Inhibitor development in nonsevere hemophilia A
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Chapter 11

Summary, Interpretation and Future Perspectives

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SUMMARY

This thesis focuses on the etiology and outcome of inhibitor development in nonsevere hemophilia A. Chapter 1 provides the introduction to the thesis by presenting an overview of hemophilia treatment and its complications. The development of inhibiting antibodies (inhibitors) towards factor VIII is currently the most severe complication of the treatment with factor VIII concentrates in hemophilia A. Inhibitors comprise the ability to manage hemorrhage in affected individuals, resulting in a greater rate of complications, costs and disability. Improved understanding of risk factors and outcome of inhibitor development in patients with nonsevere hemophilia A may indicate the areas of care that merit particular attention. The thesis consists of three parts that address genetic and environmental risk factors and the outcome of inhibitor development in nonsevere hemophilia A.

PART I GENETIC RISK FACTORS

The first part of this thesis concerns genetic risk factors for inhibitor development. In Chapter 2 the role of the factor VIII gene (F8) mutation on inhibitor risk was assessed in the INSIGHT cohort (2,709 nonsevere hemophilia patients). The analysis included 1,112 nonsevere hemophilia A patients from 14 centers in Europe and Australia that had genotyped at least 70% of their patients. During an observation period of 31 years (1980-2011), 59 of the 1,112 patients developed an inhibitor. When the risk of inhibitor development was evaluated as function of exposure to factor VIII concentrates, the lifetime prevalence was higher than previously appreciated. The inhibitor risk was 6.7% at 50 exposure days, rising to 13.3% at 100 exposure days. These findings implicate that nonsevere patients carry a lifelong risk to develop inhibitors in contrast to severe hemophilia A patients who may be considered as tolerant to exogenous factor VIII when they have not developed an inhibitor after 50 exposure days. Importantly, the inhibitor risk was only increased in patients with specific F8 mutations. Nineteen F8 missense mutations were associated with inhibitor development among a total of 214 F8 missense mutations. Patients carrying these 19 mutations had inhibitor risks that are comparable to patients with severe hemophilia A. These findings highlight the importance of genotyping in nonsevere patients as high risk patients can be identified based on the F8 genotype.

Chapter 3 describes the association between genetic variation of Fc gamma receptor genes (FCGR) and inhibitor development in severe hemophilia A. Genetic variations of the low-affinity Fc gamma receptors (FCγR), which are expressed on immune cells, have been previously associated with susceptibility for infectious and autoimmune diseases. Single nucleotide polymorphisms (SNPs) and copy number variation of the FCGR2 and FCGR3 gene
cluster were studied in an FCGR-specific multiplex ligation-dependent probe amplification assay in DNA samples of 85 severe hemophilia A patients (brother pairs from 44 families of the previously described MIBS cohort). We found that the FCGR2A polymorphism 131R>H was associated with inhibitor development, conferring a more than threefold increased risk for hemophilia A patients with the 131HH genotype. This association persisted in subgroup analysis of high titer inhibitor patients and patients with the F8 intron 22 inversion. These findings suggest that FcγRIIa may play a role in the complex pathophysiology of immunogenicity against therapeutic factor VIII, however further investigations are definitely needed to understand the immunological mechanism underlying this association.

PART II ENVIRONMENTAL RISK FACTORS

Part II of the thesis addresses environmental risk factors for inhibitor development and in particular the role of intensive treatment on inhibitor risk. Chapter 4 is a retrospective cohort study, in which we aimed to investigate the role of F8 genotype and intensive treatment with factor VIII concentrates on inhibitor risk in 138 nonsevere hemophilia patients that were treated at the hemophilia treatment center of Amsterdam. In the period of 1980-2008, ten patients had developed an inhibitor. Genotyping demonstrated that the p.Arg612Cys missense mutation was present in 52 (38%) patients. Compared to the other patients, those with the p.Arg612Cys mutation had a tenfold increased risk of developing inhibitors. Seven patients developed an inhibitor following intensive peri-operative use of factor VIII concentrates, in five of them factor VIII was administered by continuous infusion during surgery. Intensive peri-operative use of factor VIII, especially when administered by continuous infusion, was strongly associated with inhibitor development in nonsevere hemophilia A. In Chapter 5, the association between intensive treatment and inhibitor development was further explored in a prospective multicenter study. The frequency of inhibitor development after surgery was lower than the previously observed frequencies in retrospective studies, as only two patients (4%) of the 46 included in the study developed a low titer inhibitor post-operatively. This was an interesting finding, as these results suggest that intensive treatment apparently is not as strongly related to inhibitor development as previously appreciated. However, the lower incidence of inhibitors in this study might be influenced by the relatively low number of patients carrying high risk F8 mutations in combination with the selection of patients that had previous intensive factor VIII exposures without developing an inhibitor. Chapter 6 is a systematic review, in which the role of surgery on inhibitor development was assessed. A comprehensive literature search identified four cohort studies and three case control studies, comprising 342 inhibitor patients among a total of 957 severe hemophilia A patients. Surgery at first exposure was associated with a four times increased
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risk as compared to treatment for bleeding or prophylaxis in severe hemophilia A patients. Information on continuous infusion, previously treated patients and nonsevere hemophilia A was insufficient for valid meta-analyses. Considering the consistent finding of increased inhibitor incidence associated with surgical intervention across a number of heterogeneous patient groups, caution should be taken when treating patients intensively with factor VIII concentrates for surgery.

PART III TREATMENT AND OUTCOME OF INHIBITOR DEVELOPMENT

The final part of this thesis concerns the outcome of inhibitor development in nonsevere hemophilia A. Chapter 7 is an observational study, in which the outcome of different inhibitor eradication strategies was assessed in 101 nonsevere hemophilia A patients with inhibitors from the INSIGHT cohort. In total, 28 inhibitor patients (28%) received eradication therapy, in these patients the treatment strategy varied widely including both immune tolerance induction and immune suppressive therapy. In the remaining 73 inhibitor patients (72%) no inhibitor eradication treatment was given. The outcome of both strategies was comparable, the inhibitor disappeared in 75% of the patients of the treatment group and 70% of the patient in the wait and see group. In total, 52 patients were rechallenged with factor VIII concentrates after inhibitor disappearance, 36 (69%) of them remained inhibitor free (sustained success). Both high titer and low titer inhibitor patients seemed to benefit from eradication treatment, however the results of this study should be interpreted with care as patients were not randomized. Hence, we concluded that nonsevere hemophilia A patients with inhibitors require an individual eradication approach, as one single approach is unlikely to be appropriate for all patients. Chapter 8 describes the association between inhibitors development and mortality in nonsevere hemophilia A. Clinical data and vital status were collected of 2,709 nonsevere hemophilia A patients (107 with inhibitors) that were treated between 1980 and 2011 in 34 European and Australian centers in the INSIGHT cohort. Mortality rates for patients with and without inhibitors were compared. In the period of 1980-2011, 148 patients had died at a median age of 64 years. In 62 patients (42%) the cause of death was hemophilia-related, due to bleeding complications or hepatitis B/C and HIV related diseases. Sixteen inhibitor patients had died at a median age of 71 years (IQR, 60-81). Death was caused by severe bleeding complications in seven out of ten patients in whom the inhibitor was present at time of death. This substantial number of bleeding complications emphasizes the impact of inhibitor development in nonsevere hemophilia A patients. The all-cause mortality rate in inhibitor patients was more than five times increased compared to those without inhibitors. This implies that prudence is called for close follow-
up in nonsevere hemophilia A patients who need treatment with factor VIII concentrates as inhibitor development may worsen their clinical outcome substantially.

The results of part I and part II of the thesis are discussed in chapter 9 and chapter 10, respectively. Chapter 9 discusses the potential immunological mechanisms underlying the high incidence of inhibitors in patients with specific F8 missense mutations. Currently available data support the concept that inhibitor development in mild hemophilia A is due to F8 missense mutation-induced incomplete central tolerance to administered factor VIII. Integrating recent evidence from the INSIGHT study and new results on the repertoire of factor VIII derived peptides that is presented on MHC class II provides a model that potentially explains the immune reactivity to mismatched factor VIII in a subset of patients with nonsevere hemophilia A. As yet there is limited data to evaluate this model and further analyses need to confirm whether restricted CD4+ T cell responses to mismatched factor VIII are a common pathogenic mechanism for inhibitor formation in nonsevere hemophilia A. Chapter 10 addresses the association between intensive treatment and inhibitor development. Intensive treatment is administered for major bleeding episodes or surgery. As both severe bleeding and surgery are associated with tissue damage, an association between intensive treatment and inhibitor development may originate from two different triggers of the immune system: a.) a high factor VIII antigen load and b.) immunological “danger signals” arising from tissue damage and inflammation, leading to cytokine release and co-stimulatory signaling. We discuss these potential mechanisms underlying intensive treatment and inhibitor development and the methodological issues that need to be addressed in observational studies to dissect the effects of intensive treatment and immunological danger signals as risk factor for inhibitor development. This will ultimately help us to understand by which mechanisms intensive treatment causes inhibitor development and may enable further research on preventive strategies.

INTERPRETATION AND GENERAL DISCUSSION

The aims to this thesis were to gain further insight in the etiology (genetic and environmental risk factors) and outcome of inhibitor development in nonsevere hemophilia A. This final chapter discusses the evidence that has been obtained for the three objectives as stated in the introduction. The interpretation, strengths, limitations, implications for clinical practice and directions for future research will be addressed.
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GENETIC RISK FACTORS FOR INHIBITOR DEVELOPMENT IN NONSEVERE HEMOPHILIA A

The first results of the INSIGHT study demonstrated that the type of F8 mutation is an important risk factor for inhibitor development in nonsevere hemophilia A. The INSIGHT study included the largest cohort of patients with nonsevere hemophilia A to assess the association between inhibitor development and F8 mutation, taking cumulative exposure to therapeutic factor VIII concentrates into account. As inhibitor development is elicited by the exposure to therapeutic factor VIII, the risk of developing inhibitors strongly depends on the cumulative number of exposure days. This information is especially important in nonsevere hemophilia A patients as they receive factor VIII replacement therapy on an irregular basis and much less frequently than severe hemophilia A patients. This study was an advance over the registry of F8 mutations and inhibitor development in international online databases, as information about exposure is lacking in these database.\textsuperscript{1,2} Moreover, there may be a reporting bias in these databases favouring registration of those with identified inhibitors. The INSIGHT study provided valuable data enabling a more personalised inhibitor risk estimation based on F8 mutation.

Other genetic factors that have been associated with inhibitor development in severe hemophilia A patients include family history of inhibitors, ethnicity and genetic variations in immunoregulatory genes, such as HLA class II complex variations and SNPs in the genes encoding for IL-1, IL-2, IL-10, TNF-\(\alpha\), TGF-\(\beta\) and CTLA-4.\textsuperscript{3-9} Furthermore, an association between FcyRIIa-H131 and inhibitor development was demonstrated in chapter 3 of this thesis.

We were not able to evaluate any of these other genetic risk factors in nonsevere patients yet. The low incidence (2\%) of patients with a positive family history of inhibitors in the INSIGHT cohort was likely to be a function of challenges of data collection associating a particular individual with other family members (especially when they are treated in different centers) and emphasises the need for thorough family tree maintenance. As our results only cover the Caucasian population we could not investigate the effect of ethnicity on inhibitor risk in nonsevere hemophilia A. Future studies investigating the contribution of these other genetic factors may help to improve our understanding of the complex pathophysiology of immunogenicity against therapeutic factor VIII in nonsevere patients and could help to optimize identification of high risk patients based on genetic profile.
ENVIRONMENTAL RISK FACTORS FOR INHIBITOR DEVELOPMENT

Several clinical conditions have been associated with an increased risk for inhibitor development in severe hemophilia A, among them are: factor VIII product type, factor VIII dose, intensity and reason for treatment, mode of factor VIII administration (bolus injections or continuous infusion), change of factor VIII product and age at first treatment.\textsuperscript{10-15} Chapter 4 was the first cohort study that indicated an association between intensive perioperative use of factor VIII concentrates and inhibitor development in nonsevere hemophilia A, especially when it was administered by continuous infusion. Patients developed inhibitors both after recombinant and plasma products; however, the risk of individual factor VIII products was not further elucidated. There was no significant relation between factor VIII product change and inhibitor development. Future studies are definitely needed to confirm the risk of intensive treatment on inhibitor development in nonsevere hemophilia A as cumulative exposures to factor VIII concentrates were not taken into account in chapter 4 and 5. The INSIGHT cohort study is the source population for the currently ongoing nested INSIGHT case-control study. For this study detailed information for each exposure day and blood samples will be analysed to further investigate the role of all above mentioned environmental and genetic risk factors in nonsevere hemophilia A patients. The first results of this study are expected to become available by the end of 2014. In the absence of robust evidence, we propose that the potential risk of intensive treatment with factor VIII should be taken into account when treating patients with nonsevere hemophilia A. Hence, surgery should only be performed for clear indications. In order to optimize the therapeutic use of desmopressin in nonsevere patients – as an effective and safe alternative for factor VIII concentrates without the risk of inhibitor development – desmopressin response should be tested in all nonsevere hemophilia A patients. This is relevant since it appears that at least some high risk mutations respond well to desmopressin administration.\textsuperscript{16} The INSIGHT study provoked the initiative for the RISE study, an international cohort study aiming to investigate the potential role of F8 mutation and other determinants in the DDAVP response in nonsevere hemophilia A. Hopefully the results of this study will further optimize the clinical use of desmopressin that may prevent inhibitor formation in the future, at least in some patients.
THE OUTCOME OF INHIBITOR DEVELOPMENT IN NONSEVERE HEMOPHILIA A

An interesting finding in chapter 7 of this thesis was the spontaneous disappearance of inhibitors in the majority of the inhibitor patients, suggesting that not all nonsevere hemophilia A patients with inhibitors require treatment to eradicate the inhibitor. Due to the observational nature of the study, we cannot formulate any treatment recommendations for nonsevere inhibitor patients yet. The study demonstrated that some patients may benefit from eradication treatment; however one single approach is unlikely to be appropriate for all patients. Consequently, clinicians will need to continue to make these decisions on a case by case basis. Although the clinical implications of this study are limited, it provides important input for prospective studies aiming to optimize inhibitor eradication in nonsevere patients.

The importance of adequate inhibitor eradication was demonstrated by the substantial number of bleeding complications and the increased mortality rates in nonsevere hemophilia A patients with inhibitors in chapter 8. An unexpected high number of patients developed an inhibitor at an advanced age and after more than 50 exposures. Importantly, nonsevere patients seem to carry a lifelong risk to develop inhibitors in contrast to severe patients who develop inhibitors at a very young age. As life expectancy increases, the rising incidence of inhibitor development in older patients will become an important clinical challenge.

Age-related co-morbidities such as cancer and arthropathy increase the need for medical interventions and surgery. These procedures require exposure to therapeutic factor VIII concentrates that may elicit inhibitor formation. Based on these findings it should be advised that nonsevere hemophilia A patients should be monitored regularly at the hemophilia treatment center to manage hemophilia-related problems and for optimal treatment of other diseases.

CONCLUSION

This thesis emphasizes the impact of inhibitor development in patients with nonsevere hemophilia A and stresses the importance of close follow-up in patients that require factor VIII exposure. The ongoing improvement of our understanding of the pathophysiology of inhibitor development in nonsevere hemophilia A will open up new possibilities for identification of high risk patients and high risk clinical situations. This knowledge will ultimately help to implement targeted preventive measures.
REFERENCES


