Developments in modern hemophilia care

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Chapter 8

Summary and general discussion

In the last 50 years, hemophilia treatment has changed tremendously. The first aim of this thesis was to comprehensively assess the changes in health status over time of patients with hemophilia. Although hemophilia treatment has improved in many ways, inhibitor development continues to be a significant problem in patients treated with clotting factor products. Therefore, the second aim of this thesis was to evaluate different strategies to identify patients at a high risk of inhibitor development and to present an overview of anti-drug antibody strategies that could potentially be applied to these patients. We will first summarize the main results of these studies, which were reported in Chapters 2-7, and then discuss their wider implications for clinical practice and future research.

Summary of main results

Chapter 2 In this chapter, we report the results of a cross-sectional nationwide survey held in 2019 among patients with hemophilia in the Netherlands. We assessed how treatment changes have influenced major clinical outcomes over time by comparing the current results to those of similar surveys that have been previously conducted (in 1972, 1978, 1986, 1992 and 2001).[1-3] This study showed that over the course of almost 50 years the increased use of prophylactic therapy has improved joint health and that the development of effective hepatitis C treatment options has nearly eradicated hepatitis C infection. In 2019, the self-reported annual bleeding rate was zero for the majority of patients. However, patients aged › 50 years still suffered from hemophilia-related complications, especially complications arising from joint damage accrued in the past.

Chapter 3 In this chapter, we assessed overall mortality and causes of death among patients with hemophilia in the Netherlands from 1972-2018. We conducted a cohort study where 1066 patients with hemophilia who participated in a nationwide survey in 2001 were followed until July 2018. These new results were then compared with the results of similar cohort studies from earlier time periods.[4-6] The life expectancy of patients with hemophilia in the Netherlands strongly improved over time but was still lower than that of the general male population in 2018 (life expectancy estimates were 77 years versus 83 years respectively). Mortality due to HIV and HCV-related complications among patients with hemophilia has decreased over time but is still higher than that of the general population. In addition, mortality due to intracranial bleeding was also much higher among patients with hemophilia when compared to the general population. Lastly, deaths due to ischemic heart disease were consistently low during the entire 46-year follow-up period.

Chapter 4 In this chapter, we assessed the immunogenicity of recombinant-derived FVIII products in patients with hemophilia A who were exposed to FVIII for at least 50 days (also called previously treated patients or PTPs) using a meta-analysis approach. All studies that reported on de novo inhibitor development in PTPs with < 0.02 IU mL^{-1} factor activity were included. Using a random intercept Poisson regression model, we calculated the overall pooled incidence rate of inhibitor development, as well as the relative risk of inhibitor development of the different types of FVIII products included in the analysis. The overall pooled incidence rate of inhibitor development was 2.06 per 1000 person-years (95%CI: 1.06–4.01). Compared to Advate, the relative risk of inhibitor development was almost ten times higher among patients using Kogenate/Helixate and roughly 14 times higher in patients using Refacto. (both comparisons were statistically significant) These results suggest that some products may be associated with increased immunogenicity in PTPs.

Chapter 5 In this chapter, we aimed to develop and evaluate a clinical prediction model for inhibitor development. A cohort of 251 previously untreated patients (PUPs) or minimally treated patients (MTPs) previously enrolled in the SIPPET study[7] were used as the study population. Model discrimination was assessed using Harrell's C-statistic and model calibration was assessed visually using a calibration plot. The model consisted of four predictors: *F8* gene mutation, intensity of first treatment with FVIII, the presence of FVIII non-neutralizing antibodies before treatment initiation and FVIII product type (recombinant vs. plasma-derived). The C-statistic of the model was poor (0.66, 95 CI: 0.57–0.75) and calibration was moderate. Using a model cut-off point of 10%, positive- and negative predictive values were 0.22 and 0.95, respectively. Therefore, although the overall performance of the model was poor, it could be useful for identifying a small number of patients with a low risk of inhibitor formation.

Chapter 6 In this chapter, the FVIII-specific IgG epitope repertoire of 39 inhibitor-positive patients and 83 inhibitor-negative patients who were followed for 50 days of exposure to FVIII (EDs) after treatment initiation was explored by means of a novel high-throughput epitope mapping technique.[8] In short, a library of roughly 10^9 randomly generated 12-mer peptide sequences expressed on the surface of M13 bacteriophages were screened against each patient's IgG antibody repertoire. Bacteriophages with unbound peptide sequences were washed away and the DNA of the remaining bacteriophages was analyzed using next generation sequencing to identify the peptide sequences that were bound by IgG antibodies. For each patient, the assay was performed three times; pre-treatment using a standard sample, post-treatment using a standard sample and post-treatment using a FVIII-specific antibody depleted

sample. Using this method, we isolated a set of 12-mer peptide sequences with high affinity to FVIII-specific antibodies. These peptide sequences were then clustered on the basis of sequence similarity. For each cluster, a consensus motif was generated which was then aligned to the linear sequence of FVIII. The degree to which these clusters of peptide sequences could be used to discriminate between patients with and without an inhibitor was assessed using the C-statistic. We found that the FVIII-specific antibody response was highly polyclonal, with many clusters being identified and mapped onto different parts of FVIII. The most predominant clusters in inhibitor-positive patients were mapped to the heavy chain of the FVIII molecule. In the pre-treatment samples, three clusters of peptide sequences (with the consensus motifs "pxyNw", "PSLxWK" and "SWPHxxxxK") were identified that predicted inhibitor development after initiation of treatment with FVIII (with a C-statistic of 0.76, 0.80 and 0.76 respectively).

Chapter 7 In this chapter, we reviewed the literature to explore anti-drug antibody prevention strategies applied to patients with diseases other than hemophilia, with the aim of identifying anti-drug antibody prevention strategies that could provide targets for further research for immune tolerance induction (ITI) in patients with hemophilia. Several case-series reported a reduction in anti-drug antibodies using a combination of rituximab, methotrexate, and intravenous immunoglobulins in patients with Pompe's disease treated with recombinant human acid –glucosidase enzyme therapy. In patients with rheumatoid arthritis, multiple large randomized controlled trials showed that the use of methotrexate reduced the presence of anti-drug antibodies against TNF inhibitors when these two drugs where used concomitantly.

Discussion

The health status of the Dutch hemophilia population

In Chapter 2 and 3, we comprehensively assessed the current health status of the Dutch hemophilia population and compared it with the general population. We also explored how health outcomes have changed over time as a result of treatment changes and non-treatment related factors (such as the HIV/HCV epidemic). These findings underscore the successes of 50 years of hemophilia treatment, as well as highlight areas where the current treatment guidelines may be improved upon.

This is most likely the last population wide assessment of the health status of the Dutch hemophilia population where the vast majority of patients with severe hemophilia were still treated with standard or extended half-life clotting factor products.

Currently many new novel agents are entering the market or are in the process of obtaining market approval. In particular, the uptake of emicizumab[9] (a bispecific antibody mimicking FVIII) in the Dutch population is very high due to its effectiveness, the fact that it can be administered subcutaneously and its long half-life. It is to be expected that most patients with severe hemophilia A will switch to emicizumab in the next years. The main limitation of emicizumab is that clotting factor products are still needed to treat breakthrough bleeds. For patients with hemophilia B, no non-factor replacement therapy options exist at the moment. Fitusiran (a siRNA that suppresses antithrombin) and concizumab (an anti-TFPI inhibitor) are the drugs that are closest to market approval.[9] Both drugs can be used in both hemophilia A and B patients. However, both drugs have had issues with thrombotic complications, leading to temporary cessation of phase 2 clinical trials.[9] Therefore, it is as of yet unclear if these drugs will actually be available in the future.

In the coming years, it is highly likely that the first gene therapy options for hemophilia will be approved. For the first time, there is the potential for a cure for patients with hemophilia. However, there are still some challenges to be overcome before gene therapy can be readily implemented in all patients. Many patients develop transient liver toxicity in the early phase which is sometimes associated with reduced gene expression.[10] In addition, due the presence of pre-existing neutralizing antibodies against the viral vector, many patients are currently ineligible for treatment.[10] Furthermore, the application of gene therapy in children with hemophilia is challenging as loss of transgene expression may occur as the liver grows and the development of humor immunity precludes re-administration of the same viral vector.[10]

The results described in Chapters 2 & 3 may serve as a benchmark against which the effect of these novel treatment modalities on health outcomes may be compared in the future.

Our study showed that, with a median bleed rate of zero, 44% of patients with severe hemophilia still experienced at least one joint bleed per year despite prophylactic treatment. It was not clear if these bleeds were mostly spontaneous or caused by physical trauma. If spontaneous, the incidence of these bleeds could be reduced by increasing the target trough levels using the bleeding phenotype as a guide, by

pharmacokinetic-guided dosing or by switching to emicizumab (if the patient has hemophilia A). Despite this, some patients might still bleed due to non-adherence problems or due to having a target joint that is more prone to bleeding in general. Reducing physical activity to decrease the number of bleeds is not recommended, as it has not been linked to an increased bleeding rate and is beneficial to both physical health and mental health.[11] Overall, a more personalized treatment approach is needed in order to get the joint bleed rate down to zero in this group of patients.

Furthermore, our study set-up (which consisted of several cross-sectional studies) was not suitable to assess the impact of primary prophylaxis on long-term joint damage. (as this would require longitudinal follow-up of individual patients) Joint damage is a gradual process that takes decades to manifest. Current FVIII target trough levels of 1% are enough to prevent most but not all bleeds.[12] In addition, even patients without any clinically evident bleeding (i.e. an annual joint bleeding rate of zero) may still develop significant joint deterioration later in life due to subclinical joint bleeds.[13] Long-term cohort studies are needed to assess if pediatric patients in our study with subclinical joint bleeds are still at risk of developing significant joint damage in older age. If so, this might necessitate an increase in the target FVIII/FIX trough levels or a different treatment approach altogether. In the past, the number of weekly infusions needed to maintain higher trough levels would have made this strategy infeasible. However, with the newer extended half-life products or emicizumab, it is now practically feasible to increase trough levels to much higher levels.

In addition, we reported that a quarter of patients had moderate-to-severe liver fibrosis after HCV eradication. Despite clearing the virus, these patients remain at high risk of for HCV-related complications such as hepatocellular carcinoma[14] and should therefore be closely monitored.

Our data show that death due to intracranial bleeding was the second most common cause of death (after cancer) and that patients with hemophilia had a 13-fold higher chance of dying of an intracranial bleed than the general male population. In our study, all deaths due to intracranial bleeding occurred in adults (the youngest was 44 years old) and were non-traumatic. Studies have shown that the lifetime risk of intracranial bleeding in patients with hemophilia follows a bimodal distribution, with a peak around the perinatal period and infancy, as well as another peak in old age.[15]

Furthermore, our data show a high rate of hypertension among older patients with hemophilia, which is a strong independent risk factor for developing an intracranial

bleed. The biological pathways that cause the increased rate of hypertension are not clear. Some have speculated that bleeding-induced vascular remodeling in the kidneys may be the cause of the hypertension seen in these patients[16], but no studies have assessed this hypothesis as of yet. From a clinical standpoint, our findings suggest that early monitoring of patients with hemophilia for hypertension might be needed in order to reduce the risk of intracranial bleeding in adults as much as possible.

An increase in target trough levels for prophylaxis might decrease the incidence of intracranial bleeding and intracranial bleeding-related mortality in adults. In children, difficulties with venous access in the first days/months after birth precludes the use of prophylaxis with conventional clotting factor products. The fact that emicizumab that can be administered subcutaneously makes it possible to start prophylaxis almost immediately after birth to prevent intracranial bleeds in patients with severe hemophilia A. Currently, this potential use-case for emicizumab has not been implemented yet but has the support of the Scientific Advisory Council of the National Hemophilia Foundation.[17]

Identifying and treating patients at a high risk of inhibitor development

Our results show that clinical outcomes in patients with hemophilia have improved tremendously over the past decades. One major unresolved problem that still remains, however, is that many patients treated with FVIII develop inhibitors. Even patients that are switching to prophylactic treatment with emicizumab will still need FVIII to treat breakthrough bleeds. It is as of yet unclear how prophylaxis with emicizumab will impact inhibitor development. It could be possible that inhibitor development will actually be higher in these patients, as they will only be exposed to FVIII in the context of trauma or surgery. Regardless of the choice in treatment product, inhibitor development will continue to be a significant problem in the near future.

Therefore, the second aim of this thesis was to evaluate different strategies to identify patients at a high risk of inhibitor development and to present an overview of anti-drug antibody strategies that could potentially be applied to these patients. The results of these studies, which were reported in Chapters 4-7, are summarized and their wider implications for clinical practice and future research are discussed below.

Using three different study approaches, we tried to identify patients who have a high risk of inhibitor development. We first assessed how different recombinant FVIII products influenced the rate of inhibitor development in PTPs with severe or moderately severe hemophilia. In a systematic review and meta-analysis of the literature.

(Chapter 4) We found that Kogenate/Helixate and Refacto were associated with increased immunogenicity, compared to Advate.

There are several hypotheses that could explain the increased immunogenicity of these products such as differences in the amino acid sequence, culture conditions, stabilizing agents and/or the type of cell culture used for production.[18] However, due to the rarity of inhibitor development in this group, and the lack of adjustment for confounding factors (e.g. the type of *F8* gene mutation), it is difficult to draw any definite conclusions from the data. Future research on inhibitor development in PTPs should focus on creating standardized reporting systems. Good examples of this are the various national and international registries such as the Dutch HemoNED registry and the European EUHASS registry.[19]

In Chapter 4, we assessed only a single factor (product type) and its association with inhibitor development in PTPs. In contrast, Chapter 5 was aimed at combining different factors into one model to predict inhibitor development in PUPs. The newly developed clinical risk prediction model was poor at identifying patients at high risk of inhibitor development. However, the model was able to accurately identify a small number of patients with a low risk of inhibitor formation. There are several approaches to improve the accuracy of future prediction models. For example, one could incorporate information on other genetic risk factors for inhibitor development (e.g. the *CTLA-4* or *IL10* genes) into the risk score. However, if accurate prediction of inhibitor formation at baseline is impossible, then a dynamic prediction model might be more useful. This type of model could for example, incorporate the number of days of exposure to FVIII over time, transient events such as FVIII exposure during trauma or surgery, as well as changes in IgG antibody titers over time. Another interesting approach would be to use non-linear machine learning algorithms which might produce better predictions. However, the disadvantage of these models is that they are prone to overfitting (meaning that a lot of data is needed for reliable results) and that they are difficult to interpret.[20]

This prediction model could be used to identify patients with a low risk of inhibitor development who could then be safely treated with regular FVIII in countries where emicizumab is significantly more expensive than FVIII. (for example, many low-income countries) As the current prediction model has not been externally validated, we do not recommend the use of this specific model in clinical practice as of yet.

Next, the FVIII-specific IgG epitope repertoire of 122 PUPs was explored by means of a novel high-throughput epitope mapping technique using a random peptide

phage-display library. (Chapter 6) We were able to identify three clusters of highly similar peptide sequences (with the consensus motifs “pxyNw”, “PSLxWK” and “SWPHxxxxK”) that were detectable in samples taken before patients were exposed to FVIII and that were predictive for inhibitor development.

The fact that these clusters of peptide sequences with high affinity for anti-FVIII antibodies were already present in samples taken before treatment with FVIII is somewhat unexpected. However, multiple studies have reported the presence of non-neutralizing anti-FVIII antibodies in healthy people.[21] In addition, we previously reported that roughly 10% of patients enrolled in the SIPPET study had measurable anti-FVIII antibodies.[22] It could be that a certain amount of autoreactivity is common in patients as well as healthy controls. That being said, this hypothesis only holds for patients with a non-null mutation that can still produce some endogenous FVIII. It could also be that the FVIII-specific antibodies are the result of previous exposure to a pathogen (e.g. a bacteria or virus) that shares some sequence similarity with FVIII. This cross-reactivity of an antibody response has been previously reported in several auto-immune disorders and is referred to as “molecular mimicry”.[23]

To better understand the pathophysiological mechanisms underlying the association between these peptide motifs and inhibitor development, the predicted locations of these motifs on the surface of FVIII still need to be validated in further studies. (e.g. by using alanine scanning mutagenesis) This is because the final epitope motifs were aligned to the linear sequence of FVIII to find their location. However, the majority of B-cell epitopes are reported to be conformational.[24, 25] Therefore, this approach is not optimal as the majority of epitope motifs will not have been mapped to the right location.

To overcome this problem, several B-cell epitope prediction algorithms have been developed to map epitope motifs to the three-dimensional structure of FVIII, using an in-silico approach. However, all of these algorithms perform relatively poorly.[26] That being said, knowledge of the exact location of these clusters on the surface of FVIII is not necessary for risk prediction. These novel results could be used to set up tests that predict the risk of inhibitor development before starting treatment with FVIII.

Chapters 4-6 focused on predicting inhibitor development in patients treated with regular FVIII. However, it is expected that the vast majority of patients with severe hemophilia A will switch to emicizumab for prophylactic treatment in the near future. Despite this, inhibitor development will still occur as FVIII will be still be needed in the

case of an acute bleeding episode or for surgical interventions. This will make effective treatment during future bleeding episodes more difficult as the alternative would be treatment with rFVIIa or FEIBA. Thus, there is still a need for accurate inhibitor risk prediction strategies in the coming years, especially strategies to identify patients at risk of developing an inhibitor that is refractory to ITI. These high-risk patients could be candidates for preventative treatment.

Unfortunately, specific treatment strategies to prevent inhibitor development in patients with a high risk of developing inhibitors that do not respond to treatment with ITI are lacking. We therefore reviewed anti-drug antibody prevention strategies that have been used in disorders other than hemophilia in Chapter 7. Several studies have shown that a short course with rituximab, methotrexate and IVIG prevented anti-drug antibody formation in patients with classic infantile Pompe's disease. (rituximab has been used previously in rescue ITI, but only in the context of inhibitor eradication, not prevention[27]) However, the high risk of serious infections outweighs the potential benefits of implementing such a strategy in patients with hemophilia. Several large randomized controlled trials have shown that the concomitant use of methotrexate strongly reduced the proportion of patients with detectable antibodies against TNF inhibitors. As methotrexate is very cheap, it could be a promising low cost treatment option to reduce the incidence of inhibitor development. However, it is unclear if the reduction in anti-drug antibodies is due to tolerization to the drug or due to methotrexate merely suppressing the immune system temporarily. Secondly, studies assessing the risk of adverse events in methotrexate in very young pediatric patients (e.g. 1-2 years old) are scarce, which limits the practical implementation of this strategy in patients with hemophilia.

Conclusion

Our results show that clinical outcomes in patients with hemophilia have improved tremendously over the past decades. The annual bleeding rate and the proportion of patients with joint impairment have decreased strongly. In addition, HCV has almost been eradicated among patients with hemophilia in the Netherlands. As a result, life expectancy has increased to where it is almost equal to that of the general population. Furthermore, using three different study approaches, we evaluated several methods to better predict the risk of inhibitor development (which is still a significant complication of treatment with FVIII). The results of these studies are promising and could be used to improve current inhibitor prediction strategies and inform future research on this topic.

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