



TABLE OF CONTENTS

Introduction

Inhibitor Development: Risk Factors

The Importance of “Danger Signals”

Prophylaxis as Protection Against Inhibitors

Conclusion

References

Early Prophylaxis and the Influence of Immunologic “Danger Signals” on Inhibitor Development

Donald L. Yee, MD

February 22, 2011

Introduction

Medical advances of recent times have altered the ways in which many diseases are perceived and treated. [The Human Genome Project](#)

will undoubtedly change the landscape of medicine in the 21st century much like the discovery of penicillin did in the 20th century. Hemophilia care, too, has undergone changes based on discoveries that have transformed the way the disease is managed, including virus-inactivation methods for the purification of plasma-derived factor concentrates, development of recombinant factor concentrates, use of the bypassing agents FEIBA and recombinant factor VIIa, and immune tolerance induction (ITI) therapy. Despite these discoveries that have improved the quality of life for patients with hemophilia, the development of inhibitors remains the most serious complication of the disease.^{1,2}

For the 20% to 30% of patients with severe hemophilia A and the approximately 1% to 6% of patients with severe hemophilia B with inhibitors, the treatment of bleeding episodes can be a challenge.³

Current strategies in hemophilia care have emphasized the management or eradication of inhibitors through prophylactic bypassing therapy or ITI. Recently, however, there has been an added focus on prevention of inhibitors, with recent clinical data examining the potentially protective effect of early prophylaxis against their development.

Inhibitor Development: Patient- and Treatment-Related Risk Factors

Inhibitor development appears to be a multifactorial process, involving both patient- and treatment-related risk factors.^{4,5} Patient-related risk factors include hemophilia severity and genotype, costimulatory genotype-immunogenotype interactions, a positive family history for inhibitors, and ethnicity.² With respect to the factor VIII (FVIII) gene, large deletions, inversions, and nonsense mutations are associated with the highest risk for inhibitor development. Furthermore, a recent study by Viel and colleagues (2009) suggests that mismatched FVIII replacement due to FVIII gene polymorphisms may underlie some of the risk attributable to ethnicity.⁶ Treatment-related risk factors thought to be associated with inhibitor risk include the timing and intensity of FVIII exposure, method of factor administration, and FVIII product type.² This latter risk factor is currently under study by Dr. Pier Mannucci and other investigators. The SIPPET study (Survey of Inhibitors in Plasma-Product Exposed Toddlers) aims to test the hypothesis that recombinant FVIII is more immunogenic than plasma-derived vWF/FVIII concentrates.^{1,7}



Listen to noted hemophilia experts Victor S. Blanchette, Manuel Carcao, and Amy D. Shapiro discuss “[Perspectives in Hemophilia: Clinical Challenges and Current Issues in Managing Patients With Inhibitors](#).” This learning activity is derived from a presentation given at the 2010 American Society of Pediatric Hematology/Oncology (ASPHO) Annual Meeting.

The Immune System and Inhibitor Development: Importance of “Danger Signals”

The immune system plays a central role in an individual’s reaction to FVIII or FIX,³ with both genetic and

non-genetic factors influencing the immunologic response to exogenous factor exposure.⁸ Certain non-genetic risk factors may indeed exert a profound effect on inhibitor development through their influence on the immune system.⁸ The pathophysiology of inhibitor development is thought to involve a complex cascade of interactions between components of the innate and adaptive immune systems. Each step in this cascade is regulated by stimulatory and inhibitory signals that together determine the activation state of the immune cells involved. Any event that alters the balance between these signals has the potential to modulate the immune response.⁸

Certain non-genetic risk factors may exert a profound effect on inhibitor development through their influence on the immune system.⁸

Certain stimuli or triggers of the immune system are thought to convey what have been referred to as “danger signals,” and include exogenous and endogenous factors such as infection, vaccinations, severe bleeding, and surgery.^{4,8} With few exceptions, the overall quality of data supporting the significance of these and other considerations as risk factors for inhibitor development in the setting of hemophilia tends to be low, such that clinical decision-making must rest heavily on experience and expert opinion. However, **Table 1** provides a fairly inclusive list of possible stimuli that may influence danger signal generation in this context.

Mechanisms of Danger Signal Conveyance

Common belief has held that the immune system’s primary purpose is to differentiate self from non-self. However, it has been recently postulated that the immune system’s ability to sense danger is actually more critical, as part of a larger surveillance, defense, and repair system that enables multicellular organisms to recognize and respond to entities that do damage.^{4,9,10} Danger is believed to be transmitted by signals associated with pathogens or with tissue and cell damage. Pathogens express pathogen-associated molecular patterns, or PAMPs, that are recognized by pattern recognition receptors such as toll-like receptors (TLRs), Nod1-like receptors (NLRs) or Rig-I-like receptors that are expressed on a range of cells of the innate and adaptive immune system.⁴ Subsequent to being triggered, these receptors activate several signaling pathways that can induce both inflammatory responses and activation of specific anti-pathogen immune responses. It is believed that trauma, ischemia, and tissue damage also can cause inflammatory responses that are similar to those induced by pathogens. Damage-associated molecular patterns, or DAMPs, recruit and activate receptor-expressing cells, thereby promoting adaptive immune responses (see **Figure 1**).^{4,10,11}

Table 1. Aspects of Hemophilia Care With Potential to Influence Danger Signal Generation

Age at start of treatment
Blood components
Concurrent immunologic disorders
Continuous vs bolus infusions
Extravascular infusions
Infections
Intensity of treatment
Pregnancy/breast-feeding
Prophylaxis vs on-demand treatment
Reason for first infusion
Severe bleeding
Surgery
Tissue damage
Type of factor concentrate
Vaccination

Data from Astermark J, et al. *Haemophilia*. 2010;16:747-766; Kurnik K, et al. *Haemophilia*. 2010;16:256-262.

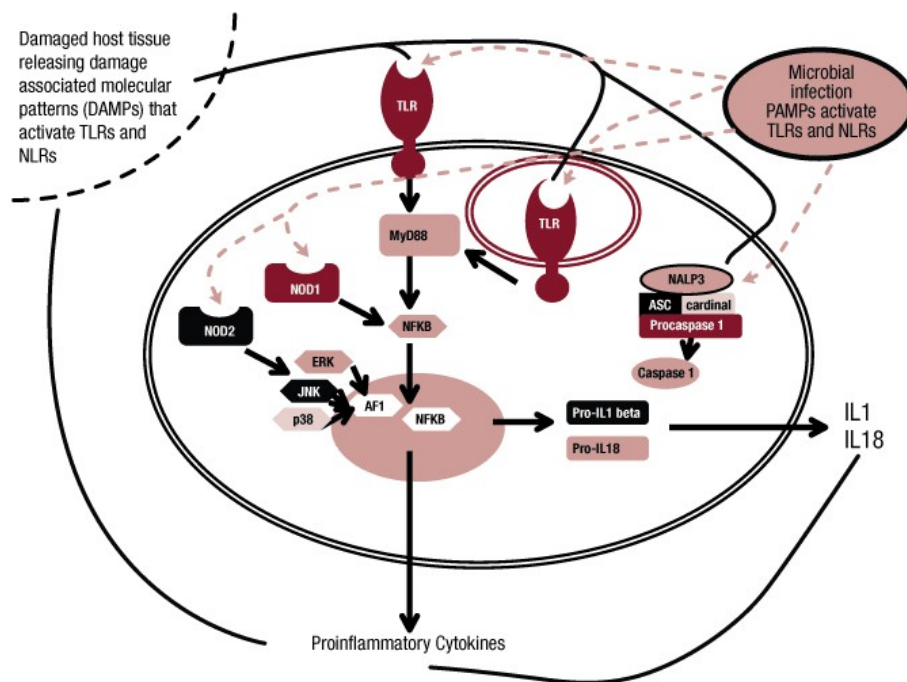


Figure 1

Signaling through pathogen-associated and damage-associated molecular patterns.

Posted with permission from McCormack WJ, et al. *Arthritis Res Ther.* 2009;11:1-8.

Supporting Evidence for Prophylaxis as Protection Against Inhibitor Development

Important studies conducted in the last few years support a protective role for early factor prophylaxis against inhibitor development.^{12,13} Santagostino and colleagues conducted a case-control study to test the interactions between genetic and environmental factors on inhibitor development in hemophilia A (2005).¹² Sixty children with inhibitors were compared with 48 inhibitor-free controls. While investigators found no association between amniocentesis/villocentesis, premature/caesarean birth, breast feeding or FVIII infusions during vaccinations or infections and inhibitor development, patients who were started on prophylaxis had a lower risk of inhibitor development than those treated on demand, even after multivariate analysis (**Table 2**). Furthermore, additional subgroup analysis confirmed that early initiation of prophylaxis (before 3 years of age) was associated with a 70% reduced risk for inhibitor development compared with those treated on demand.^{5,12}

Similar conclusions concerning the possible protective effect of early prophylaxis were reached by Gouw and investigators as part of the CANAL Study (Concerted Action on Neutralizing Antibodies in severe hemophilia A). This multicenter retrospective cohort study investigated 366 consecutive patients, more than half of whom started regular prophylaxis during the first 50 exposure days (EDs).¹³ At the start of prophylaxis, median age was 20 months and the median number of exposure days was 16. Eighty-seven patients (24%) developed inhibitors. Investigators found that inhibitor incidence was associated with surgical procedures and intensity of treatment at first FVIII exposure, or “peak treatment moments”—defined as treatment given on 5 or more consecutive days (RR 3.7, 95% CI 2.0-7.1; and RR 3.3, 95% CI 2.1-5.3, respectively). In contrast, the children receiving regular prophylaxis demonstrated a 60% lower risk of inhibitor development than those receiving on-demand treatment (**Figure 2**).¹³

Both studies, in examining conditions that stimulate an immune response in subjects, suggest that the clinical setting at the time of FVIII exposure and not FVIII exposure alone, may determine the immune response. Although different studies, they arrive at similar conclusions regarding the possible role that early factor prophylaxis under controlled conditions may play in inhibitor development and indirectly support the danger signal theory.

Table 2. Univariate and Multivariate Analysis of Prophylaxis and Other Putative Risk Factors for Inhibitor Development in a Subgroup of 25 Cases and 48 Controls

	Cases (n=25*)	Controls (n=48)	Crude OR (95% CI)	Adjusted OR† (95% CI)
Prophylaxis (%)	7 (28)	34‡ (71)	0.2 (0.06–0.5)	0.2 (0.06–0.9)
Age at first FVIII infusion				
>16 months (%)	10 (40)	16 (33)	1 (ref.)	1 (ref.)
11–16 months (%)	6 (24)	17 (36)	0.6 (0.2–1.9)	0.9 (0.2–4.7)
<11 months (%)	9 (36)	15 (31)	1.0 (0.3–3.0)	1.2 (0.2–6.9)
Breast-fed				
No. (%)	5 (20)	14 (29)	1 (ref.)	1 (ref.)
≤6 months (%)	12§ (48)	20 (42)	1.7 (0.5–5.8)	2.2 (0.4–12.8)
>6 months (%)	8§ (32)	14 (29)	1.6 (0.4–6.1)	4.0 (0.7–23.1)
Null mutations (%)	15/18 (83)	27/42 (64)	2.8 (0.7–11.2)	2.5 (0.5–12.5)
Family history of inhibitors (%)	6 (24)	1 (2)	14.8 (1.7–131.7)	4.5 (0.3–62.8)

*Number of cases who started prophylaxis prior to inhibitor development or potentially eligible for prophylaxis because they were inhibitor-free up to the age of 35 months (ie, the upper limit of the age range at prophylaxis onset in cases and the median age at prophylaxis onset in controls).

†Each variable was adjusted for all the others.

‡Within the first 150 exposure days.

§Prior to inhibitor development.

Adapted from Santagostino E, et al. *Br J Haematol*. 2005;130:422–427.

Patients at risk:

On demand	339	263	177	136	107	89
Prophylaxis	4	54	103	133	157	168

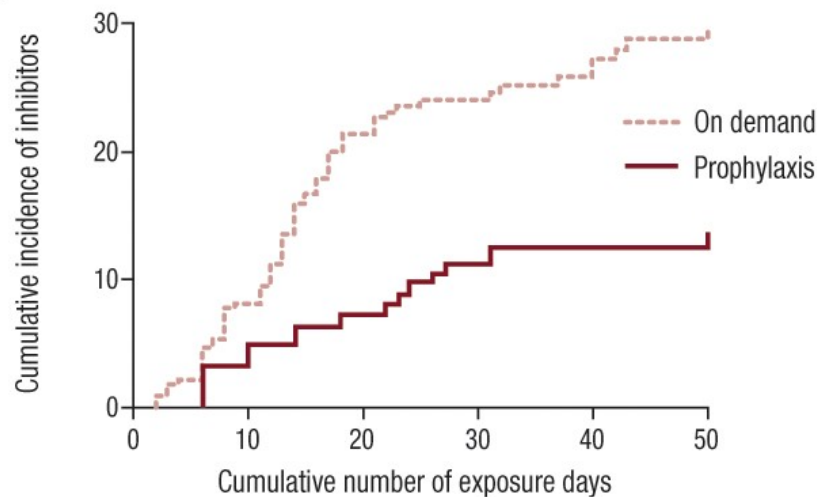


Figure 2

Cumulative incidence of inhibitor development according to treatment regimen: prophylaxis versus on demand. Reproduced with permission of the American Society of Hematology (ASH), from “Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL Cohort Study,” Gouw SC, volume 109, issue 11, copyright 2007; permission conveyed through Copyright Clearance Center, Inc.

A more recent German study, conducted by Kurnik and colleagues (2010),⁴ further tested this hypothesis. The investigators’ aim was 2-fold: 1) to design a prophylaxis regimen for the first 20 to 50 EDs to induce tolerance to administered FVIII among study subjects, with the assumption that the first 50 EDs represent a high-risk period for inhibitor development; and 2) to minimize exposure to immunologic danger signals. Accordingly, the authors report that first exposure to FVIII was avoided in bleeding situations or during infection; surgery was avoided during the first 20 EDs; vaccinations were not administered on the same day as FVIII treatment; and bleeds were treated early with higher FVIII doses to shorten time of tissue damage.⁴

The study group comprised 26 “PUPs” with severe hemophilia A who were treated with a regimen of low-dose prophylaxis (250 IU/kg weekly) titrated to increasing frequency and/or dose based on breakthrough bleeding. This cohort of patients was compared with a control group of 30 consecutive “PUPs” who were treated with a standard prophylaxis regimen (40–50 IU/kg 3x/wk). Study results indicate that while there were no significant differences between groups in patient-related factors, such as ethnicity or disease severity, there were highly significant differences between groups for prophylaxis-related factors: age at initiation of prophylaxis and number of EDs before the introduction of

the prophylaxis regimen (Table 3).

Most notably, 14 of the 30 controls (47%) developed an inhibitor on the standard regimen while only 1 of the 26 (3.8%) subjects developed an inhibitor (which was low responding) on the new regimen,⁴ as shown in Table 4 and Figure 3.

Study Conclusions

Study results suggest that minimizing danger signals during the first 20 EDs on a prophylaxis regimen that provides regular controlled exposure to a low dose of exogenous FVIII may reduce the risk of inhibitor formation.⁴ Standard prophylaxis regimens, usually initiated at or just after the first significant bleed, at a time when strong immunologic danger signals may be present, may be too little too late to prevent inhibitor formation, as the patient's immune system already has been "primed" by previous intensive treatment.⁴ The authors conclude, based on the superior inhibitor outcome in the 26 study "PUPs," that early start of low-dose prophylaxis, administered once weekly, may dramatically reduce the incidence of inhibitors.⁴

As highlighted by the authors, a low-dose regimen has several other potential advantages over the historical (40-50 IU/kg 3x/wk) regimen. In addition to the possibility of decreased bleeds and better joint scores, once-weekly administration avoids the necessity for a central venous access device and the consequent need for surgery. There is also greater initial acceptance by families due to easier compliance. Finally, there is a pharmacoeconomic benefit for patients, in that cost savings can be achieved with lower doses and less frequent treatments compared to more customary prophylactic regimens.⁴

While the results of the Kurnik study corroborate those of earlier studies,^{12,13} larger prospective clinical studies are warranted. Currently in the United States, a randomized controlled trial to address this issue is being planned, under the direction of Drs. Margaret Ragni and Craig Kessler. It is hoped that results from this study, which has been proposed to the National Heart, Lung and Blood Institute, will help answer definitively whether prophylaxis has a role in reducing risk of inhibitor development.

Table 3. Prophylaxis-Related Factors for Inhibitor Development in the Study Group Compared With the Control Group

	Age at Start of Prophylaxis* (Median/Range) (months)	Number of EDs Before Prophylaxis (Median/Range)
Control Group (standard prophylaxis regimen) (n=30)	19 (0.8-8.7)	30 (1-infinity)
Study Group (new prophylaxis regimen) (n=26)	10.7 (0.5-24.5)	1 (0-14)
Statistical Significance	Highly significant <i>P</i> <.0006	Highly significant <i>P</i> <.0001

*Age at start of prophylaxis was available for 23 of the 30 subjects in the standard prophylaxis group and all 26 subjects given the new regimen.
Adapted from Kurnik K, et al. *Haemophilia*. 2010;16:256-262.

Table 4. Inhibitor Development in the Study Group Compared With the Control Group

	Inhibitors (%)	High Responders (%)	Low Responders (%)
Control Group (standard prophylaxis regimen) (n=30)	14 (47)	8 (27)	6 (20)
Study Group (new prophylaxis regimen) (n=26)	1 (3.8)	0	1 (3.8)
Statistical Significance	Highly significant $P=.0003$ OR 0.048 (95% CI: 0.001-0.372)	Highly significant $P=.005$ OR of high response 0.00 (95% CI: 0.00-0.57)	

Adapted from Kurnik K, et al. *Haemophilia*. 2010;16:256-262.

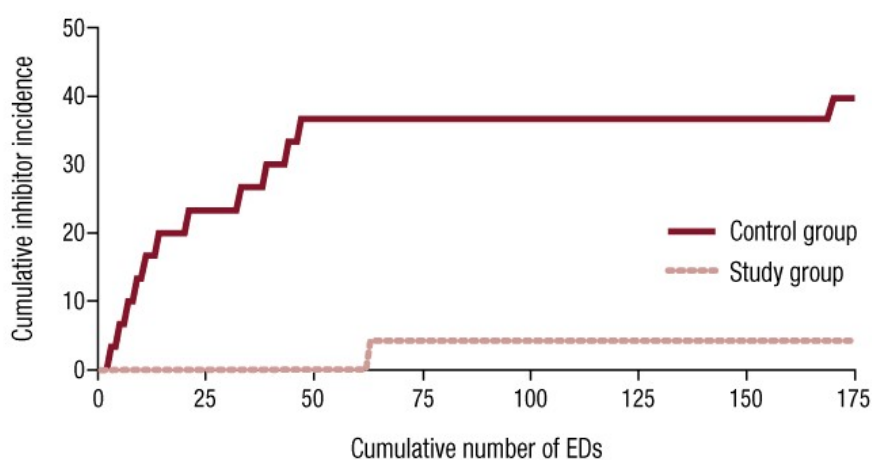


Figure 3

Cumulative inhibitor incidence with increasing number of EDs: control vs study group.

Posted with permission from Kurnik K, et al. *Haemophilia*. 2010;16:256-262.

Conclusion

Currently, inhibitors remain the major complication for patients with severe hemophilia. However, retrospective and preliminary cohort studies suggest that risk for inhibitor development may be attenuated through early and regular factor exposure under controlled conditions—that is, in the absence of immunologic “danger signals.” Before such a strategy can be incorporated into standard practice, further validation via larger prospective and, preferably, randomized clinical trials is required. In the meantime, given the lack of worldwide consensus on what constitutes the optimal initial primary prophylaxis regimen, the regimen cited by Kurnik et al holds several potential advantages over other prophylactic approaches in current use, and clinicians should consider this regimen as a viable early option for patients at risk for inhibitor formation.

REFERENCES

1. Mannucci PM, Gringeri A, Peyvandi F, Santagostino E. Factor VIII products and inhibitor development: the SIPPET study (survey of inhibitors in plasma-product exposed toddlers).

- Haemophilia*. 2007;13(suppl 5):65-68.
2. DiMichele DM. Management of factor VIII inhibitors. *Int J Hematol*. 2006;83:119-125.
 3. DiMichele DM. Inhibitors in Hemophilia: A Primer. 4th ed. World Federation of *Haemophilia*. April 2008.
 4. Kurnik K, Bidlingmaier C, Engl W, Chehadeh H, Reipert B, Auerswald G. New early prophylaxis regimen that avoids immunological danger signals can reduce FVIII inhibitor development. *Haemophilia*. 2010;16:256-262.
 5. Mancuso ME, Graca L, Auerswald G, Santagostino E. Hemophilia care in children—benefits of early prophylaxis for inhibitor prevention. *Haemophilia*. 2009;15(suppl 1):8-14.
 6. Viel KR, Ameri A, Abshire TC, et al. Inhibitors of factor VIII in black patients with hemophilia. *N Engl J Med*. 2009;360:1618-1627.
 7. SIPPET Project. Available at: <http://www.sippet.org/Source/Home.aspx>. Accessed October 13, 2010.
 8. Astermark J, Altisent C, Batorova A, et al, for the European Haemophilia Therapy Standardisation Board (EHTSB). Non-genetic risk factors and the development of inhibitors in haemophilia: a comprehensive review and consensus report. *Haemophilia*. 2010;16:747-766.
 9. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol*. 1994;12:991-1045.
 10. Kono H, Rock KL. How dying cells alert the immune system. *Nat Rev Immunol*. 2008;8:279-289.
 11. McCormack WJ, Parker AE, O'Neill LA. Toll-like receptors and NOD-like receptors in rheumatic diseases. *Arthritis Res Ther*. 2009;11:243. Available at: <http://arthritis-research.com/content/11/5/243>. Accessed November 15, 2010.
 12. Santagostino E, Mancuso ME, Rocino A, et al. Environmental risk factors for inhibitor development in children with haemophilia A: a case-control study. *Br J Haematol*. 2005;130:422-427.
 13. Gouw SC, van der Born JG, van den Berg HM, for the CANAL Study Group. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. *Blood*. 2007;109:4648-4654.

This initiative is supported by an educational grant from:



This initiative is jointly sponsored by:

