To the editor:

Insufficient evidence to suggest less stringent therapy in hemophilia B?

With great interest we read the article “Comparison of the rates of joint arthroplasty in patients with severe factor VIII and XI deficiency” and the comment by Dr Makris, “Is VIII worse than IX?” To confirm the hypothesis that severe hemophilia B is clinically milder than severe hemophilia A, Tagariello et al reported a survey of joint arthroplasty in the Italian hemophilia registry IACE. They reported a 3.38-times higher risk of joint arthroplasties in patients with hemophilia B. Based on these findings it was concluded that severe hemophilia B has a milder phenotype than severe hemophilia A and recommended that clinicians plan less primary prophylaxis in patients with hemophilia B.

More cautiously, Dr Makris commented that joint arthroplasty is an end-stage event and can hardly be used as the sole variable to describe the difference between the 2 types of severe hemophilia. A more appropriate variable for this comparison would have been bleeding frequency, but unfortunately these data were not available in the study by Tagariello et al.

At the van Creveldkliniek, treatment characteristics and bleeding episodes are documented at every routine visit, usually once every 3 to 6 months. Data on surgery and hospitalization are entered directly in the database. We have longitudinal data concerning 5094 treatment-years for 282 patients with severe hemophilia, including 30 patients with severe hemophilia B, born between 1944 and 2008. In this database, orthopedic procedures and parameters of bleeding pattern and treatment were compared across hemophilia type.

In total, 465 orthopedic procedures, including 161 arthroplasties, have been performed. Of 87 patients with severe hemophilia who underwent one or more arthroplasties, 78 had hemophilia A and 9 had hemophilia B; this difference was not statistically significant (Table 1).

The age at onset of joint bleeding is an important indicator of the clinical severity of severe hemophilia. In both groups, patients received their first treatment around the age of 1 year. In severe hemophilia A the median age at first joint bleed was 1.9 years, compared with a median of 2.4 years in severe hemophilia B, which was not significantly different due to complete overlap in 5th to 95th percentiles.

Use of prophylaxis was comparable in both groups: 77% in hemophilia A, 73% in hemophilia B. Due to the use of prophylaxis, bleeding frequencies across hemophilia type were quite similar. However, this was not caused by different treatment intensity: annual clotting factor use per kilogram of body weight was also similar across hemophilia type.

In our cohort, onset of bleeding, treatment intensity, and bleeding frequency, as well as the number of arthroplasties, were similar across hemophilia types. Any comparison of hemophlias A and B is hampered by the low numbers of patients with hemophilia B. Therefore, multinational studies are mandatory to answer this question, as was suggested by Lowe and Ludlam.

In the meantime, we find no evidence to support the suggestion of less stringent therapy and prophylaxis in patients with hemophilia B.

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Response

Comparing joint arthroplasties in severe hemophilia A with severe hemophilia B

den Uijl and colleagues found that the overall number of joint arthroplasties performed in their patients with hemophilia B (an indirect index of the severity of congenital coagulation disorder) is not different from that in patients with hemophilia A, at variance with our recent report of a significantly less frequent need of these operations in hemophilia B. Their conclusions are based on

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hemophilia A</th>
<th>Hemophilia B</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>252 (89%)</td>
<td>30 (11%)</td>
<td>—</td>
</tr>
<tr>
<td>Age at last evaluation, y</td>
<td>27.7 (2.8-51.7)</td>
<td>32.3 (2.5-52.8)</td>
<td>.738</td>
</tr>
<tr>
<td>Arthroplasty</td>
<td>78 (31%)</td>
<td>9 (30%)</td>
<td>.915</td>
</tr>
<tr>
<td>Age at 1st treatment, y</td>
<td>1.1 (0.2-2.7)</td>
<td>1.3 (0.6-2.9)</td>
<td>.065</td>
</tr>
<tr>
<td>Age at 1st joint bleed, y</td>
<td>1.9 (0.5-5.9)</td>
<td>2.4 (0.9-5.5)</td>
<td>.652</td>
</tr>
<tr>
<td>Prophylaxis†</td>
<td>194 (77%)</td>
<td>22 (73%)</td>
<td>.655</td>
</tr>
<tr>
<td>Annual joint bleeding frequency</td>
<td>4.3 (0.3-16.3)</td>
<td>3.8 (0.4-17.8)</td>
<td>.379</td>
</tr>
<tr>
<td>Annual factor use‡</td>
<td>1560 (286-3644)</td>
<td>1260 (302-5826)</td>
<td>.559</td>
</tr>
</tbody>
</table>

Values are reported as numbers (percentages) or medians (5th-95th percentiles).
†Continuous variables were tested with Mann-Whitney test, categorical variables by χ² test.
‡Use of prophylaxis was calculated over the last 3 years of treatment.
§Per kilogram of body weight.

References

78 hemophilia A and 9 hemophilia B patients who underwent arthroplasty in a single institution, whereas our conclusions stem from much larger absolute numbers of patients derived from all the hemophilia centers in Italy (253 with hemophilia A vs 15 with hemophilia B) and also from a systematic review of the literature that was consistent with our findings of fewer arthroplasties in hemophilia B. We suspect that the small sample size on which den Uijl et al based their tests of statistical significance entails a high risk of type 2 error (the error of not finding a difference that does exist), although a smaller risk of type 1 error (the error of finding a difference that does not exist) cannot be ruled out even in our much larger cohort. Furthermore, the Dutch cohort was treated early and regularly with continuous prophylaxis at a significantly higher rate than that for patients included in the Italian cohort. Obviously the efficacy of intensive prophylaxis had an impact on the natural history of the disease, making hemophilia A and B become similar in severity, and thereby explaining, at least in part, the differences between the Italian and Dutch cohorts.

With this as a preamble, we agree with the Dutch investigators that the issue of the different severity of the 2 hemophilias remains unsettled, because the rate of joint arthroplasty is only a very indirect index of disease severity and entails the potential influence of several confounders. We also agree with den Uijl et al that large multinational studies are warranted to reach a firm conclusion on the issue of varied severity, with possible clinical implications on the need for early continuous prophylaxis. In agreement with Makris,2 we believe that the different rate of gene mutation types (null vs non-null) make biologically plausible that hemophilia B is less severe than hemophilia A. Other studies3-5 are consistent with our suggestions of lesser severity of hemophilia B.

To the editor:

Delayed but functional neutrophil extracellular trap formation in neonates

Sepsis is one of the leading morbidity and mortality factors in newborns, occurring in more than 700 of every 100,000 live births.1 Newborns seem to have a unique susceptibility to early bacterial infections2 compared with adults, but the underlying pathomechanisms are still poorly defined. Neutrophils represent the first and most powerful cellular line of antibacterial host defense, as they are able to kill most bacteria within a few hours.3 These innate immune cells engulf and destroy pathogens intracellularly, a phenomenon known for more than 100 years as phagocytosis.4 In 2004, Brinkmann et al described for the first time a mechanism of how neutrophils kill bacteria extracellularly: neutrophil extracellular trap (NET) formation or NETosis.5,6 Upon stimulation, neutrophils undergo cytoplasmic and nuclear changes, the intracellular architecture is lost, and chromatin fibers are expelled that contain DNA, histones, and granular proteins to form NETs, a machinery used to trap and destroy bacteria surrounding the dying neutrophil.7

Recently, Yost and coworkers reported that neonatal neutrophils are impaired in NET formation, which may explain why newborns are prone to bacterial infections.5 Here, we present experimental data that complement and extend these findings and add to our understanding of how NETosis is regulated in newborns. Yost et al stimulated neonatal (cord blood–derived) and adult neutrophils for 1 hour with lipopolysaccharide (LPS) or platelet-activating factor (PAF) and analyzed NETosis. Their studies demonstrated an inability of neonatal neutrophils to form NETs at this early time point. We stimulated, in a similar manner, isolated neonatal and adult neutrophils with LPS, but (1) we extended the stimulation period to a maximum of 3 hours, and (2) we compared the Toll-like receptor 4 (TLR4) ligand LPS with an array of Gram-negative and Gram-positive TLR ligands. The ethical committee and the institutional review board of the Ludwig-Maximilians-Universität Munich have approved our study, which was conducted in accordance with the Declaration of Helsinki.

In line with Yost et al, we found that, upon stimulation with LPS for 1 hour, neonatal neutrophils showed no signs of NETosis compared with a moderate NET formation by adult neutrophils. However, around 2 hours of LPS stimulation, neonatal neutrophils started to form NETs and at 3 hours, neonatal neutrophils were almost equally potent in NET generation compared with adult neutrophils (Figure 1A-B). Neonatal neutrophils showed a similar delayed NETosis in response to synthetic or natural TLR2 agonists, whereas no delay was found upon TLR5, TLR8, or TLR9 activation. The identity of NETs was confirmed ultrastructurally (Figure 1C) and by immunostaining for citrullinated histone 3 (Figure 1D). Next, we challenged neonatal neutrophils with live bacteria, which elicited robust NET formation (Figure 1E). These NETs were functionally
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Giuseppe Tagariello, Alfonso Iorio and Pier M. Mannucci