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IN FOCUS

Template bleeding time and PFA-100® have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: comparative study in 148 patients

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Summary. *Objectives and patients*: We compared the template bleeding time (BT) and closure time (CT) in the PFA-100® as screening tests in 148 consecutive patients with unequivocal mucocutaneous bleeding and positive family history. Exclusion criteria: drug intake, concomitant diseases including minor infections, low platelet count, diseases of secondary hemostasis. *Results*: Type 1 von Willebrand disease (VWD-1) was diagnosed in 26 patients, primary platelet secretion defect (PSD) in 33, VWD-1 + PSD in nine, whereas 80 patients did not comply with the criteria for known hemostatic disorders (UD, unknown diagnosis). BT and CT were prolonged in 35.8% and 29.7% of all the patients, respectively ($P = 0.23$). Sensitivity increased to 48% if an abnormality of BT and/or CT was considered. Same comparisons for BT and CT in each diagnostic category were, respectively: 42 vs. 61.5% in VWD-1 ($P = 0.18$), 42 vs. 24% in platelet secretion defects ($P = 0.11$), 67 vs. 89% in VWD-1 + PSD ($P = 0.50$), and 27.5 vs. 15% in UD ($P = 0.06$). *Conclusion*: Both tests were relatively insensitive and not significantly different in detecting incoming patients with mucocutaneous hemorrhages. In patients with VWD-1, the PFA-100® performed slightly better, whereas the opposite occurred in those patients with platelet secretion defects. In the UD group, both tests lost sensitivity, but the BT detected 1.8 times more patients than the PFA-100®. Given the large proportion of undiagnosed bleeders and the overall low sensitivity of these tests, clinical decisions still rely on the medical history and etiological diagnosis of the bleeding disorder.

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Introduction

Patients presenting with non-thrombocytopenic hereditary mucocutaneous bleeding are usually difficult to diagnose. It is widely accepted that most of them have a mild disorder of primary hemostasis, which involves the successive interactions of platelets with the vessel wall and other platelets, resulting in the formation of the platelet plug [1]. However, even after repeated testing, a significant proportion of patients have no demonstrable alterations in plasma von Willebrand factor (VWF) and *ex vivo* platelet function, measurements used for the diagnosis of the most frequent and best known diseases of this system, type 1 von Willebrand disease (VWD-1) and primary platelet secretion defects. In a previous retrospective study of 589 individuals with mucocutaneous bleeding and a family history of abnormal hemorrhages, we could not diagnose more than 50% of these patients after a first laboratory workup [2]; this result is similar to that of a recent report [3]. These observations suggest that the pathogenesis of the bleeding disorder in many of these patients is still unknown.

Originally, the Ivy bleeding time (BT) was used as a test to screen functional platelet disorders, including VWD [4], and in fact, the BT is consistently prolonged in patients with severe diseases (i.e. Glanzmann thrombasthenia, Bernard–Soulier syndrome, type 3 VWD…). However, its sensitivity drops in those individuals with mild bleeding disorders, such as VWD-1 and platelet secretion defects. The routine use of BT has been questioned not only for being invasive but also for its lack of specificity and low predictive value [5,6]. Despite these drawbacks, when the BT is performed properly in a focused

patient population with positive clinical history, it is still considered a valuable tool that can yield useful, complementary information [7].

The Platelet Function Analyzer (PFA-100®) was designed as an *in-vitro* measure of primary hemostasis under conditions of high shear. The test has proved to be more sensitive than the template BT in patients with VWD, including its subtypes [8–12]. It is also abnormal in patients with severe platelet function defects, such as Glanzmann or Bernard–Soulier diseases. However, it seems to be less sensitive in bleeders with mild platelet secretion defects [13–15] who currently constitute the largest proportion of patients with hereditary functional platelet disorders [16].

Most of the published studies comparing both screening tests have been performed in selected patients with previously known diagnoses, but there is a relative lack of information on their comparative value in unselected incoming patients. Moreover, most studies have not compared the performance of both tests in that important proportion of abnormal bleeders with no laboratory evidence of a distinctive hemostatic disorder. A recent work [3] evaluated both screening tests in 346 consecutive outpatients referred for suspected or known hemorrhagic diathesis. However, they did not compare the performance of both tests in 168 (48.5%) of these patients on the basis that no diagnosis could be made in them. In this setting, the aims of our study were to compare prospectively the performance of BT and PFA-100® as screening tools in consecutive patients with unequivocal mucocutaneous bleeding of hereditary nature; moreover, we evaluated their performance in each of the diagnostic categories in which these patients were classified: VWD-1, platelet secretion defects and those without specific diagnosis.

Patients and methods

Patients and controls

The study was approved by the Medical Ethics Committee of our institution and participants gave their informed consent. Patients were referred for study to our laboratory by four physicians. These consecutive, not previously selected patients were always interviewed by the same physician (T.Q.) using a standardized questionnaire. The most frequent and typical symptoms of disorders of primary hemostasis (such as nose bleed, easy bruising, gum bleed, menorrhagia, and prolonged bleeding from small wounds) were scored from 0 to 4. For other symptoms of lesser frequency, less typical of primary hemostasis diseases, or those present only after exposure to risk, scores of 0 (= absent) or 1 (= present) were assigned. Similarly, the bleeding history of first-degree (parents, sibs and offspring of the proband) and second-degree relatives (grandparents and grandchildren, uncles and aunts, nephews and nieces, and half-sibs) was recorded. Type and frequency of bleeding symptoms are shown in Table 1. The analysis of the final score and the personal perception of the interviewing physician regarding the severity of bleeding were highly correlated ($r = 0.81$, $P < 0.00001$). The interview led to a classification of the patients into three categories: definitive bleeders, not clearly defined, and probably non-bleeders. Only the first category was included in the analysis. The controls, of similar age and sex, as well as social, economic and cultural background, were referred from the same centers for preoperative assessment for minor elective surgery (i.e. hernia, phimosis) or were voluntary controls recruited from a school with parental consent. They were subjected to the same interview, selecting only non-bleeders. Patients and controls with concurrent drug intake, other concomitant diseases, infections of any type, a platelet count $< 100\,000\ \mu L^{-1}$, clotting factor deficiencies (including hemophilia A or B), blood hemoglobin $< 11\ g\ dL^{-1}$, elevated serum creatinine and transaminases (ALT/AST), and C-reactive protein $> 1\ mg\ dL^{-1}$ were excluded. Volunteers were allowed to enter the study 1 week or 3 days after intake of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs), respectively. Finally, 148 patients (14.1 ± 9.3 years, range 4–48 years) were included in the analysis. Forty-three healthy, non-bleeder individuals (13 ± 7 years, range 4–44) served as controls to establish the normal range of PFA-100®. All the controls had normal BT and none of them presented abnormalities compatible with VWD or platelet function disorders.

Table 1 Type and frequency of bleeding and family history in 148 patients

Symptom	Frequency (%)
Epistaxis	81.1
Cauterization	26.4
Gum bleeding	62.8
Bleeding after dental extractions*	12.2
Ecchymoses	87.2
Menorrhagia*	91.1
Postpartum bleeding*	43.8
Bleeding after minor injuries	56.8
Bleeding after major trauma	17.6
Surgical bleeding*	53.8
Hematomas (mostly superficial)	38.5
Hemarthroses	4.7
Petechiae	22.3
Bridle bleeding	9.5
Hematuria	3.4
Gastrointestinal bleeding	8.1
Finding of blood in stools	18.9
Hemoptoic sputum	30.4
Hemoptyses	0.0
Umbilical bleeding	9.5
Otorrhagia	7.4
Bleeding requiring blood transfusion	23.6
Family history of bleeding	88.5

*Denotes the frequency of bleeding in those patients exposed to risk.

Laboratory tests

Fasting blood samples were drawn between 08.30 h and 09.30 h. Routine hemostatic testing included prothrombin

time (PT), activated partial thromboplastin time (APTT), thrombin time, and clot lysis in saline and urea. Plasma fibrinogen was measured by the Clauss assay (Diagnostica Stago, Asnières, France). Factor (F)VIII:C, factor (F)IX:C and factor (F)XI:C were determined by one-stage, modified APTT assays [17] using factor-depleted plasmas (Biopool Int., Ventura, CA, USA). Plasma VWF:Ag was measured by ELISA, using a capture monoclonal antibody (vW1, kindly provided by R. R. Montgomery, Milwaukee, WI, USA) and a peroxidase-conjugated rabbit antibody for detection (Dako Corp., Carpinteria, CA, USA). Ristocetin cofactor activity (VWF : RCo) was determined by the slope of aggregation of formaldehyde-fixed platelets, prepared in our laboratory [18]. VWF collagen binding assay (VWF:CB) was performed as described [19] using type III human collagen (Southern Biotechnology Associates Inc., Birmingham, AL, USA). Plasma VWF multimers were analyzed by immunoblotting using peroxidase-conjugated antibody (Affinity Biologicals Inc., Hamilton, Canada) with chemiluminiscence detection [20]. Normal values for VWF/FVIII complex had been previously established in a school-aged population ($n = 503$) [21] and in blood donors ($n = 822$) judged as non-bleeders during a selective interview.

Platelet aggregation and ^{14}C-5-HT (serotonin) secretion in platelet-rich plasma were performed as described [22]. Low concentrations of ADP (4 μM), collagen (1 μg mL^{-1}; Hormon Chemie, München, Germany) and epinephrine (10 μM) known to elicit primary and secondary waves of aggregation in most healthy subjects were employed. Higher concentrations of ADP (8 μM), collagen (2 μg mL^{-1}) and sodium arachidonate (1 mM) were used to induce full aggregation, which was measured as the maximum change in light transmission. For ^{14}C-5-HT secretion, the reaction was stopped at 5 min with cold saline–EDTA and formaldehyde. The radioactivity in supernatant platelet-poor plasma was then counted. Normal values for platelet aggregation and secretion were established from the data of 193 healthy, non-bleeder individuals (12 ± 7 years, range 4–44). For ADP, collagen and arachidonate, the observation of a reversible platelet aggregation or a decreased maximum change in light transmittance determined after 5 min following the addition of the agonist was considered as evidence of abnormality. Defective platelet aggregation with epinephrine was registered when the secondary wave of aggregation was absent. Platelet 5-hydroxytryptamine (5-HT) was measured by high-performance liquid chromatography with electrochemical detection [23].

VWD was diagnosed in patients when two or three of the following tests were abnormally low: VWF:Ag, VWF:RCo, VWF:CB. In this population, no patients were diagnosed with VWD types 2 or 3.

A primary platelet secretion defect was diagnosed when the platelet aggregation or ^{14}C-5-HT secretion was abnormal with two or more agonists or with both ADP concentrations or both collagen concentrations. A defective aggregation/secretion with arachidonate, associated with defects with all the other agonists, was initially considered evidence of aspirin, NSAIDs or even some unknown food or drug effect. Patients with this pattern of defects were included only if a repeated study confirmed these findings.

Bleeding time and PFA-100® closure time

A vertical, forearm BT was performed by only two trained technicians in our laboratory using commercial devices (Simplate® IIR and Simplate® Pediatric; Organon Teknika Corp., Durham, NC, USA). To normalize the BT in children and adults, the results were expressed as the ratio of the BT in patients to the upper normal range in controls. The upper normal limit for BT was set at mean + 2 SD in individuals > 7 years old (9.5 min, with the standard device, $n = 45$) and < 7 years old (6.5 min, with the pediatric device, $n = 35$).

The PFA-100® 'closure time' (CT) was measured as previously described [24]. Blood drawn in 3.2% sodium citrate was stored < 2 h before testing. The reference range (mean ± 2 SD) of CT values, using membranes coated with collagen-epinephrine (106, 59–156 s) and collagen-ADP (82, 47–117 s), was obtained with samples of the 43 healthy, non-bleeder individuals. The test was considered abnormal if CT was prolonged with one or both cartridges.

Statistical analysis

Results are presented as mean ± SD or as mean and range, when appropriate. An analysis of agreement of both tests was performed using the κ statistics and the disagreement was analyzed using the McNemar χ^2 test with continuity correction.

Results

Eighty (54%) of the patients did not fit the diagnostic criteria for any known hemostatic disorder. In these patients with unknown diagnosis (UD), the PT, APTT, clot lysis in saline and urea, and plasma fibrinogen concentration were normal and levels of FVIII:C, FIX:C and FXI:C were always > 50% (except for one patient with 32% of FXI:C). The remaining 68 patients were classified as VWD-1 ($n = 26$), primary platelet secretion defects ($n = 33$), or a combination of both (VWD–PSD, $n = 9$). Demographic data on these cohorts and the control population are presented in Table 2. Results of VWF:Ag, VWF:RCo, VWF:CB, and FVIII:C for each patient group are presented in Table 3. The maximum percent of platelet aggregation and secretion in these patients is shown in Table 4. As described in Patients and methods, patients with a reversible wave of platelet aggregation with some agonists were also considered to have a platelet secretion defect and always had a concomitant defective platelet 5-HT secretion; three (9%) of these patients only had a defective platelet secretion with a normal aggregation pattern. Values for platelet 5-HT concentration in this group of patients ranged between 202 and 990 ng per 10^9 platelets (normal range 204–1110 ng per 10^9 platelets). The only

Table 2 Characterization of patient and control populations

	N (% of patients)	Age	Female/male	ABO type O/ non-O	Platelet count ($\times 10^3$ μL^{-1})	Hgb (g dL^{-1})
VWD-1	26 (17.6)	14 ± 7 (4–39)	18/8	22/4	274 ± 48	12.6 ± 0.8
PSD	33 (22.3)	13 ± 8 (5–41)	18/15	24/9	243 ± 66	12.8 ± 1.0
VWD-1 + PSD	9 (6.1)	20 ± 11 (8–40)	5/4	5/4	267 ± 46	13.4 ± 1.7
UD	80 (54)	14 ± 10 (5–48)	48/32	51/29	270 ± 65	12.9 ± 0.9
Controls	43	13 ± 7 (4–44)	17/26	17/26	285 ± 57	13.0 ± 1.1

VWD-1, Type 1 von Willebrand disease; PSD, primary platelet secretion defect; UD, unknown diagnosis.

Table 3 Von Willebrand factor and factor (F)VIII:C in controls and in patients in each diagnostic category

	N	VWF:Ag %, mean (range)	VWF:RCo %, mean (range)	VWF:CB %, mean (range)	FVIII:C %, mean (range)
VWD-1	26	34 (10–53)	31 (10–47)	31 (6–64)	56 (13–101)
PSD	33	83 (43–167)	71 (36–124)	77 (28–171)	92 (42–144)
VWD-1 + PSD	9	29 (9–55)	27 (13–49)	24 (9–52)	44 (10–81)
UD	80	96 (47–210)	80 (36–143)	91 (35–210)	86 (48–150)
Controls	43	98 (49–231)	98 (45–242)	90 (44–204)	101 (46–201)

VWD-1, Type 1 von Willebrand disease; PSD, primary platelet secretion defect; UD, unknown diagnosis.

Table 4 Maximum percentages of platelet aggregation and ^{14}C-5-HT secretion in controls and in patients in each diagnostic category

		AA, 1 mM, mean (range)	EPI, 10 μM, mean (range)	ADP, 4 μM, mean (range)	ADP, 8 μM, mean (range)	C, 1 μg mL^{-1}, mean (range)	C, 2 μg mL^{-1}, mean (range)
VWD-1	PA	80 (72–89)	62 (4–88)	78 (60–94)	82 (74–105)	82 (72–89)	84 (74–100)
	PS	38 (30–72)	36 (6–66)	38 (23–55)	39 (28–55)	45 (30–58)	50 (34–66)
PSD	PA	64 (0–89)	25 (0–88)	48 (0–77)	63 (5–88)	59 (0–93)	70 (5–87)
	PS	30 (3–56)	16 (0–44)	13 (0–39)	18 (0–47)	25 (1–50)	35 (6–74)
VWD-1 + PSD	PA	75 (52–85)	26 (10–74)	53 (29–83)	66 (40–83)	61 (42–72)	75 (46–81)
	PS	35 (11–66)	23 (4–47)	22 (4–42)	27 (15–45)	30 (21–37)	38 (28–46)
UD	PA	81 (69–100)	68 (11–100)	77 (50–95)	81 (60–100)	78 (43–100)	81 (69–90)
	PS	41 (26–68)	38 (0–77)	37 (10–67)	40 (20–63)	40 (10–68)	47 (23–87)
Controls ($n = 43$)	PA	82 (69–94)	66 (6–94)	77 (46–91)	80 (62–91)	81 (64–94)	82 (57–97)
	PS	39 (27–88)	32 (10–59)	34 (14–68)	37 (29–76)	39 (27–72)	42 (32–75)

AA, Arachidonic acid; EPI, epinephrine; C, collagen; PA, platelet aggregation; PS, platelet secretion; VWD-1, type 1 von Willebrand disease; PSD, primary platelet secretion defect; UD, unknown diagnosis.

patient with platelet 5-HT slightly below normal range had normal levels of platelet ADP and ATP. Accordingly, no patients with definite storage pool disease were diagnosed in this population.

Results of BT in each group of patients are shown in Fig. 1 and results of CT with PFA-100® CT, using C-EPI and C-ADP membranes, are presented in Fig. 2. Summary and statistical comparisons of the results for the whole population of patients and for each diagnostic category are shown in Table 5. As an initial screening of defects of primary hemostasis, the performances of both tests was not significantly different, with sensitivities of 33.5% and 29.7% for BT and PFA-100®, respectively. With regard to PFA-100®, among the 44 patients with prolonged CTs, 22 were abnormal with both cartridges and 22 were abnormal with only one of them (13 were prolonged with C-EPI and nine with C-ADP). Among the 12 patients with abnormal CT in the group with UD, only one had a prolonged CT with both cartridges, seven were abnormal with C-EPI, and four with C-ADP. The overall

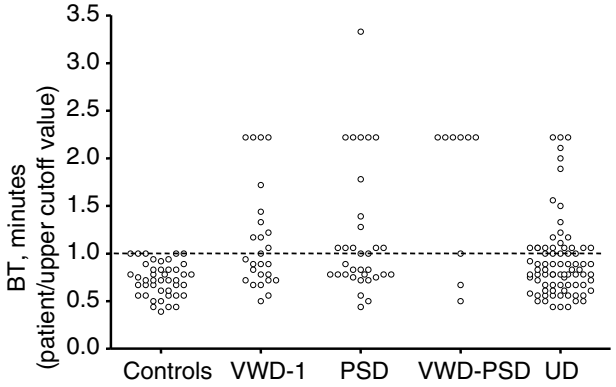

Fig. 1. Bleeding time (BT) in patients with mucocutaneous hemorrhages assorted according to final diagnosis. The ordinate reflects the ratio of the patient BT to the upper normal range of healthy individuals older than 7 years (9.5 min) and up to 7 years (6.5 min) using standard or pediatric devices, respectively. Values above the horizontal dotted line represent the abnormally prolonged BT.

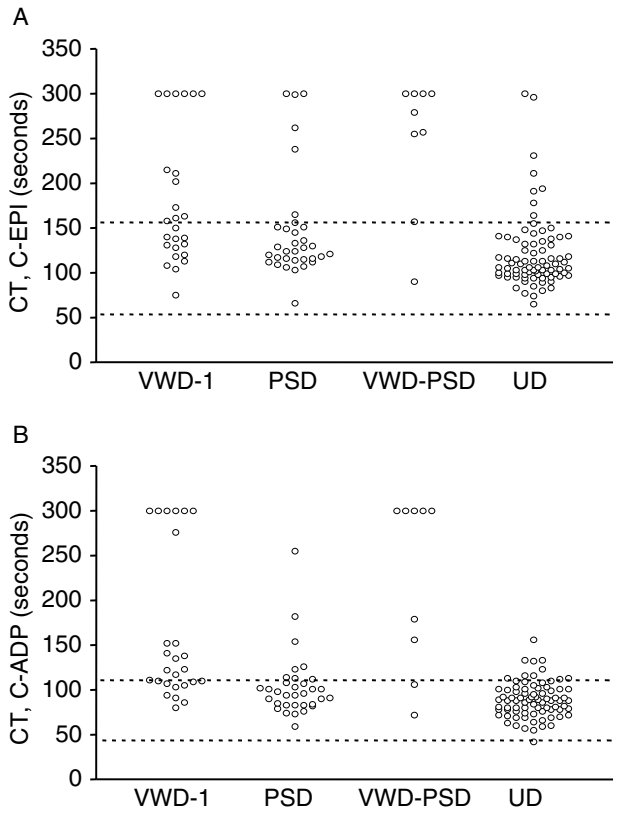

Fig. 2. Closure times using collagen-epinephrine (A) and collagen-ADP (B) membranes in the PFA-100® instrument in patients with different diagnoses. Horizontal dotted lines define the upper and lower limits of normal established in 43 healthy individuals of similar age distribution to the patients.

sensitivity increased to 48% when an abnormality of BT and/or CT was recorded. In the whole patient population, low Pearson's correlation coefficients between BT and PFA-100® were found: BT against CT with C-EPI was 0.51 and BT against CT with C-ADP was 0.39 ($n = 148$).

In patients with VWD-1 and VWD-1 + PSD, the PFA-100® system was abnormal more frequently than was the BT, even though the differences were not statistically significant. The opposite was observed in patients with PSD. In the large group of 80 patients with UD, both tests detected a lower percentage of the patients than in the other diagnostic categories; however, the BT almost doubled the detection percentage of PFA-100®, but the difference was not statistically significant. Furthermore, in this group the κ statistics ($\kappa = 0.124$) revealed a low degree of agreement beyond chance between both tests regarding the measured variable. In this group, only 24/80 (30%) of the patients had both tests abnormally prolonged.

Discussion

We compared the performance of template bleeding time and PFA-100® as screening tests in 148 consecutive patients with unequivocal mucocutaneous bleeding and similar family histories. These patients are usually suspected of having a disease of primary hemostasis, and the most frequently diagnosed diseases in these patients are VWD-1 and platelet secretion defects. In fact, 46% of the patients in this study had one or both diseases. The remaining 54% did not fit the diagnostic criteria for these diseases or other known hemostatic disorders. It must be emphasized that the characteristics of the bleeding and family history were indistinguishable among these patients and, accordingly, there is no clinical basis to select a specific disorder for laboratory diagnosis. These tests are expensive and, furthermore, it is often necessary to repeat the study in borderline cases and for confirmation of a diagnosis. In this context, an ideal screen test should detect all the patients suspected of having a primary hemostatic disorder to proceed with specific diagnostic tests. The traditional template BT is still used as a first-line screening tool despite being criticized for its invasiveness and lack of sensitivity, accuracy, reproducibility and predictive value. These drawbacks have created the need for new *in-vitro* tests as substitutes for the template BT in routine clinical practice.

The PFA-100® was introduced as a potential screening test for assessing patients with disorders of platelet function. It is a simple, rapid, non-invasive test that does not require specialized training. However, after several years of testing, it has not yet been widely accepted as a substitute for the template BT. Most published studies comparing the performance of both tests have been conducted in patients with

Table 5 Sensitivity of bleeding time (BT) and closure time (CT) (PFA-100®) in the screening of the whole population of patients with mucocutaneous bleeding and in each diagnostic category

		Prolonged values, number (%)			
	N	BT	CT-PFA-100®*	BT and/or CT-PFA 100®	*P***
Whole population	148	53 (35.8)	44 (29.7)	71 (48)	0.23
VWD-1	26	11 (42)	16 (61.5)	18 (69.2)	0.18
PSD	33	14 (42)	8 (24)	16 (48.5)	0.11
VWD-1 + PSD	9	6 (67)	8 (89)	8 (89)	0.50
UD	80	22 (27.5)	12 (15)	29 (36.3)	0.06
Controls	43	0 (0)	1 (2.3)	1 (2.3)	–

*The values include patients with abnormal CT with C-EPI and/or C-ADP cartridges. **Comparison of BT vs. CT-PFA-100® (McNemar χ^2 test). VWD-1, Type 1 von Willebrand disease; PSD, primary platelet secretion defect; UD, unknown diagnosis.

previously diagnosed VWD or platelet function disorders [8–12,14,15]. Studies are lacking for assessing outpatient clinic populations, including the analysis of individuals with mucocutaneous hemorrhages of unknown etiology. In this study, we recruited symptomatic, previously undiagnosed patients with skin and mucous membrane bleeding, excluding patients with disorders of secondary hemostasis. Even though we did not exclude patients with severe diseases of primary hemostasis in the design phase of the study, no patients were diagnosed with Glanzmann disease, Bernard–Soulier syndrome, storage pool disease or subtypes 2 and 3 of VWD during the recruitment period, confirming the lower frequency of these diseases in our population.

In our patients, the sensitivity of both BT and PFA-100® screening tests was too low to rely on them: 35.8% of the whole population had a prolonged BT and 29.7% had a prolonged CT, with one or both cartridges, a difference not statistically significant. This indicates that as a global screening test for detecting abnormalities of primary hemostasis, the PFA-100® system is not a better test than the traditional BT. The use of both screening tests increases the overall sensitivity to 48%, considering an abnormality of BT and/or CT. As the aim of this study was to compare both tests in patients with presumed defects of primary hemostasis presenting with a distinctive bleeding pattern, we did not assess the specificity of both tests. This decision was based on the fact that being designed as screening tools to detect several diseases of primary hemostasis, the BT and the PFA-100® are by definition not specific.

This global sensitivity analysis differs slightly if the patients are assorted by diagnosis. As reported in several previous studies [8–12], we confirmed that PFA-100® exhibits a better sensitivity in detecting patients with VWD-1, which is an advantage that can be used in the therapeutic monitoring of this disease [9]. In patients with PSD, these results are reversed, with the BT detecting more patients than the PFA-100® system [14,15]. As would be expected, in the small population of patients with concomitant VWD-1 + PSD (9/148 patients, 6% of the patient population), the sensitivity of both tests appears to improve (67% for BT and 89% for PFA-100®).

In the large group of patients with unknown diagnosis, the BT tended to be more sensitive than CT (prolonged in 27.5% vs. 15% of the patients, respectively, $P = 0.064$). However, given that these patients had unequivocal bleeding symptoms, these data reveal that the sensitivity of both tests is low. Moreover, both tests had a low degree of agreement, assessed by κ statistics. It could be argued that a significant proportion of the patients with unknown diagnosis represent those individuals in an otherwise normal population presenting with mucous and cutaneous bleeding [25,26]; however, the careful selection of convincingly abnormal bleeders and the finding that 30% of them had prolonged BT and/or CT strongly indicate that most of them belong to a population with a genuine disorder of primary hemostasis of still unknown nature. Our results are consistent with those of two recent studies. First, Posan *et al.* [3] found that 168/346 (48.5%) outpatients referred for suspected or known hemorrhagic diathesis had no known hemostatic disorder after a complete laboratory study. In this subpopulation, 15.8% showed an abnormal CT, which is a result almost identical to ours, except that they did not report how many of these patients had prolonged BT. Second, Wuillemin *et al.* [27] found that among 31 patients referred to their laboratory for diagnosis of a mild bleeding disorder, 17 (55%) had no laboratory abnormalities to explain the bleeding tendency. In these, six presented with prolonged BT and only two with prolonged C-EPI, whereas no patient presented with an abnormal C-ADP.

It is also unlikely that upon repeated testing a significant proportion of these patients may comply with the diagnostic criteria of VWD-1. In fact, we excluded patients who at the time of testing had acquired conditions determining a transitory increase in plasma VWF levels. Furthermore, the results of Table 2 show that the mean and range of VWF values in patients with UD are strikingly higher than those with VWD-1 and close to those of the control population, demonstrating a clear separation between both patient subgroups. Possibly, specific and still unknown defects in platelet–vessel wall interaction explain their symptoms, a process that is better taken into account by the *in-vivo* BT rather than the PFA-100®. In this regard, antiplatelet substances produced by the vascular wall (i.e. nitric oxide, prostaglandin I_2) have been shown to prolong the BT [28–30] and, due to their very short half-life, their effect is not likely to be detected by an *in-vitro* test such as CT in the PFA-100®.

In summary, once a laboratory diagnosis was made, the comparative analysis of both tests confirmed previous studies: the PFA-100® system was slightly more sensitive than the BT in patients with VWD-1 and the opposite occurred in patients with PSD. In the larger cohort of bleeders of unknown etiology constituting more than 50% of the patients, the performance of BT tended to be better than that of CT as a screening test. However, considering the whole unselected population of patients with mucocutaneous bleeding, as in the usual clinical setting, the PFA-100® system was no more sensitive than the traditional template BT. Given that most of the patients referred for hemostatic testing have no previous diagnosis, other non-technical considerations should be taken into account (i.e. cost, invasiveness, training of personnel) to use the PFA-100® as a substitute for the template BT in routine clinical practice. Considering the overall low sensitivity of both screening tests, clinical decisions still rely on a good medical history and an etiological diagnosis of the bleeding disorder.

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References

1 Schafer AI. Approach to bleeding. In: Loscalzo J, Schafer AI, eds. *Thrombosis and Hemorrhage*, Chapter 23. Cambridge, MA: Blackwell Scientific Publications, 1994: 407–22.

2 Quiroga T, Pérez M, Rodríguez S, Muñoz B, Aranda E, Morales M, Verdugo P, Pereira J, Mezzano D. Skin and mucous membrane hemorrhages: clinical assessment, study sequence and relative frequency of hereditary diseases of the hemostasis in a Chilean population. *Rev Med Chil* 1997; **125**: 409–18.

3 Posan E, McBane RD, Grill DE, Motsko CL, Nichols WL. Comparison of PFA-100 testing and bleeding time for detecting platelet hypofunction and von Willebrand disease in clinical practice. *Thromb Haemost* 2003; **90**: 483–90.

4 Burns ER, Lawrence C. Bleeding time. A guide to its diagnostic and clinical utility. *Arch Pathol Lab Med* 1989; **113**: 1219–24.

5 Rodgers RPC, Levin J. A critical reappraisal of the bleeding time. *Sem Thromb Haemost* 1990; **16**: 1–20.

6 Lind SE. The bleeding time does not predict clinical bleeding. *Blood* 1991; **77**: 2547–52.

7 Triplett DA. The bleeding time. Neither pariah or panacea. *Arch Pathol Lab Med* 1989; **113**: 1207–8.

8 Fressinaud E, Veyradier A, Truchaud F, Martin I, Boyer-Neumann C, Trossaert M, Meyer D. Screening for von Willebrand disease with a new analyzer using high shear stress: a study of 60 cases. *Blood* 1998; **91**: 1325–31.

9 Cattaneo M, Federici AB, Lecchi A, Agati B, Lombardi R, Stabile F, Bucciarelli P. Evaluation of the PFA-100® system in the diagnosis and therapeutic monitoring of patients with von Willebrand disease. *Thromb Haemost* 1999; **82**: 35–9.

10 Dean JA, Blanchette VS, Carcao MD, Stain AM, Sparling CR, Siekmann J, Turecek PL, Lillicrap D, Rand ML. Von Willebrand disease in a pediatric-based population—comparison of type 1 diagnostic criteria and use of the PFA-100® and a von Willebrand factor/collagen binding assay. *Thromb Haemost* 2000; **84**: 401–9.

11 Schlammadinger A, Kerényi A, Muszbek L, Boda Z. Comparison of the O'Brien filter test and the PFA-100 platelet analyzer in the laboratory diagnosis of von Willebrand's disease. *Thromb Haemost* 2000; **84**: 88–92.

12 Favaloro EJ, Kershaw G, Bukuya M, Hertzberg M, Koutts J. Laboratory diagnosis of von Willebrand disorder (VWD) and monitoring of DDAVP therapy: efficacy of the PFA-100® and VWF. CBA as combined diagnostic strategies. *Haemophilia* 2001; **7**: 180–9.

13 Favaloro EJ. Clinical application of the PFA-100®. *Curr Opin Hematol* 2002; **9**: 407–15.

14 Cattaneo M, Lecchi A, Agati B, Lombardi R, Zighetti ML. Evaluation of platelet function with the PFA-100® system in patients with congenital defects of platelet secretion. *Thromb Res* 1999; **96**: 213–7.

15 Kerényi A, Schlammadinger A, Ajzner E, Szegedi I, Kiss C, Pap Z, Boda Z, Muszbek L. Comparison of PFA-100 closure time and template bleeding time of patients with inherited disorders causing defective platelet function. *Thromb Res* 1999; **96**: 487–92.

16 Cattaneo M. Inherited platelet-based bleeding disorders. *J Thromb Haemost* 2003; **1**: 1628–36.

17 Langdell RD, Wagner RH, Brinkhous KM. Effect of antihemophilic factor on one-stage clotting tests. *J Lab Clin Med* 1953; **41**: 637–47.

18 Allain JP, Cooper HA, Wagner RH, Brinkhous KM. Platelets fixed with paraformaldehyde: a new reagent for assay of von Willebrand factor and platelet aggregating factor. *J Lab Clin Med* 1975; **85**: 318–28.

19 Siekmann J, Turecek PL, Schwarz HP. The determination of von Willebrand factor activity by collagen binding assay. *Haemophilia* 1998; **4** (Suppl. 3): 15–24.

20 Budde U, Schneppenheim R, Plendl H, Dent J, Ruggeri ZM, Zimmerman TS. Luminographic detection of von Willebrand factor multimers in agarose gels and on nitrocellulose membranes. *Thromb Haemost* 1990; **63**: 312–5.

21 Quiroga T, Tagle R, Pereira J, Mezzano D. Sex-related difference in plasma von Willebrand factor (VWF:Ag and VWF:RiCof) levels in adolescents. *Thromb Haemost* 1994; **71**: 800–1.

22 Mezzano D, Tagle R, Panes O, Pérez M, Downey P, Muñoz B, Aranda E, Barja P, Thambo S, González F, Mezzano S, Pereira J. Hemostatic disorder of uremia: the platelet defect, main determinant of the prolonged bleeding time, is correlated with indices of activation of coagulation and fibrinolysis. *Thromb Haemost* 1996; **76**: 312–21.

23 Kumar AM, Kumar M, Deepika K, Fernandez JB, Eisdorfer C. A modified HPLC technique for simultaneous measurement of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in cerebrospinal fluid, platelet and plasma. *Life Sci* 1990; **47**: 1751–9.

24 Favaloro EJ. Utility of the PFA-100® for assessing bleeding disorders and monitoring therapy: a review of analytical variables, benefits and limitations. *Haemophilia* 2001; **7**: 170–9.

25 Nosek-Cenkowska B, Cheang MS, Pizzi NJ, Israels ED, Gerrard JM. Bleeding/bruising symtomatology in children with and without bleeding disorders. *Thromb Haemost* 1991; **65**: 237–41.

26 Mauser Bunschoten EP, van Houwelingen JC, Sjamsoedin Visser EJM, van Dijken PJ, Kok AJ, Sixma JJ. Bleeding symptoms in carriers of haemophilia A and B. *Thromb Haemost* 1988; **59**: 349–52.

27 Wuillemin WA, Gasser KM, Zeerleder SS, Lämmle B. Evaluation of a platelet function analyzer (PFA-100®) in patients with a bleeding tendency. *Swiss Med Wkly* 2002; **132**: 443–8.

28 Gerrard JM, Duta E, Nosek-Cenkowska B, Singhroy S, Cheang M, Kobrinsky NL. A role for prostacyclin in bruising symptomatology. *Pediatrics* 1992; **90**: 33–6.

29 Simon DI, Stamler JS, Loh E, Loscalzo J, Francis SA, Creager MA. Effects of nitric oxide synthase inhibition on bleeding time in humans. *J Cardiovasc Pharmacol* 1995; **26**: 339–42.

30 Ubatuba FB, Moncada S, Vane JR. The effect of prostacyclin (PGI_2) on platelet behaviour. Thrombus formation *in vivo* and bleeding time. *Thromb Haemost* 1979; **23**: 425–35.