

Inhibitors in Hemophilia B

Cristina Santoro, MD¹ Gabriele Quintavalle, MD² Giancarlo Castaman, MD³ Erminia Baldacci, MD¹
 Antonietta Ferretti, MD¹ Federica Riccardi, PhD² Annarita Tagliaferri, MD²

¹Department of Cellular Biotechnology and Hematology, Sapienza University of Rome, Rome, Italy

²Regional Reference Centre for Inherited Bleeding Disorders, University Hospital of Parma, Parma, Italy

³Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy

Address for correspondence Cristina Santoro, MD, PhD, Hematology, Department of Cellular Biotechnology and Hematology, Sapienza University of Rome, Via Benevento, 6–00161 Rome, Italy (e-mail: santoro@bce.uniroma1.it).

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Abstract

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Hemophilia B (HB) is an X-linked bleeding disorder caused by deficiency of factor IX (FIX). Patients with the severe form (FIX <1%) account approximately for 30 to 45% of persons with HB and usually suffer from recurrent joint, soft-tissue, and muscle bleeds. The availability of safe plasma-derived and recombinant products has virtually abolished the risk of viral infections and the adoption of prophylactic regimens has attenuated the impact of hemophilic arthropathy. Therefore, the development of an inhibitor against FIX is currently the most serious complication that can still occur in the new generations of HB patients. The development of an inhibitor in HB is a rare event (1.5–3% of all patients) but is associated with a significant morbidity, related not only to the bleeding risk but also to the frequent occurrence of allergic/anaphylactic reactions and nephrotic syndrome. Due to the relative rarity of this event, few data exist about risk factors, pathophysiology, and clinical aspects of inhibitors in HB. The induction of immune tolerance is often unsuccessful and can be otherwise affected by many complications in patients with history of allergy or anaphylaxis. Therefore, alternative therapeutic strategies and new approaches are developing. The aim of this narrative review is to discuss current knowledge about risk factors, pathophysiology, and clinical aspects of this rare but serious complication.

Hemophilia B (HB) is an X-linked bleeding disorder caused by the deficiency of factor IX (FIX),¹ a vitamin K-dependent plasma protease that participates in the intrinsic blood coagulation pathway and whose function is to cleave and activate FX within the coagulation cascade.² Bleeding tendency in HB is in good accordance with the severity of factor deficiency. Thus, patients with the severe form (FIX <1%), who account approximately for 30 to 45% of persons with HB, usually suffer from recurrent joint, soft-tissue, and muscle bleedings.³ The large availability of virus-inactivated plasma-derived and recombinant concentrates allows for safer and a more widespread prophylactic approach and is

progressively leading to attenuation of hemophilic arthropathy and the risk of viral infection has been virtually abolished. Moreover, the new extended half-life (EHL) FIX concentrates are giving HB patients the chance to significantly reduce the number of required infusions, improving prophylaxis targets and treatment compliance and quality of life.^{4–6} Therefore, the development of an inhibitor against FIX is currently the most serious complication that can still occur in the new generations of HB patients.³ The reported rate of FIX inhibitors is 1.5 to 3% of all patients with HB.⁷ Since the prevalence of the disease is ~1:30,000 males,⁸ and inhibitors are anecdotally reported in nonsevere HB,⁹ the development

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of FIX inhibitors is rarely encountered in normal clinical practice. Consequently, few reliable data exist on host- and treatment-related risk factors, and in particular regarding the immunological mechanisms associated with this complication.⁷ Inhibitor development in HB is associated with significant morbidity, not only related to the risk of uncontrollable bleeding. In fact, allergic or frankly anaphylactic reactions and development of nephrotic syndrome can frequently occur with inhibitor development and further complicate attempts to eradicate FIX inhibitor as well as treatment of bleeds.^{1,10–15} The aim of this narrative review is to discuss the current knowledge about risk factors, pathophysiology, and clinical aspects of this rare but severe complication.

Epidemiology

There are no prospective studies, other than one very recent report,¹⁶ aimed to evaluate the real incidence of the inhibitor development in HB, and rates reported in the literature are referred to patient cohorts observed in a given period. The prevalence of FIX inhibitors is reported to be 1.5 to 3% of all patients with HB and 9 to 23% of severe ones.^{7,11,17–20} About 80% of the inhibitors which develop against FIX are of the high responding type, meaning titers above 5 Bethesda Units/mL (BU/mL).²¹ The great majority of studies or registries report the overall prevalence of inhibitors in cohorts of HB patients regardless of the disease severity, previous treatments with FIX concentrate, or possible relationship

with the product type.²² In the FIX International Variant Database (F9db), inhibitors are reported in approximately 2% of patients, almost exclusively with severe disease;²³ a similar rate is reported in the Universal Database Collection (UDC) project in 3,785 patients with HB analyzed.⁹ FIX inhibitor rates described in the literature by different authors are shown in **Table 1**. From a systematic review (seven studies) comprising a total of 176 severe HB previously untreated patients (PUPs), 85 treated with plasma-derived (pd) FIX concentrates and 91 with the recombinant (r) FIX product, inhibitor rates were between 5 and 14%, with an outlier value of 37% in a Swedish study, consistent with previous data, probably due to the genetic background.^{20,22} Overall, inhibitors occurred in 18 patients (10.2%). When the type of concentrate was considered, from six of seven studies providing such information, a statistically significant higher inhibitor rate in patients treated with pd versus rFIX products (11/72, 15% vs. 5/89, 5%; $p = 0.04$) was found. Among the 18 cases of inhibitors, 7 were high responding and 6 were low responding (peak inhibitor titer $>5\text{UB/mL}$ or $<5\text{UB/mL}$, after FIX exposure, respectively); in the remaining 5 patients, the titer of inhibitor was not reported. In the majority of cases with information available, inhibitors were associated with anaphylaxis (four of five patients, 80%). Finally, a high-risk mutation was found in 73% (8/11) of the inhibitor patients.²² A very recent abstract reporting data from an international prospective registry showed an inhibitor incidence of 11.8% in severe HB PUPs born in the time period 2000–2009 (10/85, 5 low- and 5 high-titer inhibitors).¹⁶ This

Table 1 Reported rates of inhibitors in HB

Study (first author, year)	All HB patients (n, %)	Severe HB patients (n, %)
Briet, 1991 ¹⁷	54/3352 1.6%	–
Katz, 1996 ¹⁹	29/1967 1.5%	28/728 3.8%
Ljung et al, 2001 ²⁰	–	11/48 23%
Key, 2004 ²¹	4–5%	–
Belvini et al, 2005 ³³	–	8/169 4.7%
Miller et al, 2012 ³¹	2/153 1.3%	2/46 4.3%
Rallapalli et al, 2013 ³⁰	59/3,721 1.5%	–
Castaman et al, 2013 ¹	8/282 2.8%	–
Puetz et al, 2014 ⁹	75/3,785 1.9%	67/1,367 4.9%
DiMichele, 2007 (review, 5 studies) ⁷	1.5–3%	9–23%
Franchini et al, 2017 (review on PUPs, 7 studies) ²²	–	18/176 10.2%
Van Den Berg et al, 2018 (PedNet registry) ¹⁶	–	10/85 11.8%

Abbreviations: HB, hemophilia B; PUPs, previously untreated patients.

incidence is similar to the data in PUPs reported in the systematic review by Franchini et al.²² No inhibitor development has been reported in previously treated patients (PTPs) exposed to the new EHL-FIX concentrates (recombinant factor IX-Fc fusion protein, rFIXFc; recombinant factor IX-albumin fusion protein, rIX-FP; Nonacog Beta Pegol N9-GP),^{4–6} while data from studies on PUPs are not available.

Risk Factors and Pathophysiology

Inhibitors are high-affinity polyclonal antibodies that neutralize the procoagulant activity of a coagulation factor. Although the incidence is lower than in HA, most HB inhibitors are “high titer.” The knowledge on determinants of inhibitor risk in HB is scarce, but at least three distinguishing features have been surmised to explain the lower frequency compared with hemophilia A.²⁴ First, it has been suggested that FIX may be a less immunogenic protein than FVIII.^{25,26} A reason for the decreased FIX immunogenicity could be the considerable conservation of amino acid sequence homology among vitamin K-dependent clotting factors (FII, FVII, FIX, FX, protein C, and protein S) which may confer some tolerance to FIX in patients with no circulating FIX.¹³ Second, as a whole, there is a lower proportion of the severe type in HB patients (30–40%) than in HA (60%).²⁷ Moreover, based on detectable FIX antigen, in HB a higher proportion of Cross Reacting Material positive (CRM+) patients who produce nonfunctional FIX protein has been found.^{20,28} So far, almost all reported inhibitors in HB have developed in patients with null mutations in FIX gene (*F9*) (deletion, stop codon, etc.) and who are anticipated to produce no protein (CRM-). The third explanation correlated to the previous feature resides in the type of *F9* defects, given the low prevalence of high-risk genotypes among HB patients compared with HA.⁷ HB is invariably caused by mutations in *F9* (Xq27, 34 kilobases, 8 exons),²⁹ and 1,094 unique molecular defects have been identified in the 3,713 patients with HB listed in the *F9db*.^{23,30} Compared with severe HA, patients with severe HB are three times more

likely to have a missense mutation (47 vs. 16%), which is associated with very low inhibitor risk.^{31,32} On the other hand, in HB patients, complete deletions or rearrangements of *F9* (~50%) and nonsense or frameshift mutations (~20%) carry an increased risk of inhibitor occurrence; gene inversions, related with inhibitor risk in HA, have not been identified in HB patients.^{12,33,34} The ISTH Scientific and Standardization Committee International FIX inhibitor registry (ISTH-SSC registry; 1997–2006) analyzed 94 patients with severe HB and an inhibitor.³ Thirty-two of 94 patients underwent genetic analysis demonstrating that large deletions and nonsense mutations were associated with both inhibitor development and risk of anaphylaxis. The *F9db* has highlighted the mutations associated with inhibitor development.^{30,35} Overall, 62 of 3,713 (1.6%) patients in the database developed inhibitors, a prevalence rate that validates the historical cohort data. Among these, only one missense mutation, Gln237Lys, was associated with inhibitor formation as was already previously reported.^{2,36,37} The frequencies of the types of mutations within the patients with inhibitor are shown in ►Fig. 1. In the Centers for Disease Control's Hemophilia B Mutation Database (CHBMP), mutations were collected from the current *F9db* combined with a systematic literature search: 1,083 variants were identified in more than 3,700 HB cases.³⁸ Moreover, a more recent subanalysis from the same database³⁴ considered the prevalence of the inhibitor within different types of mutations only in patients for whom genetic mutations and inhibitor status were available ($n = 249$). The data from these analyses are reported in ►Table 2. The systematic review from Franchini et al.²² shows that high-risk *F9* mutations are present in more than 70% of evaluable inhibitor patients. All these data suggest a strong association between absent endogenous FIX protein due to gross and complete gene mutations and inhibitor development.

The occurrence of inhibitory antibodies is a multifactorial event in which many other determinants are involved, such as immune response mechanisms. Two human cytokine genes, interleukin-10 (IL10) and tumor necrosis factor

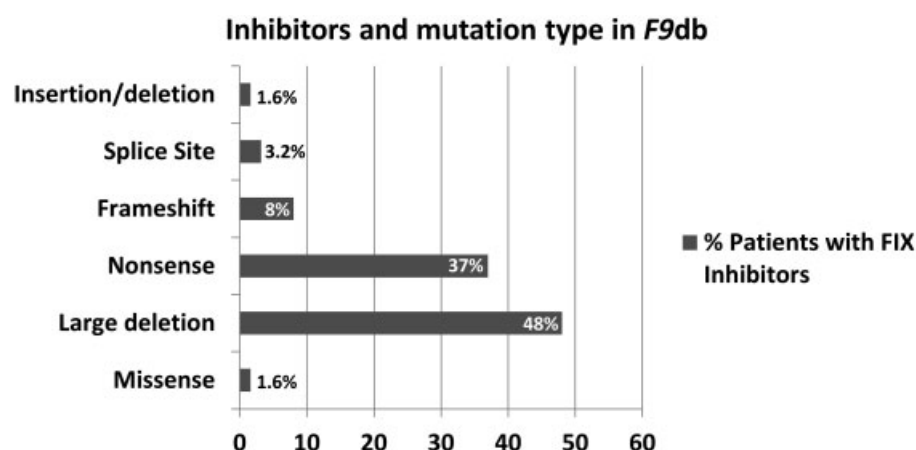


Fig. 1 Distribution of 62 patients with FIX inhibitor within *F9* gene mutations, as reported in the FIX International Variant Database (*F9db*, www.factorix.org).^{2,30}

Table 2 Rates of FIX inhibitor within the most frequent *F9* variants reported in the Centers for Disease Control Hemophilia B Mutation Project (CHBMP) mutation list

<i>F9</i> variant	Li et al: ³⁸ 3,785 pts, 1,083 mutation Inh + / total mutations (%)	Saini et al: ³⁴ 249 pts Inh + / total mutations (%)
Missense	2/633 (0.3%)	2/168 (1.1%)
Large deletions (>50 bp)	9/33 (27%)	7/18 (38.8%)
Nonsense	6/90 (6.6%)	11/32 (34.3%)
Frameshift	6/197 (3.5%)	6/22 (27.2%)
Splice site	1/102 (0.9%)	0/6 (0%)
Small structural changes ^a	0/21 (0%)	0/3 (0%)

Abbreviations: *F9*, FIX gene; pts, patients; Inh + , positive inhibitor patients.

^aSmall structural changes include small deletions (<50 bp) and insertions.

(TNF), have been linked to FVIII inhibitor development.^{39,40} No such data currently exist for FIX inhibitor patients. However, genetic linkage studies in multiple recombinant inbred strains of HB mice suggested that multiple gene loci could be linked to the inhibitor response in these animals.⁴¹ These experiments demonstrated that the major histocompatibility complex (MHC) class II (H-2) and/or K class I-a (Iak) loci were critical to the inhibitor response. The role of the HLA alleles was also highlighted in the article from Saini et al,³⁴ in which only one of two siblings with inhibitors, an identical *F9* mutation, but different HLA alleles, showed a response to immune tolerance induction (ITI). The HLA alleles of the individual patient, in conjunction with the mutation type, could be a predictor for the development of inhibitors, as well as the response to ITI.³⁴ Known linkages also include polymorphic markers from chromosomes 1 and 10, proximal to IL10 and IFN1 α (interferon- α) immune regulatory genes.⁴¹ The interesting observation of an increased rate of inhibitors in PUPs treated with pdFIX versus those receiving rFIX products (15.3 vs. 5.6%), because of the low number of events reported (i.e., inhibitor development), does not allow any definitive conclusion to be made.²² If confirmed by further studies, this finding could be, however, related to the peculiar anaphylactoid nature of most alloantibodies in HB,^{42,43} which could account for the increased immunogenic properties of intermediate and high purity pdFIX concentrates, as outlined in the report by the 10-year (1997–2006) ISTH-SSC registry.³ A similar increased inhibitor incidence in HB patients receiving pdFIX versus rFIX products (5 vs. 2%) was also found in a retrospective international study on 180 patients.⁴² Moreover, taking into account the increased clearance rate of rFIX versus pdFIX,⁴⁴ differences in the exposure to the immune system could be hypothesized to translate into a reduced immunogenicity of the former product.

As to antibody subclasses, a study on the assessment of the antibody profile of 37 HB patients showed that anti FIX IgG-4, IgA, and IgE are detected significantly more often in patients with a positive Nijmegen-Bethesda assay (NBA). All the NBA-positive samples were positive for IgG4 and showed a strong correlation with this antibody class while, although

significant, the correlation with IgG1–2 and IgA anti-FIX was not so strong. Anti-FIX IgG4 is considered particularly relevant to functional inhibition.²⁴ Similar data were previously reported by Sawamoto et al in an analysis on 10 plasma samples from six HB inhibitor patients, including five with a history of an allergic phenotype, demonstrating that the human anti-FIX antibodies were predominantly IgG4 in nature.⁴⁵ Interestingly, transient IgG1 subclass antibodies were also detected in all three allergic phenotype patients whose plasma was collected at the time of the allergic episode. However, when the plasma samples were collected from 4 days until more than 4 weeks after the acute event, IgG1 antibodies were negative in two of three of these patients, as well as in two additional allergic phenotype inhibitor patients.⁴⁵ These data suggest that the allergic response that occurs in some FIX inhibitor patients may be associated with transient IgG1-subclass antibody production. Within the FIX epitopes mostly targeted by IgG1 and IgG4 antibodies, the γ -carboxyglutamic acid (GLA) and serine protease (SP) domains were reported but not the epidermal growth factor (EGF) and activation peptide (AP) domains.⁴⁶ Functionally, these antibodies inhibited the activated FIX/FVIII intrinsic FX activation complex by interfering with FIX binding to phospholipids as well as phospholipid-independent FIX binding to FVIII light chain.⁴⁶ More in vitro and in vivo studies are required to confirm these data and to further define the immunological and biochemical features of the FIX inhibitory antibody response.

The presence of IgE functionally active on peripheral blood basophils and involved in the hypersensitivity reaction has also been demonstrated by Cugno et al.⁴⁷ The authors propose a cellular antigen stimulation test (CAST) as a screening tool to identify HB subjects who are prone to hypersensitivity reaction.

Regarding data upon the natural history of FIX inhibitor development, the ISTH-SSC registry of FIX inhibitors has added a significant contribution. Ninety-four patients with FIX inhibitors developed antibodies at a median age of 19.5 months (range: 9–156 months) after a median of 11 (range: 2–180) exposure days (EDs) to exogenous FIX replacement therapy. Antibody development occurs in all ethnic groups,

with both pdFIX and rFIX concentrates. The median historical peak inhibitor titer was 30 BU (range: 1–1,156)/mL.^{3,13} The CHBMP database was queried to determine the prevalence of inhibitors in HB subjects. In addition, disease severity, race/ethnicity, age, factor exposure, and prophylaxis usage were evaluated to determine their impact on inhibitor prevalence. Of the 3,785 male subjects with HB enrolled in the UDC database, 75 (2%) were determined to have an inhibitor at some point during the study period. Severe disease (odds ratio [OR]: 13.1, 95% confidence interval [CI]: 6.2–27.7), black race (OR: 2.2, 95% CI: 1.2–4.1), and age <11 years (OR: 2.5, 95% CI: 1.5–4.0) were found to be significantly associated with having an inhibitor.⁹

Clinical Aspects and Complications

Two unique clinical features distinguish inhibitor occurrence in HB from HA: the association with an anaphylactic response^{11–13,48} and the tendency for patients who undergo IT to develop nephrotic syndrome (kidney disorder characterized by proteinuria, hypoalbuminemia, and edema).^{3,15} Patients with severe HB are at high risk of sudden development of anaphylactic shock or other severe allergic reactions together with inhibitor development: while these two events are often closely temporally related, one may precede the other. There are several explanations for the development of allergic/anaphylactic reactions in HB, but the exact mechanisms are not yet clear:⁷ IgE-mediated and non-IgE-mediated immune responses may be involved.⁴⁸ Antibodies of subclasses IgG1 and IgG4 were detected at the occurrence of anaphylactic reactions in some patients with inhibitors and history of anaphylaxis.⁴⁵ The appearance of specific IgG inhibitors may subsequently lead to a complete activation of anaphylatoxins with the release of mediators from mast cells⁴⁹ and complement activation by transient IgG1 antibodies formation.⁷

The mast cell activation and IgE-mediated hypersensitivity could have also another explanation.³ Given its smaller molecular weight, FIX can distribute to both intra- and extravascular space compared with FVIII, which remains inside the intravascular space.²⁷ Skin and radio-allergo-sorbent testing in a few such patients supports this hypothesis, but it has yet to be further studied.⁵⁰ Another possible reason is that FIX plasma concentration is much higher than FVIII one (5 and 0.1 µg/mL, respectively).^{2,3} Therefore, the dose required to achieve adequate plasma concentrations for hemostasis with FIX infusions is also much higher and this has been hypothesized to play a role.¹³ It is indeed known that when the antigen dose is sufficient to overwhelm the binding capacity of IgG blocking antibodies, the antigen exposure itself may lead to anaphylaxis^{51,52} because IgG share antigen specificity with IgE bound to high-affinity IgE receptor (FcεRI) on mast cells, thus leading to mast cell activation.⁵³

The high concentration of exogenous FIX protein infused with treatment is probably involved in another immunologic mechanism of FIX inhibitors, which is excessive circulating immune complexes formation. Although immune complexes

have not yet been documented in allergic phenotype patients, they can initiate anaphylaxis when the individual is reexposed to FIX concentrate.^{2,7,13} Studies in mouse models support this hypothesis by showing that IgG-immune complex activation of FcγRIII may induce anaphylaxis via activation of macrophage, mast cells, or neutrophils.^{52–56}

The type of FIX product does not seem to play a role in allergic reaction induction. Data from the ISTH-SSC registry failed to detect any difference in anaphylactic and severe allergic events after exposure to intermediate-purity or high-purity (either recombinant or plasma-derived) FIX products.³ This result is supported by Recht et al, who in a retrospective study regarding the incidence of allergic reactions to FIX in 180 HB subjects did not find any difference in the frequency of allergic reactions between pdFIX and rFIX.⁴² Finally, given the risk of potentially life-threatening reactions, it has been suggested that all infants and small children with severe HB should be closely monitored over their first 20 or more infusions with any FIX-containing product in a facility equipped to treat anaphylactic shock.^{11,22,57,58}

Nephrotic syndrome may occur typically during ITI therapy and can be a severe complication. It is more frequent in patients with previous infusion reactions to FIX (allergic phenotype).¹⁵ Frequently, nephrotic syndrome is nonresponsive to steroids and requires the suspension of ITI.³ Renal biopsies demonstrated membranous glomerulonephritis in two patients, but immunohistochemical staining of tissue obtained from a single renal biopsy failed to demonstrate any association with FIX immune complexes.^{15,59,60} In general, adverse reactions can heavily affect the success rate of ITI program in HB. In the North American Immune Tolerance Registry (NAITR), 65% of patients with HB on ITI had adverse events compared with only 6% with HA.⁶¹ Allergic reactions represented 79% (11/14) of adverse events in HB, and nephrotic syndrome occurred in 3 of 16 completed ITI courses (20%).⁶¹ Within five patients who presented episodes of allergic reactions, only one underwent a successful ITI (20%). In the ISTH-SSC registry, anaphylaxis was reported in 56 of 94 patients (60%) and nephrotic syndrome affected 13 of 94 cases (14%). The nephrotic syndrome presented 8 to 9 months after the start of high dose ITI (100–325 IU/kg/day) with sepsis-like symptoms in conjunction with periorbital edema, hypoalbuminemia, and proteinuria. Both pdFIX and rFIX products were implicated. After the cessation of the FIX infusion, a clinical improvement was usually obtained, although the response to steroid therapy was historically poor.³ So far, the etiology of this phenomenon remains unclear. Only 5 of 39 (12.8%) patients who underwent ITI were successfully cleared of the inhibitor; data about the occurrence of allergic reaction within these five patients are not reported.

Inhibitor Eradication

Because of the lower prevalence and risk of inhibitor development of HB, the literature evidence to support decisions regarding inhibitor eradication in this subset of patients is

limited,⁶² and it is difficult to develop a useful and evidence-based approach to the prevention and eradication of FIX inhibitors.^{63–65} Data from the ISTH-SSC registry showed that the antibody development in susceptible patients with large or complete F9 deletions occurs early in the patient's exposure history, after a median of 11 FIX EDs, but, as also confirmed in a very recent report, may continue for up to 180 EDs.^{3,11,13,16} However, as previously discussed, allergic reactions frequently precede antibody detection, highlighting the need for a frequent surveillance protocol. In fact, severe HB patients should undergo routine early molecular diagnosis to identify high-risk mutations. Moreover, a prolonged period of hospital-based FIX infusion prior to transition to home treatment is recommended, to monitor and assess such patients closely during the high-risk period for inhibitor development. According to the UK Hemophilia Centre Doctors Organization (UKCDO) and to the Italian Association of Hemophilia Centers (AICE) guidelines, the first 20 infusions should be given in hospital with access to pediatric resuscitation facilities.^{66,67} Because of the rarity of the late development of FIX inhibitors in the heavily PTP, tests after 150 EDs are not required unless clinically indicated.⁶⁶

The general principles of ITI may be applicable to HB patients with inhibitors, but because of the potential for severe anaphylactic reactions to FIX concurrent with the appearance of an inhibitor,¹¹ the risk for nephrotic syndrome,¹⁵ and the low likelihood of successful tolerization,^{3,68} this intervention should be considered on a case-by-case basis. Patients with a low-titer inhibitor and adequate clinical response in the absence of allergic symptoms can continue to be treated with FIX, while ITI should be considered in those with a high-titer inhibitor or inadequate clinical response to FIX infusions.⁶² The historical experience with ITI in HB is limited to two small series and a total of seven patients for whom the overall success rate with high-dose FIX with or without immune modulation was 71%.^{69,70} Moreover, in six of seven HB patients treated with the Malmö regimen, the ITI was successful; however, tolerance was lost in one patient within 6 months, and a further attempt to re-induce tolerance was unsuccessful.⁷¹ In the NAITR, only 5 of 16 (31%) completed courses of ITI in HB were successful, on a median dosing regimen of 100 U/kg/day (range: 43–200).⁶¹ Daily dosing regimens and immune modulation were used in 88 and 47% of courses, respectively. Plasmapheresis was used in two courses. High purity or monoclonal FIX concentrates were used in 82% of the reported courses.⁶⁸ Similar poor outcomes were reported from the ISTH-SSC registry for FIX inhibitors.³ Only 5 of 39 (13%) patients who underwent ITI were tolerized: 25 patients were treated with FIX concentrate only, while 14 were treated with FIX combined with immune modulation. Plasmapheresis was performed on 7 of the 14 patients.³ In an Italian series, eight HB inhibitor patients were described.¹ ITI treatment was successful in four of five patients in whom it was undertaken, while it was partially successful in the remaining one patient. The median dose was 80 U/kg/day, but a wide range of dosage and schedule was used. Of interest, ITI was successful not only

in low responders but also in two high responder patients, as previously reported in the NAITR.⁶¹

To reduce the risk of anaphylactic reactions, desensitization protocols have been used. In particular, successful desensitization with gradually increased dose administration of FIX prior to ITI has been described^{48,64} in three patients, using a protocol involving skin testing to FIX-containing products. Other successful desensitization protocols combined with immune modulation, such as the use of plasmapheresis, or an addition of steroids or rituximab before, during, or after the escalating dose, have been reported.^{72–76} Currently, given the overall poor ITI success rate as well as the potential for the development of nephrotic syndrome and allergic reactions during ITI, most clinicians decide either not to proceed with ITI or proceed with extreme caution, adopting desensitization or combining the infusion of FIX concentrate with immunomodulation. This recommendation has also been made by the UKCDO and AICE guidelines.^{66,67} If the decision is made to proceed with ITI, strong consideration is given to pre-ITI immune desensitization to FIX as well as to the use of low-dose regimens which has been reported to be successful in four patients from an Italian cohort.¹ With the available information, it is not possible to support a specific regimen (dose or product type) with outcomes. In patients with a history of allergic reaction to FIX, ITI can be cautiously considered after desensitization and in conjunction with immunosuppression, but this is based on a limited number of case reports and case series and increases the burden of ITI.^{73,77} Routinely, immunosuppression in patients without a history of allergic reactions is not a part of the ITI protocol, but given poor response rates to ITI alone, it may be used during ITI after careful discussion with patients and their families. Immunosuppression regimens used have included the combination of intravenous immunoglobulin (IVIg), rituximab, dexamethasone, and mycophenolate mofetil.^{77–80} Successful tolerance was induced in a single FIX inhibitor patient using cyclosporine.⁷⁸

Monitoring for nephrotic syndrome during ITI is essential. Successful or partially successful tolerance of HB inhibitor patients with an allergic phenotype, uncomplicated by nephrotic syndrome, was reported with a regimen of mycophenolate-mofetil, IVIg, dexamethasone, and high-dose FIX.^{79,80} Few cases of severe HB patients who have failed ITI regimens and treated with rituximab for inhibitor eradication have been reported in the literature.⁸¹ Mathias et al reported the first case without clinical improvement after the 4-week standard administration of rituximab alone.⁸² However, several successful cases were subsequently reported.^{83–85} Particularly interesting are two cases^{77,86} successfully treated with a protocol combining ITI with immunosuppression with rituximab, steroids, and high-dose IVIg to enhance the response and to reduce anaphylaxis and nephrotic syndrome.

As reported in the algorithm proposed by Franchini and Mannucci,⁸³ rituximab therapy could be considered, in combination with FIX concentrate and immunosuppression therapy, for HB patients after ITI failure and the development

of allergic reactions and/or nephrotic syndrome. The administration of rituximab as first-line therapy for antibody eradication, to avoid the risk of nephrotic syndrome, is an attractive option but needs to be supported by additional clinical data. The recommendations of the panel of the international workshop on ITI highlighted the need of early genotyping to assess risk for inhibitor development with/without allergic manifestations and of consideration for desensitization to improve the control of bleeding (Level IV evidence).⁸⁷ Although no specific ITI recommendations were possible, if ITI is initiated, it has to be accompanied by close surveillance for nephrotic syndrome using frequent urinalyses.⁸⁷ According to the UKCDO guidelines, the start of an ITI should be considered carefully, given the risk of adverse reactions.⁶⁶ The addition of immunosuppression is recommended given the higher success rate (grade IIC). ▶ **Fig. 2** shows an algorithm for the management of HB patients with inhibitors.

Bleeding Treatment and Prophylaxis

Treatment of hemorrhagic episodes in HB patients with inhibitors has to be individualized. The choice depends on the type of bleeding episode, the inhibitor titer, and the history and severity of previous infusion reactions. Administration of FIX concentrates is still possible in low-titer inhibitor patients without an allergic phenotype. Otherwise, bypassing agents (activated prothrombin complex concentrate [aPCC]; activated recombinant FVII [rFVIIa]) have to be considered in the treatment (or prevention) of bleeding episodes for high-responding inhibitor patients.⁶⁴ These bypassing agents have shown similar efficacy in acute bleed-

ing control (~80%) and have both low rates of adverse events. However, approximately 30% of patients who received both products reported better efficacy with one of the two agents.^{88–90}

However, some considerations are needed in the choice of the more appropriate product. Because of the presence of FIX in aPCC, in patients with previous allergic reactions, the use of rFVIIa is recommended.^{66,91,92} Moreover, for the same reason, the use of aPCC can induce an anamnestic response. The reduced infusion volume of rFVIIa and the less frequent infusions of aPCC are adjunctive characteristics of the products that can be considered in the choice.⁹³ Finally, in case of life-threatening bleeding episodes and failure of treatment with bypassing agents, plasmapheresis or immunoadsorption with staphylococcal protein A may be considered to remove anti-FIX antibodies, and transiently restore replacement therapy with FIX.^{7,64,94}

Patients with inhibitors are at higher risk for the development of life-threatening bleeding and severe arthropathy, compared with patients without inhibitors who can undergo prophylaxis with replacement therapy.^{95,96} Thus, it would be important to try to prevent bleedings also in the inhibitor patient population. The efficacy of prophylaxis with bypassing agents in reducing joint and other types of bleeding in HA/HB inhibitor patients has been demonstrated in three prospective randomized clinical trials conducted over the past decade.^{97–99} However, a very low number of HB inhibitor patients were enrolled in two of these three studies (4/58).^{97,99} The aforementioned considerations regarding allergic reactions and anamnestic responses have to be taken into account in the choice of the product for prophylactic regimens in the HB inhibitor patients.

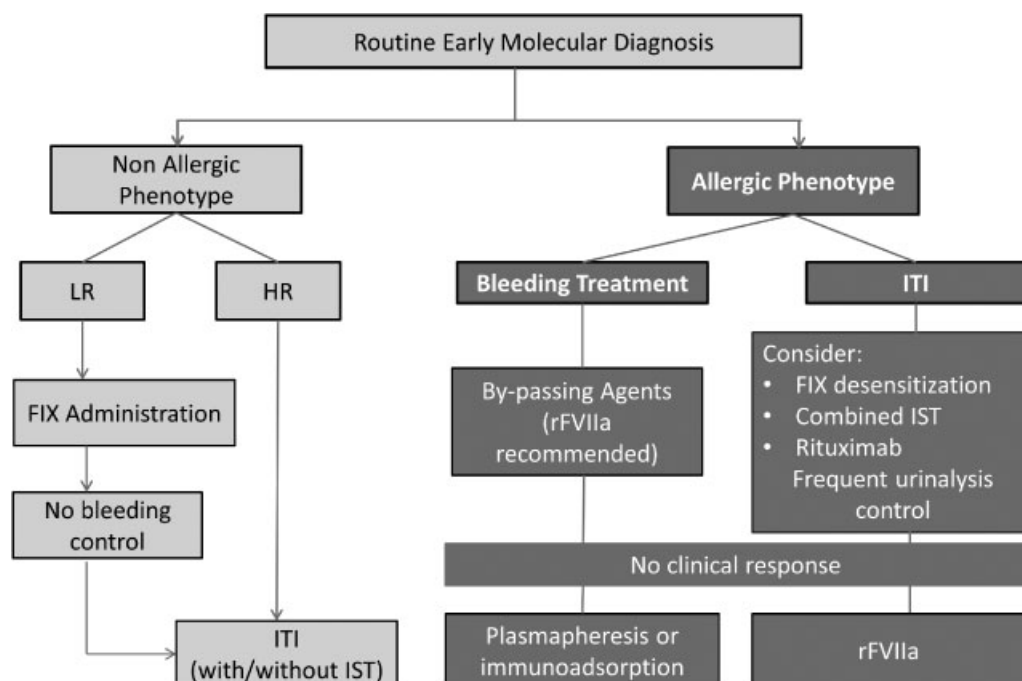


Fig. 2 Management of patients with hemophilia B and inhibitor. HR, high responding inhibitor; IST, immunosuppressive therapy; ITI, immune tolerance induction; LR, low responding inhibitor; rFVIIa, recombinant activated FVII.

Gene Therapy

Gene therapy in hemophilia allows the endogenous production of FVIII/ FIX by the transfer of a normal copy of the mutated gene through a viral or nonviral vector.¹⁰⁰ Because of their safety profile and efficacy, the most used vectors are adeno-associated virus (AAV) transfected into patient hepatocytes and inducing factor expression in the liver. Clinical trials in severe HB patients obtained a long-term FIX increase up to moderate or mild and, in one very recent study, to normal levels, and a significant reduction of bleeding episodes. Moreover, no development of inhibitor was observed.^{101–104} However, this approach is not feasible in patients with inhibitors. Furthermore, there are still some issues to be solved: the occurrence of hepatocellular toxicity with a T-cell-mediated destruction of hepatocytes producing a loss of efficacy and, for people with a previous immunity for AAV, a subsequent immune reaction to the vector injection.¹⁰⁵

With the observation of sustained plasma FIX levels after gene therapy, attention has turned to using this approach for induction of immune tolerance.^{106–108} Support for this therapeutic strategy has been shown in animal models in which liver-directed gene transfer using either AAV or lentiviral vectors can reverse preexisting anti-FIX high-titer inhibitors via T-regulatory cell induction,^{109–112} with a crucial role for CD4 + CD25 + FoxP3 + T regulatory cells in FIX tolerance.^{113,114}

Another target for gene therapy is hematopoietic stem cells (HSCs), because they provide long-term protein expression in the clinical setting.¹¹⁵ HSC-directed human FIX gene transfer was able to robustly induce tolerance in HB mice, and this was maintained also after re-exposition to protein and adjuvant challenges, which instead induced the production of high levels of anti-FIX antibodies in control mice.¹¹⁶

Finally, in megakaryocyte-directed gene transfer, FVIII or FIX is expressed in megakaryocytes and then stored into platelet α -granules.^{117,118} This promotes hemostasis protecting FVIII and FIX from circulating inhibitors until they are delivered to the site of injury upon platelet activation. Moreover, platelet FVIII or FIX generation can also be induced by ex vivo lentiviral-based gene transfer. This strategy followed by bone marrow conditioning and transplantation has resulted in hemostatic improvement and in ITI in HB mice.¹¹⁹

New Therapeutic Agents

Unique alternative approaches to bypassing therapy and agents that mimic the activity of a missing clotting factor or interfere with the natural regulators of hemostasis may provide new options for inhibitor management. Several promising alternatives for HA/HB inhibitor subset are under investigation.^{120–128} However, it has to be highlighted that the exciting data from emicizumab studies are not applicable to HB patients.¹²⁸ Inhibition of tissue factor pathway inhibitor (TFPI) promotes FX activation and thrombin generation through TF and activated FVII, without the involvement of FIX and FVIII. Concizumab is a monoclonal antibody targeting TFPI that may be effective for bleeding prophylaxis. In a

Table 3 New therapeutic agents for hemophilia in early stage of development

Product	Company	Technology	Stage of development	Main characteristics
rFVIIa-Fp ¹²³	CSL Behring (Marburg, Germany)	Fusion protein with albumin	Phase II/III study ongoing	Prolonged half-life (8.5 h)
superFVa ¹²⁴	—	Bioengineered FVa variant	Preclinical phase	Increased thrombin generation in acquired hemophilia models Synergistic effect with rFVIIa
FXa ^{116L 125}	—	Bioengineered zymogen-like FXa variant	Phase I clinical study concluded	Longer lasting plasma activity than wild-type FXa (60 vs. 1 min) Increased thrombin generation in hemophilia models
FXIII ¹²⁶	CSL Behring (Marburg, Germany)	Plasma-derived product	Preclinical phase	Long half-life (9 h) Improve clot stability alone or in association with rFVIIa
FVIIa and FX ¹²⁷	Kaketsuken (Japan)	Highly purified FVIIa and FX (MC710)	Phase III clinical studies concluded	FX substrate increases FVIIa bypassing activity duration

Note: A phase III clinical trial of a mixture agent of plasma-derived factor VIIa and factor X (MC710) in hemophilia patients with inhibitors.

first human trial, intravenous or subcutaneous administration to healthy volunteers and HB/HA patients showed a favorable safety profile and a concentration-dependent procoagulant effect.¹²⁰

Two additional anti-TFPI agents are currently under investigation in phase I studies in HA/HB patients with or without inhibitors.¹²¹ ALN-AT3 (fitusiran) is a subcutaneously administered RNAi therapeutic designed to reduce antithrombin (AT) mRNA and thus circulating AT levels with the goal of increasing thrombin generation.¹²² In the phase I study in healthy volunteers and in moderate and severe HA/HB patients, no thromboembolic events were registered. The drug achieved a dose-dependent knockdown of AT of up to 86%. A relationship was observed between reduced AT levels and increased thrombin generation. In particular, the thrombin generation values associated with the lowest AT level (reduction >75% of the baseline value) were consistent with those reported for mild hemophilia showing the possibility that fitusiran can reduce the severity of disease.¹²⁹ Although these reflect promising results, trials involving fitusiran were suspended in September 2017 after the confirmed death of a HA patient who had been receiving the therapy as part of a phase II open label extension study in association with FVIII concentrate. Very recently (December 2017), the U.S. Food and Drug Administration has lifted the hold on clinical studies.¹³⁰ Other new agents that can represent alternative strategies in the control of bleedings in patients with or without inhibitors are in earlier stages of development and are mentioned in ▶Table 3.

Conclusion

The risk of inhibitor occurrence is a rare complication of replacement therapy of HB. No particular relationship with any type of concentrate is evident, while genetic background is of utmost relevance. The onset of inhibitor may be accompanied by severe allergic/anaphylactic reactions, which in most cases prevent ITI treatment to eradicate the inhibitor because of the additional risk of nephrotic syndrome. The treatment of bleeding episodes in inhibitor-positive HB patients relies on the use of bypassing agents, with the recommendation to use rFVIIa in patients with previous allergic reactions. New therapeutic approaches are being developed to overcome the presence of the inhibitor by targeting TFPI or by silencing AT mRNA. Phase II and III clinical trials are ongoing and the results could significantly change the current options for these patients.

Conflict of Interest

None.

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