Transfusion and Apheresis Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Novel assays in the coagulation laboratory: a clinical and laboratory perspective

Jennifer Teichman[a], Hina Razzaq Chaudhry[b], Michelle Sholzberg[a,b,c,*]

[a] University of Toronto, Department of Medicine, Toronto, Canada
[b] St. Michael's Hospital Hemostasis and Thrombosis Laboratory, Toronto, Canada
[c] Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Canada

ARTICLE INFO

Keywords:
Hemophilia
Bleeding disorders
Factor VIII
Factor IX
Coagulation
One-Stage assay
Chromogenic assay
Thromboelastography
Thromboelastometry

ABSTRACT

The ability to monitor Factor VIII (FVIII) and Factor IX (FIX) levels is integral to the clinical management of hemophilia A and B patients, respectively. Factor activity levels are checked during regular follow-up, post-infusion of factor concentrates, during pre- and post-operative assessments, and when the presence of an inhibitor is suspected. However, the ability to accurately and reproducibly measure factor activity levels with standard coagulation assays has been challenging due to the emergence of recombinant factor concentrates with extended half-lives. Similarly, special considerations must be given to the type of inhibitor assay used in patients with acquired hemophilia receiving recombinant porcine FVIII replacement. Alternative approaches to achieve hemostasis with clotting factor mimetics and interference of endogenous anticoagulants lack standardized assays for monitoring hemostatic efficacy. Laboratory assays measuring dynamic clotting parameters such as thrombin generation or whole blood viscoelasticity may provide a way forward, but have yet to enter routine clinical use. This review highlights the role of specialized coagulation assays in an era where multiple new hemostatic therapeutics for hemophilia are available, and underscores the need for clear communication between bedside and laboratory clinicians.

1. Background

Hemophilia is an inherited bleeding disorder characterized by deficiencies of coagulation FVIII or FIX for Hemophilia A (HA) and B (HB), respectively. The hemophilia affect approximately 400,000 people worldwide [1]. Integral to the clinical management of HA and HB patients is the ability to monitor FVIII and FIX levels, respectively [2]. Factor activity levels are checked during regular follow-up, post-infusion of factor concentrates, during pre- and post-operative assessments, and when the presence of an inhibitor is suspected. Management of bleeding was historically achieved using factor concentrates from human donor blood plasma, and subsequently from recombinant methods. Recombinant factor concentrates with extended half-lives (EHL) have entered clinical use in the past few years, but show wide variability in potency when measured using factor assays of different methodologies and/or reagents. Despite this, the one-stage aPTT-based assay remains the most widely available test, with considerable heterogeneity in materials and methods used across laboratories [3]. To further complicate the landscape, quantification of inhibitor titers becomes critical in patients who develop neutralizing anti-FVIII or anti-FIX antibodies, yet special considerations must be given to the type of assay used in such patients receiving porcine FVIII replacement. We review the changing landscape of hemophilia treatment and the corresponding coagulation laboratory adjustments that are required.

2. Laboratory measurements of FVIII and FIX

2.1. Technical aspects

There are generally two different functional assays used to measure

Abbreviations: aFVII, activated FVII; AHA, acquired hemophilia A; anti-hFVIII, anti-human FVIII; aPCC, activated prothrombin complex concentrate; aPTT, activated partial thromboplastin time; AT, antithrombin; AUC, area under the curve; BPA, bypassing agent; BU, Bethesda unit; CAT, calibrated automated thrombogram; EHL, extended half life; ELISA, enzyme linked immunosorbent assay; FVIII, factor VIII; FIX, factor IX; HA, hemophilia A; HB, hemophilia B; HRQoL, health related quality of life; N8-GP, glycopegylated Novo Eight; NPP, normal pooled plasma; ROTEM, rotational thromboelastometry; rpFVIII, recombinant porcine FVIII; TEG, thromboelastography; TFPI, tissue factor pathway inhibitor (TFPI); TGA, thrombin generation assay; VWF, von Willibrand factor

* Corresponding author at: Li Ka Shing Knowledge Institute, St Michael's Hospital, University of Toronto, 30 Bond Street, Cardinal Carter Wing Room 2-007G,Toronto, ON, M5B1W8, Canada.

https://doi.org/10.1016/j.transci.2018.07.008

1473-0502/ © 2018 Elsevier Ltd. All rights reserved.

FVIII and FIX activity: the one-stage clotting assay and the chromogenic assay [4]. The one-stage clotting assay is the most widely used and measures the ability of patient plasma to shorten the activated partial thromboplastin time (aPTT) of a FVIII or FIX deficient plasma (obtained by immunodepletion or from a patient with severe hemophilia). The deficient plasma and patient sample are pre-incubated with the aPTT reagent containing a contact activator (e.g. ellagic acid, kaolin, silica or celite) and phospholipids; the fibrin clot formation is measured by the addition of calcium chloride. The FVIII or FIX concentration in the patient sample is assumed to be the rate-limiting determinant of the clotting time. The result is interpolated from a standard curve generated from samples containing known FVIII or FIX concentrations [5,6]. The advantages of the one-stage assays are the widespread availability of reagents, historical experience and familiarity with the techniques, and the ability to create a low-calibration curve for monitoring of low factor levels [7]. However, the wide range of available aPTT reagents – each differing in phospholipid concentrations and activators—as well as the different clot detection techniques can lead to inter-laboratory variability in measurements.

The chromogenic assay is similar to the two-stage aPTT assay and has largely replaced it. The chromogenic FVIII assay consists of adding patient plasma (containing an unknown amount of functional FVIII) to a reaction mixture of thrombin or prothrombin, FIX FX, calcium and phospholipid. This produces FVIIIa a cofactor to FIXa which activates FX. When the reaction is stopped by adding ethylenediaminetetraacidic acid and a chromogenic substrate, FXa production is assumed to be proportional to the amount of functional FVIII present in the patient sample derived from a standard curve [4–6].

The chromogenic FIX assay requires two time-dependent steps; first, diluted patient plasma is mixed with a reagent containing FVIII and FX. The reaction is initiated by adding FXIa, thrombin, calcium and phospholipids. FXIa activates FIX if present, thrombin activates FVIII and subsequently, the complete FX activating complex (FIXa, FVIIIa, calcium ions and phospholipids) rapidly activates FX. Second, the reaction is stopped by adding ethylenediaminetetraacidic acid and a chromogenic substrate. The formation of FXa is measured and is proportional to the plasma FIX concentration in the patient sample.

2.2. One-stage and chromogenic assays in the era of extended half-life recombinant factor concentrates

The chromogenic assay may be more useful than a clot-based activity assay in several scenarios. First, the chromogenic FVIII and FIX activity does not show interference from a lupus anticoagulant (LA), making it an ideal test for accurately measuring FVIII or FIX activity in patients with a LA. Second, a subset of mild hemophilia A patients with missense mutations demonstrate higher FVIII activity in one-stage compared to chromogenic FVIII activity assays [8,9]. Interestingly, the bleeding phenotype in these patients appears more closely related to the lower chromogenic-defined FVIII activity [5]. Conversely, in mild hemophilia B patients, one-stage and chromogenic assays produce discrepant FIX levels, with the chromogenic assay producing a higher result and more closely correlating with bleeding phenotype [10]. Finally, chromogenic assays have emerged as the preferred method for measuring many EHL FVIII and FIX replacement products.

Historically, both one stage and chromogenic assays have performed well with native human-derived FVIII, FIX, and full-length recombinant products [11]. However, EHL recombinant factor concentrates pose challenges to the laboratory monitoring of post-infusion levels [12]. These products contain molecular modifications (B-domain deletion (BDD) or truncation, fusion with the Fc region of IgG or with albumin, linkage to polyethylene glycol) intended to extend the half-life and thereby reduce the frequency of injections. Many of these truncated products show wide variation in measured activity levels between different one-stage assays, and between one-stage and chromogenic assays [13]. For example, BAY 94–9027, a BDD rFVIII with site-specific PEGylation shows underestimation of factor activity levels in one-stage assays using silica-based aPTT reagents compared to ellagic acid reagents, presumably due to interference of the PEG moiety in silica activation of the contact pathway [14]. AFSTYLA is a novel rFVIII with fusion of the heavy and light chains that enhances binding to von Willebrand Factor (VWF). During development of AFSTYLA, an approximate 50% discrepancy in measured activity levels was noted between one-stage and chromogenic assays [15–17]. Subsequent clinical studies confirmed consistent and reproducible underestimation of AFSTYLA by one-stage assays [18], and utilization of a 2-fold conversion factor has been proposed where chromogenic assays are not readily available [19].

However, not all recombinant products have this issue. Glycopegylated Novo Eight (N8-GP), a b-domain truncated rFVIII appears to have acceptable recovery across various one-stage assays with the exception of one reagent [20]. Similarly, N8-GP levels are consistently measured across six distinct chromogenic assays. [21].

A comprehensive review of assay performance among each EHL factor concentrate is beyond the scope of this paper, and can be found elsewhere [7]. However, in general one-stage and chromogenic assays can vary in their estimations of factor activity levels by 20–50% for most products [22]. This discrepancy can be clinically relevant particularly in individuals undergoing treatment for a major bleed or surgery. In fact, the National Hemophilia Foundation recommends that laboratories routinely performing factor assays for hemophilia patients should strongly consider using chromogenic assays [23]. Despite this, a recent survey of coagulation laboratory scientists from seven countries across Europe, Asia and North American revealed widespread use of one-stage aPTT assays with a lack of consistency in materials and practices used to test for factor activity in hemophilia [3]. Chromogenic assays were available in 68% of laboratories, but only 23% reported frequent use [3].

The current era of new and emerging therapies for hemophilia demands an integration of clinical and laboratory perspectives in the management of these patients. Laboratories will require systems in place for identifying the product being measured. In tandem, clinicians must be aware of the performance characteristics of each assay as they pertain to the particular replacement product prescribed to the patient. Furthermore, baseline factor activity levels should be interpreted with the patient's bleeding phenotype in mind.

3. Inhibitor assays

A major complication of congenital Hemophilia is the development of inhibitory alloantibodies following exposure to factor replacement therapy. These mainly IgG antibodies bind to functional domains on FVIII or FIX and inhibit their coagulant activity [24,25]. While inhibitors to FIX are uncommon (occurring in approximately 3% of HB patients), anti-human FVIII (anti-hFVIII) antibodies develop in approximately 30% of patients with severe HA following exposure to factor replacement. The presence of an inhibitor makes bleeding episodes considerably more difficult to control, with poor response to standard doses of clotting factor replacement [26]. As a consequence, compared to those without inhibitors, those with inhibitors have more frequent, poorly controlled hemarthroses resulting in more severe joint disease, are at greater risk for life- or limb-threatening hemorrhage, and have reduced health-related quality of life (HRQoL) [27–29].

De novo inhibitors to FVIII can also manifest in non-HA patients, in a rare condition called acquired hemophilia A (AHA). The majority of AHA cases are idiopathic, while the remaining are associated with autoimmune disease, malignancy, and the post-partum state [30,31]. The bleeding phenotype of AHA differs from congenital, with soft tissue bleeding being more common than hemarthroses.

Download English Version:

https://daneshyari.com/en/article/10218347

Download Persian Version:

https://daneshyari.com/article/10218347

Daneshyari.com