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References

1 Warkentin TE, Greinacher A, Koster A, Lincoff AM. American College of Chest Physicians. Treatment and prevention of heparin-induced thrombocytopenia: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008 ; **133** (6 Suppl.): 340S–80S.
2 Greinacher A, Warkentin TE. Recognition, treatment, and prevention of heparin-induced thrombocytopenia: review and update. *Thromb Res* 2006; **118**: 165–76.
3 Francis JL. A critical evaluation of assays for detecting antibodies to the heparin-PF4 complex. *Semin Thromb Hemost* 2004; **30**: 359–68.
4 de Larrañaga G, Martinuzzo M, Bocassi A, Fressart MM, Forastiero R. Heparin-platelet factor 4 induced antibodies in patients with either autoimmune or alloimmune antiphospholipid antibodies. *Thromb Haemost* 2002; **88**: 371–3.
5 Martin-Toutain I, Piette JC, Diemert MC, Faucher C, Jobic L, Ankri A. High prevalence of antibodies to platelet factor 4 heparin in patients with antiphospholipid antibodies in absence of heparin-induced thrombocytopenia. *Lupus* 2007; **16**: 79–83.
6 Jang IK, Marcie JH. When heparin promote thrombosis: review of heparin-induced thrombocytopenia. *Circulation* 2005; **111**: 2671–83.
7 Price EA, Hayward CPM, Moffat KA, More JC, Waerkentin TE. Laboratory testing for heparin-induced thrombocytopenia is inconsistent in North America: a survey of North American specialized coagulation laboratories. *Thromb Haemost* 2007; **98**: 1357–61.
8 Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part I: platelets. *Int J Lab Hematol* 2007; **29**: 4–20.
9 Bartels PC, Schoorl M, Lombarts AJ. Screening for EDTA-dependent deviations in platelet counts and abnormalities in platelet distribution histograms in pseudothrombocytopenia. *Scand J Clin Lab Invest* 1997; **57**: 629–36.
10 Amiral J, Bridey F, Dreyfus M, Vissoc AM, Fressinaud E, Wolf M, Meyer D. Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. *Thromb Haemost* 1992; **68**: 95–6.
11 Chong BH, Burgess J, Ismail F. The clinical usefulness of the platelet aggregation test for the diagnosis of heparin-induced thrombocytopenia. *Thromb Haemost* 1993; **69**: 344–50.
12 Schwarzinger I, Speiser W, Lubenow N, Greinacher A, Panzer S. Heparin-platelet factor (PF) 4 antibodies in patients with pseudothrombocytopenia: coincidence or association? *Thromb Haemost* 2000; **84**: 1123–4.

Evaluation of the diagnostic utility for von Willebrand disease of a pediatric bleeding questionnaire

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The accurate assessment of hemorrhagic symptoms, which is a critical component in the diagnosis of mild bleeding disorders such as von Willebrand disease (VWD), can be particularly difficult in children. Although normal children frequently report bruising and nosebleeds, a child with VWD may not have had the opportunity to manifest hemorrhagic symptoms such as postoperative bleeding or menorrhagia. To the best of our knowledge, a pediatric-specific bleeding score has never been published, and we felt that there was value in validating a quantitative bleeding score for children that could be used as a screening tool and that was related to an adult scoring system.

The MCMDM-1VWD Bleeding Questionnaire [1] (which is based on the bleeding questionnaire published by Rodeghiero *et al*. [2]) was modified by including pediatric-specific bleeding symptoms in the 'Other' category; these symptoms are umbilical stump bleeding, cephalohematoma, post-circumcision bleeding, post-venipuncture bleeding, and macroscopic hematuria. The scoring system was kept consistent with the original, and is summative. The resulting Pediatric Bleeding Questionnaire (PBQ) and scoring key can be found as SupportingInformation. The range of bleeding scores in normal children was determined, and the questionnaire was prospectively tested on children being investigated for VWD.

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Table 1 Characteristics of 151 prospectively investigated children

	VWD (n = 6)	Positive bleeding score, no VWD (n = 31)	Negative bleeding score, no VWD (n = 114)	P-value
Females, no. (%)	4 (67)	17 (55)	52 (46)	0.439
Mean age, years (range)	9.5 (1–15)	8.9 (18 months to 17 years)	8.0 (6 months to 17 years)	0.585
Age groups (years)				
0–3	1	5	24	
4–6	1	7	29	
7–9	0	7	20	
10–12	1	2	13	
13–15	3	7	14	
16–18	0	3	14	
Blood group O, no. (%)	4 (67)	11 (44)	54 (50)	0.077
Mean VWF:Ag, U/mL (range)	0.45 (0.32–0.57)	1.04 (0.50–1.84)	0.94 (0.41–3.97)	0.009
Mean VWF:RCo, U/mL (range)	0.26 (0.12–0.40)	0.77 (0.47–1.34)	0.71 (0.32–2.72)	0.005
Mean FVIII:C, U/mL (range)	0.77 (0.51–1.15)	1.07 (0.19–1.91)	1.08 (0.50–2.54)	0.188
Median bleeding score (range)	4 (1–11)	4 (2–14)	0 (− 2 to 1)	< 0.001

The P-values were obtained using chi-squared tests for categorical data and one-way analysis of variance (ANOVA) for linear data. The non-parametric Kruskal-Wallis test was used to compare bleeding scores as they were not normally distributed.
Tukey's *post hoc* testing, antigen: von Willebrand disease (VWD) vs. non-VWD bleeders, $P = 0.006$; VWD vs. negative bleeding score, $P = 0.019$; non-VWD bleeders vs. negative bleeding score, $P = 0.461$. Ricof: VWD vs. non-VWD bleeders, $P = 0.003$; VWD vs. negative bleeding score, $P = 0.006$; non-VWD bleeders vs. negative bleeding score, $P = 0.709$. Mann–Whitney U-testing, *post hoc*, bleeding score: VWD vs. non-VWD bleeders, $P = 0.794$; VWD vs. negative bleeding score, $P \leq 0.001$; non-VWD bleeders vs. negative bleeding score, $P \leq 0.001$.
In the VWD column, the upper end of the range of von Willebrand factor antigen (VWF:Ag) is 0.57 U/mL. This is from a 15-year-old female (bleeding score = 11) who also had a VWF ristocetin cofactor (VWF:RCo) = 0.12 U/mL and abnormal multimers (type 2A VWD). There is another type 2 VWD individual included in this column; an 18-month-old female (bleeding score = 3) with VWF:Ag = 0.50 U/mL, VWF:RCo = 0.21, and genetic testing results that confirm type 2B VWD (R1306W). In the positive bleeding score, no VWD column, there was one individual with VWF:RCo = 0.47 U/mL, but the VWF:Ag and multimers were normal in this case (did not meet the criteria for VWD). In the same column, the lower end of the FVIII range is 0.19 U/mL. This is from a 9-year-old male (bleeding score = 4) who was diagnosed with mild hemophilia. In the negative bleeding score column, there are VWF:Ag and VWF:RCo levels < 0.50, but in each of these cases only one was low (not both).

A total of 142 multiethnic children (80 females, 62 males) with a mean age of 9 years (range 6 months to 18 years) were recruited for the determination of a normal pediatric bleeding score from parent–teacher association meetings in Oakland, California. Blood work was not performed on this group. Informed consent was obtained for all participants, and the study was approved by the Institutional Review Board of the Children's Hospital of Oakland. In this group, the mean bleeding score was 0.5. Given that the data were normally distributed, we determined the normal range to be − 1.5 to 2.5 (mean ± 2 standard deviations); to increase sensitivity (and given that scores in increments of 0.5 are not possible), we determined a positive (abnormal) bleeding score to be ≥ 2. Of the 142 children, 19 (13%) had bleeding scores ≥ 2; the symptoms in these children included bleeding from minor wounds, epistaxis, easy bruising, and menorrhagia (females > 12 years old). Bleeding scores were not affected by age, gender, or ethnicity.

For the prospective validation, 151 children were recruited from the waiting room of the Children's Outpatient Centre (COPC), a busy primary care pediatric clinic at the Hotel Dieu Hospital in Kingston, Ontario, and were investigated for VWD because of a personal history of hemorrhagic symptoms and/or a family history of VWD and/or for preoperative screening. Informed consent was obtained for all subjects, and the study was approved by the Research Ethics Board of Queen's University. The PBQ was administered, blood samples were collected, and von Willebrand factor (VWF) laboratory testing was performed on all subjects, as previously described [3].

Of the 151 children, 36 (24%) had positive bleeding scores (≥ 2), leaving 115 with negative bleeding scores. Of the 36 with positive bleeding scores, five met the laboratory criteria for VWD (Table 1). One of the 115 children with negative bleeding scores also met these criteria. The laboratory criteria used for this study are VWF antigen (VWF:Ag) and VWF ristocetin cofactor (VWF:RCo) between 0.05 and 0.50 U/mL on at least two occasions, a VWF:RCo/VWF:Ag ratio > 0.50, and a normal VWF multimer pattern for type 1 VWD. For type 2A VWD, the diagnostic definition is VWF:Ag and/or VWF:RCo between 0.05 and 0.50 U/mL on at least two occasions, a VWF:RCo/VWF:Ag ratio < 0.50, and an abnormal VWF multimer pattern. Type 2B VWD is defined similarly to type 2A VWD, with the additional requirement for positive genetic testing results.

Additional, standardized hemostasis investigations on the 31 children with positive bleeding scores but no VWD were not performed in the context of this study. Certainly, other bleeding disorders are possible in this group, including platelet function disorders or mild coagulation factor deficiencies (such as in the boy diagnosed with mild hemophilia A, as shown in Table 1). Prospective investigation of this cohort is ongoing.

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Given the difficulty in obtaining blood samples from children for research purposes only, samples for study blood work on the children with negative bleeding scores had to be collected at the time of phlebotomy ordered by their regular physician. In some instances, this meant that extra tubes were taken during routine clinic phlebotomies; however, in some cases, we obtained blood samples from children recruited through the COPC who were having minor, elective surgery during the course of the study. In these cases, blood samples were obtained in the operating room immediately following the induction of anesthesia, but before the start of the surgery. Of the 115 children with negative bleeding scores, samples for blood work were collected for 59 during clinic phlebotomies and for 56 in the operating room. A subgroup of 10 children had baseline VWF testing performed at the time of their preoperative clinic assessments, as well as an intraoperative sample to allow for comparison. Analysis of the data from the subset of 10 children who had preoperative and intraoperative VWF testing performed showed no statistically significant difference in VWF or factor VIII levels, suggesting that the laboratory values in the entire control group are reflective of outpatient phlebotomy results.

The sensitivity, specificity, positive predictive value and negative predictive value of the PBQ are 83%, 79%, 0.14, and 0.99, respectively. Although the 95% confidence intervals (CIs) for specificity are reasonable (72–86%), they are quite wide for sensitivity (42–124%), because of the small number of true positives. The likelihood ratio of a positive bleeding score (≥ 2) for VWD is 3.9 (95% CI 2.4–6.3). A receiver operator curve (ROC) analysis produced an area under the curve of 0.88 ($P < 0.002$ against the null hypothesis that the true area is 0.5), showing that the questionnaire can accurately distinguish between affected and unaffected children. None of the children being prospectively investigated reported any of the pediatric-specific bleeding symptoms, so none of the above-mentioned parameters changed if the 'Other' category was eliminated. This category did not add value in this study, perhaps because the affected cohort is at the mild end of the spectrum, and these questions could therefore be eliminated if the questionnaire is being used for screening in a primary care setting. It is possible, however, that the pediatric-specific symptoms may be of greater value in a more severely affected population, such as in a tertiary care setting [4].

Given that there is no consensus laboratory definition of type 1 VWD, we have used a definition from our center that is commonly used in both the clinical and research settings. We acknowledge that different definitions are used at other centers, and have therefore also analyzed our data using decreasing VWF:RCo level cut-offs. The sensitivity and specificity for VWF:RCo < 0.40 IU/mL are 80% and 100%, respectively. The sensitivity for VWF:RCo < 0.30 IU/mL is 75% (this is lower than the higher cut-off values because of the small sample size; specificity cannot be calculated below 0.40) and that for VWF:RCo < 0.20 IU/mL is 100%.

We also evaluated each individual item from the PBQ to determine which were useful in distinguishing affected from unaffected children. The items significantly associated with VWD were as follows: epistaxis – longer duration, lack of seasonal correlation, and requiring medical intervention to be stopped; bruising brought to medical attention; increased number and duration of episodes of bleeding from minor wounds; oral cavity bleeding requiring medical attention; increased number of episodes of gastrointestinal bleeding; and bleeding following dental extraction (P-values all < 0.05, chi-squared).

In conclusion, the high negative predictive value and strong ROC data support the inclusion of the PBQ in the screening of a child for VWD. The common scoring system between this questionnaire and validated adult questionnaires is a significant strength, and lays the groundwork for a common method of communication in this field in the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Pediatric Bleeding Questionnaire.
Table S1. The scoring key for the Pediatric Bleeding Questionnaire.

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References

1 Tosetto A, Rodeghiero F, Castaman G, Goodeve A, Federici AB, Batlle J, Meyer D, Fressinaud E, Mazurier C, Goudemand J, Eikenboom J, Schneppenheim R, Budde U, Ingerslev J, Vorlova Z, Habart D, Holmberg L, Lethagen S, Pasi J, Hill F, *et al*. A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). *J Thromb Haemost* 2006; **4**: 766–73.

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2 Rodeghiero F, Castaman G, Tosetto A, Batlle J, Baudo F, Cappelletti A, Casana P, De Bosch N, Eikenboom JC, Federici AB, Lethagen S, Linari S, Srivastava A. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. *J Thromb Haemost* 2005; **3**: 2619–26.
3 Bowman M, Mundell G, Grabell J, Hopman WM, Rapson D, Lillicrap D, James P. Generation and validation of the Condensed MCMDM-1VWD Bleeding Questionnaire for von Willebrand disease. *J Thromb Haemost* 2008; **6**: 2062–6.
4 Biss TT, Blanchette VS, Bowman M, Clark DS, Silva M, Lillicrap D, James P, Rand ML. Quantitation of bleeding symptoms in children with von Willebrand disease or a platelet function disorder: use of a standardized pediatric bleeding score. *Blood* 2007; **110**: 636a.

Microparticle-associated tissue factor activity in cancer patients with and without thrombosis

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In a previous study [1], we found that the tissue factor (TF) activity of microparticles (MP) isolated from platelet-poor plasma (MP-TF activity) was markedly increased in patients with breast and pancreatic adenocarcinoma who presented with venous thromboembolism (VTE). Based on these findings, we questioned whether MP-TF activity is also associated with VTE and survival in unselected patients with a broad spectrum of different types of cancer. We addressed this question by studying MP-TF activity in consecutive patients with various types of malignancies and irrespective of stage of the disease, who presented with VTE in our hospital. Exclusion criteria for entry into the study were any other serious diseases such as diabetes or renal insufficiency and use of anticoagulants, in order to preclude that any such factor could affect the MP-TF activity, our primary endpoint. Between mid 2003 and mid 2006, 52 consecutive cancer patients who presented with a first episode of VTE were invited to participate; one patient refused and thus 51 patients were studied. VTE was ascertained by echo Doppler and/or spiral computed tomography (CT). Blood was collected in the acute phase at the time the diagnosis of VTE was assessed and just before anticoagulation was started. After inclusion of a case (i.e. a cancer patient presenting with VTE) the next cancer patient who matched for age ± 5 years, sex, type of cancer, stage of the disease and type of cancer-specific treatment, including the same chemotherapy regimen and previous cancer-specific treatments, was asked to participate and included in the study as a control. To avoid genetic stratification, these cancer patients were also matched for ethnicity and geographical area. We included 49 cancer patients as controls. One control was not matched for sex and age. For two cases with an adenocarcinoma of the lung we were not able to identify an appropriately matched control. All cancer patients were followed-up in our hospital at regular intervals until death or end of study, with no patients lost to follow-up, thus enabling us to precisely assess the time of death. In all patients, mortality was due to cancer-specific death; none of the patients died as a consequence of the thrombotic event. The Medical Ethics Committee approved investigation of blood MP in patients with various types of cancer at different stages of their disease. All 100 patients gave informed consent.

The distribution of malignancies in the 51 unselected cases, who presented with thrombosis, were as follows: 27 had gastrointestinal carcinomas (two oesophageal, 14 colorectal, 10 pancreatic, and one cholangiocarcinoma), 12 patients had genito-urinary tract tumours (four renal cell, two prostate carcinomas and six germ cell tumours of the testis), two had respiratory tract (lung) tumours, two had bone tumours, two had tumours of the larynx, two had breast tumours, three had ovarian carcinoma and one had adrenal carcinoma.

Citrated plasma was prepared immediately after blood collection by centrifugation at $1550 \times g$ for 20 min, snapfrozen in liquid nitrogen and stored at −80 °C. The number of Annexin-V^+ MP was measured by flow cytometry as previously described [1]. The TF activity in MP preparations (MP-TF activity) was measured by determining the FVII-dependent factor Xa (FXa) generation as previously described [1]. In all samples FXa generation was measured both in the presence and absence of FVII and in the presence and absence of

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