Molecular and clinical predictors of inhibitor risk and its prevention and treatment in mild hemophilia A

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Introduction

Mild hemophilia is an X-linked bleeding disorder defined by factor VIII or IX (FVIII/FIX) levels between 5 and 40 U/dL. Until recently, the disorder has received relatively little attention because of its purported low morbidity rate, resulting in an apparently uncomplicated quality and duration of life in the absence of virally induced comorbidities.1-3 In a recent cohort study, 23% of patients with mild hemophilia were positive for hepatitis C virus, a proportion substantially lower than in patients with severe hemophilia.3,4

In patients with mild hemophilia A (MHA), excessive bleeding usually occurs after minor trauma, dental procedures, or surgery. This is unlike patients with severe deficiency (FVIII < 1 U/dL), who frequently bleed spontaneously without preceding trauma.2 Diagnosis of MHA usually occurs later in life, and a significant proportion of cases may be diagnosed during subsequent family investigation.5 However, because of the delayed presentation of bleeding, sometimes these patients could be first seen by physicians who are not used to interpreting symptoms of bleeding.6 Thus, these symptoms could be more dramatic at a time of initial assessment, with the risk for intensive treatment with FVIII concentrates potentially increasing the risk for inhibitor development.

This review focuses on the emerging issue of inhibitor development in MHA, its molecular and clinical predictors, and preventive strategies and treatment.3,4

Inhibitor development risk in MHA: not so early, not so low

Some patients with MHA may develop inhibitory antibodies after treatment with FVIII concentrates, with a prevalence of 5% to 10%.5,7,8 When exposure days (ED) are taken into account, the risk for inhibitor development clearly increases with the number of ED to exogenous, therapeutic FVIII concentrates.5 The International Study on Etiology of Inhibitors in Patients with a Moderate or Mild Form of Hemophilia A, Influences of Immunogenetic and Hemophilia Treatment Factors (INSIGHT) study in a large population of patients with nonsevere HA, including a large proportion of MHA cases, showed that the inhibitor risk was 6.7% (95% confidence interval, 4.5%-8.9%) at 50 ED, rising to 13.3% (95% confidence interval, 9.6%-17%) after 100 ED.8 This indicates that inhibitor development may occur throughout the lifetime in MHA, in contrasting to patients with severe hemophilia A, who have the highest risk for inhibitor development at 10 to 15 days, which becomes almost negligible at 50 ED or more.4

Molecular and clinical predictors of inhibitor risk in MHA

FVIII missense mutations are the main cause of MHA, although about 5% to 10% of patients may have splicing defects, point deletions, deep intronic changes, or promoter mutations.9 Of interest, it has been definitively demonstrated that among more than 150 different causative missense mutations for MHA, some relatively frequent mutations are associated with a high risk for inhibitor development on replacement therapy.7,8,10 In particular, p.Arg612Cys (Arg593Cys) in the A2 domain and p.Tyr2124Cys (Tyr2105Cys) and p.Arg2169His (Arg2150His) clustered in the C1 and C2 domains of the light chain represent the most frequent mutations associated with this risk, with an inhibitor development risk after 20 ED from 0% to 9.1% of patients.7,8,10 However, some rarer mutations (p.Asp2093Gly [Asp2074Gly] and p.Trp2248Cys [Trp2229Cys]) are particularly important because the risk for inhibitor development at 20 ED (21.2% and 41.7%, respectively) parallels that of severe patients.8 It is not entirely clear why these particular mutations carry an increased risk for inhibitors. For some missense mutations occurring at particular residues of FVIII molecule (Arg2169, Arg2178 and Ala2220), it has been demonstrated that antibodies elicited by treatment with exogenous therapeutic FVIII concentrate can discriminate the therapeutic wild-type FVIII and the patient’s
endogenous FVIII, reflecting the specificity of the T-cell epitope.\textsuperscript{11,12} Recently, it has been suggested that the risk for inhibitor formation associated with FVIII missense mutations is significantly higher when amino acid substitution belongs to another physicochemical class than the original residue.\textsuperscript{13} However, the recent description of an association between an intronic mutation (IVS10-18 G$\rightarrow$A) and inhibitor occurrence after intensive replacement treatment and more than 90 ED again suggests that the pathogenesis may be heterogeneous.\textsuperscript{14} In conclusion, genetic testing at diagnosis would be useful for identifying subjects with high-risk mutations before planning FVIII replacement therapy.

Inhibitors may appear, especially after a period of intensive treatment or continuous infusion with FVIII concentrate, and no association with a particular concentrate is evident.\textsuperscript{15-17} Two retrospective Dutch studies\textsuperscript{17,18} demonstrated that p.Arg612Cys was a strong risk factor, together with intensive perioperative FVIII administration.\textsuperscript{17} The presence of an inhibitor in patients with MHA is generally suggested by a sudden change of the bleeding pattern. In a majority of the patients, the FVIII plasma levels are reduced below 1 U/dL, as the inhibitor cross-reacts with the patients’ endogenous FVIII.\textsuperscript{17} Bleeding may be severe and potentially life-threatening. It often occurs in muscles and joints, but large cutaneous bruising and gastrointestinal and urogenital bleeding may occur, as in acquired haemophilia.\textsuperscript{17}

**Inhibitor treatment**

Inhibitor disappearance may occur spontaneously when no further treatment with FVIII concentrates is given (A.S. van Velzen, C.L. Eckhardt, D.P. Hart, et al, manuscript in preparation),\textsuperscript{7,15} but most patients are at risk for severe bleeding complications. Bleeding episodes in patients with inhibitors can be prevented or treated with FVIII bypassing agents, such as recombinant FVIIa (Novoseven, 90 $\mu$g/kg intravenously every 2-3 hours) or activated prothrombin complex (FEIBA, 50 U/kg every 8-12 hours). Desmopressin may have a role for patients who have circulating endogenous FVIII levels.\textsuperscript{19,20}

Data on immune tolerance induction in patients with MHA and inhibitors are mostly anecdotal and heterogeneous, so a definite conclusion and guidance on the best regimen is not feasible. Immunomodulatory drugs such as corticosteroids, cyclophosphamide, and anti-CD20 monoclonal antibody rituximab have also been used (A.S. van Velzen, C.L. Eckhardt, D.P. Hart, et al, manuscript in preparation),\textsuperscript{5,17,21} as well as avoidance of reexposure to exogenous FVIII, using desmopressin and bypassing agents to treat bleeding episodes (A.S. van Velzen, C.L. Eckhardt, D.P. Hart, et al, manuscript in preparation). Immune tolerance induction could be more effective than no specific treatment or immunomodulating drugs in preventing risk for anamnesis of the inhibitor after reexposure to factor VIII.\textsuperscript{7} The INSIGHT study showed that in half of the patients with nonsevere HA, the inhibitor disappeared without eradication treatment (A.S. van Velzen, C.L. Eckhardt, D.P. Hart, et al, manuscript in preparation). However, this does not imply sustained success, as the inhibitor may return after the patient is treated again with FVIII concentrates (anamnestic response). Both patients with high-titer and patients with low-titer inhibitors seemed to benefit from eradication treatment, but in patients with low-titer inhibitors, sustained success without eradication was also likely (A.S. van Velzen, C.L. Eckhardt, D.P. Hart, et al, manuscript in preparation).

### Table 1. FVIII mutations associated with MHA and risk of inhibitors, and for which a biologic response to desmopressin has been reported

<table>
<thead>
<tr>
<th>Amino acid substitution (previous nomenclature)</th>
<th>Domain</th>
<th>Cases/tested reported with FVIII level ≥30 U/dL after desmopressin, n (%)</th>
<th>Cases/tested reported with FVIII level ≥50 U/dL after desmopressin, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS10-18 G$\rightarrow$A*‡</td>
<td>A2</td>
<td>0/3 (0%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>p.Arg550Cys (Arg531Cys)‡</td>
<td>A2</td>
<td>5/5 (100%)</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>p.Arg612Cys (Arg593Cys)‡</td>
<td>A2</td>
<td>26/27 (96%)</td>
<td>15/27 (56%)</td>
</tr>
<tr>
<td>p.Asn637Ser (Asn618Ser)‡</td>
<td>A2</td>
<td>10/10 (100%)</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>p.Pro1873Leu (Pro1854Leu)‡</td>
<td>A3</td>
<td>33 (100%)</td>
<td>2/3 (66%)</td>
</tr>
<tr>
<td>p.Tyr2124Cys (Tyr2105Cys)†</td>
<td>C1</td>
<td>4/4 (100%)</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>p.Arg2169His (Arg2159His)†</td>
<td>C1</td>
<td>11/11 (100%)</td>
<td>7/11 (64%)</td>
</tr>
<tr>
<td>p.Arg2178Cys (Arg2159Cys)‡</td>
<td>C1</td>
<td>9/9 (100%)</td>
<td>9/9 (100%)</td>
</tr>
</tbody>
</table>

*Intronic mutation.
†Including 3 unpublished cases.
‡All cases with a basal level of 5 U/dL or higher.

Table adapted from Refs. 3, 16, 19, 24-27, 29, and unpublished observation.

**Figure 1.** Factor VIII missense mutations in MHA consistently associated with poor response (upper part of figure; FVIII postadministration <30 U/dL) or short FVIII half-life (lower part of figure; <3 hours) after desmopressin. #Mutations associated with dysfunction of protein.
Table 2. Recommendations for MHA

1. Patients with suspected MHA should be referred to a specialized hemophilia treatment center;
2. Perform genetic testing to identify patients with mutations potentially at risk for inhibitor;
3. Perform a desmopressin challenge in all patients unless a contraindication exists;
4. Use desmopressin where possible, and use caution with high-dose/prolonged courses of FVIII replacement therapy, especially in patients with mutations associated with inhibitor development;
5. Test for inhibitor after 4-6 wk from intensive treatment with FVIII concentrates, before surgery, or at least every 6 or 12 mo if sporadically treated with FVIII concentrates;
6. Record accurately the progressive number of ED to anticipate the onset of inhibitor, especially in patients with high-risk mutations.

How to reduce the risk for inhibitor development in MHA

Desmopressin represents the therapeutic option of first choice in MHA, as it is cheap and safe and carries no risk for bloodborne virus transmission. Desmopressin (typically at 0.3 μg/kg body weight) is usually administered intravenously, diluted in 50 to 100 mL saline, either infused over 20 to 30 minutes or subcutaneously when concentrated formulation is available, which could be more convenient for home treatment. The drug is also available with an intranasal spray administration; this can, however, result in variable adsorption with less FVIII and von Willebrand factor (VWF) increase. Desmopressin induces a 2- to 5-fold increase of plasma FVIII and VWF levels after administration. It has been suggested that FVIII levels of at least 30 U/dL are adequate for the treatment of spontaneous or posttraumatic bleeding, whereas FVIII levels higher than 50 U/dL are required to cover major surgery, although in the latter case, levels of 80 to 100 U/dL should be achieved. However, no randomized or controlled clinical trial is available, and treatment modality remains almost empirical. Although FVIII increase occurs in most cases, only 50% to 60% of patients achieve FVIII levels higher than 50 U/dL.

The peak postdesmopressin depends in part on the patient’s basal FVIII level and age. Young children often have markedly lower responses to desmopressin than adults, but they may become responsive at an older age. The FVIII half-life, typically around 6 to 8 hours, is positively associated with basal and peak FVIII antigen levels and patient age. Some mutations are consistently associated with favorable responses (in particular, several of those at risk for inhibitor development; Table 1), whereas promoter, splicing, or intronic mutations respond poorly, and some missense mutations show a reduced FVIII survival (Figure 1). Although there is a certain consistency of the response within the same mutation, the response to desmopressin is somehow heterogeneous. Therefore, the individual response should always be assessed by a test infusion of desmopressin with FVIII measured at least 1 and 4 hours after its administration to ascertain the pattern of response and the rate of clearance.

In von Willebrand disease, rapid clearance of VWF after desmopressin is an important pathophysiological mechanism associated with some mutations (eg, R1205H), especially those located in the D3 domain of VWF. Although there is scarce published evidence for possible increased clearance of FVIII after desmopressin in MHA, nevertheless, as in a few patients there is evidence of fast FVIII clearance (Figure 1), it seems advisable to test at least after 4 hours after desmopressin. Tachyphylaxis (ie, a reduced response on repeated administration) should be considered when using desmopressin at closely spaced intervals during surgical procedures.

Desmopressin should be used whenever possible in the treatment of MHA not only to reduce the cost of treatment but also to minimize the exposure to exogenous FVIII, thereby reducing the risk for inhibitor development. For major surgery, a combined use of desmopressin and FVIII concentrates could be suggested to reduce exposure to FVIII concentrates and the associated risk for inhibitors in patients with high-risk mutations. Most frequent mutations associated with inhibitor risk respond well to desmopressin, but published information is scanty for other, rarer mutations (Table 1).

The frequency of desmopressin administration should be guided by monitoring FVIII levels at 12-hour intervals during surgery. As an adjunct to desmopressin, antifibrinolytics can be used as a concomitant treatment, especially for mucosal bleeding.

Hyponatremia and volume overload resulting from the antidiuretic effect of desmopressin occur rarely, but small children who have received closely repeated infusions are particularly at risk. To avoid this complication, fluid intake should be limited during desmopressin treatment. Finally, this drug should be used cautiously in patients with uncontrolled hypertension or recent myocardial infarction or stroke, or who suffer from angina, all of which have been reported to occur after the use of desmopressin. FVIII concentrates remain the mainstay of treatment in patients unresponsive to desmopressin, if sustained long-term correction of FVIII levels is mandatory, or when contraindications to the use of desmopressin are present.

Conclusions

There is a growing interest in MH. The elucidation of molecular basis and the evaluation of pathophysiological mechanisms of several mutations causing MHA have provided interesting insights in the response to desmopressin and the risk for inhibitor development. The identification of several high-risk mutations emphasizes the need to adopt preventive measures in patients carrying these mutations. The use of desmopressin is an important clinical strategy to reduce exposure to therapeutic FVIII concentrates, thereby mitigating inhibitor risk, as well as for patients who do not carry high-risk mutations. Avoidance of intensive courses of treatment with FVIII concentrates should be considered, especially in those patients known to carry a high-risk mutation or with a relative who developed an inhibitor. Table 2 summarizes some practical recommendations for the management of patients with mild hemophilia.

Authorship

Contribution: G.C. and K.F. wrote and reviewed the manuscript.

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