Case report

Inhibitor development and management in three non-severe haemophilia A patients with T295A variant

V. Ivaskevicius1*; G. Goldmann1*; S. Horneff1; N. Marquardt1; C. Klein1; T. Albert1; H. Zeitler2; J. Oldenburg1
1Institute of Experimental Haematology and Transfusion Medicine, University Hospital Bonn, Germany;
2Internal Medical Clinic I, Center of Extracorporeal Therapy and Autoimmunity (CETA), Bonn, Germany

Keywords
Factor VIII, haemophilia A, FVIII inhibitors, F8 gene, missense mutation

Summary
Missense mutations are the most common F8 gene defects among the patients with non-severe haemophilia A. This type of mutation is typically associated with low (5%) inhibitor risk. In the present retrospective study we analysed the clinical data of 16 haemophiliacs with the T295A missense mutation treated at Bonn Haemophilia Centre. In total, three patients developed inhibitors: two patients experienced low-titer and one high-titer inhibitors. Both patients with low titer inhibitors underwent successful ITI. The third patient, at the age of 81, developed initially low-titer inhibitors (3 BU/ml) after rFVIII therapy because of knee surgery. He experienced spontaneous multiple large skin haematomas and haemarthrosis. Immunosuppressive therapy was not applicable because of the infectious origin of discitis (Th1-Th4). Immunoadsorption was performed, but the inhibitor titer increased up to 42 BU/ml nine weeks after termination. A successful treatment of discitis with antibiotics finally allowed a weekly therapy (four times) with rituximab (375 mg/m²). This resulted in a decrease of inhibitor titre to 0.7 BU/ml eight weeks after the fourth rituximab application. Patient had endogenous FVIII levels of 3–5%. Twelve months after rituximab therapy (after B cells recovery) he relapsed with low-titer inhibitors and therefore was treated with single rituximab dose (375 mg/m²) again. This resulted in his depletion of B cells, measurable endogenous FVIII levels and non measurable inhibitors. This study demonstrated T295A variant to be associated with significantly increased (3/16 patients, 17%) inhibitor development.

Schlüsselwörter
Faktor VIII, Hämophilie A, FVIII-Inhibitoren, F8-Gen, Missense-Mutation

Zusammenfassung

Correspondence to:
Vytautas Ivaskevicius
Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn,
Sigmund-Freud-Str. 25, 53127 Bonn, Germany,
Tel. +49/(0)228/28 71 51 75, Fax +49/228/28 71 43 20
E-mail: vytautas.ivaskevicius@ukb.uni-bonn.de

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* contributed equally

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Mild haemophilia A is a rare disease which is usually caused by a missense mutation in the factor VIII (FVIII) gene (F8), mainly in the A domains, resulting in a decreased clotting FVIII activity between 0.05 and 0.40 IU ml\(^{-1}\) (1–3).

In mild haemophilia A FVIII replacement is required in most of cases due to trauma or surgery providing factor correction for a longer period. Risk factors for inhibitor development in haemophilia are a

- family history of inhibitors,
- period of intensive FVIII infusion,
- so-called peak treatment (4), and
- certain missense mutations (5).

The exact incidence of inhibitor development in mild haemophilia is unknown, but is reported to be between 3 and 13% (6–8). Inhibitors may cross-react with endogenous FVIII resulting in a decrease of endogenous FVIII to \(<0.01\) IU mL\(^{-1}\), converting mild into moderate or severe haemophilia (9, 10).

Inhibitor development is associated with a high bleeding tendency and mortality. Classical immune tolerance induction (ITI, Bonn-protocol) with success rates of 60–90% has limitations in older patients (11, 12). Additive immunosuppressive therapy is a problem in patients with underlying infections.

Previously, it was demonstrated that the risk of inhibitors is higher in patients with mutations in A3, C1 and C2 domains. A recently detected T295A (HGVS: p.T314A) variant in A1 domain resulting in mild FVIII deficiency was associated with an increased inhibitor risk (2/15), too (13).

In the presented retrospective study we analysed the clinical data of 16 haemophiliacs with the T295A variant treated at Bonn Haemophilia Centre.

**Patients, methods**

Blood was collected by peripheral venous puncture as a 1:10 volume ratio in 3.8% trisodium citrate. At the Bonn centre factor VIII activity was determined using the modified Nijmegen Bethesda unit (BU) has to be verified at least 0.6 Bethesda unit (BU). A value \(\geq 0.6\) Bethesda unit (BU) has to be verified at least twice (15).

F8 Genotyping of the patients was done in the previous studies (13, 15).

**Patients**

The patients’ data was evaluated in 01/2014. Among 16 patients (of them 10 unrelated) 13 were adults and 3 children. The data of exposure days, inhibitor titer, type of concentrate, invasive procedures, age at inhibitor development and HIV/ HBV/HCV status has been analysed (Table 1).

The first patient developed inhibitors (3.3 BU/ml) at the age of nine under the plasma derived product treatment due to traumatic ankle joint distortion. The second patient developed low titer (3 BU/ml) inhibitors at the age of 56 due to substitution with rFVIII for sinus surgery. Both patients underwent successful ITI (Bonn-protocol) with inhibitor eradication in less than six months.

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**Tab. 1**

Data of the patients

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Year of Birth</th>
<th>Year of Death</th>
<th>Bethesda Unit/ml</th>
<th>Total Exposure Days</th>
<th>FVIII Activity</th>
<th>Surgery</th>
<th>Age at Inhibitor Formation</th>
<th>HCV Status</th>
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<tr>
<td>Family A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1997</td>
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<td>no</td>
<td>-</td>
<td>chronic</td>
</tr>
<tr>
<td>I: 2</td>
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<td></td>
<td>0</td>
<td>(&lt;100)</td>
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<td>II: 3</td>
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<td>(&lt;35)</td>
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<tr>
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<td>&gt;250</td>
<td>p &gt; r</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>II: 5</td>
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<td>0</td>
<td>&lt;10</td>
<td>r</td>
<td>no</td>
<td>-</td>
<td>-</td>
<td></td>
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<td>D</td>
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<td>-</td>
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<tr>
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<td>0</td>
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<td>no</td>
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<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* prior to inhibitor formation; p: plasma derived; r: recombinant
The third patient was an 81-year-old patient with mild haemophilia A, FVIII:C 7%. He received FVIII concentrate twice daily due to replacement of the retropatellar facet four years after knee joint endoprosthesis and had more than 250 exposure days before. Two weeks later, despite of continued FVIII substitution, he developed a knee joint bleeding. An irrigation was performed, FVIII was applied for one more week when he suddenly started to suffer from a severe lumbalgia and multiple large skin haematomas necessitating admission to our hospital. Discitis (Th3-Th4) was diagnosed, antibiotic treatment was started immediately. Coagulation tests revealed a FVIII activity <1% and a low titer inhibitor of 2.4 BU/ml with a type 2 kinetics. Haemoglobin was decreased to 7.5 mg/dl. A re-bleeding into the knee joint occurred with subsequent synovitis.

Initially, ITI (Bonn protocol) was performed for two weeks, FVIII trough levels after 12 hours were below 3%. Immunosuppressive therapy was not applicable because of discitis. To increase effectiveness, additional immunoadsorption was done for five weeks resulting in FVIII 12 hours trough levels of 60–80% initially, a decrease to 40–60% in the course and to <10% ultimately. The inhibitor titer increased to 3.8 BU/ml. Intravenous immunoglobulins were applied, too. Compliance problems after the long hospitalization and difficult venous access finally resulted in a treatment only with bypassing agents, FEIBA 50 IU/kg BW twice daily. A d-dimer increase led to a reduction of the FEIBA dose to 25 IU/kg BW. In the meantime, discitis and synovitis as well as inflammatory parameters have improved. Antibiotic treatment was continued. The inhibitor titer increased up to 21 BU/ml three weeks after the fourth rituximab application. The patient relapsed with low titer inhibitors and therefore was treated with a single rituximab dose (375 mg/m²) again (Fig. 2). This resulted in depletion of B cells, measurable endogenous FVIII levels and non-measurable inhibitors.

The majority of patients (n = 10) with the T295A variant had more than 100 EDs with no detectable inhibitors against FVIII. Two children (8 and 12 years old) with less than 25 EDs are still at risk to develop inhibitors. None of the patients suffered from HIV or chronic HBV infection. Two patients had chronic Hepatitis C and unfortunately died due to liver cirrhosis complications at the age of 42 and 79 (Table 1).

Results and discussion

Thr295Ala or p.T314A (according to a new Human Genome Variation Society [HGVS] nomenclature) missense mutation in A1 domain was previously reported by Schwaab et al. 1995 (13) and recently described to be associated with inhibitor formation in two patients from the Bonn Haemophilia Centre (15). Thr295Ala mutation has not been so far described in other populations besides Germans. Although a detailed pedigree analysis among the 16 patients was not performed it is very likely all of them to have a common ancestor.

Thr295Ala (p.T314A) seems to be associated with a high risk (17%) of inhibitor development. This is an unexpected finding since in general, missense mutations outside the C1 and C2 domains were found to have low inhibitor incidences (3.6% versus 8.7%) (15). Although Thr295 is located in the terminal part of A1 domain it is not exposed on FVIII molecule surface (data...
not shown) thus has no higher exposition to the immune system.

The family A (▶Fig. 1 and ▶Tab. 1) is of special interest. In total 5 individuals carried Thr295Ala (p.T314A) mutation in this family. Low titer inhibitors were diagnosed only in one (II:3) of them, despite of the fact that further three patients had more than 50–250 EDS.

In multivariate analyses of patients with non-severe haemophilia A patients with FVIII inhibitors demonstrated rituximab alone (n = 6) and other immune-modulating treatments alone (n = 2) to be significantly associated with an increased likelihood of inhibitor clearance [hazard ratio (HR) = 4.4 (95% CI = 1.06–20.03) and 10.21 (95% CI = 1.17–78.28), respectively], whereas ITI alone (n = 9) was not [HR = 1.35 (95% CI = 0.44–4.07)] (16). In opposite, our data demonstrates successful ITI treatment in two younger patients and recurrence of inhibitors in an elderly patient after rituximab treatment.

It remains unclear what was the most important trigger of inhibitor development in the elderly patient (patient D) – FVIII exposure and surgery or discitis or both.

**Conclusion**

The T295A variant seems to be associated with a significantly increased (3/16 patients, 17%) inhibitor development. Inhibitor treatment in elderly patients with moderate to mild haemophilia A is challenging especially when infections are present. In the 81 year old PTP patient inhibitor development might have been triggered by the knee surgery followed by discitis. Alternative therapy strategies are necessary to control bleeding and reduce mortality and hospitalization.

**Conflict of interest**

J. Oldenburg received reimbursement for attending symposia and congresses and/or honoraria for speaking and/or honoraria for consulting and/or funds for research from Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma and Pfizer. The other authors declare that they have no conflict of interest.

**References**