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**ORIGINAL ARTICLE**

# A multicenter clinical evaluation of the Clot Signature Analyzer

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**Summary.** *Background*: The Clot Signature Analyzer (CSA) was designed to assess global hemostasis as a screening assay using non-anticoagulated whole blood. Three different measurements are produced by the instrument: platelet hemostasis time (PHT), clot time (CT), and collagen-induced thrombus formation (CITF). *Objectives*: The purpose of the present study was to determine normal ranges for these measurements and assess the performance of the CSA in patients with well-characterized hemostatic disorders and in normal subjects. *Patients and methods*: Four institutions participated in the study. Each established their own normal reference ranges. Patients with well-characterized hemostatic disorders and concurrent normal controls were subsequently examined. *Results*: Normal ranges between institutions were similar although statistically different. One hundred and eight patients were examined: 46 individuals with von Willebrand disease (VWD) (type 1, 26; type 2A, 11; type 2B, six; type 3, three); 38 patients with a coagulation factor deficiency; 13 individuals with platelet function defects; 10 patients taking warfarin; and one individual on low-molecular-weight heparin. Of these patients, 89% had at least one abnormality by CSA: 42/46 VWD patients, 35/38 coagulation protein defect patients, 9/13 patients with platelet function defects, 9/10 patients on warfarin and 1/1 patient on low-molecular-weight heparin. Of 116 normal subjects, 103 (89%) fell within the centers' normal range. These data suggest that the CSA has a good sensitivity for bleeding disorders.

**Keywords**: bleeding disorders, Clot Signature Analyzer, screening, testing.

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## Introduction

The traditional approach to the laboratory evaluation of patients with suspected abnormal bleeding has been to use a battery of screening tests including the prothrombin time, activated partial thromboplastin time, platelet count, and bleeding time [1–3]. Unfortunately, these tests suffer from poor sensitivity, specificity, and positive and negative predictive value [4–10]. The clinical applicability of the results is often unclear and there is often uncertainty regarding further evaluation and treatment based upon the results. These results may lead to frequent use of tests for specific protein or platelet defects such as factor assays and platelet function tests. These tests also have variable sensitivity and specificity which makes interpretation of the results difficult. Moreover, this approach is usually not cost effective due to the expense of these assays.

In the past few years, several new test systems have been developed to screen for hemostatic abnormalities. These include the Platelet Function Analyzer (PFA)$^{TM}$ [11,12], thromboelastography [13], Platelet Analysis System$^{TM}$ [14], and the Clot Signature Analyzer (CSA)$^{TM}$ [15]. The PFA is primarily a test of platelet function and has been evaluated in von Willebrand disease (VWD) and various platelet function disorders, both inheritable and acquired [12,16–18]. The CSA is intended as a global hemostasis screen for assessing both platelet function and fibrin clot formation. It includes three component tests: the platelet hemostasis time (PHT), clot time (CT), and collagen-induced thrombus formation (CITF). The PHT is the time required for occlusion of a puncture in a tube of flowing blood. The CT is the time for a hemostatic plug to occlude completely the lumen of the tube. The CITF is the time required for a collagen-induced thrombus to occlude completely a tube of flowing blood. To measure the PHT, whole, non-anticoagulated blood is passed through a small plastic tube under pressure. Two holes are punched in the tube and the resulting drop in pressure is recorded. The PHT is the time required for the holes to be plugged by a platelet and fibrin clot and is measured by recovery of the pressure. As the platelet plug propagates and a fibrin clot is formed, the tube

becomes occluded and pressure at the distal end of the tube falls and is measured as the CT. Whole blood is also passed through another tube containing collagen fibrils. Platelets adhere to the collagen and aggregation, release, and fibrin clot formation is initiated. The time to a 50% decrease in pressure is the CITF.

A multicenter evaluation of the CSA was performed to assess its utility in patients with various hemostatic disorders. The goal of the study was to determine normal ranges for the PHT, CT, and CITF and then examine both patients with previously diagnosed bleeding disorders and additional normal subjects. The results of these investigations are reported here.

## Methods

### *Participating institutions*

Four institutions, Rochester General Hospital, Georgetown University, the University of Michigan, and St Louis University participated in the study. At each institution, the study to collect blood from normal individuals and patients with hemostatic disorders was approved by the respective Institutional Review Board.

### *Normal subjects*

Normal subjects from each institution were recruited for determining local normal reference ranges and as concurrent controls for the patient study of previously diagnosed individuals with disorders of hemostasis. All individuals who were classified as normal subjects had values for the prothrombin time (PT), activated partial thromboplastin time (APTT), platelet count, hemoglobin, and hematocrit that fell within the 95% confidence interval for normal subjects at that institution. Subjects with test results outside of the normal range at each institution were excluded from the study. Subjects were excluded for use of aspirin or any drug known to affect platelet function within the preceding 10 days and non-steroidal anti-inflammatory drugs within the prior 48 h. Other exclusion criteria for individuals considered normal included personal or family history of a bleeding disorder, prior cardiac or vascular procedures, or diabetes mellitus.

Normal control subjects participating in the study of patients with previously diagnosed bleeding disorders were required to meet the same entrance criteria as the subjects participating in the normal range study. In addition, they were tested for ristocetin-induced platelet aggregation and, at some institutions, for aggregation in response to collagen, ADP, epinephrine, gamma thrombin, and arachidonic acid. The agonist concentrations and preparations used were different at each institution. Furthermore, each institution used their own normal values for an appropriate response to each of these agonists. Efforts were made to match normal subjects in the patient study for age and sex with the patient group. All subjects participating in both the normal range study and as controls in the patient study were tested twice (once for each study) and required to have normal screening tests on both occasions.

### *Patient subjects*

Patients with previously diagnosed hemostatic disorders were recruited from the population followed at each institution. These patients included individuals with VWD, plasma coagulation factor deficiencies, e.g. hemophilia A and B, platelet function disorders, and various other hereditary or acquired hemostatic abnormalities. All patients had been previously diagnosed at the respective institution using generally accepted criteria and were well known to the investigators. The severity of coagulation abnormality varied from mild to severe. They were required to have coagulation test abnormalities at the time of the study.

### *Laboratory assays*

Tests on patients were performed on samples concurrently drawn with the CSA sampling. Citrated plasma specimens were drawn for PT, APTT, and platelet aggregation studies using collagen and arachidonic acid in all instances. Each institution was allowed to use their usual collagen reagent although it was required that a high and low final concentration be used. The final collagen concentrations ranged from 1 $\mu g\ mL^{-1}$ to 10 $\mu g\ mL^{-1}$ among the institutions. The final concentration of arachidonic acid was required to be at least 1 mM. Ristocetin-induced platelet aggregation was performed on all patients with a diagnosis of VWD using final concentrations of ristocetin of 0.5–0.6 and 1.0–1.2 $mg\ mL^{-1}$. Some institutions also performed platelet aggregation studies assessing threshold responses to ADP (1.0–7.5 μM), epinephrine (0.1–10 μM), and gamma thrombin (35–146 nM). Relevant coagulation factor assays were performed to confirm the diagnosis in patients with clotting factor deficiencies. Only those patients who had an abnormal assay in accordance with their previous diagnosis at the time of CSA testing were included in the analysis. Local normal ranges for all assays were used to include/exclude patients from the study.

### *Clot Signature Analyzer testing*

The CSA was operated as per the manufacturer's instructions. Briefly, 3 mL of non-anticoagulated whole blood was drawn into each of two polypropylene syringes prewarmed in a special device supplied by the manufacturer. These syringes were immediately inserted into prewarmed test cassettes followed by loading the cassettes onto the CSA and starting the instrument. The instrument automatically performs the tests from this point. In all cases of testing, the subject was brought to the location where the instrument was housed. The time from collection of the blood until loading into the instrument cassettes was always $< 3$ min. The CSA is a two-channel instrument. One channel measures the PHT and CT and the other measures the CITF. The results are presented on a printout in both tabular and graphic form (Fig. 1). The data

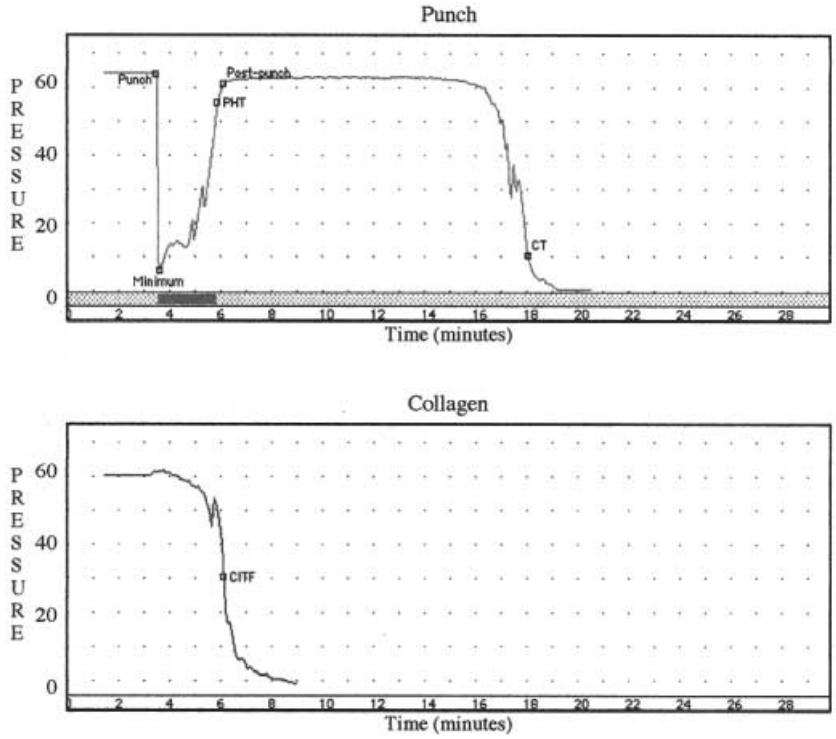


**Fig. 1.** Top: Representative Clot Signature Analyzer (CSA) tracing of a normal subject showing loss of pressure when the tube is punctured, normalization vof pressure when a platelet plug fills the puncture (PHT), and complete occlusion of the tube by subsequent clot (CT). Bottom: Representative tracing of a normal subject showing collagen-induced thrombus formation (CITF).

was analyzed based upon the printout of the results. CSA tests on the subjects in the normal range study were performed in duplicate. Test subjects in the other study were tested once.

### *Data analysis*

Normal reference ranges for each CSA measurement at each institution were calculated as 2 SD from the mean [19]. Analysis of variance was determined on the reference range study using an Excel spreadsheet. Sensitivity and specificity were calculated as described [19].

## Results

### *Normal ranges*

A total of 172 normal subjects were tested at the four institutions (range 39–53 per institution) (Table 1). Analysis of variance showed no statistical difference between the centers for PHT ($P = 0.12$) or for CITF ($P = 0.09$). CT showed somewhat greater variability ($P = 0.05$). Because of the borderline variability, subsequent normal and patient values were analyzed using the normal range obtained at each institution. The time from phlebotomy to testing varied from just under 1 min to about 2 min. No effect on CSA testing could be discerned. No tests were unable to be performed because of clotting prior to CSA testing.

### *Patient study*

A total of 108 patients were tested at the four institutions (Table 2). These patients included 46 individuals with VWD, 38 subjects with hereditary coagulation factor deficiency, 13 patients with hereditary platelet function defects, and 11 individuals with various other hereditary and acquired coagulation abnormalities. As described in Methods, all

**Table 1** Normal Clot Signature Analyzer (CSA) reference ranges for each center

| Center | Subjects (male/female) | Age (range) | PHT (range) | CT (range) | CITF (range) |
|---|---|---|---|---|---|
| 1 | 41 (14/27) | 37 (20–58) | 6.0 (2.6–9.4) | 23.9 (14.7–33.1) | 5.8 (3.6–8.0) |
| 2 | 52 (25/27) | 30 (19–71) | 6.8 (2.2–11.4) | 22.9 (14.1–31.7) | 5.8 (4.0–7.2) |
| 3 | 40 (20/20) | 39 (25–58) | 6.3 (1.7–10.9) | 22.9 (14.7–29.9) | 5.8 (3.4–8.2) |
| 4 | 39 (19/20) | 37 (22–54) | 5.9 (2.3–9.5) | 21.3 (14.5–28.1) | 5.4 (3.6–7.2) |

Included are the number of subjects, their sex, and age. Values for platelet hemostasis time (PHT), clot time (CT), and collagen-induced thrombus formation (CITF) are the mean and range (±2 SD) in minutes. $P = 0.12$ for PHT; $P = 0.05$ for CT; $P = 0.09$ for CITF.

**Table 2** Results of Clot Signature Analyzer (CSA) tests in patients with hereditary and acquired hemostasis disorders (number of patients)

| Disorder | Patients | Abnormal CSA | Abnormal PHT | Abnormal CT | Abnormal CITF |
|---|---|---|---|---|---|
| VWD-1 | 26 | 22 | 14 | 14 | 9 |
| VWD-2A | 11 | 11 | 9 | 6 | 11 |
| VWD-2B | 6 | 6 | 3 | 3 | 6 |
| VWD-3 | 3 | 3 | 3 | 2 | 2 |
| All VWD | 46 | 42 | 29 | 25 | 28 |
| FVIII def. | 22 | 20 | 15 | 18 | 10 |
| FIX def. | 9 | 9 | 6 | 9 | 4 |
| FVII def. | 1 | 1 | 0 | 1 | 0 |
| FXI def. | 4 | 4 | 4 | 4 | 3 of 3 |
| FXII def. | 1 | 1 | ND | ND | 1 |
| FXIII def. | 1 | 0 | 0 | 0 | 0 |
| All deficiencies | 38 | 35 | 25 of 37 | 32 of 37 | 18 of 36 |
| Glanzmann's | 2 | 2 | 2 | 2 | 2 |
| SPD/HPS | 3 | 2 | 2 | 2 | 1 |
| SPD/not HPS | 3 | 2 | 2 | 2 | 2 |
| PFD, NOS | 5 | 3 | 0 | 2 | 3 |
| Total | 13 | 9 | 6 | 8 | 8 |
| Warfarin | 10 | 9 | 1 | 8 | 5 |
| LMW heparin | 1 | 1 | 1 | 1 | 0 |
| Total | 11 | 10 | 2 | 9 | 5 |
| Total | 108 | 96 | 62 of 107 | 74 of 107 | 59 of 106 |

VWD, Von Willebrand Disease; Glanzmann's, Glanzmann's thrombasthenia; SPD/HPS, storage pool disease/Hermansky–Pudlak Syndrome; PFD/NOS, platelet function defect, not otherwise specified; LMW heparin, low-molecular-weight heparin; PHT, platelet hemostasis time; CT, clot time; CITF, collagen-induced thrombus formation.

patients with normal coagulation tests at the time of CSA testing were excluded from the study.

### *Von Willebrand disease*

Forty-two of the 46 patients with VWD had at least one abnormal CSA result (Table 2). All four of the patients that were not detected as abnormal by the CSA have type 1 VWD, three of whom are mildly affected. All type 2A, type 2B, and type 3 patients were recognized by at least one abnormal CSA test. Overall, the sensitivity of any abnormal CSA test for VWD was 91%. However, no individual test performed by the CSA was particularly sensitive (PHT 63%; CT 54%; CITF 61%).

### *Coagulation factor deficiencies*

Thirty-five of the 38 patients with a coagulation factor defect had at least one abnormal CSA test for an overall sensitivity of 92% (Table 2). The CT appeared to be the most sensitive of the three assays since it was abnormal in 32 of the 37 patients (86%) in which it was performed. Neither the PHT nor the CITF were as sensitive. The PHT was abnormal in 25 of the 37 (68%) patients and the CITF was abnormal in 18 of the 36 (50%) patients tested. Two patients with factor VIII:C deficiency had all normal CSA results, whereas all the other factor-deficient patients, with the exception of the patient with factor (F)XIII deficiency, had at least one abnormality. No clear-cut correlation between factor level and CSA test results was found; some patients with severe factor deficiency had little to no prolongation of CSA tests, whereas some CSA tests were significantly prolonged in patients with mild factor deficiency.

**Table 3** Clot Signature Analyzer (CSA) results in normal subjects (number of patients)

| Normals | Abnormal CSA | Abnormal PHT | Abnormal CT | Abnormal CITF |
|---|---|---|---|---|
| 116 | 13 | 1 | 5 | 7 |

PHT, Platelet hemostasis time; CT, clot time; CITF, collagen-induced thrombus formation.

### *Platelet function disorders*

Thirteen patients with various platelet function disorders were examined (Table 2). Of these, nine had at least one abnormal CSA test for a sensitivity of 69%. Both patients with Glanzmann's thrombasthenia were detected, as were four of six patients with a storage pool disorder. Three of five patients with 'platelet function defect, not otherwise classified' had abnormal CSA results. No individual CSA measurement appeared to be more sensitive in recognizing platelet function defects (PHT 46%; CT 62%; CITF 62%).

### *Other disorders*

Ten patients on long-term, stable warfarin therapy were tested, of whom nine had abnormal CSA results (Table 2). The CT test was the most sensitive to warfarin therapy. One patient receiving low-molecular-weight heparin was examined and had an abnormal CSA.

### *Normal study*

One hundred and sixteen concurrent normal individuals were also tested with the CSA during the patient study (Table 3). These included many of the subjects used in the normal range study. All of these subjects were demonstrated to be normal at the time of sample collection for CSA by other standard coagulation and platelet function tests. Thirteen (11%) of the 116 had at least one abnormal CSA result for a specificity of 89%. The CITF assay was found to be the most frequently abnormal test in this group.

## Discussion

We have carried out a multicenter study of the performance of the CSA to establish normal ranges and to assess both patients with previously diagnosed heritable or acquired coagulation disorders and normal subjects.

The normal ranges reported by each institution were similar overall with borderline statistically significant differences between the CT and CITF. This finding is encouraging, given

the well-recognized discrepancies that have been reported between laboratories for tests such as the bleeding time, PT, and APTT [20,21]. Additional experience with the CSA may reduce the interlaboratory variation. Because of the differences between the CSA normal ranges, test results on patients with previously diagnosed coagulation abnormalities were interpreted using each laboratory's normal range.

The CSA detected 89% of the heritable and acquired hemostatic abnormalities. This finding included 91% of patients with VWD, 92% of patients with a coagulation factor deficiency, 69% of patients with platelet function defects, 90% of patients taking warfarin, and the one patient receiving low-molecular-weight heparin. As expected, the CSA was normal in one patient with FXIII deficiency. The specificity for each type of coagulation abnormality is obviously quite low. On the other hand, the CSA was normal in 103 of the 116 normal subjects, yielding a specificity of a negative test for no coagulation abnormality of 89%.

The performance of the individual measurements, PHT, CT, and CITF, is harder to assess but appears to be less robust. The CT was abnormal in 92% of the coagulation factor-deficient patients and in eight of 10 patients taking warfarin. This result presumably reflects its proposed role in measuring fibrin clot formation. In contrast, neither the PHT nor the CITF, tests supposedly sensitive to platelet function disorders, were more sensitive to platelet abnormalities than the CT, although the number of patients tested was small.

We did not directly compare the CSA with standard screening tests, but these are well known to lack sensitivity and specificity. The bleeding time, for example, is abnormal in only about 46% of patients with storage pool disease and in about 50% of patients with documented VWD [17,18,22]. Similarly, other screening tests such as the PT and APTT have well-recognized limitations [23]. Other instruments, including the PFA-100, thromboelastograph, and Hemodyne Platelet Analysis System, have been designed to assess all or part of the hemostatic system. While they show promise, there are still unresolved validation issues with each.

Tests with more specificity, such as ristocetin cofactor activity and von Willebrand factor antigen, show considerable temporal variation and may, on occasion, be normal in patients ultimately shown to have VWD [24]. Platelet aggregation testing is highly sensitive to agonist concentration, platelet preparation, and numerous other factors and not uncommonly gives false-positive or false-negative results and misses up to 25% of platelet release defects [25–28]. By comparison, the CSA, which was abnormal in 89% of patients with bleeding disorders and normal in 89% of normal subjects, performed quite well.

It is unclear what role the CSA could play in coagulation testing. Our results indicate that it may be valuable as a screening instrument, although in some aspects it falls short in this regard. The ideal screening test should consistently identify patients with possible bleeding disorders and provide direction as to further evaluation. Unfortunately, the CSA, despite its overall good sensitivity, does not adequately distinguish between platelet and coagulation factor disorders. This is interesting, in that the different CSA tests were specifically intended to assess platelet function and coagulation function as discrete activities. The cause of this failure is not clear. Possible explanations could include the attempt to compartmentalize a process that functions as a continuum, and use of a non-physiological substrate (a plastic tube) as the vascular component.

The instrument itself is easy to use, but is not without problems: mainly, the requirement for non-anticoagulated whole blood. Although it has some intellectual appeal, it presents a significant practical issue in that the blood must be tested within 3 min. Consequently, patients must come to the instrument to be tested as it is too large to be moved and must go through warming and calibrating procedures before use. This makes the CSA impractical for use on inpatients.

The large number of coagulation tests available, their high cost, and the frequent difficulty in interpreting the results have been a potent stimulus in the search for tests that are sensitive and specific and will appropriately direct further testing and treatment. Our results indicate that the CSA partially meets this goal, as it has good sensitivity to a variety of bleeding disorders. Its specificity appears to be low, since an abnormal result on any of the three measurements does not offer guidance with respect to further laboratory evaluation. In addition, the instrument uses non-anticoagulated whole blood that must be processed within minutes of venepuncture. Nonetheless, the technology appears sound and further development of this technology is appropriate.

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## References

1 Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 1984; **194**: 927–9.
2 White MM, Foust JT, Mauer AM, Robertson JT, Jennings LK. Assessment of lumiaggregometry for research and clinical laboratories. *Thromb Haemost* 1992; **67**: 572–7.
3 Mielke CH. Measurement of the bleeding time. *Thromb Haemost* 1984; **52**: 210–1.
4 Lind SE. The blooding time does not predict surgical bleeding. *Blood* 1991; **77**: 2547–52.
5 Rodgers RPC & Levin J. A critical reappraisal of the bleeding time. *Sem Thromb Haemost* 1990; **16**: 1–20.
6 Eika C, Havig O, Godal HC. The value of preoperative haemostatic screening. *Scand J Haematol* 1978; **21**: 349–54.
7 Eisenberg JM, Clarke JR, Sussman SA. Prothrombin and partial thromboplastin time as preoperative screening tests. *Arch Surg* 1982; **117**: 48–51.
8 Gravlee GP, Arora S, Lavender SW, Mills SA, Hudspeth AS, Cordell AR, James RL, Brockschmidt JK, Stuart JJ. Predictive value of blood clotting tests in cardiac surgical patients. *Ann Thorac Surg* 1994; **58**: 216–21.

9 Burk CD, Miller L, Handler SD, Cohen AR. Preoperative history and coagulation screening in children undergoing tonsillectomy. *Pediatrics* 1992; **89**: 691–5.
10 Suchman AL & Griner PF. Diagnostic uses of the activated partial thromboplastin time and prothrombin time. *Ann Int Med* 1986; **104**: 810–6.
11 Kundu SK, Heilmann EJ, Sio R, *et al*. Description of an *in vitro* platelet function analyzer—PFA-100$^{TM}$. *Semin Thromb Haemost* 1995; **21** (Suppl. 2): 106–12.
12 Jilma B. Platelet function analyzer (PFA-100): a tool to quantify congenital or acquired platelet dysfunction. *J Lab Clin Med* 2001; **138**: 152–63.
13 Salooja N & Perry DJ. Thrombelastography. *Blood Coagul Fibrinolysis* 2001; **12**: 327–37.
14 Carr ME Jr. Development of platelet contractile force as a research and clinical measure of platelet function. *Cell Biochem Biophys* 2003; **38**: 55–78.
15 Li CKN, Hoffmann TJ, Hsieh P-Y, Malik S, Watson WC. The Xylum Clot Signature Analyzer: a dynamic flow system that simulates vascular injury. *Thromb Res* 1998; **92**: S67–S77.
16 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.
17 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.
18 Kerenyi 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.
19 Elston RC & Johnson WD. *Essentials of Biostatistics*. Philadelphia, PA: FA Davis Co., 1987.
20 Ng VL, Valdes-Camin R, Gottfried EL, Echaves S, Stead A, Ebert RF. Highly sensitive thromboplastins do not improve INR precision. *Am J Clin Pathol* 1998; **109**: 338–46.
21 Ng VL, Levin J, Corash L, Gottfried EL. Failure of the International Normalized Ratio to generate consistent results within a local medical community. *Am J Clin Pathol* 1993; **99**: 689–94.
22 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.
23 Bajaj SP & Joist JH. New insights into how blood clots. Implications for the use of the aPTT and PT as coagulation screening tests and in monitoring of anticoagulant therapy. *Sem Thromb Hemost* 1999; **25**: 407–17.
24 Abildgaard CF, Suzuki, Harrison J, Hiller E. Serial studies in von Willebrands's disease: variability versus 'variants'. *Blood* 1980; **56**: 712–6.
25 Riess H, Braun G, Brehm G, *et al*. Critical evaluation of platelet aggregation in whole human blood. *Am J Clin Pathol* 1986; **85**: 50–6.
26 Day JH & Rao AK. Evaluation of platelet function. *Sem Hematol* 1986; **23**: 89–101.
27 Nieuwenhuis HK, Akkerman J-WN, Sixma JJ. Patients with a prolonged bleeding time and normal aggregation tests may have storage pool deficiency: studies on one hundred six patients. *Blood* 1987; **70**: 620–3.
28 Israels SJ, McNicol A, Roberson C, Gerrard JM. Platelet storage pool deficiency: diagnosis in patients with prolonged bleeding times and normal platelet aggregation. *Br J Haematol* 1990; **75**: 118–21.