MOLECULAR AND CLINICAL PREDICTORS OF INHIBITOR RISK, ITS PREVENTION AND TREATMENT IN MILD HEMOPHILIA A

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Running title: Inhibitors in Mild haemophilia A

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SUMMARY

The risk of inhibitor development in mild hemophilia A (factor VIII levels between 5 to 40 U/dL) is larger than previously anticipated, continues throughout life and is particularly associated with certain mutations in $F_8$. Desmopressin may reduce inhibitor risk by avoiding exposure to FVIII concentrates, but the heterogenous biological response to desmopressin, showing large inter-individual variation, may limit its clinical use. However, predictors of desmopressin response have been recently identified, allowing the selection of the best candidates to its use.
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 relative little attention because of its purported low morbidity rate, resulting in an apparently uncomplicated quality and duration of life, in absence of virally induced comorbidities\textsuperscript{1-3}. In a recent cohort study, 23% of patients with mild hemophilia were HCV positive, a proportion substantially lower than in severe patients\textsuperscript{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\textsuperscript{2}. Diagnosis of MHA usually occurs later in life and a significant proportion of cases may be diagnosed during subsequent family investigation\textsuperscript{3,5}. However, because of delayed presentation of bleeding, sometimes these patients could be firstly seen by physicians who are not used to interpreting symptoms of bleeding\textsuperscript{6}. Thus these symptoms could be more dramatic at time of initial assessment, with the risk of intensive treatment with FVIII concentrates, potentially increasing the risk for inhibitor development (see below).

This review will focus on the emerging issue of inhibitor development in MHA, its molecular and clinical predictors, preventive strategies and treatment\textsuperscript{3,4}.

INHIBITOR RISK IN MILD HEMOPHILIA A: NOT SO EARLY, NOT SO LOW

Some patients with MHA may develop inhibitory antibodies after treatment with FVIII concentrates, with a prevalence of 5-10\%\textsuperscript{5,7,8}. When exposure days are taken into account, the risk of inhibitor clearly increases with the number of exposure days to exogenous, therapeutic FVIII concentrates\textsuperscript{8}. The INSIGHT study in a large population of nonsevere HA patients, including a large proportion of MHA cases, showed that the inhibitor risk was 6.7\% (95\% CI 4.5-8.9) at 50 exposure days, rising to 13.3\% (95\% CI 9.6 – 17) after 100 exposure days\textsuperscript{8}. This indicates that inhibitor development may occur throughout life in MHA, contrasting with patients with severe hemophilia A who have the highest risk of inhibitor development at 10-15 days and the risk becomes almost negligible at 50ED\textsuperscript{4}. 
MOLECULAR AND CLINICAL PREDICTORS OF INHIBITOR RISK IN MILD HEMOPHILIA A

FVIII missense mutations are the main cause of MHA, although about 5-10% of patients may have splicing defects, point deletions, deep intronic changes or promoter mutations. Of interest, it has been definitely demonstrated that among more than 150 different causative missense mutations for MHA, some relatively frequent mutations are associated with a high risk of inhibitor development upon replacement therapy. In particular p.Arg612Cys (Arg593Cys) in A2 domain, p.Tyr2124Cys (Tyr2105Cys), p.Arg2169His (Arg2150His) clustered in C1 and C2 domains of the light chain represent the most frequent mutations associated with this risk, with an inhibitor risk after 20 ED from 0 to 9.1% of patients. However, some rarer mutations (p.Asp2093Gly [Asp2074Gly] and p.Trp2248Cys [Trp2229Cys]) are particularly important since the risk of inhibitor at 20 ED (21.2 and 41.7%, respectively) parallels that of severe patients. It is not entirely clear why these particular mutations carry an increased risk of 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. Recently it has been suggested that the risk of inhibitor formation associated with FVIII missense mutations is significantly higher when amino acid substitution belongs to another physicochemical class than the original residue. However, the recent description of an association between an intronic mutation (IVS10-18 G>A) and inhibitor occurrence after intensive replacement treatment and more than 90 ED again suggests that the pathogenesis may be heterogeneous. In conclusion, genetic testing at diagnosis would be useful to identify subjects with high risk mutations before planning F VIII 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. Two retrospective Dutch studies demonstrated that p.Arg612Cys was a strong risk factor together with intensive perioperative FVIII administration. 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’s endogenous FVIII\(^7,15\). Bleeding may be severe and potentially life-threatening. It often occurs in muscles and joints, but large cutaneous bruising, gastrointestinal and urogenital bleeding may occur as in acquired haemophilia\(^17\).

**INHIBITOR TREATMENT**

Inhibitor disappearance may occur spontaneously when no further treatment with FVIII concentrates is given\(^7,15,19\), but most patients are at risk of severe bleeding complications. Bleeding episodes in inhibitor patients can be prevented or treated with FVIII bypassing agents, such as recombinant FVIIa (Novoseven®, 90 μg/kg iv every 2-3 h) or activated prothrombin complex (FEIBA®, 50 U/kg every 8-12 h). Desmopressin may have a role for patients that have circulating endogenous FVIII levels\(^20,21\).

Data on immune tolerance induction in patients with MHA and inhibitors are mostly anecdotal and heterogeneous so that a definite conclusion and guidance on the best regimen is not feasible. Immunomodulatory drugs such as corticosteroids, cyclophosphamide, anti-CD20 monoclonal antibody rituximab have also been used\(^17,19,22\) as well as avoidance of re-exposure to exogenous FVIII using desmopressin and bypassing agents to treat bleeding episodes\(^19\). Immune tolerance induction could be more effective than no specific treatment or immunomodulating drugs in preventing risk of anamnesis of the inhibitor after re-exposure to factor VIII\(^7\). The INSIGHT study showed that in half of the nonsevere HA patients the inhibitor disappeared without eradication treatment\(^19\). However, this does not imply sustained success, as the inhibitor may return after the patient is treated again with FVIII concentrates (anamnestic response). Both high titer and low titer inhibitor patients seemed to benefit from eradication treatment, but in patients with low titer inhibitors sustained success without eradication was also likely\(^19\).

**HOW TO REDUCE THE RISK OF INHIBITOR IN MILD HEMOPHILIA A**

Desmopressin represents the therapeutic option of first choice in MHA since it is cheap, safe and carries no risk of blood-borne virus transmission\(^23,24\). Desmopressin (typically at 0.3 μg/kg body weight) is usually administered intravenously diluted in 50-100 mL saline infused over 20-30 min or subcutaneously, when concentrated formulation is available, which could be more convenient for home-
treatment. The drug is also available as intranasal spray administration, which can however result in variable adsorption with less FVIII/VWF increase. Desmopressin induces a 2-5 fold increase of plasma FVIII and VWF levels after administration\textsuperscript{23,24}. It has been suggested that FVIII levels of at least >30 U/dL are adequate for the treatment of spontaneous or post-traumatic bleeding, while FVIII levels >50 U/dL are required to cover major surgery\textsuperscript{23,24}, though in the latter case levels of 80-100 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 50-60 % of patients achieve FVIII levels > 50 U/dL\textsuperscript{3,24-27}. The peak post-desmopressin depends in part on the patient’s basal FVIII level\textsuperscript{25,26} and age. Young children often have markedly lower responses to desmopressin than adults, but they may become responsive at an older age\textsuperscript{28-30}. The FVIII half-life, typically around 6-8 h, is positively associated with basal and peak VWF antigen levels and patient age\textsuperscript{25}. Some mutations are consistently associated with favourable responses, and in particular several of those at risk of inhibitor (Table 1), while promoter, splicing or intronic mutations respond poorly and some missense mutations show a reduced FVIII survival\textsuperscript{3,25-28} (Figure 1). Although there is a certain consistency of the response within the same mutation, the response to desmopressin is somehow heterogeneous\textsuperscript{25-28,30}. 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 (e.g., R1205H), especially located in D3 domain of VWF\textsuperscript{31}, while there is scarce published evidence for possible increased clearance of FVIII after desmopressin in MHA. Nevertheless, since in a few patients there is evidence of fast FVIII clearance\textsuperscript{25} (Figure 1), it seems advisable to test at least after 4 hours after. Tachyphylaxis (i.e. a reduced response upon repeated administrations) should be considered when using desmopressin at closely-spaced intervals during surgical procedures\textsuperscript{24,32}.

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 and thereby reducing the risk of 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 of inhibitors in patients with high-risk mutations. Most frequent mutations associated with inhibitor risk respond well to desmopressin but published information are scanty for other more rare mutations (Table 1).

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

Hyponatremia and volume overload due to the antidiuretic effect of DDAVP occur rarely, but small children who have received closely repeated infusions are particularly at risk\(^3\). To avoid this complication, fluid intake should be limited during DDAVP treatment. Finally, this drug should be used cautiously in patients with uncontrolled hypertension, recent myocardial infarction or stroke, or suffering from angina, which have been reported to occur following its use\(^34,35\). 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 of 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, also for patients that 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.
Authorship

Contribution: GC and KF wrote and reviewed the manuscript

Conflict of interest disclosures: The authors declare no competing financial interests.
REFERENCES


Table 1. FVIII mutations associated with mild hemophilia A and risk of inhibitors\textsuperscript{8,15}, and for which a biologic response to desmopressin has been reported\textsuperscript{3,16,20,25-28,30} and unpublished observation.

<table>
<thead>
<tr>
<th>Amino acid substitution (previous nomenclature)</th>
<th>Domain</th>
<th>N° and percentage of cases/tested reported with FVIII level $\geq 30$ U/dL after desmopressin</th>
<th>N° and percentage of cases/tested reported with FVIII level $\geq 50$ U/dL after desmopressin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS10-18 G$&gt;$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>3/3 (100 %)</td>
<td>2/3 (66 %)</td>
</tr>
<tr>
<td>p.Tyr2124Cys (Tyr2105Cys)$§</td>
<td>C1</td>
<td>4/4 (100 %)</td>
<td>4/4 (100 %)</td>
</tr>
<tr>
<td>p.Arg2169His (Arg2150His)$@</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>
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* Intronic mutation
§ including 3 unpublished cases
@ all cases with basal level $\geq 5$ U/dL
**Table 2. Recommendations for mild hemophilia A**

<table>
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<th>Recommendations</th>
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<tbody>
<tr>
<td>- Patients with suspected mild hemophilia A should be referred to a specialized hemophilia treatment center</td>
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<tr>
<td>- Perform genetic testing to identify patients with mutations potentially at risk of inhibitor</td>
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<td>- Perform a DDAVP challenge in all patients unless a contraindication exists</td>
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<tr>
<td>- Use DDAVP where possible and use caution with high dose/prolonged courses of FVIII replacement therapy, especially in patients with mutations associated with inhibitor development</td>
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<tr>
<td>- Test for inhibitor after 4-6 weeks from intensive treatment with FVIII concentrates, before surgery, or at least every 6 months or 12 months if sporadically treated with FVIII concentrates</td>
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<td>- Record accurately the progressive number of exposure days to anticipate the onset of inhibitor, especially in patients with high risk mutations</td>
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LEGEND TO FIGURE

**Figure 1.** Upper part: Factor VIII missense mutations in mild Haemophilia A consistently associated with poor response (FVIII post-administration < 30 U/dL) or, lower part, short FVIII half-life (< 3 hours) after desmopressin.

# Mutations associated with dysfunctional protein.
Molecular and clinical predictors of inhibitor risk, its prevention and treatment in mild hemophilia A

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