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ORIGINAL ARTICLES

Coagulation disorders in Duchenne muscular dystrophy? Results of a registry-based online survey

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Different complications of hemostasis have been reported in patients with Duchenne Muscular Dystrophy (DMD). These comprise an increased rate of bleeding-symptoms during scoliosis surgery but also thromboembolic complications such as pulmonary embolism, cerebral infarction, deep vein thrombosis or cardiac thrombus.

For this cross-sectional study, personalized online survey-links were forwarded to 682 registered patients with a genetically confirmed diagnosis of DMD via the German-Austrian DMD patient registry (www.dmd-register.de). The questionnaire enquired data regarding the degree of mobility, disposition to hematoma, epistaxis and gum bleeding, occurrence of peri- and postsurgical hemorrhage, stroke, deep vein thrombosis, and cardiac thromboembolism. Further data on regular medication and age were recorded.

Three-hundred-fifty-one DMD-patients completed the questionnaire (response rate of 51.5%). Of those, 164 (46.7%) were ambulatory and 187 (53.3%) were non-ambulatory. Age distribution was homogeneous. Two participants had a history of thromboembolic events (0.6%). Correlations analysis revealed no coherence with the degree of mobility, age or regular medication. A bleeding tendency was reported by 76 participants (21.7%). No significant correlations with age or degree of mobility were found. We found no association with underlying genetic variants. Results of this patient registry-based survey do not indicate a distinct DMD-specific risk for thromboembolic events that exceeds the risk by typical comorbidities of chronic immobility and cardiac insufficiency in advanced stages of the disease. The results of this survey suggest a mild bleeding tendency in this DMD cohort, whereas a selection bias cannot be excluded.

Key words: Duchenne muscular dystrophy, coagulopathy, bleeding tendency

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Conflict of interest
The Authors declare no conflict of interest

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Introduction

Duchenne muscular dystrophy (DMD) is a rare genetic disease leading to chronic and progressive degeneration of muscle tissue. First symptoms of muscle weakness typically occur in pre-school age. With further

progression, loss of ambulation occurs in teenage years, followed by development of scoliosis, respiratory insufficiency, and dilated cardiomyopathy. DMD is caused by mutations in the dystrophin gene, which lead to a loss of function of the dystrophin protein [1]. Along with beta-dystroglycan, dystrobrevin and syntrophin, dystrophin is part of the dystrophin-associated protein complex (DAPC), connecting extracellular matrix to muscular cytoskeleton. While full-size dystrophin (427kDa) is expressed predominantly in skeletal muscle cells and myocardial cells, smaller isoforms are also expressed in other tissues. In most cases, only male individuals are affected by DMD as mutations in dystrophin follow an X-linked pattern of inheritance. Prevalence of the disease was estimated to be about 4.8 in 100,000 male individuals worldwide, while incidence ranges between 15.9 to 19.5 per 100,000 live born males per year [2].

In patients with DMD both an increased risk of bleeding as well as thromboembolic events have been discussed. Especially bleeding-complications during scoliosis surgery in DMD have been described repeatedly [3,4]. Several case-reports or smaller retrospective studies reported pulmonary embolism [5], cerebral infarction [6-9], deep vein thrombosis [10] or cardiac thrombus [11]. The incidence of cerebral infarction in patients with DMD has been estimated to be around 0.75-1.8% and is thereby notably higher than in the general population [8]. As the smaller dystrophin isoform dp71 is also expressed in platelets, a disease-specific disorder of thrombocytic function and hence in primary hemostasis in DMD appears possible [12-14]. The aim of this cross-sectional survey was to explore if disease-specific complications due to undetected coagulation disorders are present in patients with DMD and eventually depend on age and degree of mobility.

Methods

We used a two-step approach with an initial screening questionnaire and specific follow-up questions. The screening questionnaire consisted of 9 questions assessing (1) age, the degree of mobility and long-term medication, (2) bleeding tendency (disposition to hematoma, epistaxis or gum bleeding and occurrence of peri- or postsurgical hemorrhage) and (3) thromboembolic events in the past (stroke, deep vein thrombosis, cardiac thromboembolism). During a pilot phase, neuromuscular and hemostaseologic specialists from the University of Freiburg reviewed, tested and optimized the questionnaire (for complete questionnaire see supplemental Table II). Inclusion criteria of this study were (1) registration in the German-Austrian DMD patient-registry (*www.duchenne-register.de*, based at the Friedrich-Baur-Institute, Ludwig-Maximilians-University of Munich, Germany), which involves deposition of genetic confirmation and (2) present residence in Germany. No exclusion criterion was defined. No personal data from the patient registry were forwarded to the study center. The corresponding Ethics Committee and the oversight committee of the DMD patient-registry approved the project. For distribution of the questionnaire, we used the online platform "SurveyMonkey.com" and generated personalized links. Registry curators sent these links by e-mail or surface mail to each registered patient. The link also provided the option to decline participation. In case of no answer we sent two reminders. Double use of individual online-questionnaires was traceable. Patients were offered to provide their consent and contact details for further follow-up questions.

Patients giving consent for follow up were contacted differently: In case of a reported bleeding tendency, patients received a more precise questionnaire based on the answers provided in the initial survey. If perioperative or postoperative hemorrhages in the past had been indicated in the initial survey, patients were contacted by phone and corresponding medical reports were requested.

To analyze whether disorders of coagulation are associated with the type of genetic mutations, the underlying genetic findings of all participants were assessed and grouped in large mutations (deletions or duplications of 1 exon or larger), small mutations (deletions or insertions < 1 exon, splice site mutations, point mutations), and intronic mutations according to previous studies [15]. Mutations downstream of exon 63 are known to disrupt the expression of the shortest dystrophin isoform dp71 [16], so that mutations were further grouped by localization within the dystrophin gene (upstream of exon 30; exons 31 to 62; downstream of exon 63).

We analyzed clinical data descriptively and processed them with absolute frequencies and percentage values. For statistical analysis we used SPSS (version 22.0) and performed correlation analysis using a two-sided approach for ordinal scaled parameters (Kendall-Tau-b).

Results

The survey was conducted between October 2017 and January 2018. In October 2017, 1459 patients were registered in the DMD patient-registry. Of those 682 fulfilled the inclusion criteria and were included in this study (see Figure 1 for a flowchart of the study). A total of 351 DMD-patients/caregivers completed the questionnaire (response rate of 51.5%). Age distribution was homogeneous (< 10 years = 36.1%; 11-15 years = 20.5%; > 15 years = 39.4%). Of all participants 164 (46.7%) were ambulatory and 187 (53.3%) were non-ambulatory. Regular medication was taken by 259 (73.8%) participants. No information on reg-

ular medication was available for 14 participants (4.0%); see Table I for further characterization of participants regarding degree of mobility, age and regular medication.

Thromboembolic events in the past were identified in two participants (0.7%). One patient with known cardiac insufficiency and EF of 30% had a history of acute chest pain at the age of 31 years. Pulmonary embolism was confirmed by thoracal computer tomography and elevated D-Dimers. Concomitant deep vein thrombosis of the lower extremities was excluded by sonography and the patient was started on life-long oral anticoagulation. Another patient reported a history of an ischemic insult of the left mid cerebral artery and a left-ventricular thrombus that was diagnosed at the same time. Unfortunately, consent for follow-up of this patient was not available, so that additional information could not be collected. Correlation analysis revealed no significant coherences of past thromboembolism with the degree of mobility, age at event, regular medication, regular intake of steroids or cardiac medication; see Figure 2 for illustration of replies to questions enquiring signs of thrombophilia and bleeding tendency.

Interestingly, four other participants reported a history of past thromboembolic events in the initial survey, but were excluded from respective analysis after medical reports were available:

One patient was found to have a history of a perinatal hemorrhagic stroke with resulting unilateral spastic hemiplegia; according to the available medical information the patient and his mother were both found to have a heterozygous factor V mutation. Two patients (0.6%) reported a history of cerebral fat embolism after precedent femoral fracture at age of 14 and 15 years, respectively. A patent fo-

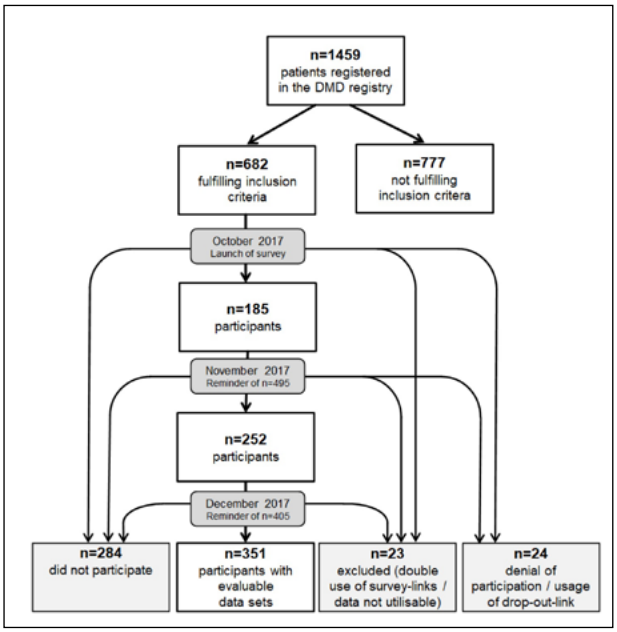

Figure 1. Flowchart of the survey.

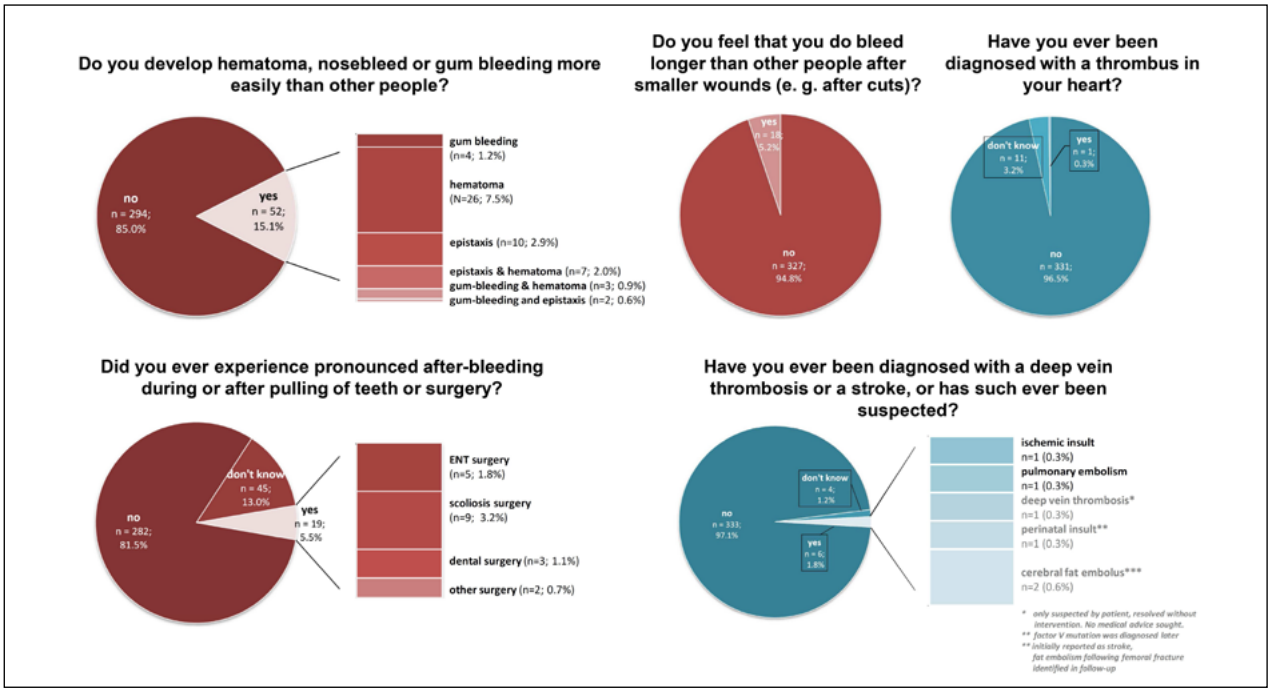

Figure 2. Illustration of questions enquiring signs of thrombophilia and bleeding tendency and given answers by survey participants.

Table I. Characterization of participants by degree of mobility, age and regular medication.

Question	Valid answers	Possible answers	All	Mobility	
				Ambulatory	Non- ambulatory
Degree of mobility					
		Fully ambulatory	n = 110 (31.3%)	-	-
		Wheelchair, partially	n = 37 (10.5%)	-	-
		Wheelchair, predominantly	n = 17 (4.8%)	-	-
		Wheelchair, solely	n = 185 (57.6%)	-	-
	n = 351 (100.0%)	*Bedridden*	n = 2 (0.6%)	-	-
Age					
		< 6 ys	n = 44 (12.5%)	n = 44 (12.5%)	n = 0 (0%)
		6-10 ys	n = 83 (23.6%)	n = 76 (21.6%)	n = 7 (2.0%)
		11-15 ys	n = 72 (20.5%)	n = 31 (8.8%)	n = 41 (11.7%)
		16-20 ys	n = 69 (19.6%)	n = 10 (2.8%)	n = 59 (16.8%)
	n = 337 (96.0%)	*> 20 ys*	n = 69 (19.6%)	n = 0 (0%)	n = 69 (19.6%)
Medication					
		None	n = 78 (22.2%)	n = 34 (9.7%)	n = 44 (12.5%)
		Steroids	n = 147 (41.9%)	n = 111 (31.6%)	n = 36 (10.3%)
		Cardiac medication	n = 102 (29.1%)	n = 15 (4.3%)	n = 87 (24.8%)
		Oral anticoagulation	n = 3 (0.9%)	n = 1 (0.3%)	n = 2 (0.6%)
		Ataluren	n = 17 (4.8%)	n = 12 (3.4%)	n = 5 (1.4%)
	n = 337 (96.0%)	*Eteplirsen*	n = 1 (0.3%)	n = 1 (0.3%)	n = 0 (0%)

ramen ovale could be excluded by echocardiography in both patients. Another single patient reported a suspected deep vein thrombosis in the past, whereas clinical symptoms resolved without therapy and medical advice was not sought.

A bleeding tendency was reported by 76 participants (21.7%). Of those 52 (14.8%) reported a disposition to hematoma, epistaxis or gum bleeding, or a combination of those symptoms. Occurrence of peri- or postoperative hemorrhage or hemorrhage after extraction of teeth was reported by 19 patients (5.4%), and a prolonged bleeding after cuts was declared from 18 patients (5.1%) (see Table III in the supplemental material for an overview of all reported perioperative, postsurgical or post interventional bleeding episodes that were reported). No significant correlations with age, degree of mobility, cardiac medication or preceding or present intake of blood-thinners were found.

Those participants that gave consent for follow-up were contacted for more detailed information. In short, a disposition to bruises was reported more often (79%) than development of nose-bleeding (50%) or gum-bleed (14%).

Bleeding-episodes after smaller wounds were of short duration (< 5 minutes) in most participants (78%) and need for medical intervention to stop bleeding had been necessary in only one patient. See Table IV in the supplemental material for a more detailed synopsis of follow-up data.

Analysis of genetic findings of participants showed a similar distribution of underlying genetic mutations compared to previous studies in larger cohorts of DMD

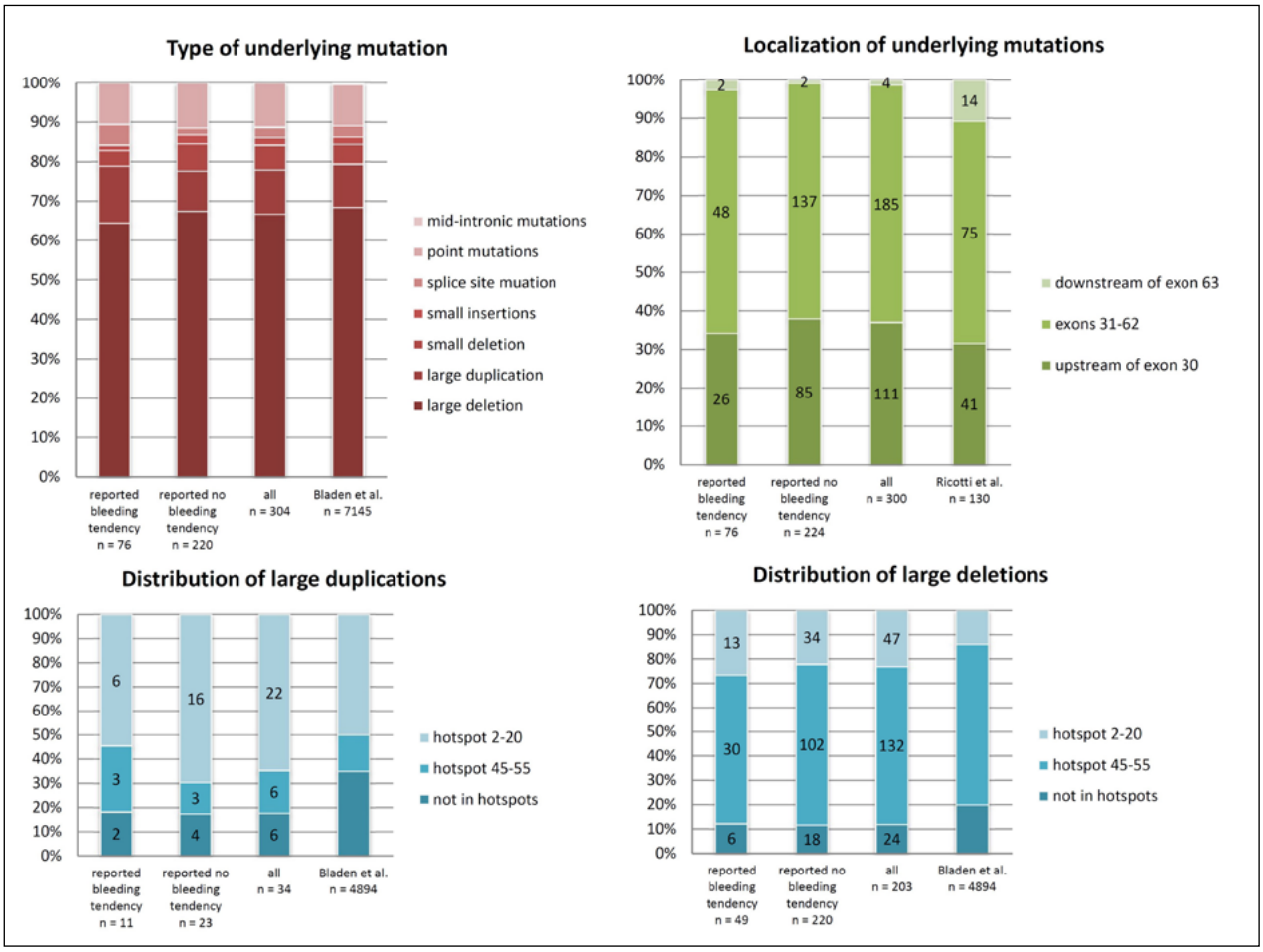

Figure 3. Genetic findings of participants.

patients [15] and did not differ between those patients reporting an increased bleeding tendency and those that did not (see Figure 3). Furthermore, there was no association with mutations affecting the expression of dp71 and a reported bleeding tendency.

Discussion

This study was initiated to assess clinically relevant thromboembolic events or increased bleeding symptoms in patients with DMD. The overall incidence for pediatric venous thromboembolism (VTE) has been estimated between 0.07 to 0.49 per 10,000 children [17-19] and approximately tenfold higher in hospitalized children [20], while the incidence in adulthood has been estimated to be about 5.6-16 per 10,000 adults per year [21]. While the overall incidence of VTE in the pediatric population is lower when neonates are excluded from epidemiologic analyses [22], there is consensus that other typical risk factors include presence of central catheters, intake of oral contraceptive pills, systemic bacterial infection and immobilization [19,23,24]. The role of cardiac disease as a risk factor for VTE is well established in adulthood, but has so far not been highlighted by retrospective analyses in children. Chronic immobility and cardiac insufficiency are well known risk-factors for thromboembolic vascular obstruction – both typical for advanced stages of DMD. Among the initial reports of thromboembolic events in the past were two cases of fat embolism following bone fracture and one case of a perinatal insult with subsequently diagnosed factor V mutation – all three not clearly attributable as primary VTEs without underlying medical condition or risk factor. Another patient was excluded as a real thromboembolism in the past was found to be unlikely as symptoms resolved quickly without intervention. Thus, despite reports of venous thromboembolism in DMD, findings of this registry-based survey do not support an increased risk for thromboembolic events that exceeds the usual risk of immobilized patients due to different medical conditions [19]. An increased risk for

cerebral infarction in DMD has been suggested repeatedly; most recently by authors of a retrospective analysis in which arterial ischemic strokes were identified in 4 out of 54 analyzed patients [9]. However, in this study, only one participant reported a history of left-ventricular thrombus and ischemic insult, so that we do not conclude an increased risk for cerebral infarction in DMD.

In contrast, the high number of patients reporting a bleeding tendency is striking. Bleeding complications can be caused by a primary or a secondary hemostasis defect. Typical symptoms of an impaired hemostasis include prolonged bleeding after injuries, mucocutaneous bleedings, such as epistaxis and gum bleed and hematoma. Secondary hemostasis comprises a complex cascade of different coagulation factors and can be activated either intrinsically or extrinsically. Secondary hemostasis has been investigated in DMD before and these analyses did not show any disease-specific abnormalities [25,26]. The primary hemostasis on the other side relies on platelet function and on the von Willebrand Factor (vWF). A prolonged bleeding time, considered to be a reliable indicator of dysfunctional cellular hemostasis, has been reported in DMD patients in different studies [4,25-27]. Analyses of platelet aggregometry and vWF-antigen, as well as flow cytometry of platelet receptors in patients with DMD gave very heterogeneous and inconclusive results [4,26,27]. As the smaller dystrophin isoform dp71 is also expressed in thrombocytes and has been shown to be important for changes in thrombocytic configuration and contractile properties [13,14], a possible disease-specific impairment of thrombocytic function thus appears possible for DMD. Dp71 is using an alternative promotor upstream of exon 63, but mutations in this part of the dystrophin gene are overall rare in DMD-patients. The analysis of the genetic findings of participants did not reveal differences between those reporting an increased bleeding tendency and those that did not regarding the type or localization of underlying mutations.

Apart from being prone for a possible selection bias, there are other limitations of this cross-sectional survey: The design of the survey did not allow discriminating between primary and secondary hemostasis. For example, the question "Do you develop hematoma, nosebleed or gum bleeding more easily than other people?" embraces both possible disorders of primary (gum bleed, epistaxis) and secondary (bruises) hemostasis. The overall response-rate of the follow-up questionnaires was too low to reliably classify the initial data retrospectively. Furthermore, the design of the questions did not allow for a separate indication of fat embolism, so that two cases of this well-known complication of bone fractures in DMD were initially reported as 'stroke' and correctly identified only in follow-up. Finally, reporting an increased bleeding tendency is certainly not the same as really suffering from it. The approach by questionnaire was chosen to capture the self-evaluation of DMD patients and to assess bleeding events in daily life that do not necessarily lead to medical attendance and thus may not be detectable by review of medical records. As in every self-reporting questionnaire based survey, this approach carries a risk of imprecise data.

Further research is needed to clarify whether a disease-specific dysfunction of coagulation is associated with the phenotypic spectrum in DMD. The results of this survey do not prompt a DMD-specific risk for thromboembolic events exceeding the risk of typical thrombophilia-associated conditions such as immobility or cardiac insufficiency. DMD patients are known to have higher blood losses during scoliosis surgery. Results of this survey suggest an additional bleeding tendency in daily life of DMD-patients that is not determined by type or localization of the underlying genetic mutations.

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Appendix

Table II. Questionnaire with indication of all questions in full-text and results according to degree of mobility.

	Question		Valid answers	Possible answers	All	Mobility	
					Ambulatory	Non- ambulatory	
	#1	**"How do you move in daily life?"**	n = 351 (100.0%)	*Ambulatory*	n = 110 (31.3%)	-	-
				Wheelchair, partially	n = 37 (10.5%)	-	-
				Wheelchair, predominantly	n = 17 (4.8%)	-	-
				Wheelchair, solely	n = 185 (57.6%)	-	-
				Bedridden	n = 2 (0.6%)	-	-
	#2	**"Do you develop hematoma, nosebleed or gum bleeding more easily than other people?"**	n = 346 (98.6%)	*No*	**n = 294** (83.8%)	n = 132 (37.6%)	n = 162 (46.2%)
				Yes	**n = 52** (14.8%)	n = 29 (8.3%)	n = 23 (6.5%)
	#3	**"Did you ever experience pronounced after-bleeding during or after pulling of teeth or surgery (e. g. spinal surgery, tonsillectomy, adenotomy)?"**	n = 346 (98.6%)	*I don't know*	**n = 45** (12.8%)	n = 25 (7.1%)	n = 20 (5.7%)
				No	**n = 282** (80.3%)	n = 133 (37.9%)	n = 149 (42.4%)
				Yes	**n = 19** (5.4%)	n = 3 (0.8%)	n = 16 (4.6%)
	#4	**"Do you feel that you do bleed longer than other people after smaller wounds (e. g. after cuts)?"**	n = 345 (98.3%)	*No*	**n = 327** (93.2%)	n = 154 (43.9%)	n = 173 (49.3%)
				Yes	**n = 18** (5.1%)	n = 7 (2,0%)	n = 11 (3.1%)
Bleeding tendency	**"Yes" in questions 2, 3 or 4?**				**n = 76 (21.6%)**	n = 34 (9.7%)	n = 42 (11.9%)

	#5	**"Have you ever been diagnosed with a thrombus in your heart in former examinations (heart ultrasound)?"**	n = 343 (97.7%)	*I don't know*	**n = 11** (3.1%)	n = 7 (2.0%)	n = 4 (1.1%)
				No	**n = 331** (94.3%)	n = 154 (43.9%)	n = 177 (50.4%)
				Yes	**n = 1** (0.3%)	n = 0 (0%)	n = 1 (0.3%)
	#6	**"Have you ever been diagnosed with a deep vein thrombosis or a stroke, or has such ever been suspected?"**	n = 343 (97.7%)	*I don't know*	**n = 4** (1.1%)	n = 2 (0.5%)	n = 2 (0.5%)
				No	**n = 333** (94.9%)	n = 159 (45.3%)	n = 174 (49.6%)
				Yes	**n = 6** (1.7%)	n = 0 (0%)	n = 6 (1.7%)
Thrombophilia	**"Yes" in questions 5 or 6?**				**n = 6 (1.7%)**	n = 0 (0%)	n = 6 (1.7%)
	#7	**"Have you taken blood-thinning medication in the past?"**	N = 343 (97.7%)	*I don't know*	**n = 337** (96.0%)	n = 161 (45.9%)	n = 176 (50.1%)
				No	**n = 6** (1.7%)	n = 0 (0%)	n = 6 (1.7%)
	#8	**"Which medication do you take frequently?"**	N = 337 (96.0%)	*None*	**n = 78** (22.2%)	n = 34 (9.7%)	n = 44 (12.5%)
				Following (selected free-text indications):	**n = 259** (73.8%)	n = 127 (36.2%)	n = 132 (37.6%)
				Steroids	**n = 147** (41.9%)	n = 111 (31.6%)	n = 36 (10.3%)
				Cardiac medication	**n = 102** (29.1%)	n = 15 (4.3%)	n = 87 (24.8%)
				Oral anticoagulation	**n = 3** (0.9%)	n = 1 (0.3%)	n = 2 (0.6%)
				Ataluren	**n = 17** (4.8%)	n = 12 (3.4%)	n = 5 (1.4%)
				Eteplirsen	**n = 1** (0.3%)	n = 1 (0.3%)	n = 0 (0%)
	#9	**"How old are you?"**	N = 337 (96.0%)	*< 6 ys*	**n = 44** (12.5%)	n = 44 (12.5%)	n = 0 (0%)
				6-10 ys	**n = 83** (23.6%)	n = 76 (21.6%)	n = 7 (2.0%)
				11-15 ys	**n = 72** (20.5%)	n = 31 (8.8%)	n = 41 (11.7%)
				16-20 ys	**n = 69** (19.6%)	n = 10 (2.8%)	n = 59 (16.8%)
				> 20 ys	**n = 69** (19.6%)	n = 0 (0%)	n = 69 (19.6%)

Table III. Synopsis of reported events of perioperative and postsurgical hemorrhage in the initial survey.

Patient ID	Type of surgery	Medical letter accessible?	Age at surgery	Comments
Orthopedic surgery (n = 9)				
423	Spondylodesis Th4-L5	Yes	15	Postsurgical transfusion of red blood cells
456	Spondylodesis Th3-L4	Yes	14	Anamnestic report of postsurgical hemorrhage. No respective findings in medical letter, apart from wound healing deficits
631	Spondylodesis Th5-L5	Yes	14	Postsurgical transfusion of red blood cells
659	Spondylodesis Th3 -Th5, sublaminar fusion Th6-Th12, pedicle screws L1-S1	Yes	14	Intraoperative and postsurgical transfusion of red blood cells
661	Spondylodesis Th3-S1	Yes	20	Postoperative hematothorax, transfusions of red blood cells and plasma, substitution of FXIII and antithrombin. Diagnosis of FXIII-deficiency was made postoperative.
437	Spondylodesis Th3-S1	Yes	15	Intraoperative transfusion of red blood cells
323	Spondylodesis	No	NA	NA
404	Spondylodesis	No	NA	NA
599	Spondylodesis	No	NA	NA
ENT surgery (n = 4)				
192	Tonsillectomy adenotomy, paracentesis	Yes	4	Increased bleeding with need for surgical control on postoperative day #2, transfusion of red blood cells
377	Tonsillectomy	Yes	5/6	Increased bleeding with need for surgical control on postoperative day #11
290	Tonsillectomy	No	NA	Increased bleeding with need for surgical control on postoperative day #3
326	Tonsillectomy	No	NA	NA
Dental surgery (n = 3)				
223	Molar tooth extraction	No	4	No need for medical intervention
417	Dental surgery (2 episodes)	No	3 and 14	No need for medical intervention
433	Molar tooth extraction	No	17	Anamnestic report of wound healing deficiency and need for antibiotic therapy
Other types of surgery (n = 2)				
549	Frenuloplasty, phimosis surgery	Yes	18	Anamnestic report of post-surgical bleeding, no respective findings in medical letter

NA = information not available

Table IV. Synopsis of follow-up data for patients giving account of an increased bleeding tendency in the initial survey.

	Question	Positive answer in initial survey	Consent for follow-up	Follow-up (response-rate)	Method of follow-up	Questions in follow-up:	(Possible) answers	
Bleeding tendency	**Frequent hematoma, nosebleed or gum bleeding?**	n = 52	n = 42	n = 24 (57.1%)	*Questionnaire*	"Do you develop bruises more easily than others?"	Yes	N = 19 (79%)
							No	N = 5 (21%)
						"Do you develop nose-bleed more easily than others?"	Yes	N = 12 (50%)
							No	N = 12 (50%)
						"Do you develop gum-bleed more easily than others?"	Yes	N = 3 (14%)
							No	N = 18 (86%)
	Pronounced after-bleeding after pulling of teeth or surgery?	n = 17	n = 14	n = 14 (100.0%)	*Phone call/ medical letters*	Dental surgery	Molar extraction	N = 5 (35%)
							Dental procedure	N = 4 (26%)
						Spinal surgery	Spondylodesis	N = 6 (43%)
						ENT surgery	Tonsillectomy/ adenotomy	N = 5 (36%)
						Others	Phimosis surgery	N = 1 (7%)
	Prolonged bleeding?	n = 18	n = 14	n = 9 (64.3%)	*Questionnaire*	"How many episodes of prolonged bleeding after cuts do you have per year?"	< 1/year	N = 4 (44.4%)
							1-5/year	N = 4 (44.4%)
							6-12/year	N = 0 (0%)
							> 12/year	N = 0 (0%)
						"How long lasts an episode in average?"	< 5 minutes	N = 7 (77.8%)
							> 5 minutes	N = 2 (22.2%)
						"Has ever been need for medical measures to stop bleeding?"	Yes	N = 8 (88.9%)
							no	N = 1 (11.1%)

Note that more cases of bleeding complications after dental surgery were reported in follow-up than in the initial survey.