CRITICAL REVIEW

Shifting landscape of hemophilia therapy: Implications for current clinical laboratory coagulation assays

Hanny Al-Samkari1,2 | Stacy E. Croteau2,3

1 Center for Hematology, Massachusetts General Hospital Cancer Center, Boston, Massachusetts; 2 Harvard Medical School, Boston, Massachusetts; 3 Boston Children’s Hospital, Boston Hemophilia Center, Boston, Massachusetts

Correspondence
Dr. Stacy E. Croteau, 300 Longwood Ave., BCH 3141, Boston, MA 02115.
Email: Stacy.Croteau@childrens.harvard.edu

Clinical coagulation assays are an integral part of diagnosing and managing patients with hemophilia; however, in this new era of bioengineered factor products and nonfactor therapeutics, problems have arisen with use of traditional coagulation tests for the quantification of several of these new products. Discussion over the use of one-stage clotting assays versus chromogenic substrate assays for clinical decision making and potency labeling has been ongoing for many years. Emerging factor concentrates have heightened concern over assay selection and availability. Emicizumab interferes with all aPTT based assays, rendering them unreliable and potentially falsely reassuring to the unaware provider. This review explores considerations for coagulation assays in the clinical setting and highlights how awareness of institutional coagulation assays and potential limitations have never been more critical for providers caring for patients with bleeding disorders.

1 | INTRODUCTION

Clinical coagulation laboratory assays serve an important role in diagnosing and supporting treatment decisions for patients with bleeding disorders, yet current factor assays have gone largely unchanged for several decades. The prothrombin time (PT) and activated partial thromboplastin time (aPTT) serve as screening tests for an underlying factor deficiency, while specific factor activity assays by one-stage clotting assays (OSA) or chromogenic substrate assays (CSA) are used for diagnosis and monitoring factor levels following infusion of clotting factor concentrates for individuals with hemophilia A or B, factor VIII (FVIII) or factor IX (FIX) deficiency, respectively. Licensure of new, bioengineered factor concentrates to provide half-life extension1–5 and an emerging group of nonfactor therapeutics, such as emicizumab-kxwh (Hemlibra, Roche),6,7 have heightened awareness of the limitations of our current clinical coagulation assays and fueled debate over the most clinically relevant assays. With these novel therapies, and several more products currently in various stages of clinical trial investigation,8–10 problems have arisen for some products that cannot be measured accurately with traditional factor assays. Bioengineered factor products have modifications distinct from endogenous coagulation proteins for which the existing factor assays were designed.

OSA and CSA are used routinely in the U.S. and Europe for potency labeling of factor concentrate vials as well as clinical monitoring of patients.

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While both types of assays indirectly measure FVIII activity (FVIII:C), they utilize different methodologies and are thus each subject to unique sources of variability and inaccuracy. The number of commercial reagents, and therefore the potential for interlaboratory variability, is more pronounced for OSA in comparison to CSA. In a 2006, UK National External Quality Assurance Scheme (NEQAS) survey of UK clinical laboratories, 25 different aPTT reagents, 20 different FVIII-deficient plasmas, and 13 different reference plasmas were in use for OSA, in contrast to only 5 different CSA kits.11 Discrepancy between OSA and CSA is increasingly recognized with newer bioengineered factor products and certain F8 pathogenic variants.12 This is not a new problem, however. Emergence of the first B domain-deleted (BDD) recombinant FVIII (rFVIII) clotting concentrate highlighted the clinical challenges that can arise when OSA and CSA are discordant. Potency labeling assigned by CSA was 20% higher than when assayed using OSA. Since OSA underestimated factor activity it was recommended to either use CSA or OSA calibrated with the B-domain deleted product. To overcome this challenge in the U.S. market, the amount of factor protein in each vial was increased 20%.13,14

Global hemostasis assays, such as the thrombin generation assay (TGA), thromboelastography (TEG) [and its modification, rotational thromboelastometry (ROTEM)], aPTT waveform analysis, and others are not currently in routine clinical by hematologists but in the age of novel hemostatic agents may offer alternative assessment of hemostasis in patients with bleeding disorders.15 Currently, there are no CLIA-approved laboratory assays to quantify the hemostatic impact of emicizumab, and indeed the presence of even small quantities of emicizumab in patient plasma interferes with all aPTT based assays rendering them unreliable. Given the long half-life of emicizumab, 27.8 ± 8.1 days, this interference can persist...
for up to 6 months after emicizumab discontinuation. Providers caring for hemophilia patients in the age of bioengineered factor products and emicizumab must have a deeper understanding of the nuances of coagulation laboratory testing than ever before.

Here, we review methodology and limitations of commonly-used laboratory clotting tests for hemophilia, and how conventional and extended half-life factor concentrates, as well as nonfactor therapies, impact assay interpretation with particular attention to relevance when providing clinical care.

2 | QUANTIFYING FACTOR VIII AND IX ACTIVITY

Routine diagnosis and management of patients with hemophilia depend on factor activity assays. Additionally, these assays support accurate potency labeling of clotting factor concentrates. Assays must be able to accurately reflect baseline FVIII or FIX activity levels to establish the diagnosis and severity of individuals with hemophilia. Increasingly, postinfusion factor levels in conjunction with population pharmacokinetic (PK) models are being employed to generate individual PK profiles and personalize prophylaxis regimens. Accurate results are important for quality care. OSA is the most widely used assay used for clinical laboratory monitoring, potency labeling for FIX clotting factor concentrates, and in the United States for potency labeling of FVIII clotting factor concentrates. While CSA is the preferred test for FVIII potency labeling in Europe, a 2013 survey revealed that over 90% of European coagulation laboratories utilized OSA for clinical monitoring of FVIII replacement. The U.S. FDA presently endorses OSA for FVIII replacement potency labeling to complement the predominant modality used for clinical monitoring, though the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation has recommended widespread adoption of CSA in clinical coagulation laboratories for those circumstances in which OSA is unreliable.

2.1 | One-stage clotting assays

The OSA was first described in 1953 by Langdell and colleagues. It is performed via mixing serial dilutions of patient plasma with FVIII-deficient plasma followed by addition of aPTT reagents and calcium to initiate coagulation. The same process is carried out with patient plasma. The aPTT results are plotted on logarithmic-linear scale graph paper; all lines should run in parallel (parallelism). The FVIII activity of the sample is determined by identifying the dilution of the reference plasma that results in the same aPTT result as the sample plasma at the dilution designated 100 IU/dl for the reference plasma. In modern laboratory practice, the OSA is frequently automated. Many different aPTT reagents (multiple types of phospholipids and activators) and various analyzers are in routine clinical use worldwide. OSA is the most widely used assay used for clinical laboratory monitoring, potency labeling for FIX clotting factor concentrates, and in the United States for potency labeling of FVIII clotting factor concentrates. While CSA is the preferred test for FVIII potency labeling in Europe, a 2013 survey revealed that over 90% of European coagulation laboratories utilized OSA for clinical monitoring of FVIII replacement. The U.S. FDA presently endorses OSA for FVIII replacement potency labeling to complement the predominant modality used for clinical monitoring, though the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation has recommended widespread adoption of CSA in clinical coagulation laboratories for those circumstances in which OSA is unreliable.

2.2 | Two-stage clotting assay

Although now largely superseded by CSA, the traditional two-stage clotting assay may still be found in some specialized hemostasis laboratories. First described in 1955 by Biggs and colleagues, the two-stage clotting assay...
The CSA was originally developed in the 1970s with subsequent modifications. In the first stage, which is similar methodologically to the two-stage clotting assay, dilutions of patient plasma are incubated with FIXa, factor X, phospholipids and calcium, resulting in formation of FXa proportional to the FVIII:C of the patient sample. The second stage involves subsampling this mixture into normal plasma containing prothrombin and fibrinogen and measurement of the clotting time, which is correlated indirectly with the FVIII:C of the original sample. From a laboratory perspective, the primary advantages of the two-stage clotting assay in comparison with the OSA are the lack of requirement for FVIII-deficient plasma and less interlaboratory variation. The disadvantages include complexity, lack of automation, and lack of commercial reagents.

2.4 | Sources of variation/error in OSA and CSA

Each of these assays is susceptible to different sources of preanalytic and analytic errors. Those of key interest to clinicians are summarized in Table 1. The OSA is susceptible to hirudin contamination and lupus anticoagulants which can alter results; the CSA is not sensitive to either of these potential sources of error. Both assays are sensitive to the presence of direct oral anticoagulants, which result in a falsely low measurement of FVIII activity, and both will report falsely low FVIII activity if the specimen is clotted because FVIII is consumed during clotting. OSA, but not CSA, is susceptible to "preactivation" of FVIII that can occur due to thrombin activation during venipuncture because of differences in the assay methodology. In contrast to OSA, CSA has an incubation period which allows for activation of all FVIII present making preactivation irrelevant. Preactivation of FVIII in OSA results in an overestimation of FVIII activity. OSA is generally less accurate in the lower ranges of FVIII activity, making it less sensitive in the initial diagnosis and differentiation of moderate and severe hemophilia A than CSA.

2.5 | Sources of interlaboratory variation in OSA

Several variables may impact OSA measurement and result in substantial inaccuracy or interlaboratory variation. Numerous different commercial FVIII-deficient plasma products are available, which may be chemically-depleted, immuno-depleted, or naturally deficient; moreover, they may contain von Willebrand Factor (VWF). Certain commercial FVIII-
TABLE 1  Extrinsic sources of assay interference for OSA and CSA

<table>
<thead>
<tr>
<th>Consideration</th>
<th>Affects OSA</th>
<th>Affects CSA</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin contamination</td>
<td>✓</td>
<td>*</td>
<td>CSA contains a heparin neutralizer; if a very high amount of heparin is present, it may overcome the neutralizer and affect the assay</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct oral anticoagulants</td>
<td>✓</td>
<td>✓</td>
<td>Both direct factor Xa inhibitors and direct thrombin inhibitors</td>
</tr>
<tr>
<td>Emicizumab</td>
<td>✓</td>
<td></td>
<td>Even low plasma concentrations of emicizumab normalize aPTT-based assays; CSA using nonhuman reagents does not recognize emicizumab</td>
</tr>
<tr>
<td>Preactivation of FVIII from venipuncture</td>
<td>✓</td>
<td>✓</td>
<td>Results in overestimation of FVIII activity</td>
</tr>
<tr>
<td>Range of FVIII activity</td>
<td>✓</td>
<td></td>
<td>Less accurate in 1–3% range</td>
</tr>
<tr>
<td>Variant FVIII protein structure/stability</td>
<td>✓</td>
<td>✓</td>
<td>Pathogenic variant-dependent; both assays used in diagnostic evaluation</td>
</tr>
</tbody>
</table>

deficient plasma products may have small residual FVIII resulting in flattening of the standard curve at low activity ranges (0–3 IU/dL). This affects the accuracy of the test at low FVIII concentrations. As with a standard aPTT assay, results of the OSA may be impacted by the specific aPTT reagent used; dozens are commercially available. Each aPTT reagent contains a source of phospholipid and a surface activator, such as kaolin, ellagic acid, or silica. Specific activators may cause inaccurate measurements of the FVIII:C or FIX:C when assaying vial potency or recovery of certain modified FVIII or FIX proteins (see Table 2). The buffer used for dilution may influence results and numerous buffers are available commercially. Finally, the instrument (analyzer) used to assess for clotting may be optical, measuring turbidity as clot forms, or mechanical, measuring increased viscosity as clot forms, which provides yet another potential source of inter-laboratory variation. Therefore, from a laboratory perspective, OSA may be less expensive and easier to automate than CSA, but it has numerous possible sources of inaccuracy. If several recommended OSA technical standardization procedures are followed, such as use of FVIII-deficient plasma with normal VWF levels and addition of albumin to all assay buffers, reduction in inter-laboratory variation can be achieved.

2.6 | Potency labeling of factor concentrate vials with OSA vs. CSA

Debate continues over the best approach for factor concentrate vial potency labeling. Accurate potency labeling is critical for proper dosing of patients and valuation of factor vials. Studies comparing potencies measured by OSA and CSA often demonstrate significant discrepancy, with some demonstrating potencies measured at up to 40% higher for the OSA and others demonstrating potencies measured at approximately 20% higher for the CSA. When utilizing the OSA for labeling, the specific aPTT reagent used for OSA testing has been found to impact the measured potency results.

3 | DISCREPANT OSA AND CSA RESULTS DUE TO FVIII PATHOGENIC VARIANTS

In the assessment of FVIII:C, clinically relevant discrepancy between assays is generally considered >20%. If only the OSA or CSA is performed, accurate diagnosis of hemophilia may be compromised. Certain F8 genotypes can result in substantial FVIII:C discrepancy between OSA and CSA measurements. FVIII activity should be assayed by both during the initial diagnostic evaluation because approximately one-third of patients with mild hemophilia A secondary to single point mutations show a significant discrepancy in FVIII activity between OSA and CSA. This discrepancy can result in up to 10% of patients with mild hemophilia A being misdiagnosed as normal if only one of the two assays is performed.

3.1 | Point mutations at the A1-A2-A3 interfaces

Appreciation of these pathogenic variants and their resultant impact requires a basic understanding of FVIII structure and function. FVIII is a heterodimer composed of a heavy chain (A1, A2, and B domains) and light chain (A3, C1, and C2 domains). In the process of thrombin-catalyzed conversion of FVIII to FVIIIa, the B domain is cleaved off and the result is a heterotrimer comprised of an A1 subunit, A2 subunit, and A3-C1-C2 subunit. The A2 subunit is only weakly associated with A1 and A3-C1-C2 subunits via an ionic interaction and is therefore susceptible to dissociation from the rest of the protein; this is how physiologic inactivation of FVIIIa occurs. In patients with variants at the interfaces between where the A2 subunit interacts with the A1 and A3-C1-C2 subunits, such as those that disrupt potential intersubunit hydrogen bonds, Figure 3, the measured FVIII:C by OSA is over twice that measured by CSA. This results from the longer incubation duration of CSA, which allows for more time for unstable FVIIIa to dissociate, resulting in lower measured FVIII activity.

3.2 | Point mutations at thrombin cleavage sites

By contrast, mutations that affect activation of FVIII by thrombin or the binding of FVIII to FIXa or to VWF result in discrepancy such that OSA results are lower than CSA results. Any variant affecting thrombin activation of FVIII or its physiologic binding to FIX is unlikely to be problematic in the CSA, where thrombin and FIX have been added in excess and additional time for necessary reactions to occur (time not present in the short incubation period of the OSA).
### TABLE 2
Recombinant factor VIII and factor IX products available in the United States and reliability of one-stage clotting assays (OSA) and chromogenic substrate assays (CSA) for each

<table>
<thead>
<tr>
<th>Recombinant Factor Product</th>
<th>Features</th>
<th>OSA/CSA Generally Reliable</th>
<th>Caution warranted</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FVIII Concentrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advate (rurioctocog alfa)</td>
<td>Full-length FVIII, CHO cell line</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adynovate (rurioctocog alfa pegol)</td>
<td>Full-length FVIII, random pegylation with branched 20kDa PEG</td>
<td>✓</td>
<td></td>
<td>Consistent underestimation of FVIII:C by all types of aPTT reagents, FDA recommends conversion factor (multiply OSA result by 2)</td>
</tr>
<tr>
<td>Afstyla (lonoctocog alfa)</td>
<td>Single-chain, B-domain truncated FVIII</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAY 94–9027 (danoctocog alfa pegol)</td>
<td>B-domain deleted FVIII, site-directed pegylation with 60kDa PEG</td>
<td>✓</td>
<td>Underestimation of FVIII:C may occur with silica-based aPTT reagents, manufacturer recommends ellagic aPTT reagents as the preferred</td>
<td></td>
</tr>
<tr>
<td>Eloctate (rFVIII-Fc, efmoroctocog alfa)</td>
<td>B-domain deleted FVIII, fusion with IgG1 Fc at carboxy-terminus</td>
<td>✓</td>
<td>Field study</td>
<td></td>
</tr>
<tr>
<td>Kogenate (octocog alfa)</td>
<td>Full-length FVIII, BHK cell line</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kovaltry (octocog alfa)</td>
<td>Full-length FVIII, BHK cell line</td>
<td>✓</td>
<td>Field study</td>
<td></td>
</tr>
<tr>
<td>NovoEight (turoctocog alfa)</td>
<td>B-domain truncated FVIII</td>
<td>✓</td>
<td>Field study</td>
<td></td>
</tr>
<tr>
<td>NovoEight-GP (N8-GP, turoctocog alfa pegol)</td>
<td>B-domain truncated FVIII, site-directed pegylation with 40kDa PEG</td>
<td>✓</td>
<td>Underestimation of FVIII:C (&gt;30%) may occur with some ellagic acid and silica-based aPTT reagents; overestimation of FVIII:C with some chromogenic kits observed</td>
<td></td>
</tr>
<tr>
<td>Nuwiq (simoctocog alfa)</td>
<td>B-domain deleted FVIII, HEK-293 cell line</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xyntha/ReFacto AF (moroctocog alfa)</td>
<td>B-domain deleted FVIII</td>
<td>✓</td>
<td>May underestimate FVIII:C unless product-specific laboratory standard used</td>
<td></td>
</tr>
<tr>
<td><strong>FIX Concentrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BeneFix (nonacog alfa)</td>
<td>rFIX</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ixinity (trenonacog alfa)</td>
<td>rFIX, Thr-148 polymorphism</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rixubis (nonacog gamma)</td>
<td>rFIX</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alprolix (rFIX-Fc, eftrenonacog alfa)</td>
<td>rFIX, fusion with IgG1 Fc at carboxy-terminus</td>
<td>✓</td>
<td>Underestimation of FIX:C was observed with some silica-based reagents and consistently for kaolin-based aPTT reagents, OSA (all reagents) tended to overestimate FIX:C at low concentrations</td>
<td></td>
</tr>
<tr>
<td>Idelvion (rFIX-FP, albutrepenonacog alfa)</td>
<td>rFIX, fusion with recombinant human albumin at cleavable linker peptide</td>
<td>✓</td>
<td>Underestimation of FIX:C observed with kaolin- and ellagic acid-based aPTT reagents (by up to 50%)</td>
<td></td>
</tr>
<tr>
<td>Rebinyn (N9-GP, nonacog beta pegol)</td>
<td>rFIX, site-directed pegylation with branched 40kDa PEG</td>
<td>✓</td>
<td>Underestimation of FIX:C observed with kaolin-based aPTT reagents. Substantial overestimation of FIX:C observed with silica-based aPTT reagents. Most ellagic acid-based aPTT reagents accurate, but some underestimate FIX:C</td>
<td></td>
</tr>
</tbody>
</table>

Note not all aPTT reagents have been studied for use with each factor concentrate; magnitude of discrepancy between OSA result and expected may vary along the range of concentrates.
FIGURE 3  F8 pathogenic variants associated with alteration of hydrophobic or charged amino acids decreasing the stability of FVIII, resulting in discrepant FVIII activity reported by OSA and CSA. While the OSA is not sensitive to this reduced FVIII stability due to the time course of the assay, the incubation time of the CSA permits more accurate assessment of FVIII activity and detection of mild deficiency. FVIII molecule (with B domain removed) with heavy chain (purple) and light chain (blue) illustrated; most mutations are clustered at A1-A2, A1-A3, and A2-A3 interfaces.12 The opposite relationship occurs for pathogenic variants located in FIX- binding and thrombin cleavage sites (not shown in this figure); OSA is more sensitive to detecting resultant mild deficiencies compared to CSA. 3D render of FVIII protein created using Cn3D macromolecular structure viewer, NCBI Structure Group59 [Color figure can be viewed at wileyonlinelibrary.com]

5 | EMICIZUMAB AND COAGULATION TESTING

Emicizumab is a recombinant, humanized bispecific monoclonal antibody which serves the function of activated FVIII, bringing together FXa and factor X (FX). Emicizumab is currently approved for bleeding prophylaxis in hemophilia A patients with inhibitors by the FDA, EMA, Japan and several other countries.6,7 It is administered subcutaneously once weekly with a longer dosing interval presently under investigation. Considerations for dosing in an acute bleed setting or perioperatively are also being explored as is safety and efficacy of emicizumab prophylaxis in hemophilia A patients without inhibitors.64

5.1 | Conversion of emicizumab plasma concentrations to “equivalent” factor VIII activity measurements

While emicizumab represents a major advancement in the management of patients with hemophilia complicated by inhibitors, coagulation laboratory testing methods have not yet caught up with this novel therapy. The ability of current clinical assays to quantify the hemostatic potential of an individual on emicizumab prophylaxis is limited. A conversion factor of emicizumab (µg/mL) to “equivalent” FVIII:C of approximately 0.3, such that a concentration of 33 µg/mL of emicizumab would be expected to correspond to a FVIII:C of approximately 10% based on cynomolgus monkey studies has been suggested65 and subsequently used in human emicizumab studies to inform initial dose selection; however, correlative human studies are not available.66

5.2 | Limitations of current clinical coagulation assays

Emicizumab interacts with all intrinsic pathway clotting-based laboratory assays, Table 3, which include all aPTT-based assays, such as the OSA and activated clot time (ACT). The binding characteristics of emicizumab are such that even low plasma concentrations normalize the aPTT, abolishing the typical relationship between FVIII activity and aPTT used to monitor hemostatic effect. Under normal circumstances,

<table>
<thead>
<tr>
<th>TABLE 3 Coagulation assays to be avoided or used with caution in individuals actively or recently receiving emicizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated partial thromboplastin time (aPTT)</td>
</tr>
<tr>
<td>One-stage clotting assay (aPTT-based)</td>
</tr>
<tr>
<td>Bethesda or Nijmegen-Bethesda assay (aPTT-based)</td>
</tr>
<tr>
<td>Activated clotting time</td>
</tr>
<tr>
<td>Chromogenic substrate assay for FVIII (utilizing bovine protein reagents)</td>
</tr>
<tr>
<td>Chromogenic substrate assay for FVIII (utilizing human protein reagents)</td>
</tr>
<tr>
<td>Activated protein C resistance assay (aPTT-based)</td>
</tr>
</tbody>
</table>
aPTT-based clotting assays measure the total clotting time of the intrinsic pathway of coagulation, including activation of FVIII to FVIIIa by thrombin; emicizumab does not require activation by thrombin and will therefore result in a supra-physiologically short clotting time. Therefore, OSA performed in the presence of emicizumab would be expected to grossly overestimate FVIII:C. While this is less clinically relevant in the inhibitor population for whom laboratory monitoring of FVIII:C is not generally clinically useful, standard inhibitor assays and monitoring of use of FVIII in low-titer inhibitor patients with breakthrough bleed events is not feasible with local laboratories.

5.3 | Quantifying hemostasis with emicizumab

The current FDA-approved CSA products commercially available in the U.S. are unable to assay the hemostatic impact of emicizumab as they use bovine coagulation proteins and thus are unable to detect emicizumab, a humanized antibody. Therefore, while these assays cannot be used to estimate emicizumab activity, they could be used to quantify both exogenous and endogenous FVIII activity even in the presence of emicizumab, for example in the noninhibitor patient population. Use of emicizumab in this population will require thoughtful education of not only hemophilia providers but also patients, families, and other medical providers (particularly in operative and emergency settings) and careful communication with the local coagulation laboratory about available and appropriate assays.

In contrast, human CSA reagents are available in the research laboratory setting and thus can provide a readout of FVIII activity that includes the emicizumab contribution. Available data suggests the measured FVIII activity in patients on emicizumab overestimates the patient's actual hemostatic potential. With co-administration of emicizumab and FVIII concentrates, hemostasis shortly after FVIII infusion are likely to be driven by the exogenous FVIII because at high concentrations FVIII will outcompete emicizumab for FIXa binding (FVIIIa binds FIXa with a K_D of approximately 15 nM, in comparison with emicizumab, which has a much lower affinity for FIXa, binding it with a K_D of 1.5 μM). The duration of this effect will depend on the individual's FVIII clearance which is highly variable among patients and often not known a priori.

6 | NOVEL APPROACHES TO ASSAYING HEMOSTASIS

The global hemostasis assays including thrombin generation assays and viscoelastic assays may ultimately emerge as an option for the monitoring of hemophilia patients, particularly those treated with emicizumab or other nonfactor hemostatic agents currently in early phase clinical trials. The reported clinical utility of these assays varies in part due to poor standardization of protocols and dependence on skill of individuals performing the assays as well as the intended application of the results. Novel strategies to capture hemostatic capacity in the broader context of the vessel microenvironment incorporating the complexity of interactions between endothelial cells, platelets, and coagulation factors are necessary. Microfluidic technologies may also provide an opportunity for point of care testing and reduction of blood volume required.

7 | CONCLUSIONS

The aPTT-based OSA is the primary assay used for routine monitoring and diagnosis of patients with hemophilia; however, use of CSA is gaining in popularity given the expanding armamentarium of factor concentrates available and the numerous product-specific considerations to keep in mind when using different analyzers and reagents for OSA. First, some of the new bioengineered factor products and now the novel hemostatic agents such as emicizumab cannot be accurately measured by OSA and in some cases CSA. Knowing the specific situations in which OSA is unreliable and CSA should be used is critical for the safe use of factor concentrates in the modern era. Dialogue to share knowledge of aPTT reagents and patient factor products used locally benefits both hemophilia providers and the coagulation laboratory team. Emicizumab is a breakthrough therapeutic in the management of patients with hemophilia A, particularly those with inhibitors, but it renders standard coagulation assays used in this population unreliable and therefore unusable for clinical care, particularly in emergent clinical settings. As data emerge for potential use of emicizumab in the noninhibitor hemophilia A population, recognition of its impact on routine clinical laboratories will become even more critical to ensure safe, effective care. New assays or modifications of currently employed assays are on the horizon, potentially including global hemostasis assays and thrombin generation assays, to describe the hemostatic potential of individual patients and optimize the management of hemophilia patients receiving novel therapeutics.

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AUTHOR CONTRIBUTIONS

H. Al-Samkari wrote the first draft and subsequent revisions of the manuscript and contributed to concept and design, critical writing of the intellectual content, and final approval; S.E. Croteau contributed to concept and design, critical writing and revising the intellectual content, and final approval.

ORCID

Hanny Al-Samkari http://orcid.org/0000-0001-6175-1383
Stacy E. Croteau http://orcid.org/0000-0001-6122-6166

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