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Monitoring of factor VIII or IX levels in patients receiving extended half-life products.

Hemophilia A and B are rare bleeding disorders caused by mutations in the factor VIII (FVIII) and IX (FIX) genes. Frequency and severity of bleeding symptoms are correlated with clotting factor levels and treatment is primarily based on replacing the missing factor. Over the past decade, a new generation of FVIII and FIX recombinant products with extended plasma half-life (EHL) have been introduced. These new products have improved the quality of life of patients by reducing injection frequency for prophylaxis and increasing trough levels. The modifications of these new factor products may however induce discrepancies in results between chromogenic and one-stage clotting assays, and between the various available one-stage clotting assays, and may not reflect the actual factor level.

In this information letter, we aim at providing guidance on which STAGO reagent is the best suited for laboratory monitoring of FVIII or FIX depending on the recombinant EHL product being used. The proposals are based on the European Medicines Agency summary of product characteristics (SPC), guidelines from scientific societies, and data published in the literature or presented at a scientific conference.

As of today, five EHL recombinant FVIII products and one FVIII-mimetic bispecific antibody are available on the market (table 1):

- Efmoroctocog alfa (ELOCTA®) is a recombinant fusion protein linking a B-domain deleted FVIII to the FC domain of human immunoglobulin G1. The SPC indicates that the FVIII activity can be significantly affected by the reagent used but does not give precise information on which one to use[1]. We recommend using the chromogenic assay TriniCHROM FVIII:c as a first-line test[2]. FVIII levels obtained using different one-stage clotting assays show minor differences that are clinically acceptable[3–6]. STA-PTT Automate, STA-C.K. Prest, TriniCLOT Automated aPTT, and TriniCLOT aPTT S are an

acceptable alternative and can be used interchangeably for monitoring FVIII levels.

- Lonoctocog alfa (AFSTYLA®) is a recombinant single chain FVIII. The TriniCHROM FVIII:c kit can be used to monitor this drug after local verification[5–7]. One-stage clotting assays, regardless of the activator used, generate values approximately 50% lower than expected. The SPC indicates that chronometric assays can be used if the result is multiplied by a factor of two[7]. Several guidelines oppose the use of this conversion factor because of the risk of overestimation at low Lonoctocog alfa plasma levels[4,5,8]. We recommend using one-stage clotting assays only as a last resort and after comparing it locally with the chromogenic assay.
- Turoctocog alfa pegol (ESPEROCT®) is a recombinant B-domain truncated FVIII with a site-specific pegylation. One-stage clotting assays using silica-based activators generate spuriously low FVIII levels, which may be due to a decelerated activation of the turoctocog alfa pegol by thrombin in presence of such an activator[3–5,9,10]. STA-PTT Automate, TriniCLOT Automated aPTT, and TriniCLOT aPTT S should be avoided. The chromogenic assay TriniCHROM FVIII:c shows very good correlations with spiked concentrations of turoctocog alfa pegol and is the recommended first-line assay[2]. STA-C.K. Prest has not been evaluated in this indication but could be an acceptable alternative after local verification[3,4].
- Damactotog alfa pegol (JIVI®) is a recombinant B-domain deleted FVIII with a site-specific pegylation. FVIII levels are underestimated by most of the one-stage clotting assays using Silica-based activators[3–5,11]. Conversely, the use of kaolin reagents seems to overestimate the FVIII activity[3,5,6,11]. STA-PTT Automate, STA-C.K. Prest, TriniCLOT Automated aPTT, and TriniCLOT aPTT S should not be used for the biological monitoring of damactotog alfa pegol treatments. Several on market chromogenic kits have been validated in a field study and recommended by several guidelines[5,6,12]. The TriniCHROM FVIII:c kit has not yet been evaluated but information on the other chromogenic assays suggests that it could be used after local verification.
- Rurioctocog alfa pegol (ADYNOVI®) is a recombinant full-length FVIII with a non-specific pegylation. Available data are inconsistent, and it is unclear whether one-stage clotting are appropriate for measuring FVIII levels or overestimating it[3,5,6]. Use of a product-specific calibrator appears to reduce overestimation and may yield acceptable results[13]. Recently, our research and

development department evaluated the performances of the TriniCHROM FVIII:c kit and found an excellent correlation with spiked concentration of rurioctocog alfa pegol[2]. We recommend using the chromogenic assay TriniCHROM FVIII :c as a first-line test. STA-PTT Automate, STA-C.K. Prest, TriniCLOT automated aPTT, and TriniCLOT aPTT S may be used to measure FVIII activity after calibration with a product-specific standard and local verification[5].

- Emicizumab (HEMLIBRA®) is a recombinant humanized bispecific antibody that binds both activated factor IX (FIXa) and factor X (FX) and mimics activated FVIII cofactor function. The interaction of this drug with human FIXa and FX is responsible for interferences on all tests involving these two human factors. Emicizumab shortens aPTT and falsely increases FVIII levels measured by one-stage aPTT-based assays and chromogenic assays using human-derived factor components[14]. Kershaw *et al* have demonstrated that this interference can be used to measure emicizumab levels[15]. STA-PTT Automate, STA-C.K. Prest, TriniCLOT automated aPTT, TriniCLOT aPTT S and TriniCHROM FVIII:c may be used as emicizumab assays after replacing plasma calibrator with emicizumab calibrator.

<table>
<tr><th>FVIII products</th><th>STA-C.K. Prest</th><th>STA-PTT Automate</th><th>TriniCLOT Automated aPTT</th><th>TriniCLOT aPTT S</th><th>TriniCHROM FVIII:c</th></tr>
<tr><td>Efmoroctocog alfa (ELOCTA®)</td><td colspan="4">Acceptable*</td><td>Recommended✝</td></tr>
<tr><td>Lonoctocog alfa (AFSTYLA®)</td><td colspan="4">May be used with a conversion factor in the absence of alternative#</td><td>May be used after local verification#*</td></tr>
<tr><td>Turoctocog alfa pegol (ESPEROCT®)</td><td>May be used after local verification*</td><td colspan="3">Should be avoided#*</td><td>Recommended✝</td></tr>
<tr><td>Damactotog alfa pegol (JIVI®)</td><td colspan="4">Should be avoided#*</td><td>May be used after local verification*</td></tr>
<tr><td>Rurioctocog alfa pegol (ADYNOVI®)</td><td colspan="4">May be used after calibration with a product specific standard in the absence of alternative✝</td><td>Recommended✝*</td></tr>
<tr><td>Emicizumab (HEMLIBRA®)</td><td colspan="5">May be used after replacing plasma calibrator with emicizumab calibrator✝</td></tr>
</table>

Table 1. Proposals for the monitoring of factors VIII levels in patients receiving extended half-life products. *: position of learned societies, #: as per SPC, ✝: data published in the literature or presented at a scientific conference.

As of today, three EHL recombinant FIX products are available on the market (table 2):

- Eftrenonacog alfa (ALPROLIX®) is a recombinant fusion protein linking FIX to the FC domain of human immunoglobulin G1. The chromogenic assay Rossix FIX is recommended for monitoring patients treated with eftrenonacog alfa[3,5,6]. FIX levels are underestimated by one-stage clotting assays using kaolin-based activators[16]. FIX activity appears close to the target value with most of the silica-containing reagents. STA-PTT Automate is associated with contradictory information; the World Federation of Hemophilia states that it should not be used for monitoring replacement therapy with ALPROLIX®[3–6,17].TriniCLOT Automated aPTT and TriniCLOT aPTT S may be used after comparison with the recommended method.
- Albutrepenonacog alfa (IDELVION®) is a recombinant fusion protein linking FIX to albumin. The Rossix FIX kit overestimates FIX levels and should be avoided for the determination of albutrepenonacog alfa activity. STA-C.K. Prest affect FIX activity by underestimating it up to 50% in some case[3–6,18]. albutrepenonacog alfa can be accurately measured by one-stage clotting assays using silica-based activators[3,4,6]. Field studies support this claim for STA-PTT Automate, but data are lacking for TriniCLOT Automated aPTT and TriniCLOT aPTT S. We recommend using STA-PTT Automate as the first-line test to measure FIX activity. TriniCLOT Automated aPTT and TriniCLOT aPTT S may be used after local verification.
- Nonacog beta pegol (REBINYN®) is a recombinant full-length FIX with a non-specific pegylation. The SPC recommends using chromogenic assays, such as the Rossix FIX kit[19]. A large overestimation of FIX levels has been reported with most one-stage clotting assays using silica-based activators[3,4,19]. This may be due to an accelerated conversion of the nonacog beta pegol to FIXa by the PEG group[20]. Spuriously low levels of FIX are generated by reagents containing kaolin[3,4]. STA-PTT Automate, STA-C.K. Prest and TriniCLOT Automated aPTT should not be used to monitor patients treated with Nonacog beta pegol. A recent study demonstrated acceptable results for TriniCLOT aPTT S when using a calibration curve derived from the nonacog beta pegol vial[21].

FIX products	STA-C.K. Prest	STA-PTT Automate	TriniCLOT Automated aPTT	TriniCLOT aPTT S	Rossix FIX:c
Eftrenonacog alfa (ALPROLIX®)	Should be avoided ŧ#		May be used after local verification*		Recommended*
Albutrepenonacog alfa (IDELVION®)	Should be avoided*#	Acceptable*	May be used after local verification*		Should be avoided*
Nonacog beta pegol (REBINYN®)	Should be avoided*#			May be used after calibration with a product specific standard ŧ	Recommended#

Table 2. Proposals for the monitoring of factors IX levels in patients receiving extended half-life products. *: position of learned societies, #: as per SPC, ŧ: data published in the literature or presented at a scientific conference.

We hope that this additional information will be useful to your daily practice.

Asnières-sur-Seine, December 7, 2024

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