Hemophilia on the treshold of the 21st century

Plug, I.

Chapter 3.1

Bleeding in carriers of hemophilia

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Summary

A wide range of factor VIII and IX levels is observed in heterozygous carriers of hemophilia, as well as in non-carriers. In female carriers extreme lyonisation may lead to low clotting factor levels. We studied the effect of heterozygous hemophilia carriership on the occurrence of bleeding symptoms.

A postal survey was performed among the majority of all women who were tested for carriership of hemophilia in the Netherlands between 1985 and 2001. The questionnaire included items on personal characteristics, characteristics of hemophilia in the affected family members, carrier testing and history of bleeding problems such as bleeding after tooth extraction, bleeding after tonsillectomy and operations. Information on clotting factor levels was obtained from the hospital charts. Logistic regression was used to assess the relation of carrier status and clotting factor levels with the occurrence of hemorrhagic events.

In 2004, 766 questionnaires were sent, 546 women responded (80%). Of these 274 were carriers of hemophilia A or B. The median clotting factor level of carriers was 0.60 IU/ml (range 0.05-2.20 IU/ml) compared to 1.02 IU/ml (range 0.45-3.28 IU/ ml) in non-carriers. Clotting factor levels between 0.60 and 0.05 IU/ml were increasingly associated with prolonged bleeding from small wounds, prolonged bleeding after tooth extraction, tonsillectomy and operations.

Carriers of hemophilia bleed more than other women, especially after medical interventions. Our findings suggest that not only clotting factor levels at the extreme of the distribution, resembling mild hemophilia but also mildly reduced clotting factor levels between 0.41 and 0.60 IU/ml are associated with bleeding.

Introduction

Hemophilia is an X-linked hereditary bleeding disorder caused by a deficient or defective coagulation factor VIII (hemophilia A) or factor IX (hemophilia B). Resulting from the recessive X-chromosomal inheritance pattern mostly men are affected and their female relatives can be heterozygous for the mutation, often referred to as carriers of hemophilia. Previously, pedigree analysis and clotting factor VIII or IX levels were used to diagnose carriership hemophilia[1]. In the early nineteen eighties it became possible to ascertain the carrier status by means of DNA analysis, which has evolved from haplotyping, to mutation analysis offering certainty about the carrierstatus[2]. During the last three decades genetic counseling, carrier testing and prenatal diagnosis of hemophilia have become an integrated part of the comprehensive care for hemophilia[3].

Female carriers are expected to have a plasma concentration of FVIII or IX corresponding to half the concentration found in healthy individuals, which is generally sufficient for normal hemostasis. However, in carriers a wide range in clotting factor levels is seen, from very low, resembling affected males, to the upper limit of normal [4]. This range has been attributed to X-chromosome inactivation, which takes place in the early embryonic life[5]. Other genetic factors, such as ABO blood group, may also affect factor VIII and FIX plasma concentrations in carriers, as they do in non-carriers, where a wide distribution is observed, too[6].

Although Merskey et al[7] already reported excessive bleeding after tooth extraction in 47% of known carriers (n=19) in 1952, this first publication was not followed by larger studies. Some case series showed joint bleeds, prolonged bleeding after tonsillectomy or tooth extractions or post-partum bleeding[8-13]. It is important to assess the risk of bleeding in carriers of hemophilia, to assist help physicians in improving care for hemophilia carriers, for instance by the implementation of prophylactic intervention in carriers at risk for bleeding.

While extensive information on bleeding in men with hemophilia is available, only a few studies have focused on bleeding in carriers. We present a large national cross-sectional study examining bleeding in women in whom genetic testing for hemophilia was performed within the last decade. We focused on spontaneous bleeding and bleeding following surgical interventions.

Objectives:

The aim of this study was to examine the risk of bleeding among carriers of hemophilia A or B compared to non-carriers.

Methods

Subjects

We contacted all women who had been tested for carriership of hemophilia A or B before 2001 in the Netherlands. All women had to be 18 years or over to participate. Diagnosis of carriership of hemophilia consisted of DNA diagnostics, pedigree analysis or the assessment of clotting factor levels. Carriers of hemophilia were women in whom the genetical defect related to hemophilia was established through DNA analysis (haplotype or mutation analysis) or, before 1985, through the determination of clotting factor levels in combination with pedigree analysis. Non- carriers were women in whom testing showed that they were not carrying the mutation that caused hemophilia A or B in their family. By comparing carriers and non-carriers both from hemophilic families we excluded the possible bias introduced by knowledge on hemophilia.

Assessments

Questionnaires were sent by postal mail, followed by two reminding letters. The questionnaire included items on personal characteristics, type and severity of hemophilia in affected relatives, carrier testing and several bleeding problems. We assessed if patients ever reported spontaneous bleeding and bleeding after trauma: bruising, nose bleeds, gum bleedings and joint bleeds. Questions on bleeding after medical interventions included bleeding after tooth extractions, (adeno) tonsillectomy and operations. Prolonged bleeding after medical interventions was defined as bleeding for over three hours after tooth extractions, (adeno) tonsillectomy or operations. The topics on bleeding were based on a validated questionnaire developed by Šrámek et al, validated by means of sensitivity analysis[14]. Restrictions in daily life due to excessive blood loss during the menstrual period were measured on a seven-point scale in which the score one represented no restrictions and seven points severe restrictions. To evaluate the questionnaire a pilot study was performed in 12 carriers. Informed consent was obtained to allow us to verify the diagnosis and to obtain information on factor VIII and IX activity from the hemophilia treatment centres. In most women clotting factor activity had been determined at several time points, in which case the lowest value was used for evaluation in this study. Severity of hemophilia in the male family members was classified according to residual percentage of factor VIII or IX clotting activity: severe (<0.01 IU/ml), moderate (0.01-0.05 IU/ml) or mild (>0.05-0.40 IU/ml). The Committee of Medical Ethics of the Leiden University Medical Center approved this study.

Data analysis

Women with clotting disorders due to other causes than hemophilia or who used antithrombotic medication were excluded from the analysis. The prevalence of bleeding symptoms in women who were carriers of hemophilia A or B was compared to that of women

not carrying a hemophilia mutation. Due to the limited number of women reporting hemophilia B in the family we could not distinguish between the two types of hemophilia in the analysis. The risk of bleeding related to the carrier status and clotting factor levels was determined, and we tested for a graded response using a Wald test. If in the comparison between carriers and non-carriers a specific bleeding event showed a relative risk (RR) above 1 and a 95% confidence interval (CI) not including one, its association with clotting factor levels is also presented. In the analysis of bleeding risk caused by specific interventions only women who ever underwent this intervention were included in the analysis. Women who were treated with cyclokapron (tranexamic acid), desmopressin or clotting factor preparations before the medical intervention were excluded from the analysis. Clotting factor levels were analyzed as a categorical variable, the studied categories were <=0.40 IU/ml, 0.40-0.60 IU/ml and >0.60 IU/ml. In the analysis of excessive blood loss during the menstrual period only women were included who were pre-menopausal. To exclude the effect of referral for carrier testing because of bleeding problems we repeated the determination of the risk of bleeding after (adeno) tonsillectomy or operations among women who were not tested because of an increased bleeding tendency.

Results

Response and patient characteristics

A total of 766 questionnaires were sent, and 546 questionnaires were completed and returned (response of 80%). Excluded from analyses were women who reported other clotting disorders than hemophilia (19 women of whom 13 had Von Willebrands disease) and 10 women in whom the carrier status was not conclusive or unknown. This resulted in 274 carriers and 245 non-carriers for the current analyses. Of these, 73% (n=384) reported hemophilia A, 9% (n=48) hemophilia B, whereas of the of the other 95 (18%) women the type

of hemophilia in the family was unknown. Table 1 shows the general characteristics according to hemophilia carrier status. The median age of the carriers and non-carriers was similar, 39 years (yrs) (range 18-77 yrs) and 40 years (range 20-90 yrs), respectively.

Table 1. General characteristics of study population

	Carrier of hemophilia	
	Yes (n=274)	No (n=245)
Mean age at questionnaire (range)	39 (18-77)	40 (20-90)
Use of oral contraceptives*	79 (29)	65 (27)
Family		
Severe hemophilia in family	140 (51)	113 (46)
Hemophilia A in family	230 (84)	151 (62)
Clotting factor		
Clotting factor levels available	225 (82)	143 (58)
Median FVIII activity (IU/ml)	0.60 (0.05-2.19)	1.02 (0.45-3.28)

Data presented are numbers (percentages) of median (range)
*Current use of oral contraceptives
** hemophilia in family, not including own children

Current use of oral contraceptives in carriers and non-carriers was similar, 29 % and 27% respectively. Past use of oral contraceptives was reported by 159 carriers (51%) and by 119 non-carriers (56%). Severe hemophilia in the family was reported by 140 (51%) of carriers and 113 (46%) non-carriers. Thirty-one carriers (11%) and 42 non-carriers (21%) were not aware of the severity of hemophilia within their family. Carrier testing by means of DNA diagnostics, available in the Netherlands since 1985, was performed in 177 carriers (57%) and in 122 non-carriers (58%).

Factor VIII and IX characteristics

Clotting factor levels were missing for 18 % (49/274) of carriers and 43% (103/245) of non-carriers. The median clotting factor level in carriers was 0.60 (range 0.05-2.19) IU/ml compared to 1.02 (range 0.45-3.28 IU/ml) in non-carriers. Sixty-two women had a clotting factor levels lower than 0.40 IU/ml, all carriers. Figure 1 shows the distribution of clotting factor levels in carriers and non-carriers.

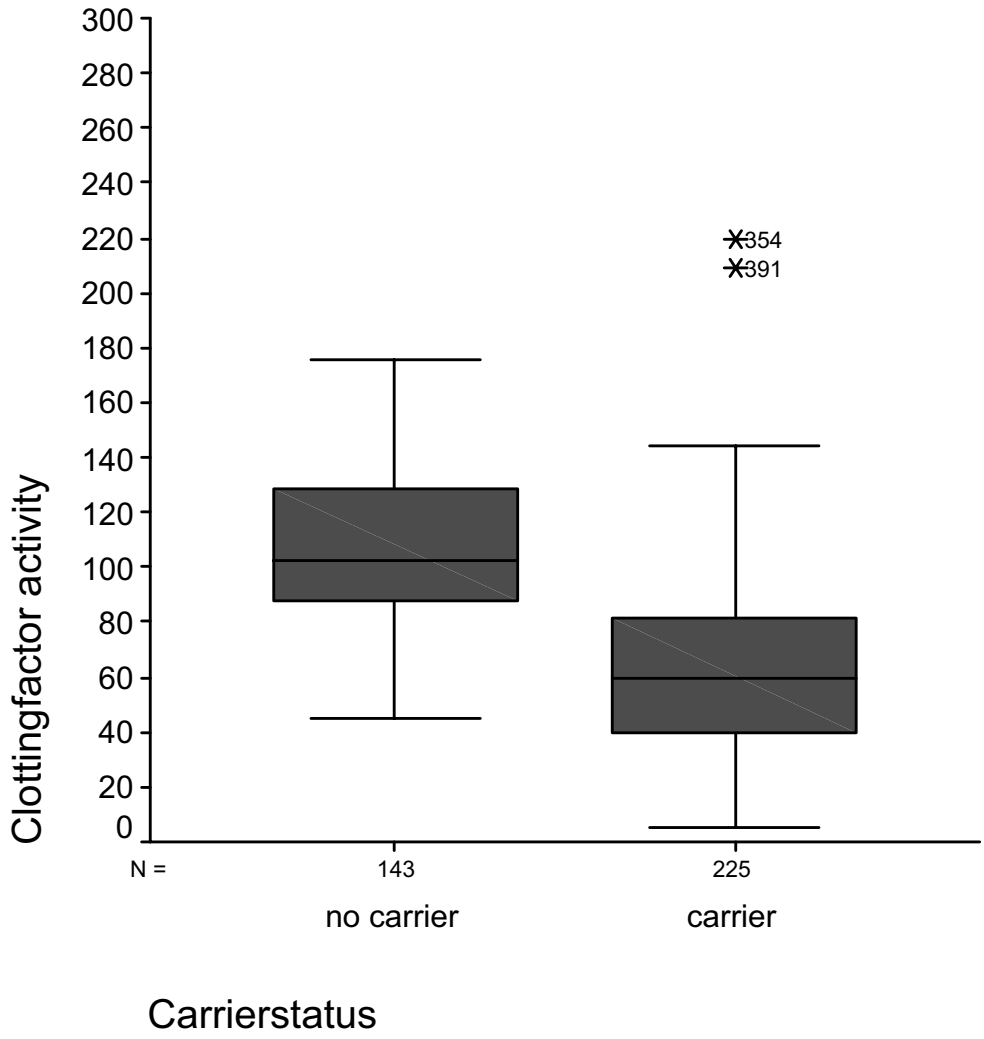

Figure 1. Clotting factor level in relation to carrier status shown for participants of whom clotting factor level is known.
This box-whisker plot shows the median and the interquartile range of clotting factor activity levels in carriers and non-carriers. The box is marked by the first and the third quartile, the whiskers extend to the range

Bleeding symptoms

Spontaneous bleeding and bleeding after trauma

Table 2a presents the risk of (ever having experienced) bleeding of carriers compared to non-carriers. The risk of prolonged bleeding (>5 minutes) from small wounds was two times higher (relative risk (RR) 2.2, 95% confidence interval (CI) 1.4-3.5) in carriers compared to non-carriers.

Table 2a. Spontaneous bleeding or bleeding after trauma in carriers and non-carriers of hemophilia

Risk moments	Carriers	Non-carriers	Relative Risk (CI)*
Nose bleeds			
Ever reported	115/270 (43)	105/237 (44)	1.0 (0.8-1.2)
Bruising	50/269 (19)	42/243 (17)	1.1 (0.7-1.6)
Small wounds	56/262 (21)	23/237 (9)	2.2 (1.4-3.5)
Gum bleeding			
Present (yes/no)	164/271 (60)	148/243 (61)	1.0 (0.9-1.1)
Joint bleeds	23/271 (8)	11/240 (5)	1.9 (0.9-3.7)

Data presented are numbers(percentage)
*CI= 95% Confidence Interval

Table 2b. Risk of bleeding after medical interventions

	Carriers	Non-carriers	RR (CI)*
Tooth extraction†			
Prolonged bleeding (> 3 hours)	61/228 (27)	26/219 (12)	2.3 (1.5-3.4)
Treatment after intervention	24/228 (11)	1/219 (0.5)	23.1 (3.1-169)
Tonsillectomy or adenotomy†			
Prolonged bleeding (>3 hours)	29/123 (24)	16/122 (13)	1.8 (1.0-3.1)
Treatment after intervention	10/123 (8)	1/122 (0.8)	9.9 (1.3-76.3)
Operations†			
Prolonged bleeding (> 3 hours)	52/170 (31)	19/146 (13)	2.9 (1.6-5.3)
Treatment (ever)	16/174 (9)	6/149 (4)	2.3 (0.9-5.7)
Blood transfusion	29/174 (17)	18/149 (12)	1.4 (0.8-2.4)

Data presented are frequencies (percentage)
*RR (CI)= Relative Risk (95% Confidence Interval)
†Participants who had been treated prior to the clinical intervention with clotting factor preparations, cyclokapron or desmopressin were excluded from the analysis.

Low clotting factor levels were associated with an increased occurrence of prolonged bleeding from small wounds and joint bleeding (Table 3). Joint bleeds were reported by 8% of carriers and by 5% of women not carrying hemophilia, which was a two times increased risk (RR1.9 CI 0.9-3.7). Although no higher risk of nose bleeds was observed in carriers compared to non-carriers, prolonged nose bleeding occurred more often in carriers: 9% of carriers had nose bleeds that lasted longer than 10 minutes compared to in 2% of non-carriers. Seventeen percent of carriers had received treatment for nosebleeds, compared to 10% of non-carriers. Carriers of hemophilia did not have a higher risk for large bruising and gum bleeding.

Table 3. Bleeding tendency according to decreasing clotting factor level

	> 0.60 IU/ml	0.40-0.60 IU/ml	<0.40 IU/ml	p for trend
Small wounds				
Frequency (%)	28/233 (12)	25/64 (39)	11/60 (18)	
RR (CI)*	1	3.3 (2.0-5.2)	1.5 (0.8-2.9)	0.009
Joint bleeds				
Frequency	12/241 (5)	9/65 (14)	6/62 (10)	
RR (CI)	1	2.8 (1.2-6.3)	1.9 (0.8-4.9)	0.06
Tonsillectomy				
Frequency (%)	21/124 (17)	6/26 (23)	11/31 (35)	
RR (CI)	1	1.4 (0.6-3.0)	2.1 (1.1-3.9)	0.06
Tooth extraction				
Frequency (%)	18/139 (13)	14/51 (27)	15/36 (42)	0.00
RR (CI)	1	1.8 (1.0-3.0)	2.5 (1.5-4.2)	
Operations				
Frequency (%)	18/139 (13)	14/49 (29)	15/36 (42)	0.00
RR (CI)	1	2.2 (1.2-4.1)	3.2 (1.8-5.7)	
Bleedingscore>=2	1	3.0 (1.5-5.8)	4.0 (2.1-7.7)	0.00

Women who ever received treatment with clotting factor concentration, cyclokapron or desmopressin before tooth extraction, tonsillectomy of operations were excluded from the analysis.
*RR (CI)= Relative Risk (95% Confidence Interval)

Bleeding after medical interventions

Tooth extractions had been performed in 228 carriers and 219 non-carriers, and the risk of bleeding for more than three hours after tooth extraction was two times higher in carriers compared to non-carriers (RR 2.3 CI 1.5-3.4) (Table 2b). In 24 of 228 carriers additional treatment due to bleeding after tooth extractions had been required, compared to one of 219 non-carriers. Treatment included intervention by a dentist or the use of cyclokapron, desmopressin, or administration of clotting factor concentrate. Clotting factor levels below 0.60 IU/ml were associated with prolonged bleeding after tooth extraction. A total of 123 carriers and 122 non-carriers underwent tonsillectomy and or adenotomy, 24% of carriers and 13% of non-carriers reported bleeding for more than three hours following tonsillectomy (RR=1.8, CI 1.0-3.1). Six carriers and one non-carrier (1%) were treated prophylactically with either cyclokapron or desmopressin before the intervention. In eight carriers (3%) a blood transfusion was required after (adeno) tonsillectomy compared to none of the non-carriers. Eleven per cent of women carrying hemophilia needed treatment for bleeding following surgery. In the majority of cases a second intervention to treat bleeding had to be performed. Decreasing clotting factor level were also associated with prolonged bleeding after (adeno) tonsillectomy. Women with a clotting factor level of 0.4 IU/ml or below had a 2.1 times (RR=2.1, CI 1.1-3.9) increased risk compared to women with a clotting factor level above 0.6 IU/ml. Prolonged bleeding for more than three hours after one or more operations was reported by 52 of 170 carriers and by 19 of 146 non carriers, RR=2.9 (CI 1.6-5.3). Women with a clotting factor level of 0.4 IU/ml or below had a three times (RR=3.2, CI 1.8-5.7) increased risk of prolonged bleeding after operations compared to women with a clotting factor level of 0.6 IU/ml and above.

Additional treatment during or after surgery due to bleeding problems was necessary in 12%(51/421) of the carriers and in 5% (13/270) non-carriers. One or more blood transfusions

were required in 11% of operations in participants carrying hemophilia compared to 7% in non-carriers. Other additional treatment consisted of infusion of clotting factor concentrate or a second operation.

After the exclusion of women in whom bleeding problems were not the indication for carrier testing the risks of prolonged bleeding after operations (RR=2.8 CI 1.6-5.0) or tonsillectomy (RR=1.8 CI 0.9-3.4) was similar to the finding in the whole study population.

Excessive bleeding during the menstrual period

Women with lower clotting factor levels reported more often excessive blood loss during the menstrual period (menorrhagia) (Table 4).

Table 4. Characteristics of the menstrual period in relation to clotting factor level

	>0.60 IU/ml	0.4-0.60 IU/ml	0-0.40 IU/ml	p for trend
Excessive blood loss				
Frequency	93/195 (48)	31/54 (57)	31/51 (61)	
Risk	1	1.2 (0.9-1.6)	1.3 (1.0-1.7)	0.07
Anemia				
Frequency	16/195 (8)	9/54 (17)	8/51 (16)	
Risk	1	2.0 (1.0-4.3)	1.9 (0.9-4.2)	0.07
Iron suppletion				
Frequency	15/195 (8)	11/54 (20)	7/51 (14)	
Risk	1	2.6 (1.3-5.4)	1.8 (0.8-4.1)	0.07
Hysterectomy[*]				
Frequency	7/44 (16)	2/10 (20)	2/11 (18)	
Risk	1	1.3 (0.3-5.2)	1.1 (0.3-4.8)	0.9
Restrictions in daily life[†]	17/189 (9.0)	9/53 (17)	9/49 (18)	
	1	2.0 (0.8-4.9)	2.3 (1.0-5.6)	0.04

[*]Reported by postmenopausal women due to excessive blood loss.
[†] Moderate to severe restrictions in daily life due to excessive blood loss during the menstrual period

Similarly, the risk for requiring iron suppletion was 80% increased (RR 1.8 CI 0.7-5.0) in women with a clotting factor level of 0.40 IU/ml or below compared to women with a clotting factor level of 0.6 IU/ml and above. Sixty-two (23%) women carrying hemophilia visited the general practitioner for excessive bleeding during the menstrual period, compared to 20% (n=47) of non-carriers. Fifty-eight women (31 carriers (11%) and 21 non-carriers (9%)) consulted a gynecologist and in 18 women a hysterectomy was performed for this reason. Mild to severe restrictions in daily life due to excessive blood loss during the menstrual period was reported by 18% of women with a low clotting factor level compared to 9% of the women with a clotting factor level above 0.60 IU/ml (RR=2.3 CI 1.0-5.6).

Discussion

Although hemophilia is a well-known bleeding disorder in men, it is seldom recognized that female carriers of hemophilia might not only have an increased bleeding tendency, but that the symptoms may be frequent and severe. We studied the risk of bleeding among carriers of hemophilia A or B compared to that of non-carriers. Although usually a level of 0.40 IU/ml is used as the upper limit defining hemophilia, we found an increased risk of bleeding in women with clotting factor levels between 0.40 and 0.60IU/ml. Carriers of hemophilia experience more spontaneous and provoked hemorrhages than non-carriers, with a higher risk of prolonged bleeding after operations, tooth extractions, tonsillectomy. The risk is highest in those with the lowest clotting factor levels.

Strengths and limitations

In this study we approached all women who had been counseled and tested for carriership of hemophilia A or B in the Netherlands. Eighty per cent of women responded to the questionnaire. We included 519 women, which makes this currently the largest survey into

the hemorrhagic risk in female relatives of men with hemophilia. Non-responders were somewhat older than the responders. Yet, age did not modify the association between the clotting factor levels and the risk of bleeding. We therefore assume our results to be generalizable to carriers of hemophilia in general. Recall bias may have influenced the reporting of bleeding symptoms as questions were asked after carrier testing. Yet, Mauser-Bunschoten et al showed by comparing bleeding tendency in obligatory carriers with normal factor VIII levels to non-carriers that the awareness of carrier status has little influence on the reported frequency of bleeding[10]. A more serious problem is that women, who grew up in families with hemophilia patients, may judge bleeding symptoms differently from women in the general population. Selection bias may have been introduced if carrier testing was done because of bleeding problems. To counter this problem, as well as the possible incomparability of carriers from hemophilia families to women from the general population, we only included women from families known with hemophilia. The carriers and non-carriers in our study had grown up in the same environment and had not been aware of their carrier status until testing. Therefore, even if carrier testing was done because of bleeding symptoms, which was the case in 7% of participants, this would not have affected the comparison. Clotting factor levels were missing for 18% of carriers and 43% of non-carriers. Although the group of women in whom clotting factor levels had been measured is most likely to be different from the group in whom these are missing this will not affect the observed relation between clotting factor levels and bleeding.

In the literature mild hemophilia is defined as a clotting factor level of below 0.40 IU/ml. This cut-off point is also described in the guidelines set by the subcommittee of the ISTH[15] and is used in clinical practice. Our study shows that the risk of bleeding is increased in women who would be defined as mild hemophilia, but also in women with clotting factor levels

between 0.40 and 0.60 IU/ml. These findings could have implications for the currently used definition of clotting factor levels considered to be "reliable" to perform medical interventions.

We found a moderately increased risk of joint bleedings in carriers. As joint bleedings are relatively rare the reported joint bleeds may be overestimated and they may have been confused with superficial bleeding of tissue in the joint region.

Our study mainly shows an increased risk of bleeding after trauma and medical interventions, which is similar to the clinical profile of mild hemophilia, and in line with a previous study in carriers[10]. Our findings underline the importance for clinicians and carriers to be aware of the complications that may occur after operations in carriers of hemophilia. Both the clinician and the carrier should be informed on the clotting factor level, which is strongly related to the hemorrhagic risk.

However in many carriers the clotting factor levels were either not measured or not known to the woman. It is clear that not only in obligatory carriers, like daughters of hemophilia patients, but also in potential carriers clotting factor levels should be measured preceding a medical intervention, also at a young age. The clotting factor levels in carriers are independent of severity of hemophilia within the family and vary from person to person[16] . This indicates the importance of clotting factor measurement during carrier testing in all women related to men with hemophilia, independent of the severity of hemophilia and family history.

In conclusion our study suggests a higher risk of bleeding in carriers of hemophilia related to clotting factor levels especially after medical interventions. This implicates the importance of the measurement of clotting factor levels before interventions in all carriers and potential carriers of hemophilia.

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