestimate the product recovery by approximately 50% in spiked plasma [3]. In this present study, medians of less than 10% difference from Pathromtin SL were observed with Actin, APTT SP and SynthASil in Idelvion-treated patients. Median underestimation by greater than 35% was observed with AFS and Actin FSL and overestimation by greater than 40% was observed with SynthAFax and both CSA. These latter methods also may not be suitable for routine monitoring of Idelvion *in vivo* but more data is required to substantiate these findings. A trend of increasing percentage difference with increasing FIX:C was observed with both CSA and Synthafax OSA. Similar non-linear relationships between some modified FVIII and FIX molecules at measured at increasing plasma concentrations with certain reagents have been reported in spiked plasma [2, 7]

Field studies that used plasma artificially spiked with Alprolix concluded that the product may be measured by OSA with APTT reagents comprising ellagic acid or silica activators. The kaolin-based reagent, CK Prest, which underestimated the expected recovery by 20-40% depending upon the FIX:C activity, would not be acceptable for monitoring [2, 7].

In HB patients treated with Alprolix, the target FIX:C was determined using OSA and Actin APTT reagent. In our study, Rossix CSA demonstrated the best agreement with Actin FIX:C. The remaining reagents demonstrated approximately 30% difference compared to Actin although this was extremely variable for each patient sample. Alprolix samples with FIX:C below 15 IU/dL demonstrated much greater percentage difference of up to 63%, than those with higher activity. All samples with Actin FIX:C of greater than 50 IU/dL demonstrated less than 36% difference with the remaining reagents. A clear dose dependent trend of decreasing percentage difference at increasing FIX:C was observed with SynthAFax OSA and Hyphen Biomed CSA.

Currently, there is no international consensus of the degree of variation between reagents or assay methods measuring the same parameter which could be expected to alter the clinical management of patients. 20-30% difference has been considered acceptable in several studies.[2, 8, 9]. At FIX:C below 10 IU/dL, the calculation of percentages can magnify small differences in activity which may not be clinically significant. Since FIX:C trough levels can be used to tailor patient dosing regimens, it is important that these can be accurately determined, however, field studies have reported significant variability at low FIX:C concentrations [2, 3, 6, 7]. The HB patients included in this study demonstrated up to two fold difference in FIX:C with some reagents, irrespective of rFIX concentrate, a disparity which may influence clinical decisions.

Results in this *ex vivo* study confirm and extend *in vitro* spiking data for both Idelvion and Alprolix. The activator present in the APTT reagents tested, either ellagic acid or silica-based, did not appear to consistently influence results for either EHL FIX product but other reagents should be tested locally before use. Each patient included in this present study had samples from more than one venepuncture and no patient-

specific trends were observed within the cohort. Our data confirms that the accurate monitoring of patients on Idelvion or Alprolix is dependent upon the use of the appropriate assay. It is therefore essential that laboratories and clinicians are aware of the characteristics of the specific FIX:C assays used in their centres and how these interact with different EHL products. Our data support the recommendation that laboratories should use an assay that has been validated for use with the specific extended half life concentrate being used. (10)

REFERENCES

1. Chowdary P, Kearney S, Regnault A, Hoxer CS, Yee DL. Improvement in health-related quality of life in patients with haemophilia B treated with nonacog beta pegol, a new extended half-life recombinant FIX product. Haemophilia. 2016;22:e267-74.
2. Sommer JM, Buyue Y, Bardan S, Peters RT, Jiang H, Kamphaus GD, Gray E, Pierce GF. Comparative field study: impact of laboratory assay variability on the assessment of recombinant factor IX Fc fusion protein (rFIXFc) activity. Thromb Haemost. 2014;112:932-40.
3. St Ledger K, Feussner A, Kalina U, Jacobs I, Viogt C, Bensen-Kennedy D. Performance of a recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in the one-stage assay. haemophilia. 2016;22:60.
4. Europe Co. Human Coagulation factor IX. Monograph 1644. Strasbourg: Ph. Eur. 8th Edition 2014.
5. EMA, editor Workshop report: Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples. EMA; 2014; London.
6. Bowyer AE, Hillarp A, Ezban M, Persson P, Kitchen S. Measuring factor IX activity of nonacog beta pegol with commercially available one-stage clotting and chromogenic assay kits: a two centre study. J Thromb Haemost. 2016;14:1428-35.
7. Geske FJ, Sadeghi-Khomami A. Recombinant factor IX Fc fusion protein (rFIXFc) clotting activity assessment in internation haemophilia treatment centres. THSNA; Chicago2016.

8. Tiefenbacher S, Bohra R, Amirel J, Bowyer A, Kitchen S, Lochu A, Rosen S, Ezban M. Qualification of a select one-stage activated partial thromboplastin time-based clotting assay and two chromogenic assays for the post-administration monitoring of nonacog beta pegol. J Thromb Haemost. 2017;15:1-12.
9. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. J Thromb Haemost. 2016;14:248-61.
10. Collins P, Chalmers E, Chowdary P, Keeling D, Mathias M, O'Donnell J, Pasi KJ, Rangarajan S, and Thomas A. The use of enhanced half-life coagulation factor concentrates in routine clinical practice: guidance from UKHCDO. Haemophilia (2016), 22, 487–498