Hemophilia management: Tips on monitoring modified replacement therapies

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April 2017—Some modified recombinant factor VIII and IX products for hemophilia prophylaxis show significant reagent-dependent recovery in the one-stage assay, while recovery in the chromogenic assay appears to be more consistent, especially for modified recombinant factor IX. The variable results can lead to over- or underestimating the factor level, warn Stefan Tiefenbacher, PhD, of Colorado Coagulation, and Rajiv K. Pruthi, MBBS, of Mayo Clinic.

They spoke in a recent webinar, hosted by CAP TODAY and sponsored by Novo Nordisk, on the laboratory diagnosis of hemophilia and the pitfalls associated with monitoring factor VIII and IX replacement therapy.

Dr. Tiefenbacher

Dr. Tiefenbacher reviewed the status of five modified recombinant factor VIII products: Bax 855 (full length), rFVIII-Fc (B-domain deleted), and CSL627 (B-domain deleted), which have been FDA approved; and N8-GP (BDtrunc PEGylated) and BAY 94-9027 (B-domain deleted), which are in late-stage development.

"Approaches to extend the half-life and to decrease the immunogenicity of these factor VIII products include changes to the protein expression systems, such as, for example, the use of human instead of animal cell lines as well as modifications to the actual therapeutic protein, such as pegylation or Fc fusion," said Dr. Tiefenbacher, who is technical director and vice president of Colorado Coagulation, Englewood, Colo., a member of the LabCorp Specialty Testing Group.

Three of the products—Bax 855, N8-GP, and BAY 94-9027—use attachment of hydrophilic polyethylene glycol chains to increase the molecular site or mass of the therapeutic protein, thereby reducing glomerular filtration and hepatic clearance through the LRP receptor. The Fc-fusion product uses recombinant DNA technology to link a therapeutic protein such as a recombinant factor VIII to the Fc region of IgG1, allowing the therapeutic protein to bind to the neonatal Fc receptor and thereby protecting the protein from the lysosomal degradation pathway.

"Last but not least, there is also a protein sequence modification in which a covalent bond is introduced between the heavy and light chain of factor VIII, resulting in a single chain circulating factor VIII molecule that demonstrates improved affinity to von Willebrand factor and therefore is thought to demonstrate improved stability compared to native factor VIII," Dr. Tiefenbacher said. The half-life extensions that these modifications achieve vary from 1.2-fold for the single chain modification (when compared with a recombinant full-length factor VIII) to 1.5- to 1.6-fold for the pegylated and Fc-fusion modifications.

On the factor IX side, there are three new modified products: rFIX-Fc and CSL654, both of which are approved in the United States; and N9-GP. "Similar to the factor VIII side, modifications to extend the half-life of the IX protein include Fc fusion utilized in factor IX Fc, or pegylation—in this particular case, glycopegylation of recombinant factor IX, which is utilized in N9-GP," Dr. Tiefenbacher said. Referring to the CSL654 product, he added, "Here we, in addition, have a recombinant factor IX protein that is genetically fused to recombinant albumin, which results in

half-life extension due to [the] size and long half-life of the albumin." That effect is related to albumin's interaction with the neonatal Fc receptor, "protecting the therapeutic protein from the lysosomal degradation pathway, similar to the mechanism of halfway extension for Fc fusion."

Whereas factor VIII's half-life is largely determined by von Willebrand factor, the half-life extension achieved with these modifications is much greater for factor IX. That extension ranges from 2.4-fold for the recombinant factor IX Fc-fusion protein to fivefold or more for the glycopegylated and albumin fusion protein.

Dr. Tiefenbacher presented his own laboratory's in vitro data to demonstrate the aPTT-reagent dependent recovery that occurs when measuring some of these modified recombinant factor proteins in commonly used IVD approved one-stage factor assays. The laboratory compared the recovery of four recombinant factor IX replacement products, one of which was the established recombinant factor IX product BeneFIX. Recovery was compared over the reportable range of the factor assay between 80 and one percent factor IX activity, and the samples were created by spiking the recombinant factor into congenital factor IX deficient plasma.

The results showed that, consistent with the existing literature, glycopegylated factor IX (N9-GP) is significantly overestimated (recovering at around 1,000 percent from expected) when tested using a silica-based aPTT reagent. In contrast, "the other modified factor IX product recovers appropriately in this reagent," Dr. Tiefenbacher said. "If a laboratory uses this particular silica-based reagent [to measure N9-GP], it will greatly overestimate the activity, and this would likely result in significant under-dosing and mismanagement of the patient.

"On the other hand," he continued, "when glycopegylated factor IX is tested in one of the commonly used ellagic acid-based aPTT reagents, the product under-recovers at around 50 percent of expected." This underestimation, he said, "would likely result in unnecessary use of additional product that is not needed."

The lesson in all this: The recovery for at least some of the modified recombinant factor IX products can vary greatly according to the aPTT reagent used for factor activity determination, and that can result in both over- and underestimation of factor level, depending on the factor IX replacement product and the particular aPTT reagent used.

"On the other hand," Dr. Tiefenbacher pointed out, "when we look at the recovery of the same recombinant products in a chromogenic factor IX assay, you can immediately see that the modified and the undisclosed, as well as the established recombinant product [BeneFIX], all recover within about 25 percent of each other." The two modified recombinant factor IX products recovered within established limits across the entire concentration range tested, while BeneFIX, in accordance with other reports in the literature, "slightly under-recovered" across all the concentrations tested.

Dr. Tiefenbacher mentioned, too, that all of the recombinant factor IX products that the laboratory tested in this study recovered at 100 percent at the one percent factor activity level. "This might actually have been an artifact of the assay setup and protocol for this particular factor IX chromogenic assay on this specific coagulation analyzer," he said. "Additional testing will have to be performed to determine whether the values at the one percent factor activity levels are valid or not."

Regarding the recombinant factor VIII product, most products—including the B-domain deleted and some of the modified recombinant factor VIII products—recover within about 25 percent of expected in the silica activated PTT reagent. That said, one of the modified recombinant VIII products under-recovers at 50 percent of expected in the silica activated PTT, but recovers appropriately in the ellagic acid-activated PTT.

He pointed out that the B-domain deleted recombinant factor VIII products in the two aPTT reagents shown did not show reagent dependent under-recovery, as previously reported for Refacto, and thus could be expected to be found for both modified and unmodified recombinant B-domain deleted factor VIII. "Also, the established full-length recombinant factor VIII product slightly over-recovers in the ellagic acid-activated PTT used in our study," he added.

Meanwhile, recovery of the recombinant factor VIII products in the factor VIII chromogenic assay was more variable, with both of the modified recombinant products demonstrating over-recovery across the factor activity concentration range tested. That's in contrast to some of the existing published data that suggest all modified recombinant products can be measured adequately in the chromogenic assay.

"I should also point out," Dr. Tiefenbacher said, "that the over-recovery observed for the two modified products could be related to the over-recovery that was observed for the SSC standard, which is the secondary standard to the WHO 6 international standard that was run as a control to verify the assigned value of the plasma calibrator."

Dr. Tiefenbacher reviewed the challenges clinical laboratories face when using the existing factor activity assays to measure some of these modified recombinant factor products. One of those challenges pertains to the difference between potency assays (those used to assign potency to a factor product) and the clinical factor activity assay used in the laboratory to measure the product.

Factor potency assays commonly use a product-specific standard, a calibrator, that has been verified against the WHO concentrate international standard, whereas clinical laboratories often use a pooled normal plasma standard that the manufacturer has verified against the WHO plasma international standard. With a product-specific standard—but *not* with a pooled normal plasma standard—any potential nonlinearities for a modified product in a particular reagent system are likely to be masked.

For factor VIII products, potency assignment in accordance with recommendations of the European Pharmacopoeia is commonly performed using the factor VIII chromogenic assay. To date in the U.S., factor product activity in clinical laboratories is still performed predominantly using a one-stage aPTT-based assay. To account for that potential difference, Dr. Tiefenbacher advises, it's important to make sure that the factor assay used for post-infusion monitoring aligns with either (or both) the assay used to assign the potency to the product or the assay used to demonstrate clinical efficacy of the product in clinical studies. "This is often easier said than done," he pointed out, "as it is often not common knowledge what assay system and/or reagent was used during registration of a particular product."

A second challenge is the inherent variability that exists for one-stage factor VIII and IX activity assays between different clinical laboratories, as demonstrated in CAP Surveys and other proficiency testing. Significant interlaboratory variability (for example, around 30 percent at normal factor levels, with up to 70 percent at factor levels below 20 percent) for both the factor VIII and IX one-stage assay has been observed.

In a recent CAP Survey, he said, "a normal plasma sample with an anticipated value of 100 percent yielded results ranging between 72 and 161 percent," depending on the aPTT reagent used and the laboratory performing the testing. That inherent variability in the one-stage factor activity assays only makes it more difficult to evaluate and interpret factor activity data for the modified products when generated across different one-stage assay systems and laboratories.

Yet another challenge: Data are limited regarding the behavior of some of the modified recombinant factor replacement products in the one-stage and chromogenic factor assay reagents commonly used in U.S. clinical labs. In fact, such data are currently available for only two of the five modified recombinant factor VIII products, namely, Bax 855 and factor VIII Fc, and for only one of the three modified recombinant factor IX products for factor IX Fc. Ideally, information regarding whether a modified replacement product demonstrates aPTT reagent dependent recovery would be addressed before the product is launched so it can be included on the product label as required.

Finally, although the medical and scientific advisory council of the National Hemophilia Foundation recommends the use of chromogenic factor activity assays for monitoring the modified products, factor VIII and IX chromogenic assays are still infrequently used in clinical laboratories. "Only three of the five available factor VIII chromogenic assays—more specifically the Coatest SP, the Coamatic, and the Siemens factor VIII chromogenic assay—are currently IVD approved for clinical use in the U.S.," Dr. Tiefenbacher said. "Of these three, only the Siemens [assay] is IVD approved for use on an automated coagulation platform. The remainder of the factor VIII

chromogenic kits are currently IVD approved for manual plate-based use only."

On the factor IX side, only two chromogenic assays are currently marketed in the U.S., neither of which the FDA has evaluated and both of which are thus RUO-labeled. Furthermore, for the factor IX chromogenic assays, only limited validated instrument applications and/or coagulation instrument protocols are currently available, restricting their use to what he called "more expert-level labs."

Dr. Rajiv Pruthi, director of Mayo Clinic's Comprehensive Hemophilia Center in Rochester, Minn., used two case examples to illustrate potential issues that laboratories may encounter when monitoring the new modified recombinant factor concentrates. Prophylaxis via scheduled administration of concentrates has become the standard of care for hemophilia management, and patients are generally taught to self-administer factor concentrates at home.

Dr. Pruthi

"The half-life of the current generation of unmodified factor concentrates varies," said Dr. Pruthi, who is also an associate professor, Mayo Clinic College of Medicine, and co-director of Mayo Clinic's special coagulation laboratory. For factor VIII, the half-life is between eight and 12 hours, whereas the half-life for factor IX is between 18 and 20 hours. "Based on this, the typical practice is to infuse the unmodified factor VIII concentrate about three times weekly, and the factor IX concentrate is typically administered two times weekly."

The target trough factor level is usually greater than one percent. "Typically, we like to target between one and five percent," Dr. Pruthi said. "Targeting that level completely changes the frequency of bleeding that severe hemophilia patients experience." However, there's wide variability between patients in the half-life of these factors. To provide the most cost-effective therapy, individualized pharmacokinetic studies are usually performed so the dosing can be tailored to each patient.

He shared a slide illustrating the results of a typical pharmacokinetic study for an unmodified and a modified factor VIII concentrate. In this trial, the half-life of the standard factor VIII was compared with that of a modified factor VIII. The data illustrate the time it takes for the factor levels to decrease from a post-infusion level of about 100 percent to a trough of between one and three percent. For the unmodified concentrate, it takes about three days to reach that level; for the modified factor VIII concentrate, it takes about five days. It takes about four days for the standard factor IX concentrate to get down to between one and three percent, but with the modification of the factor IX molecule, the time to a trough level of one to three percent is extended to about 10 days.

"There is a wide inter-individual variability," Dr. Pruthi reminded the audience. "One of the consequences of inaccurate measurements is that with under-dosing of the factor concentrate, you may increase the risk of bleeding. However, if you over-dose the factor concentrate, you may increase the risk of thrombosis. You certainly will be increasing the cost of care."

He presented the case of a 20-year-old male with severe hemophilia A who was switched to a modified factor VIII concentrate, and whose pre-infusion baseline factor level was less than one percent (normal range, 55 to 200 percent). "We calculated the dose he would require to target a peak factor VIII level of approximately 60 percent," Dr. Pruthi explained. "However, when we measured his post-infusion level, it was actually only measured at 30 percent using the one-stage assay. So when this happens, there are several questions one has to address." For example: Were the sample collection and transportation done correctly? Was the assay the right assay for this

concentrate? Did he receive the ordered dose?

“Once the preanalytic and analytic aspects of the assay have been investigated and the assay’s result is not felt to be erroneous, the typical next step is to increase the dose of the recombinant factor concentrate and recheck the pharmacokinetics,” he continued. “Now, if that result was inaccurate and we would be increasing the dose, then definitely we would be increasing the cost of care and potentially putting the patient at a higher risk of thrombosis.”

His team realized the reagents used for the one-stage assay performed on this plasma sample underestimated the true factor level by about 50 percent for this modified factor concentrate. In fact, the package insert for the modified concentrate recommends that the one-stage assay result be multiplied by a factor of two. In other words, the patient was on the right dosage—it was just that the one-stage assay result had to be multiplied by two. The result of a chromogenic factor VIII assay confirmed such.

In the second case, a 12-year-old male with severe hemophilia B was referred to Mayo Clinic’s hemophilia center for help switching to the new modified recombinant factor IX concentrate. His dosing was calculated to achieve a trough level of five percent, and indeed his pre-infusion factor IX was five percent. An hour post-infusion, he reached a peak level of 80 percent.

“So the patient was referred back to his local care provider with the advice that the pharmacokinetics should be rechecked at some point, and between going back to his provider and coming in for a recheck of his pharmacokinetics, the patient experienced no bleeding events since initiation of the prophylaxis,” Dr. Pruthi said.

But when the pharmacokinetics were rechecked at one point, the results—obtained in a local laboratory—demonstrated that his pre-infusion or trough level was less than one percent, while his post-infusion peak level was only 40 percent.

The patient’s primary care provider had planned to increase the dose of the modified factor IX concentrate but contacted the Mayo Clinic hemophilia center for advice. The center determined that the local laboratory was using an aPTT reagent based on a kaolin activator for the one-stage assay, and this kaolin activator was known to underestimate the true factor IX level for this particular product. A sample was mailed to the laboratory affiliated with the hemophilia center, which confirmed the results of the original pharmacokinetic study.

“So the underestimation of the true factor level has a significant consequence,” Dr. Pruthi stressed. “You may be increasing the dose of factor infusion, overdosing the patient, increasing the cost, and putting the patient at risk for thrombotic complications. Whereas if you overestimate the true factor level, the potential consequence is you would reduce the dosage of the factor concentrate and potentially increase the risk of bleeding.”

This is a complex situation with multiple potential solutions, some more practical than others. For example, each laboratory could have an individualized calibrator for each concentrate for which it will potentially perform assays. However, the information regarding which concentrate the patient is on might not be communicated to the laboratory. And maintaining assays with different calibrators poses special challenges to both low- and high-volume laboratories.

“What about chromogenic assays?” he said, referring to Dr. Tiefenbacher’s outline of the available factor VIII kits. “There are no currently FDA-approved factor IX kits. And so each lab would have to validate a kit as a laboratory-developed test, which poses unique regulatory challenges and is very time-consuming and expensive.” Finally, one could multiply the one-stage assay result by a correction factor, as one of the cases showed. “However, each hemophilia care provider would have to be aware of such recommendations to ensure that the correct correction factor is being applied.”

Dr. Pruthi concluded by stressing again that exclusively using one type of assay may lead to misclassification of non-severe hemophilia or even a missed diagnosis, and when monitoring factor concentrates, may lead to over- or

underestimating factor levels. “Hemophilia care providers should be made aware of these assay-related issues,” he said, so as to avoid risking incorrect dosage adjustments of the factor concentrates.
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