

The Pathophysiology, Pathogenesis, and Management of Inhibitors in Patients with Hemophilia A and B

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In addressing the topic on the pathogenesis and management of inhibitors in patients with hemophilia, one must begin by addressing the risk factors for inhibitor development, the incidence of inhibitors, the type of antibody response, the kinetics of inhibitor neutralization of either Factor VIII and IX, the epitopes of Factors VIII and IX recognized by the inhibitors, the mechanism of action of the inhibitors, their diagnosis, and, finally, the management of patients with bleeding who have inhibitors to either Factor VIII or IX.

The factors that play a role in inhibitor development include the severity of the hemophilia. Most of the patients who developed inhibitors to either Factor VIII or IX have either severe hemophilia A or severe hemophilia B, respectively. The genetic abnormalities underlying severe hemophilia usually include deletions, which is particularly true in Factors VIII and IX. In Factor VIII deficiency, however, the inversion and crossing over of intron 22 is a major cause of inhibitor development, and this accounts for a large number of severe hemophiliac patients. There is some correlation with HLA types, but this has not been clearly delineated. Other genetic risk factors must be present since there is an increased incidence of inhibitors in brother pairs. It is also known that local factors such as sepsis and inflammation may also influence the development of inhibitors. In patients with either Factor VIII or IX, all inhibitors develop after transfusion of Factor VIII- and IX-containing products, respectively.

There have been one or two outbreaks of inhibitor development that have obviously been caused by the type of product used. The prototype of this kind of inhibitor development is exemplified by the report from Belgium, where it was noted that a Dutch Red Cross concentrate resulted in an increase in inhibitor development significantly above the usual and a return to baseline values after cessation of use of this particular concentrate.

The incidence of inhibitors to Factor VIII have been a matter of controversy recently, especially following the development of new recombinant Factor VIII products. The incidence of Factor VIII to inhibitor development has been reported to range from 2% to over 40%. But in an extensive study of 451 patients observed over 18 years who were receiving intermediate- to high-purity Factor VIII concentrates, the incidence of inhibitors was about 20% in hemophilia A patients. Currently there is no real evidence that there is an increased risk of inhibitor development using the newer recombinant Factor VIII concentrates.

The incidence of inhibitors in hemophilia B is about

1–3% of severely affected patients. Again, these patients have all received Factor IX, either in plasma, intermediate-purity concentrates, or even high-purity concentrates. Some of these patients who developed inhibitors also may develop anaphylactic reactions as well as the nephrotic syndrome. This is significantly different from the inhibitors in hemophilia A in which neither anaphylaxis nor the nephrotic syndrome has been reported.

The type of antibody response in hemophilia A and B is virtually identical. Mostly the antibodies consist of the immunoglobulin G class and of the kappa light chain type. However, it should be noted that immunoglobulin classes A and M have been noted along with either kappa- or lambda-light chains. In both hemophilia A and B, there may be some restriction in antibody response. For example, in many cases, the antibody responses are of the IgG-4 kappa light chain type. Even though the antibody response may be restricted, the antibodies are not monoclonal since INV and GM markers are different in the antibodies.

Inhibitor kinetics in the Factor VIII-deficient patients who develop these antibodies have been interesting. These antibodies do not cause immunoprecipitation of the factor VIII antigen. The inhibition of Factor VIII by these antibodies is quite slow, requiring incubation of the clotting test. The inhibitors to Factor VIII are dependent on time, temperature, and pH. To identify a Factor VIII inhibitor, one mixes inhibitor plasma with normal plasma and incubates for two to four hours at 37 °C and measures the disappearance of Factor VIII over that time period. The reason for the time- and temperature-dependence of the Factor VIII inhibitors is because of the low concentration of the Factor VIII antigen and its association with the von Willebrand factor protein. Factor IX inhibitors, on the other hand, are not time- and temperature-dependent, even though many times they are measured in the same way as Factor VIII inhibitors, that is, after incubation for two hours at 37 °C.

The epitopes recognized by the Factor VIII antibodies are generally on the A2 and the C2 domain. The epitope on the A2 domain has been recognized to be between the arginyl residue at position 484 and the isoleucyl residue at position 508 in the A2 domain. The epitope of the C2 domain is between residues 2303 and 2332. In addition, some antibodies have interfered with the arginyl 322 thrombin cleavage site. However, the main mechanism of inhibition of the Factor VIII function is to inhibit the tenase complex or the inhibition of binding to phospholipids. The epitope on Factor IX has not been as clearly identified, but at least one epitope, known from the work of investigators in Chapel

Hill, is between residues 155 and 176 of the protein.

Inhibition of Factor IX Inhibits Tenase Activity of the Factor IXa Molecule

The diagnosis of inhibitors of either hemophilia A or B can be suspected, based on the lack of response of the patient's bleeding episodes to infusions of either Factor VIII or IX, respectively. For laboratory diagnosis, the usual approach is to take one part of the patient's plasma and one part of normal plasma and incubate for two hours; if this is prolonged, one can suspect an inhibitor. One should always be sure that the inhibitor that is seen in these patients is specific for the clotting factor such as Factor VIII or Factor IX. To do this, one must take a dilution of the patient's inhibitor plasma, add it to normal plasma, and determine which specific factor is inhibited in the normal plasma. With the knowledge of specificity, one can then titrate both the Factor VIII and Factor IX inhibitors by using the Bethesda assay, that is, taking a dilution of the patient's plasma, which will neutralize 50% of a standard solution of either Factor VIII or Factor IX in normal plasma after two hours at 37 °C. This is one Bethesda unit. When this is multiplied by the dilution, one can get the total number of Bethesda units inhibited. Other units of inhibitor activity, the Oxford unit and the Malmö unit. There is also the new Nijmegen assay, which is similar to the Bethesda assay except that during the two-hour incubation the plasma is buffered so the pH does not change.

One can also specifically measure these antibodies using enzyme-linked immunosorbent assay. The clinical management of inhibitors need not be complex, but for successful management, one needs to know whether the patient is a high or low responder. A high responder is arbitrarily defined as that patient whose inhibitor titer increases above ten Bethesda units after challenge with either Factor VIII or Factor IX antigen. A high responder can present clinically with bleeding with an inhibitor titer below ten Bethesda units and sometimes undetectable inhibitor. This often occurs when the patient has not been treated with the Factor VIII or IX for a number of months. However, after challenge with antigen, a high responder will always have an anamnestic response after three to five days to above ten Bethesda units per milliliter. A low responder is, by definition, either a hemophilia A or B patient whose inhibitor titer does not increase above ten Bethesda units even after challenge with Factor VIII or Factor IX, respectively. However, it is important to note that some historically low responders will occasionally convert to high responder status.

Once one can establish whether a patient is a high or low responder, one then needs to establish what the patient's

initial titer is as well as the severity of the bleeding episode. In the case of low responders with a bleeding episode considered minor, such as a simple hemarthrosis, the best form of treatment is to use Factor VIII or IX inhibitor bypassing agents such as prothrombin complex concentrates or activated prothrombin complex concentrates. In those countries where Factor VIIa is available, the activated partial thromboplastin time is perhaps an even better approach than the prothrombin complex concentrates or the activated PCCs. One could also treat a low responder with very high doses of Factor VIII or porcine Factor VIII, but given the fact that some low responders occasionally convert to high responders after challenge with antigen, many clinicians will not treat a minor bleeding episode with these antigens. On the other hand, in a low responder with a major bleeding episode, it is best to begin with a large loading dose of human Factor VIII. This may require a loading dose of up to 15,000 units of Factor VIII as a bolus followed by 1,000 units of Factor VIII per hour. Such high doses of Factor IX may be also given to hemophilia B patients if they are low responders, but one should remember that the Factor IX concentrate should be pure and should not be Factor IX in prothrombin complex concentrates. In inhibitors of Factor IX, if one uses prothrombin complex concentrates, one should not go over 75 units per kilogram of body weight because of the danger of thromboembolic episodes.

High responders who experience minor bleeding episodes who are admitted with a low titer or a high titer can be treated with inhibitor bypassing agents. Most clinicians now prefer to treat these with preparations of recombinant Factor VIIa, although prothrombin complex concentrates or activated prothrombin complex concentrates may be tried. For major bleeding episodes, however, in high responder patients whose initial titer is low, one should administer human Factor VIII in high doses since one can achieve hemostatic levels of Factor VIII, for the anamnestic response occurs after three to five days. In high responder patients who present with a high titer inhibitor (above 10 Bethesda units), it is best then to determine whether the inhibitor crossreacts with porcine Factor VIII; if it doesn't, porcine Factor VIII in a dose of 50 to 100 units per kilogram of body weight should be administered. In those high responder patients who have a high titer inhibitor (above 10 Bethesda units), human Factor VIII will not be efficacious, although one might try high doses of porcine Factor VIII. In case the inhibitor crossreacts with porcine Factor VIII, one would then have to retry recombinant Factor VIIa, prothrombin complex concentrates, or activated prothrombin complex concentrates.