Study regarding pathogenesis of the symptom gingival bleeding in patients suffering from von Willebrand Disease type 2 and 3

1. Sponsor and investigators of the study:

1.1 Sponsor

Prof. Dr. med. dent. Peter Eickholz
Poliklinik für Parodontologie, Zentrum der Zahn-, Mund- und Kieferheilkunde,
Johann Wolfgang Goethe-Universität Frankfurt
Theodor-Stern-Kai 7 (Haus 29)
60596 Frankfurt am Main
Tel.: 069-6301-5642
Email: eickholz@med.uni-frankfurt.de

1.2 Vice Sponsor

Priv.-Doz. Dr. med. Wolfgang Miesbach
Hämophiliezentrum
Med. Klinik III / Institut für Transfusionsmedizin
Klinikum der Johann Wolfgang Goethe-Universität Frankfurt
Theodor-Stern-Kai 7
60590 Frankfurt am Main
Tel.: 069/6301-7788
Fax.: 069/6301-6738
Email: wolfgang.miesbach@kgu.de

1.3 Investigators:

Dr. Lisa Weickert, Dr. Katrin Nickles, MSc., Dr. Susanne Scharf, MSc.
Poliklinik für Parodontologie, Zentrum der Zahn-, Mund- und Kieferheilkunde,
Johann Wolfgang Goethe-Universität Frankfurt
Theodor-Stern-Kai 7
60596 Frankfurt am Main

Dr. Stefanie Krekeler
Hämophiliezentrum
Med. Klinik III / Institut für Transfusionsmedizin
Klinikum der Johann Wolfgang Goethe-Universität Frankfurt
Theodor-Stern-Kai 7
60590 Frankfurt am Main

1.4 Laboratory

Gerinnungslabor der Med. Klinik III / Institut für Transfusionsmedizin
Klinikum der Johann Wolfgang Goethe-Universität Frankfurt
Theodor-Stern-Kai 7
60590 Frankfurt am Main
Tel.: +49-69-6301-6326

2. Background

2.1 Introduction and state of research

Von Willebrand Disease (VWD) is the most common inherent bleeding disorder [1]. The disease is caused by deficiency or dysfunction of von Willebrand factor (VWF), a plasma protein that mediates platelet hemostatic function and stabilizes blood coagulation factor VIII. VWF is also the carrier of factor VIII in plasma. Thus, its deficiency may also result in low levels of factor VIII.

Estimates for prevalence of VWD range between 0.6 and 1.3% [2]. Inherent VWD is classified into three main types (1, 2 and 3) with type 2 divided into four subtypes (A, B, M, N) [3]. The most prevalent and mildest form is VWD type 1 (about 75%) representing a partial quantitative deficiency of VWF [1]. VWD type 2 (qualitative defects of VWF) accounts for around 20 to 25% of cases [1]. Subtype 2A exhibits impaired VWF multimer assembly (group 1 mutations) or increased proteolysis of VWF in circulation (group 2 mutations). Subtype 2B shows increased, type 2M decreased affinity of VWF for platelet GPIb receptor. Subtype 2N is characterized by decreased factor VIII because of VWF deficiently binding to factor VIII [1]. VWD type 3 (0.6 to 6% of cases) represents complete quantitative deficiency of VWF [1, 3, 4]. These three major types of VWD may affect both males and females. Type 1 and type 2A and 2B are inherited in an autosomal dominant manner while type 2M, 2N and 3 are inherited in an autosomal recessive manner [3].

People with VWD bruise easily, have recurrent mucocutaneous bleeding, or bleed after tooth extraction, tonsillectomy or other surgery [5]. A common symptom of VWD is epistaxis (nose bleeding). Furthermore, women can have increased menstrual bleeding. Also a frequently reported symptom of VWD is gingival bleeding [6-8].

Gingival bleeding is also a leading symptom of plaque-induced gingivitis and untreated periodontal disease [4, 9, 10]. Interestingly case reports on VWD reporting spontaneous gingival bleeding as symptom did not look into periodontal health in detail. Abbas & Prabhu report “fair oral hygiene” and the gingival tissues to “look otherwise healthy” [6]. In another case continual bleeding 12 hours after scaling and polishing of the lower teeth is reported [8]. However, plaque indices or periodontal

variables as probing pocket depths (PPD) and vertical probing attachment level (PAL-V) [6, 8].

2.2 Previous work

A case control study comparing type 1 VWD with controls matched for sex, age, number of teeth, severity of periodontal disease, and smoking failed to detect increased gingival bleeding in VWD [4].

3. Aim

3.1 Hypothesis

The sypmtome gingival bleeding in patients suffering from VWD type 2 and 3 is not primarily due to VWD but due to inflammatory periodontal diseases (gingivitis, periodontitis).

3.2 Aim of the study,

assessment whether in patients suffering from VWD type 2 and 3 the regularly occuring symptome gingival bleeding is caused by the impaired coagulation due to VWD or due to inflammatory periodontal diseases (gingivitis, periodontitis).

If patients are diagnosed with gingivitis or periodontitis patients who want so may have periodontal treatment at the Dept. of Periodontology.

4. Outcomes

Primary outcome (bleeding symptome):

- BOP (Bleeding on probing)

Secondary outcome:

- GBI (Gingival Bleeding Index) (Ainamo & Bay 1975)

Control variables:

- PCR (Plaque Control Record) (O'Leary 1972)
- Probing pocket depths (PPD)

5. Material and Methods

5.1 Test (VWD patients)

In the present prospective case control study patients suffering from type 2 and 3 VWD (VWF Ristocetin cofactor [VWF:RCo] < 30%) were compared to hematologically healthy controls matched for sex, age, number of teeth, severity of periodontal disease, and smoking.

This study applies the protocol of a case control study comparing type 1 VWD with controls matched for sex, age, number of teeth, severity of periodontal disease, and smoking to type 2 and 3 VWD patients [4]. All patients with type 2 and 3 VWD consecutively consulting the Haemophilia Centre, Medical Clinic II/Institute for Transfusion medicine, Hospital of the Johann Wolfgang Goethe-University Frankfurt/Main were asked to participate in this study as cases. They were asked for bleeding and subjective symptoms indicating periodontal disease. This study is a human observational study and conforms to the STROBE guidelines.

The study complied with the rules of the Declaration of Helsinki and was approved by the Institutional Review Board for Human Studies of the Medical Faculty of the Goethe-University Frankfurt/Main (Application# 143/15). All participating individuals were informed on risks and benefits as well as the procedures of the study and gave written informed consent. The study is registered under the number NCT03078595 at http://www.clinicaltrials.gov.

Inclusion criteria:
- Age between 18 and 80 years,
- Written informed consent,
- Formerly diagnosed type 2 and 3 VWD (according to VWF multimer analysis and VWF:RCo < 30 %)

Exclusion criteria:
- Requirement of systemic antibiotics for measures that may cause transitory bacteremia (e.g. pocket probing),
- VWD type 1 (VWF:RCo > 30 %),
- Additional bleeding disorders (e.g. hemophilia A or B),
- Anticoagulation or antiplatelet treatment (e.g. acetylsalicylic acid, warfarin)

5.2 Controls

For each case (VWD) a respective hematologically healthy control was recruited from the gingivitis and periodontitis patients of the Department of Periodontology, Center for Dentistry and Oral Medicine (Carolinum), Johann Wolfgang Goethe-University Frankfurt/Main. Each control was matched to one of the respective cases for sex, age (±5 years), self-reported smoking status (current smoker/non-smoker), number of

remaining teeth (±2 teeth), and periodontal diagnosis (gingivitis, chronic or aggressive periodontitis).

Smoking may interfere with gingival bleeding [11, 12]. Thus, all participants were asked about current and past cigarette smoking habits. Patients who reported smoking or had quit smoking for less than five years were classified as smokers [13]. Additionally the amount of carbon monoxide (CO) in exhaled air was measured using a Smokerlyzer® (Bedfont Smokerlyzer EC50-Micro; Bedfont Scientific Ltd, Rochester, Great Britain).

5.3 Fallzahlschätzung

A sample size of n = 62 (31 VWD cases and 31 controls) was required to detect an inter-group difference of 5.5% [4] GBI or BOP with a type 1 error $\alpha < 0.05$ and a test power of 80% (http://jumbo.uni-muenster.de/fileadmin/jumbo/applets/falla.html). After inclusion of 24 type 2 and 3 VWD cases no further type 2 and 3 VWD cases could be recruited at the Haemophilia Centre, Medical Clinic III/Institute for Transfusion medicine, Hospital of the Johann Wolfgang Goethe-University Frankfurt/Main. Thus, the study was analyses with 24 cases and 24 matched controls.

5.4 Study type

Prospective clinical case control study

5.5 Hematologic examinations

Twenty ml of blood was sampled from an arm vein. The following data were assessed at the Haemophilia Centre for clinical routine during VWD patient care and due to study design as well as to determine whether haemotological disease was present or not in the controls:

- von Willebrand parameters (VWF antigen [VWF:Ag], VWF activity [VWF:Act], coagulation factor VIII [FVIII:C])
- Current medication if any

Also diabetes mellitus and glycemic control may interfere with systemic as well as periodontal inflammation and, hence, gingival bleeding [14, 15]. Even so called prediabetes may contribute to inflammation. However, the state of prediabetes may not be known by the affected individual and not detected by medical history [15]. Thus, HbA1c as a measure for glycemic control was assessed also.
VWF:Ag and VWF:Act were measured turbidimetrically using a BCS (Siemens, Marburg, Germany). FVIII:C was assessed with specific agents on a coagulation analyzer (ACL-700®, IL Instrumentation Laboratory, Kirchheim, Germany).

5.6 Periodontal examinations

For all participants, a commercially available test (PerioSafe®, Dentagnostics, Jena, Germany) was performed to detect activated matrix metalloproteinase 8 (aMMP-8) from the gingival sulcus. First, patients rinsed with tap water for 30 seconds then spat the water out. After 1 minute, the patients rinsed with 5mL of purified water for 30 seconds and spat the sample back into the test cup. A syringe was used to gather 2mL of the sampled saliva and water mixture. A filter was then put onto the syringe and 3 drops of the saliva was pressed through the filter into the ELISA kit. After 5-10 minutes, the result was read from the test kit [16]. If both the control and test strips were visible (≥ 25 ng aMMP 8 per mL), the respective test was positive.

The following clinical parameters were assessed at 6 sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual) [4]:

- modified Gingival Bleeding Index (GBI) [17]
- modified Plaque Control Record (PCR) [18]
- PPD and recession to the nearest 0.2 mm using an electronic probe (Florida Probe, Version 3.2, Gainesville, FL, USA). Recession was assessed from the cemento-enamel junction (CEJ) to the gingival margin. At sites where the CEJ was destroyed by restorations the restoration margin (RM) was used as reference. At sites where the CEJ or RM was located apically from the gingival margin the value for recession was negative
- Bleeding on probing (BOP) recorded as positive when bleeding occurred within 30 seconds from probing. For each patient a BOP index was calculated providing the amount of sites with positive BOP in % per patient.
- Attachment loss was calculated as sum of PPD and recession. At sites where the gingival margin was located coronally of the CEJ recession was scored as a negative value.

All individuals were classified into the following diagnoses [14, 19]:

- plaque-induced gingivitis (PPD < 3.6 mm; PAL-V ≤ 2 mm),
- generalized mild, localized moderate chronic periodontitis (PPD ≥ 3.6 mm; PAL-V 3 to 4 mm ≤ 30% of sites; 1 to 2 mm > 30% of sites),
- generalized mild, localized severe chronic periodontitis (PPD ≥ 3.6 mm; PAL-V ≥ 5 mm ≤ 30% of sites; 1 to 2 mm > 30% of sites)
- generalized moderate chronic periodontitis (PPD ≥ 3.6 mm; PAL-V 3 to 4 mm > 30%)
- generalized moderate localized severe chronic periodontitis (PPD ≥ 3.6 mm; PAL-V 3 to 4 mm > 30%; ≥ 5 mm ≤ 30%)

In all individuals hematological and periodontal examinations were obtained within 24 hours. After dental and periodontal examination all patients received oral hygiene instructions and professional tooth cleaning. In cases of untreated periodontal disease periodontal treatment was offered. Increased bleeding may occur at home after patients had already left the clinic. Thus, only subjects with VWD were asked to

report any bleeding complications after periodontal probing and professional tooth cleaning.

5.7 Statistical analysis

The individual patient was used as statistical unit. All analyses were performed on patient level. BOP was defined as the main outcome variable and GBI as secondary outcome variable. All other parameters were control variables.

For all individuals, cigarette pack years were calculated. Group frequencies (VWD, control) were expressed for sex, current smoking. Group means and standard deviations were calculated for GBI, BOP, age, number of remaining teeth, pack years, CO, PCR, Body weight, VWF:Ag, VWF:RCO, FVIII:C, Hb1c. Further, for each individual the following variables were calculated to describe the periodontal status:

- Mean±standard deviation of PPD and PAL-V
- Percentage of PPD < 4 mm, 4 to 6.8 mm, ≥ 7 mm
- Sum of all PPD (Wohlfeil et al. 2009), i.e. the sum the PPD measured at all sites within a patient
- Sum of all PPD with BOP (Wohlfeil et al. 2009), i.e. the sum the PPD measured at all sites exhibiting BOP within a patient
- Periodontal inflamed surface area (PISA) [20, 21]. For each patient PPD were entered into an Excel sheet that can be downloaded freely (http://www.parsprototo.info/pisa.html).

From these group means and standard deviations were calculated. Comparisons between groups for dichotomous parameters were made by χ^2 or Fisher's exact test and for all other parameters by Mann-Whitney-U test. A post-hoc analysis was performed to estimate the test power that would be required to find a clinically relevant inter-group difference (δ) of 5.5% for BOP and GBI index with a type 1 error (α) of 0.05 for the actual sample size.

Using stepwise linear backward multiple regression analysis, factors should be identified that influenced BOP and GBI. The following independent variables were entered into the model for BOP: group (VWD/control), VWD type 2 or 3, sex, age, body weight, HbA1c, PCR, CO, aMMP-8, number of remaining teeth, PISA. The following independent variables were entered into the model for GBI: group (VWD/control), VWD type 2 or 3, sex, age, body weight, HbA1c, PCR, CO, aMMP-8, number of remaining teeth, PISA. Due to the fact that mean PPD is mathematically coupled to sum of PPD, sum of PPD with BOP, and PISA these 4 variables were not entered into the regression model at the same time. PISA provides the best representation of the subgingival inflamed area. Thus, PISA was chosen for the final model. The following parameters were described by dummy variables: group (control = 0, VWD = 1), sex (male = 0, female = 1), smoking status (never and former smoker = 0, current smoker = 1). All factors with $p < 0.05$ were kept in the models. For statistical analysis a PC program was used (Systat™ for Windows Version 13, Systat Inc., Evanston, USA).

6. Time line

Laufzeit:	June 2015 to December 2016
Clinical and laboratory examination:	June 2015 to December 2015
Analysis and publication:	January 2016 to December 2016

7. References

1. Nichols WL, Hultin MB, James AH, Manco-Johnson MJ, Montgomery RR, Ortel TL, et al. von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA). Haemophilia. 2008;14(2):171-232. doi: 10.1111/j.1365-2516.2007.01643.x. PubMed PMID: 18315614.
2. Federici AB, Rand JH, Bucciarelli P, Budde U, van Genderen PJ, Mohri H, et al. Acquired von Willebrand syndrome: data from an international registry. Thromb Haemost. 2000;84(2):345-9. PubMed PMID: 10959711.
3. Wilde JT. Von Willebrand disease. Clin Med (Lond). 2007;7(6):629-32. PubMed PMID: 18193717.
4. Weickert L, Miesbach W, Alesci SR, Eickholz P, Nickles K. Is gingival bleeding a symptom of patients with type 1 von Willebrand disease? A case-control study. J Clin Periodontol. 2014;41(8):766-71. doi: 10.1111/jcpe.12274. PubMed PMID: 24814158.
5. Israels S, Schwetz N, Boyar R, McNicol A. Bleeding disorders: characterization, dental considerations and management. J Can Dent Assoc. 2006;72(9):827. PubMed PMID: 17109803.
6. Abbas KE, Prabhu SR. Gingival bleeding as a presenting symptom in Von-Willebrands' disease--review of literature and report of cases. J Oral Med. 1980;35(4):87-90. PubMed PMID: 6970798.
7. Sandoval C, Dong S, Visintainer P, Ozkaynak MF, Jayabose S. Clinical and laboratory features of 178 children with recurrent epistaxis. J Pediatr Hematol Oncol. 2002;24(1):47-9. PubMed PMID: 11902740.
8. Zakrzewska J. Gingival bleeding as a manifestation of von Willebrand's disease. A review of the literature and management. Br Dent J. 1983;155(5):157-60. doi: 10.1038/sj.bdj.4805170. PubMed PMID: 6610434.
9. Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. Acta Odontol Scand. 1963;21:533-51. PubMed PMID: 14121956.
10. Theilade E, Wright WH, Jensen SB, Loe H. Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. J Periodontal Res. 1966;1:1-13. PubMed PMID: 4224181.
11. Scott DA, Singer DL. Suppression of overt gingival inflammation in tobacco smokers - clinical and mechanistic considerations. Int J Dent Hyg. 2004;2(3):104-10. doi: 10.1111/j.1601-5037.2004.00079.x. PubMed PMID: 16451473.
12. Farina R, Tomasi C, Trombelli L. The bleeding site: a multi-level analysis of associated factors. J Clin Periodontol. 2013;40(8):735-42. doi: 10.1111/jcpe.12118. PubMed PMID: 23713685.

13. Lang NP, Tonetti MS. Periodontal risk assessment (PRA) for patients in supportive periodontal therapy (SPT). Oral Health Prev Dent. 2003;1(1):7-16. PubMed PMID: 15643744.
14. Mariotti A. Dental plaque-induced gingival diseases. Ann Periodontol. 1999;4(1):7-19. doi: 10.1902/annals.1999.4.1.7. PubMed PMID: 10863371.
15. Abduljabbar T, Al-Sahaly F, Al-Kathami M, Afzal S, Vohra F. Comparison of periodontal and peri-implant inflammatory parameters among patients with prediabetes, type 2 diabetes mellitus and non-diabetic controls. Acta Odontol Scand. 2017;75(5):319-24. doi: 10.1080/00016357.2017.1303848. PubMed PMID: 28325134.
16. Izadi Borujeni S, Mayer M, Eickholz P. Activated matrix metalloproteinase-8 in saliva as diagnostic test for periodontal disease? A case-control study. Med Microbiol Immunol. 2015;204(6):665-72. doi: 10.1007/s00430-015-0413-2. PubMed PMID: 25841875.
17. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J. 1975;25(4):229-35. PubMed PMID: 1058834.
18. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol. 1972;43(1):38. doi: 10.1902/jop.1972.43.1.38. PubMed PMID: 4500182.
19. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999;4(1):1-6. doi: 10.1902/annals.1999.4.1.1. PubMed PMID: 10863370.