



# Hemophilia A and B: molecular and clinical similarities and differences

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## Introduction

Hemophilia A and B are rare X-linked bleeding disorders caused by mutations in the genes encoding coagulation factor VIII (FVIII) and factor IX (FIX). Hemophilia A (HA) is more common than hemophilia B (HB), with a prevalence of one in 5,000 male live births compared to one in 30,000, respectively.<sup>1</sup>

The disease severity in hemophilia is classified according to the plasma level of FVIII or FIX activity. The severe form is defined as a factor level <1% of normal, the moderate form as a factor level of 1-5%, and the mild form with a factor level >5 and <40%.<sup>2</sup> Patients with severe hemophilia frequently develop hemorrhages into joints, muscles or soft tissues without any apparent cause. They can also suffer from life-threatening bleeding episodes such as intracranial hemorrhages. Persons with mild and moderate factor deficiency rarely experience spontaneous hemorrhages, and excessive bleeding mostly occurs only following trauma or in association with invasive procedures.

The residual factor activity generally correlates well with clinical characteristics; however, heterogeneous bleeding phenotypes among individuals with the same factor levels can occur.<sup>3</sup> Furthermore, although HA and HB have been usually considered clinically indistinguishable with negligible differences in severity and outcomes, several recent studies are challenging this concept, suggesting that patients with HB could have a less severe bleeding tendency compared to HA patients with the same residual plasma level.<sup>4</sup>

In this review, we provide an up-to-date summary of evidence highlighting the similarities and differences of these two clotting factor deficiencies.

## Comparison of gene defects in hemophilia A and hemophilia B

Both *F8* and *F9* genes are located on the X chromosome, *F8* gene being at the end of the long arm at Xq28<sup>5</sup> and *F9* IX gene on the long arm, more towards the centromere, at Xq27.<sup>6</sup>

*F8* gene is extremely large (approx. 180 kb) and structurally complex (26 exons), while *F9* gene is considerably smaller (approx. 34 kb in length) and structurally simpler, containing only eight exons, the largest of which is only 1,935 bp long.

The mutations causing hemophilia A and B have been characterized in several thousands of patients. What is immediately evident from the enormous number of mutations that have been elucidated is that the molecular basis of the hemophilias is extremely diverse.

Point mutations, deletions, insertions, and rearrangements/inversions have all been found either in *F8* and *F9* genes. However, the relative frequency of these mutations differs between HA and HB. In particular, gross genetic abnormalities account for approximately 7% of HB cases in contrast to HA in which gene rearrangements account for almost half of severe cases, with intron 22 inversion being the most common defect. A summary of the differential characteristics of hemophilia A and B is presented in Figure 1.

Previous studies have shown that the mutation type in the FVIII and FIX genes correlates with the residual factor activity in plasma and the bleeding tendency in hemophilia patients, with larger gene defects generally associated with a more severe clinical phenotype.<sup>3,7</sup> Although one could intuitively argue that HA and HB patients with null mutations could experience a similar bleeding history, such a comparison has never been systematically carried out.

The different prevalence of mutations predicting a null allele also explains a higher proportion of HB patients that can be classified as cross-reacting material positive (CRM<sup>+</sup>). The presence of null mutations prevents the synthesis of any detectable FVIII or FIX antigen. Approximately 5% of HA patients are CRM<sup>+</sup> and

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have circulating FVIII protein levels at almost 30% of normal.

The mutations thought to be responsible for CRM<sup>+</sup> HA are generally missense mutations found in the A2-domain of FVIII.<sup>8</sup> At variance with HA, almost one-third of patients with HB are classified as CRM<sup>+</sup> and can produce variable amounts of FIX protein.

The higher prevalence of less severe mutations (missense mutations) in HB could provide a biological basis for a milder bleeding phenotype compared to HA, although clinical evidence is limited. Furthermore, this could also explain the lower prevalence of inhibitors in HB, mostly associated with stop codon or partial/whole gene deletion, probably together with the fact that FIX is smaller than FVIII, with less antigenic epitopes. Interestingly, it is well known that some missense mutations in mild HA are associated with inhibitor occurrence,<sup>9</sup> while this has never been reported in patients with mild HB.

It is also interesting to note that for some FIX nonsense gene mutations in HB, usually categorized as null mutations, the mechanism of ribosome readthrough could restore translation impaired by mutations and could account for minimal full-length protein biosynthesis. This mechanism could be a modifier of clinical outcomes in this specific patient population.<sup>10</sup>

Finally, although rare, being implicated in just a small proportion of severe HB cases, it is worth noting the possibility of a particular variant of HB: the hemophilia B Leyden.<sup>11</sup> The molecular mechanism is likely to involve disruptions of sites in the proximal promoter of the *F9* gene. In this condition, abnormal hemostasis is present after birth but spontaneously ameliorates at puberty, with a progressive recovery of FIX expression and normaliza-

tion of FIX level in adulthood.<sup>12</sup> This effect is associated with rising post-pubertal growth hormone levels.<sup>13</sup>

Similar molecular mechanisms that can potentially improve the clinical presentation or outcomes, such as these two mechanisms just discussed for HB, have not yet been identified in HA patients.

### Similarities and differences in hemophilia A and B clinical phenotype

The numerous bleeding episodes that individuals with severe hemophilia experience can lead to long-term disability. Recurrent joint bleedings can result in severe arthropathy, muscle atrophy, pseudo-tumors, and lead to chronic pain and impaired mobility that often requires surgery and arthroplasty to improve joint function. HA and HB display similar clinical characteristics; however, several studies have reported on possible differences in bleeding frequency and factor consumption,<sup>14</sup> clinical scores,<sup>15</sup> and the need for orthopedic surgery.<sup>16,17</sup>

The possible different clinical evolution of HB was initially suggested in 1959 by Quick<sup>18</sup> and was based on 24 HB cases he had personally studied. He observed that HB, even in its most severe form, can be less incapacitating and disabling than HA, and that this difference was especially pronounced after adolescence. It should be kept in mind, however, that historically, in some studies, severe HB has been defined with a FIX <2% that could contribute to a less severe bleeding tendency compared to HA, usually defined with a FVIII <1%. However, forty years after Quick, a retrospective study reporting demographic characteristics, hospital admissions, and causes of death of patients with hemophilia was carried out in Scotland by Ludlam *et al.*<sup>19</sup> They retrospectively studied 282 patients

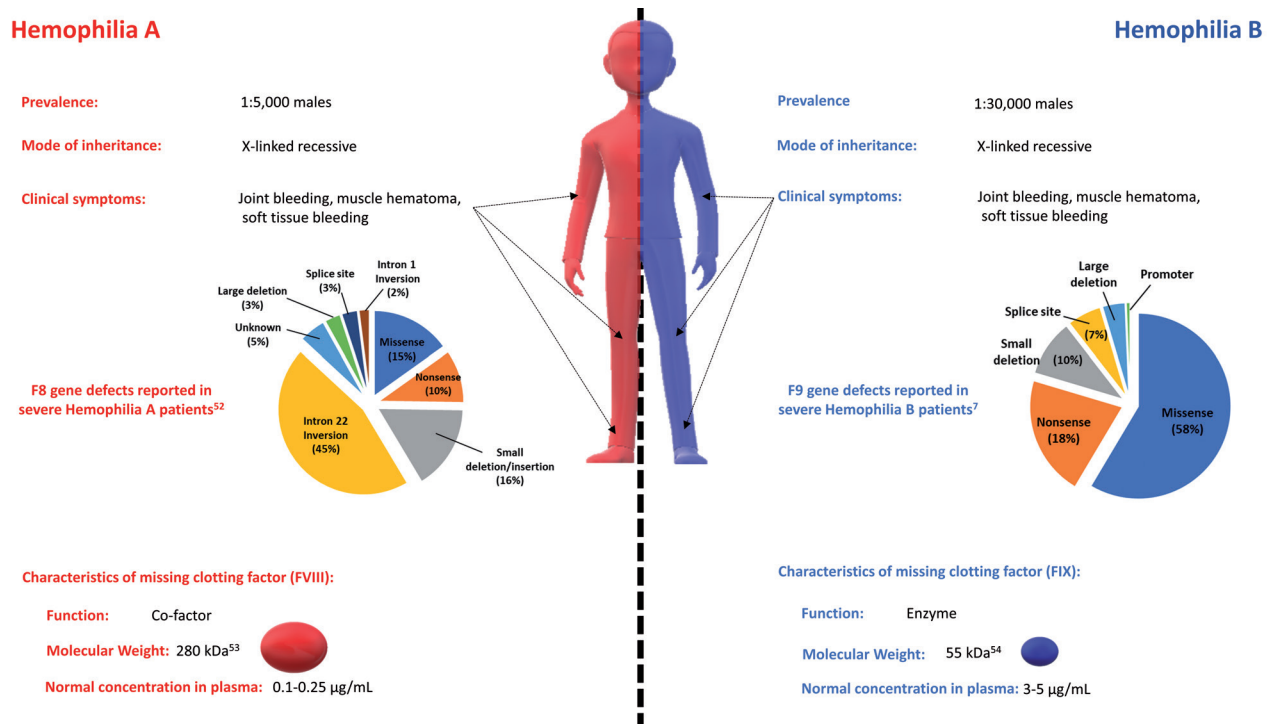


Figure 1. Comparison of characteristics of hemophilia A and B. FIX: factor IX. <sup>52</sup>Gouw *et al.* (2012); <sup>7</sup>Belvini *et al.* (2005); <sup>53</sup>Brummel-Ziedins and Mann. (2014); <sup>54</sup>Nazeef and Sheehan (2016).

with hemophilia during the period between 1980 and 1994 who were treated predominantly with on-demand therapy. The authors found a lower rate of hospital admissions for patients with HB at all levels of severity, suggesting that these individuals have a milder bleeding phenotype compared to HA patients.

Results consistent with these were obtained in the US a few years later in a cross-sectional study conducted between May 1998 and May 2002.<sup>20</sup> Data collected from 4,343 males with hemophilia aged 2-19 years included age, bleeding frequency, family history, insurance status, orthopedic procedures, prophylaxis use, age at diagnosis and first hemophilia treatment center (HTC) visit, frequency of visits, hemophilia type, inhibitor status, race/ethnicity, body mass index. The authors highlighted the fact that overall, individuals with HB consistently reported fewer bleeding episodes, regardless of age or severity. Interestingly, among individuals with moderate factor deficiency, those affected by HA had a greater degree of range of motion limitation compared to persons with HB.

A survey conducted in 2006 aiming to describe prophylaxis use in patients of all ages and severities with HA or HB in Canada also showed some differences between HA and HB treatment.<sup>21</sup> Data on 2,663 individuals (2,161 hemophilia A, 502 hemophilia B), were returned by 22 Canadian HTC, totaling 98% of the Canadian hemophilia population. When comparing the use of prophylaxis, the authors reported that 32% of patients with severe HB were receiving prophylaxis compared with 69% of patients with severe HA. However, it is not clear if this difference is the result of a real or perceived difference in the clinical phenotype or just reflect the traditional therapeutic approach to HB patients.

However, a subsequent study reported similar results. In a project aimed at constructing a composite score (Hemophilia Severity Score, HSS) to assess the severity of the disease, Schulman *et al.* evaluated 100 patients affected by HA (n=67) and HB (n=33).<sup>15</sup> This was intended as a comprehensive measurement of the clinical severity of the disease and took into account the number of joint bleeds per year, the orthopedic joint score, and the annual consumption of FVIII. Interestingly, the HSS was higher for severe HA [median=0.50; interquartile range (IQR)=0.41-0.68] than for severe HB (median=0.29; IQR=0.23-0.45) ( $P=0.031$ ). This result was not replicated in a subsequent external validation of the score in a smaller, single-center study in Italy. In this case, 65 consecutive hemophilia patients (57 with HA, 8 with HB) were enrolled, and no differences in HSS score were found between HA and HB (median=0.87 for severe HA vs. 0.91 in severe HB patients).<sup>22</sup>

An additional study that indirectly showed a possible difference in the clinical phenotype of severe HA compared to HB was published a few years later. This single-center, case-control study was carried out in Italy to evaluate the role of genotype and endogenous thrombin potential (ETP) as possible predictors of the clinical phenotype of patients affected by severe hemophilia.<sup>3</sup> The authors evaluated patients displaying an extremely mild bleeding tendency (n=22) in comparison with those showing a typical bleeding tendency (n=50). In this study, the odds of having a milder form of the disease was five times higher in HB patients compared to persons affected by severe HA.<sup>3</sup>

More recently, a Canadian single-institute retrospective study evaluating possible differences between bleeding frequency and use of factor concentrate among adult patients with severe and moderate HA and HB was published.<sup>14</sup> Sixty-eight HA patients (58 severe, 10 moderate) and 20 patients with HB (15 severe, 5 moderate) were studied between 2001 and 2003. Although no significant difference in terms of factor consumption was observed between the two groups, 10 of 68 (14.7%) HA patients had surgical procedures to correct musculoskeletal complications compared to only 1 of 21 (4.7%) in the HB patient group. The bleeding events were also more frequent in the HA group. A total of 2,800 bleeding events were reported in the severe HA group (average 16/patient/year) while 502 total bleeds were reported among the severe HB patients (average 11/patient/year). The difference in the average number of bleeds per year was even more pronounced when considering patients with moderate factor deficiency: 4.6 for HA (n=10) and 1.06 for HB (n=5) patients.

However, a few years later, a study of pediatric HA and HB patients showed apparently contrasting results;<sup>23</sup> overall, this study showed a similar severity in the bleeding phenotype during the initial stage of the disease in severe and in moderate hemophilia A and B.

The cohort of patients in this analysis was made up of consecutive severe and moderate HA and HB patients from the PedNetHaemophilia Registry study and patients with severe HA from the RODIN study. A total of 582 patients with severe HA and 76 with severe HB were included and there was no difference in age at first exposure to clotting factor (0.81 vs. 0.88 years;  $P=0.20$ ), age at first bleed (0.82 vs. 0.88 years;  $P=0.36$ ), or age at first joint bleed (1.18 vs. 1.20 years;  $P=0.59$ ).<sup>23</sup> However, one should bear in mind that this study differed substantially from the others with respect to: a) age (pediatric population vs. adults); b) extensive use of prophylaxis (the authors reported a uniform intention to treat with continuous prophylaxis in 90% of patients born between January 1<sup>st</sup> 2000 and January 1<sup>st</sup> 2010); c) type of outcomes evaluated (bleeding characteristics during the early stage of the disease compared to later-in-life bleeding phenotype and musculoskeletal complications). It is interesting to consider that for all parameters in this study there was a non-significant trend towards earlier age at bleeding in HA *versus* HB patients.

A robust support to the different frequencies of bleeding episodes among the two comes from two recent trials recruiting patients with HA and HB, all treated on demand, for phase III studies with recombinant long-acting products.<sup>24,25</sup> These studies clearly showed that, at enrollment, the annualized bleeding rates in the year before entering the studies were significantly greater in HA patients.

A significant contribution to understanding the possible different evolution of the hemophilic arthropathy in HA and HB was produced by Melchiorre *et al.* in 2016.<sup>26</sup> In this study, including mostly adult patients, the authors showed that the ultrasound score was significantly worse in HA when matched for age and frequency of hemarthrosis. Likewise, the World Federation of Hemophilia clinical score in the HB group was lower [mean and Standard Deviation (SD): 48.6±16.2 vs. 22.6±16.4;  $P<0.0001$ ], indicating a less severe arthropathy than in HA patients with a similar total number of hemarthrosis. In addition, the

analysis of circulating osteoprotegerin (which plays a protective role for the subchondral bone) and receptor activator of nuclear factor- $\kappa$ B and RANK ligand (involved in osteoclast activation and bone erosions) showed a more favorable profile in HB patients. Consistent results were obtained with the histological analysis performed on synovial tissue collected from these patients. Taken together, these data confirmed a less severe evolution of the arthropathy in HB patients and widened our understanding of the pathophysiological mechanisms underlying the different rate of joint deterioration and severity of disease.

Data published in 2018 by Mancuso *et al.*,<sup>27</sup> reporting a study aimed at the development and validation of criteria to define clinically severe hemophilia (CSH), showed again that FIX deficiency is associated to a milder clinical phenotype when comparing patients with the same residual factor activity. In this study, the authors evaluated the ability of residual circulating FVIII/FIX measured at diagnosis using a one-stage clotting assay to discriminate a severe clinical phenotype (defined *a priori* as a CSH score  $>3$ ). Importantly, the results showed a sensitivity of 0.87 [95% Confidence Interval (CI): 0.81-0.91] for FVIII but only 0.68 (95% CI: 0.43-0.87) for FIX, considering a cut-off of 1 IU/dL. In this study, 65.5% (156 of 238) of severe HA patients and 41.2% (13 of 31) of severe HB patients had a CSH score  $>3$ . The higher proportion of patients with HA with a severity score  $>3$  suggests also in this cohort of patients the possible milder phenotype in patients with HB. Among patients with severe disease, the odds of having a clinically more severe form of bleeding symptoms in HA was 2.63 (95% CI: 1.23, 5.64). These results have been recently confirmed also in a study on HA and HB patients with mild disease.<sup>28</sup>

### Orthopedic surgery

The need for orthopedic surgical treatment can be considered a surrogate of severity of hemophilia disease. Chronic arthropathy is a consequence of recurrent bleeding into joints, hemarthrosis, which is a hallmark of severe hemophilia. The higher the number of bleeds in the joints, the higher the chance that a patient will develop permanent bone and cartilage damage requiring surgical intervention. In a retrospective national collection of data on hemophilia patients who underwent joint arthroplasty, Tagariello *et al.*<sup>17</sup> found an Odds Ratio of 3.38 (95% CI: 1.97-5.77;  $P < 0.001$ ) when considering the risk of undergoing orthopedic surgery in HA compared to HB. This difference was confirmed after adjustment for human immunodeficiency virus, hepatitis C virus, and inhibitor status [Hazard Ratio (HR): 2.65; 95% CI: 1.62-4.33;  $P < 0.001$ ]. It is important to note that neither HA nor HB patients had been on regular primary prophylaxis during their lifetime before arthroplasty.

A study on a smaller cohort of patients from the Netherlands could not confirm these results.<sup>29</sup> However, this Dutch analysis was based on a substantially lower number of arthroplasty interventions and patients were mostly on factor prophylaxis (77% in HA, 73% in HB).

A more recent study from the hemophilia treatment centers in the USA collected data on mild and moderate hemophilia patients who were exclusively treated with on-demand therapy.<sup>16</sup> Patients with inhibitors were excluded. A total of 4,771 patients were included in the analysis; 289 (6%) had had orthopedic surgery, such as synovectomy ( $n=75$ ), joint fusion or joint replacement

( $n=126$ ), and 123 had a different type of invasive orthopedic procedure. Interestingly, in the regression analysis, the predicted number of joint bleeds for patients with factor activity  $<30\%$  was greater for patients with HA. Also, the likelihood of undergoing an invasive orthopedic procedure was lower for HB patients (OR: 0.7, 95% CI: 0.5-0.9). These data are consistent with the Italian experience which has also suggested a more frequent progression to orthopedic surgery among patients with HA. Taken together, these results suggest a milder natural history of the disease in the individuals affected by HB. Table 1 summarizes the main clinical findings reported in the studies.

### Potential mechanisms affecting the variability in disease severity between hemophilia A and hemophilia B

Several possible underlying biological explanations for differences in disease severity can be hypothesized and are presented here. A summary of these mechanisms is reported in Figure 2.

*Associated prothrombotic abnormalities* - the variable severity and frequency of bleeding that patients with hemophilia can experience, even at the same measured factor activity, has long been reported. The presence of an associated hypercoagulable state, such as gain-of-function mutations (FV Leiden or prothrombin G20210A) and other coagulation abnormalities (deficiencies of antithrombin, protein C, protein S), has been hypothesized to modulate the bleeding phenotype. However, the clinical relevance of such factors in modifying the clinical phenotype of severe hemophilia patients is still uncertain. In fact, a low prevalence of such prothrombotic factors in severe HA and HB patients with a milder phenotype<sup>30,31</sup> has been reported and conflicting results from different studies have been seen.<sup>32-35</sup> A more recent study investigating ETP as predictor of the clinical phenotype in severe hemophilia patients showed no differences in the distribution of FV Leiden or prothrombin G20210A mutations between severe HA and HB patients.<sup>3</sup>

*Extravascular distribution of FIX* - a possible explanation for a milder phenotype in HB patients may lie in differences in the molecular characteristics and different pharmacokinetics of FVIII and FIX proteins. FVIII resides exclusively in the intravascular space, and its residence time is determined exclusively by the rate of plasma clearance. In contrast, FIX also distributes extravascularly. Since the first pharmacokinetic studies of FIX concentrates, it has become evident that the volume of distribution of FIX, unlike FVIII, is around four times greater than the estimated patient plasma volume and is similar to the central compartment plus the volume of the extracellular fluid. Significant extravascular FIX compartmentalization may increase the apparent volume of distribution, and potentially constitutes a mechanism for extended levels of biologically active FIX. In fact, pharmacokinetic studies showed that the PK of FIX is not linear and is most likely best represented by 3-compartment modeling. Assuming a multi-compartmental model implies that the drug in question, here specifically FIX, follows a complex disposition, with receptor binding or compartmentalization in some extra-vascular space, with potential pharmacodynamic implications.<sup>36</sup>

Even though there has not yet been a direct demonstration of any clinical impact of such a mechanism, pre-clin-

ical studies have now provided several lines of evidence of the ability of FIX to distribute extravascularly,<sup>37</sup> of binding to the extracellular matrix<sup>38</sup> (and in particular to type IV collagen<sup>39</sup>), an *in vivo* effect of the binding to collagen IV,<sup>40</sup> and of tissue distribution of FIX.<sup>41</sup> Although there is some

evidence of tissue distribution in humans,<sup>42,43</sup> in hemophilia patients, the extravascular compartment is not readily accessible and further studies are needed in order to obtain robust evidence of the clinical impact of these specific characteristics of FIX.

**Table 1.** Summary of main clinical findings reported in the studies.

	Patients with severe hemophilia		Patients with moderate hemophilia	
	Hemophilia A	Hemophilia B	Hemophilia A	Hemophilia B
<b>Ludlam <i>et al.</i><sup>19a</sup></b>				
N of patients	99	24	69	33
Hospital admission rate (bed days/pt/year)	7.3	3.1 <sup>o</sup>	3.5	2.1 <sup>o</sup>
<b>Biss <i>et al.</i><sup>21</sup></b>				
N of patients	681	134	250	188
N of patients receiving prophylaxis (%)	424/617 (32)	42/131 (32) <sup>o</sup>	43/244 (18)	9/187 (5) <sup>o</sup>
<b>Schulman <i>et al.</i><sup>15</sup></b>				
N of patients	37	6	21	8
Bleeding score, median (IQR)	0.15 (0.1–0.25)	0.12 (0.6–0.19) <sup>†</sup>	0.05 (0–0.15)	0.05 (0.019–0.11) <sup>†</sup>
Joint score, median (IQR)	0.079 (0.033–0.144)	0.057 (0.024–0.122) <sup>†</sup>	0.020 (0–0.033)	0.054 (0.016–0.096) <sup>†</sup>
Factor score, median (IQR)	0.15 (0.086–0.267)	0.039 (0.028–0.112) <sup>*</sup>	0.004 (0.001–0.015)	0.004 (0.002–0.011) <sup>†</sup>
HSS, median (IQR)	0.50 (0.41–0.68)	0.29 (0.23–0.45) <sup>*</sup>	0.073 (0.024–0.253)	0.115 (0.039–0.349) <sup>†</sup>
<b>Tagariello <i>et al.</i><sup>17</sup></b>				
N of patients	1770	319	Na	Na
Rate of patients undergoing arthroplasty, % (95% CI)	14.3% (12.7%–15.9%)	4.7% (2.4%–7.0%)	Na	Na
Odds of undergoing arthroplasty, OR (95% CI)	3.38 (1.97–5.77)	1 <sup>**</sup>	Na	Na
<b>Den Uijl <i>et al.</i><sup>29</sup></b>				
N of patients	252	30 <sup>o</sup>	Na	Na
Incidence of arthroplasty (%)	78 (31%)	9 (30%) <sup>†</sup>	Na	Na
Age at 1 <sup>st</sup> treatment (years), medians (5 <sup>th</sup> –95 <sup>th</sup> percentiles)	1.1 (0.2–2.7)	1.3 (0.6–2.9) <sup>†</sup>	Na	Na
Age at 1 <sup>st</sup> joint bleed (years), medians (5 <sup>th</sup> –95 <sup>th</sup> percentiles)	1.9 (0.5–5.9)	2.4 (0.9–5.5) <sup>†</sup>	Na	Na
Patients on prophylaxis, N (%)	194 (77%)	22 (73%) <sup>†</sup>	Na	Na
Annual joint bleeding frequency, medians (5 <sup>th</sup> –95 <sup>th</sup> percentiles)	4.3 (0.3–16.3)	3.8 (0.4–17.8) <sup>†</sup>	Na	Na
Annual factor use (IU Kg-1), medians (5 <sup>th</sup> –95 <sup>th</sup> percentiles)	1560 (286–3644)	1260 (302–5826) <sup>†</sup>	Na	Na
<b>Santagostino <i>et al.</i><sup>3</sup></b>				
N of patients	61	11 <sup>o</sup>	Na	Na
Mild bleeding phenotype, N (%)	15 (25)	7 (64) <sup>*</sup>	Na	Na
Median age at first bleed, months (IQR)	12 (12–36)	24 (12–48) <sup>†</sup>	Na	Na
Median age at first joint bleed, months (IQR)	24 (24–48)	66 (51–102) <sup>**</sup>	Na	Na
Median number of bleeds per year (IQR)	11 (2–25)	0 (0–12) <sup>*</sup>	Na	Na
<b>Clausen <i>et al.</i><sup>23</sup></b>				
N of patients	582	76	97	26 <sup>o</sup>
Age at diagnosis (years)	0.42 (3 days–0.88)	0.43 (0 days–0.88) <sup>†</sup>	0.77 (2 days–1.62)	2.5 days (0 days–1.17) <sup>†</sup>
Age at 1 <sup>st</sup> treatment	0.81 (0.43–1.11)	0.88 (0.60–1.18) <sup>†</sup>	1.42 (0.90–2.92)	1.74 (0.99–4.10) <sup>†</sup>
Age at 1 <sup>st</sup> bleed	0.82 (0.50–1.12)	0.88 (0.60–1.19) <sup>†</sup>	1.47 (0.98–2.82)	1.76 (0.94–3.23) <sup>†</sup>
Patients without bleeding without prophylaxis < 2 years of age, N	105 (17.5)	12 (15.4) <sup>†</sup>	34 (34.7)	15 (57.7) <sup>*</sup>
Median factor (IU Kg-1/year) (IQR)	1280 (369–2170)	333 (38–487) <sup>**</sup>	Na	Na
Median orthopedic joint score (range)	13 (6–20)	5 (2–9) <sup>*</sup>	Na	Na
Median Pettersson score (range)	28 (20–45)	23 (9–31) <sup>†</sup>	Na	Na
<b>Nagel <i>et al.</i><sup>14</sup></b>				
N of patients	58	15	10	5 <sup>o</sup>
Total bleeds over 36 months, N	2 800	502 <sup>o</sup>	138	16 <sup>o</sup>
Joint bleeds, N	1 491	332 <sup>o</sup>	Na	Na
<b>Melchiorre <i>et al.</i><sup>26</sup></b>				
N of patients	70	35	Na	Na
Hemarthrosis, N (%)				
<10	11 (15.7)	15 (42.9) <sup>**</sup>		
10–50	16 (22.8)	3 (8.5) <sup>**</sup>		
>50	43 (61.4)	17 (48.6) <sup>*</sup>		
Pettersson score, mean ± SD	6.81±3.99	5.64±4.02 <sup>†</sup>	Na	Na
WFH score, mean ± SD	36.6±21.6	20.2±14.6 <sup>**</sup>	Na	Na
US score, mean ± SD	10.91±4.05	4.34±3.39 <sup>**</sup>	Na	Na

<sup>o</sup>Severe hemophilia was defined as a factor VIII/IX level of <2 U/dL; moderate hemophilia was defined as 2–9 U/dL. +Number (N) of patients at study end in 1994. \* $P \leq 0.05$ , HA versus HB. \*\* $P \leq 0.001$ , HA versus HB. <sup>†</sup>NS: not statistically significant, HA versus HB. <sup>o</sup>Comparison of HA versus HB not available. IQR: interquartile range; CI: Confidence Interval; HSS: Hemophilia Severity Score; Pt: patient; SD: Standard Deviation; WFH: World Federation of Hemophilia; US: ultrasound; Na: not available.

It is interesting to note that the results of a recent study by Cooley *et al.*<sup>59</sup> also suggest that the total amount of FIX is approximately three times larger than what can be measured in the intravascular compartment.

The accessible intravascular compartment from which we collect our plasma samples to measure FIX activity might not, therefore, be reflecting the overall coagulative capacity of the endogenous FIX, but may only reveal information about the fraction that is freely circulating. This adds up to the inter-laboratory differences in FVIII:C and FIX:C measurements and it should be borne in mind that also misclassification might explain, at least in part, the discrepancy in the bleeding tendency between severe HA and HB patients.

Altogether, the information available so far on the pharmacokinetic and pharmacodynamic characteristics of FIX suggest some potential mechanisms that could explain a difference in bleeding tendency between HB and HA patients.

**Inhibitor development** - the occurrence of high affinity anti-FVIII or anti-FIX antibodies that neutralize the activity of the infused clotting factor is a major complication of replacement therapy in hemophilia patients. Inhibitor antibodies against FVIII can develop in approximately 25-30% of severe HA patients; in contrast, in patients with HB, inhibitors develop in only 3-5% of patients treated with factor concentrates.<sup>44</sup> Immune tolerance induction (ITI) via long-term, intravenous administrations of factor concentrates is the only proven strategy to eradicate inhibitors. However, this approach is expensive and impractical for the patients.<sup>45</sup> Overall, it is successful in approximately 60-70% of HA patients. The results of a

randomized, controlled study comparing high-dose (200 IU/Kg/day) and low-dose (50 IU/Kg/3 times a week) protocols in a cohort of severe HA patients with high-titer inhibitor showed a similar overall success rate and no statistically significant differences in time to achieve tolerance, but the median time to negative inhibitor titer was 4.6 months (range: 2.8-13.8).<sup>46</sup>

Although the development of inhibitors to FIX is a much less common event, ITI treatment is not as successful (only approx. 30%), and the development of anti-FIX antibodies may be associated with anaphylactic reactions which may prevent or complicate ITI regimes in a significant proportion of cases.<sup>47</sup> HB patients undergoing ITI can also develop nephrotic syndrome.<sup>48,49</sup> However, a few anecdotal experiences suggest that ITI could be successfully achieved by adding immunosuppression treatment, especially in patients with anaphylactic reactions.<sup>50</sup>

## Conclusions

Different lines of evidence seem to support a difference in bleeding severity between HA and HB. The pathophysiology of the two disorders is, indeed, diverse, with a different distribution of the factors in the body and, in keeping with this, the PK characteristics of infused factors are significantly different. However, because of the rarity of the disorders, no prospective, head-to-head comparative studies have been carried out, and the modern approach in the pediatric hemophilia population (i.e. starting prophylaxis very early) does not allow us to acquire a better understanding of the possible clinical differences during follow up. Improved replacement therapy with extended half-life concentrates with 10- to 14-day intervals between infusions and sustained high FIX troughs<sup>51</sup> are greatly

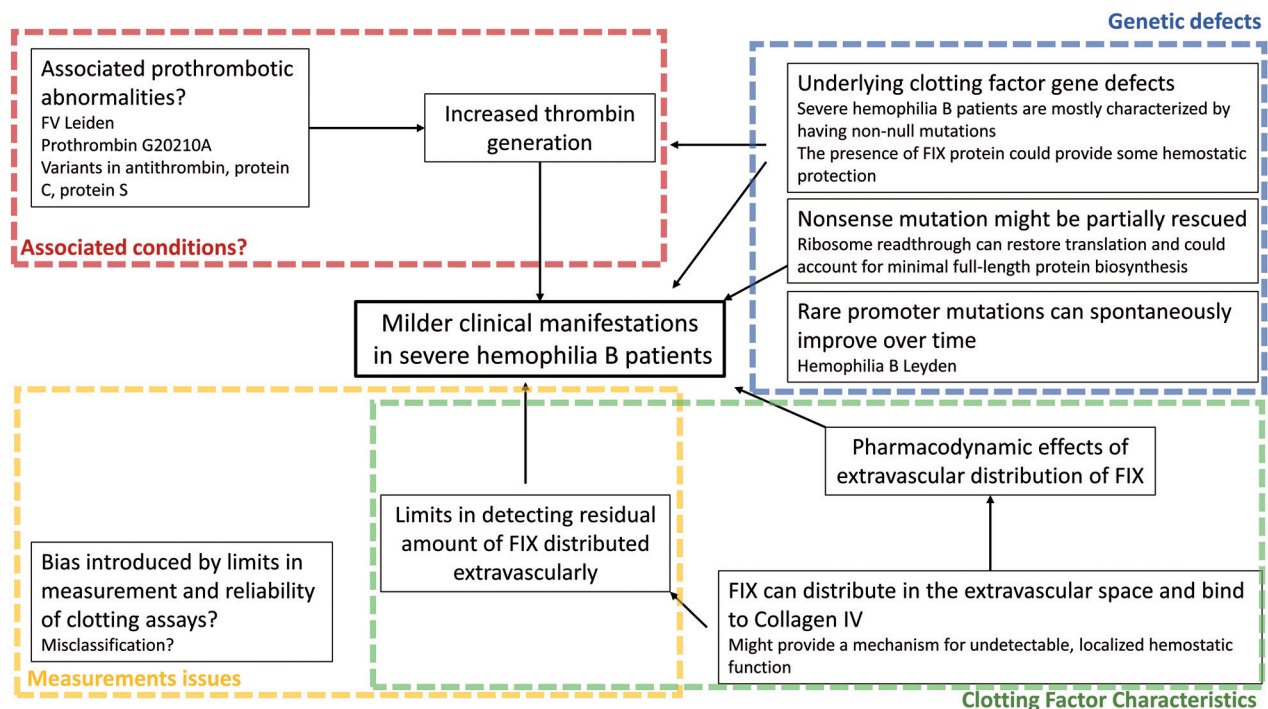


Figure 2. Possible mechanisms involved in the differences in clinical evolution between hemophilia A and hemophilia B patients. FIX: factor IX.

improving the clinical outcome and, even more so, the quality of life of HB patients when compared to HA patients. The promising perspectives of gene therapy are painting a future scenario in which the decision to offer this option to patients with HB and very mild clinical problems could be challenging considering the costs involved and the yet unknown long-term effects.

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