Received: 12 March 2019 | Accepted: 11 April 2019

DOI: 10.1111/jth.14454

ORIGINAL ARTICLE

# Diagnostic utility of the ISTH bleeding assessment tool in patients with suspected platelet function disorders

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## Abstract

**Background:** Bleeding assessment tools (BATs) have been widely implemented in the evaluation of patients with suspected bleeding disorders. However, diagnostic BAT utility regarding platelet function disorders is still elusive.

**Aim:** We aimed to assess the diagnostic value of the International Society on Thrombosis and Haemostasis BAT (ISTH-BAT) for platelet function disorders in clinical practice.

**Methods:** The clinical characteristics and laboratory data of all consecutive patients with a suspected bleeding disorder referred between January 2012 and March 2017 to an outpatient unit of a university hospital were prospectively collected. The diagnostic evaluation was performed according to current recommendations following a prespecified protocol and platelet function was tested using light transmission aggregometry as well as flow cytometry.

**Results:** Five hundred and fifty-five patients were assessed; 66.9% were female, median age was 43.7 years (interquartile range [IQR] 29.3, 61.7). Confirmed platelet function disorder was diagnosed in 54 patients (9.7%), possible platelet function disorder in 64 patients (11.5%), and other disorders in 170 patients (30.6%). Median scoring of the ISTH-BAT was 2 in patients without a bleeding disorder (IQR 1, 3), 4 in patients with a possible platelet function disorder (2, 7), and 7 in patients with confirmed platelet function disorder (5, 9). Area under the receiver operating characteristic curve (the area under the curve [AUC]) was 0.75 (95% CI 0.70, 0.80).

**Conclusions:** Presence of a platelet function disorder was associated with substantially higher BAT scorings compared to patients without. Our data suggest that the ISTH-BAT provides a useful screening tool for patients with suspected platelet function disorders.

KEYWORDS

blood platelet disorders/diagnosis, hemorrhagic disorders/diagnosis, platelet function tests, predictive value of tests, prognosis, surveys and questionnaires

Manuscript handled by: David Lillicrap

Final decision: David Lillicrap, 11 April 2019

## 1 | INTRODUCTION

Platelet function disorders (PFDs) are among the most common hereditary bleeding disorders, exposing affected patients to the risk of bleeding, particularly in the context of trauma and medical interventions.[1,2] Diagnosing PFD is, however, cumbersome, and many patients are probably not identified.[3] While severe PFD such as Glanzmann thrombasthenia, Bernard-Soulier syndrome, or syndromic disorders are easier to recognize, diagnosis is much more elusive in the majority of PFD patients. The diagnostic evaluation requires laboratory assays that are technically challenging, time-consuming, and difficult to interpret.[4] These tests tend also to be available in specialized laboratories only.[3] In addition, complex preanalytic requirements often necessitate blood drawing on site at the laboratory rather than having the sample shipped from farther afield.[5,6] It is therefore important to select the patients for referral adequately.

Structured BATs such as from the International Society on Thrombosis and Haemostasis (ISTH-BAT) may be utilized as a screening instrument in specific patients and implemented in the diagnostic evaluation, thereby triggering a referral. The ISTH-BAT is an instrument to record both the presence and the severity of bleeding symptoms covering 14 important sites of bleeding in patients.[7,8] It has been validated extensively in patients with suspected von Willebrand disease (VWD).[9–11] The diagnostic utility of the ISTH-BAT with regard to platelet function disorders is, however, still elusive.[12] With respect to adult patients, it has never been studied within the appropriate target population, namely, consecutive patients referred for evaluation of a suspected bleeding disorder.

With the present study, we aimed to assess the diagnostic value of the ISTH-BAT of the International Society on Thrombosis and Haemostasis for platelet function disorders in a representative cohort of patients.

**Essentials**

- The utility of bleeding assessment tools regarding platelet function disorders is still elusive.
- We studied consecutive patients in a prospective cohort study in a tertiary hospital.
- Substantially higher scorings were observed in patients with platelet function disorders.
- Bleeding assessment tools might provide a useful screening tool.

## 2 | METHODS

### 2.1 | Study design, setting, and population

We included all patients older than 18 years of age referred with a suspected bleeding disorder to a specialized outpatient clinic of Inselspital University Hospital in Bern, Switzerland, between January 2012 and March 2017. Reasons for the referral were (a) a bleeding tendency, (b) a family history of bleeding disorders, or (c) abnormal laboratory test results. Inselspital Bern is a tertiary university hospital in Switzerland covering a region with 1.5 million inhabitants. The catchment area of the hospital is representative for Switzerland with regard to German-speaking and French-speaking residents, as well as patients living in either urban or rural areas. Inselspital University Hospital comprises the only specialized laboratory and its hematological outpatient clinic functions as a reference center for coagulation disorders.

Clinical data were prospectively recorded using an established in-house questionnaire as well as the ISTH-BAT sheet in the electronic hospital database. Laboratory data were stored accordingly. Patient files were coded using an in-house identification system and data were retrieved by two investigators working in parallel (MA/JK, MN). All patients signed informed consent forms and the ethics committee approved the study protocol (No. 02289).

### 2.2 | Evaluation of patients and determination of ISTH-BAT

The evaluation of patients was done using a standardized protocol as proposed in previous recommendations.[4,7,13–15] Before consultation, patients completed a 13-item, in-house questionnaire assessing the presence and severity of bleeding at specific organs, including the skin, nose, oral cavity, gastrointestinal and urogenital systems, joints and muscles, bleeding in association with minor injuries, dental procedures, surgery, transfusion requirements, bleeding after ingestion of drugs known to affect hemostasis, and family history.[16] During the consultation, trained resident physicians took detailed history using a standardized form and applied the ISTH-BAT.[7,8] The scores were applied prior to laboratory testing and physicians were not aware of any laboratory test results. The ISTH-BAT scorings were challenged by a second experienced physician (attending). Patients were instructed to stop anticoagulant treatment, antiaggregant treatment, nonsteroidal antirheumatic drugs, and/or selective serotonin reuptake inhibitors 10 days prior to consultation. Potential bleeding stigmata including petechia, hematomas, as well as signs of amyloidosis, telangiectasia, or joint hyperflexibility, were documented after a thorough physical examination.

### 2.3 | Handling of samples

To prevent preanalytical errors, a standardized protocol was implemented for blood drawing and preparation of blood samples.[17] Blood was collected by standard venipuncture with a 21-gauge needle in EDTA tubes (Monovette; Sarstedt, Nümbrecht, Germany) for blood cell count; in 0.106-mol/L trisodium citrate (9:1, v/v) tubes (Monovette; Sarstedt) for standard coagulation testing and platelet flow cytometric assays; and buffered citrate (0.13 mol/L trisodium citrate, pH 5.5) (Monovette; Sarstedt) for platelet aggregation studies. Samples were transported manually to the laboratory.

## 2.4 | Laboratory evaluation

Laboratory evaluation was done in a stepwise manner with the following initial tests conducted simultaneously: blood count with mean platelet volume, a blood smear analyzing platelet morphology, prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen concentration (Clauss's method); and coagulometric determination of factor II, factor V, factor VII, factor X, factor VIII, factor IX, factor XI, factor XIII, von Willebrand factor (VWF) antigen, VWF ristocetin cofactor activity, α2-antiplasmin, and platelet function analyzer 100. Secondary tests included light transmission aggregometry (LTA) and platelet flow cytometry if the initial test results were normal. In selected cases, chromogenic factor VIII, VWF multimer analysis, and VWF factor VIII binding capacity were also performed. Lumiaggregometry and molecular diagnostics were conducted only in a few cases. Determination of LTA and platelet flow cytometry are described later; all other laboratory tests are reported in detail in the Supplementary Material.[18]

## 2.5 | Determination of light transmission aggregometry and platelet flow cytometry

Light transmission aggregometry was done inline with current recommendations[5,19] and as previously described[16] using the aggregometer APACT® 4004V (LABiTec GmbH, Ahrensburg, Germany). Platelet aggregation was induced by increasing concentrations of adenosine diphosphate (ADP) (Sigma-Aldrich, St. Louis, MO; 4, 6, and 10 μmol/L for male patients and 3, 4, and 6 μmol/L for female patients); collagen (HORM®; Nycomed, Linz, Austria) at 1.5, 3, and 4 μg/mL; arachidonic acid at 2 mmol/L (Bio Data/Medonic Servotec AG, Interlaken, Switzerland); and ristocetin at 1.5 and 0.5 mg/mL (Sococchim SA, Lausanne, Switzerland). Platelet-rich plasma (PRP) was prepared (centrifuged at 150 *g* for 15 min) and platelet count was adjusted to $250 \times 10^9$/L. Then, 200 μL of PRP prewarmed at 37°C for 1 min was added to the aggregometer cuvette and run for an additional minute to exclude spontaneous aggregation; 20 μL of the agonist was added and the response was recorded. If the response to one agonist was outside the limits of the normal range, the test was repeated. The LTA was performed 1 h after collection of venous blood samples from the patient and was completed within 2.5 h. The in-house reference values have been previously established.[20] A sample from a healthy volunteer was analyzed as an internal control; LTA was not performed when the platelet count was <100 G/L.

Platelet flow cytometry was conducted as previously described.[16] Surface glycoproteins (GPs) were analyzed using antihuman antibodies: Ibα (CD42b-PE; Ibα; Dako), GPIIb/IIIa (CD41-FITC, Becton Dickinson; CD61-FITC, Becton Dickinson), baseline P-selectin expression (CD62P-PE, Becton-Dickinson), and PAC-1 binding (PAC1-FITC, Becton Dickinson). FACSCanto® (Becton Dickinson, Heidelberg, Germany) flow cytometer was used. The dose response of platelet reactivity was investigated with ADP (0.5, 5.0, and 50 μmol/L), convulxin (5, 50, and 500 ng/mL), and thrombin (0.05, 0.5, and 5 nmol/L) with anti-CD62P and PAC1. The surface expression of negatively charged phospholipids was investigated using Annexin V-FITC (Roche, Rotkreuz, Switzerland) after incubation with either Ionophore A 23187 or the combination of convulxin (500 ng/mL) and thrombin (5 nmol/L). To evaluate the content and secretion of dense granules, platelets were loaded with mepacrine (0.17 as well as 1.7 μmol/L) and analyzed with thrombin. The in-house reference values had been previously established.[16] As a control, a sample from a healthy volunteer was analyzed in parallel with each run. Flow cytometric analysis was repeated once with different control platelets to confirm the results.

## 2.6 | Definition of diagnoses

Bleeding disorders were diagnosed following current recommendations. Type 1 VWD was diagnosed with repeatable (two times) VWF:GPIbM levels of 0.05 to 0.4 U/mL and VWF:Ag of 0.05 to 0.4 U/mL, a VWF:GPIbM/VWF:Ag ratio of >0.7, a normal multimer pattern, and an appropriate bleeding history.[21–25] The threshold of 0.4 U/mL was chosen rather than a 0.3 in order to simplify treatment decisions in clinical practice.[26] Type 2 VWD was diagnosed according to ISTH criteria.[23] Low VWF was diagnosed in patients with VWF:GPIbM or VWF:Ag below 0.5 U/mL, not meeting the criteria mentioned, and associated with blood group O.[14] Hemophilia and other single-factor deficiencies were diagnosed according to current definitions.[27]

Interpretation of LTA and flow cytometry was done according to previous recommendations and established in-house reference ranges [16] by three experienced individuals; discrepancies were resolved by discussion.[3,4,6,28–30] Lumiaggregometry was additionally considered if available (in a few patients only). We categorized PFD into "confirmed platelet function disorder" in cases with repeated abnormal LTA and/or flow cytometry measurements in the absence of other disorders and "possible platelet function disorder" if only one measurement was available or there were inconclusive results, or concomitant disorders were present. Patients were categorized into one of the following PFD subgroups: (a) Glanzmann's thrombasthenia, defined as a defect in GPIIb/IIIa associated with a severely diminished aggregation of all agonists except ristocetin, reduced expression of GPIIb/IIIa, and/or markedly reduced activation of PAC1-binding[1,3,31,32]; (b) Gi-like defects, defined as an accentuated deficiency in aggregation to the Gi-coupled receptor antagonists ADP and adrenaline, associated with corresponding flow cytometry results[1,3,32]; (c) thromboxane $A_2$ pathway defects, defined as an absent aggregation in response to arachidonic acid, and possibly associated with an impaired response to other agonists[1,3,19,31,32]; (d) dense granule secretion defects, defined as a defect in storage and/or secretion of mepacrine[1,3,16,31,32]; (e) collagen receptor defects, defined as an isolated reduction in aggregation and secretion after stimulation with collagen and convulxin[1,16,32]; (f) α-granule disorders, defined as a reduced expression and/or secretion of P-selectin, associated with varying impaired aggregation after stimulation with collagen and epinephrine [1,16]; (g) decreased generation of procoagulant (COAT) platelets, defined as an impaired binding of Annexin-V after incubation with convulxin and thrombin[16]; (h) complex disorders, defined as defects in a

number of agonists (LTA) and/or several flow cytometry results that cannot be attributed to any of the disorders mentioned.

We defined a "bleeder of undefined cause" as a patient with an abnormal ISTH-BAT (male ≥4 points; female ≥6 points) if results of all other tests mentioned in the diagnostic evaluation were normal and no bleeding disorder was identified.[33–35] Patients who did not have hemostatic disorders but did have systemic disorders associated with bleeding symptoms (e.g. hereditary telangiectasia and thrombocytopenia) were categorized as having "systemic disorders."

### 2.7 | Statistical analysis

Descriptive statistics were used to characterize the study population (numbers/percent or median/interquartile range as appropriate). To evaluate the ISTH-BAT ability to discriminate between patients with or without a demonstrable platelet defect on platelet function testing, a receiver-operating characteristic curve analysis was performed and simple logistic regression was computed with the Stata 14.2 statistics software package (StataCorp. 2014. Stata Statistical Software: Release 14 College Station, TX: StataCorp LP). Figures were created using Prism 6 (GraphPad Software, Inc., La Jolla, CA).

## 3 | RESULTS

### 3.1 | Patient characteristics

Between January 2012 and March 2017, 555 patients were referred with a suspected bleeding disorder and were included in the study cohort (Figure 1A). Patients were referred from general practitioners in 200 cases (36.0%), from gynecologists in 121 (21.8%), and from other medical specialists in 207 cases (37.2%). The reason for referral was a bleeding tendency in 453 cases (81.6%), abnormal coagulation tests in 35 cases (6.3%), family history in 42 cases (7.6%), and verification of a known hemostatic disorder (reevaluation) in 8 cases (1.5%). The median age was 42.9 years (interquartile range [IQR] 28.0, 64.8) and 371 patients were female (66.9%). Of all patients, 55 were being treated with antiplatelet drugs (9.9%; stopped 10 days before assessment) and 34 were receiving anticoagulant treatment (6.1%; predominantly vitamin K-antagonists). The ISTH-BAT was abnormal in 153 patients referred for a bleeding tendency (35%) and in 156 patients referred for any reason (28.1%). Detailed patient characteristics are displayed in Table 1.

### 3.2 | Type of bleeding disorders

A bleeding disorder was diagnosed in 288 patients (51.9%; Figure 1; Table 1). The underlying type of bleeding disorder was a possible platelet function disorder in 64 cases (11.5%), confirmed platelet function disorder in 54 cases (9.7%), von Willebrand disease and low von Willebrand factor in 50 cases (9.1%), mild hemophilia in 5 cases (0.9%), deficiency of other coagulation factors in 7 cases (1.3%; including 6 patients with factor XI deficiency and 1 patient with factor X deficiency), fibrinogen disorders in 6 cases (1.1%), disorders of fibrinolysis in 7 cases (1.3%; including 1 patient with α2-antiplasmin deficiency, 3 patients with PAI1 deficiency, and 3 patients with

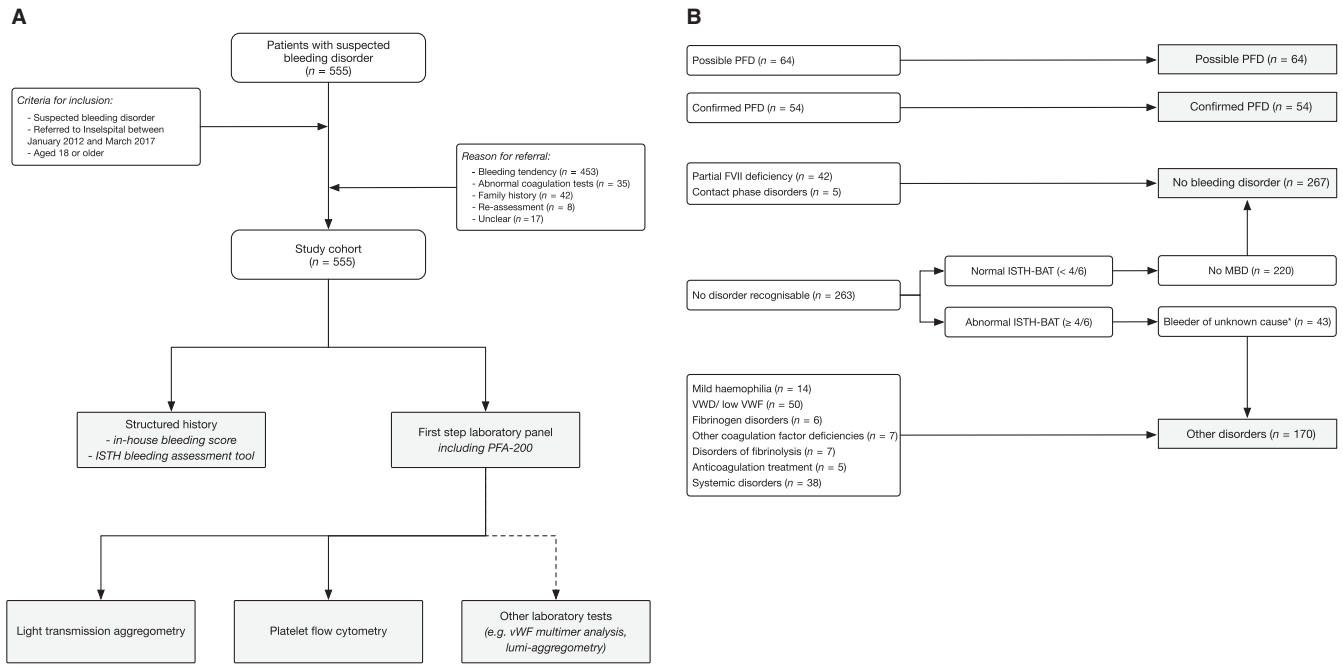


**FIGURE 1** A, Flow of the patients. The clinical characteristics and laboratory data of all consecutive patients referred between January 2012 and March 2017 to an outpatient unit of a university hospital with a suspected bleeding disorder were collected prospectively. The diagnostic evaluation was performed according to current guidelines and platelet function was tested using light transmission aggregometry as well as flow cytometry. B, Classification of patients according to diagnosis. FVII, factor VII; MBD, mild bleeding disorder; PFD, platelet function disorder; VWD, von Willebrand disease; VWF, von Willebrand factor

**TABLE 1** Characteristics of patients referred with a suspected bleeding disorder (n = 555; 2012-2017)

| Characteristics | Numbers (%) or median (IQR) as appropriate | | | | |
|---|---|---|---|---|---|
| | No bleeding disorder | Possible platelet function disorder[a] | Confirmed platelet function disorder[a] | Other bleeding disorders | Missing data |
| Patients | 267 (48.0) | 64 (11.5) | 54 (9.7) | 170 (30.6) | 0 |
| Age (y) | 40.2 (27.3, 60.4) | 49.3 (34.9, 63.9) | 49.8 (33.5, 64.0) | 44.3 (30.7, 61.7) | 0 |
| Sex | | | | | |
| Female | 186 (69.7) | 52 (74.3) | 32 (66.7) | 101 (59.4) | 0 |
| Male | 81 (30.3) | 18 (25.7) | 16 (33.3) | 69 (40.6) | 0 |
| Reason for referral | | | | | |
| Bleeding tendency | 191 (75.5) | 63 (98.4) | 51 (94.4) | 148 (88.6) | 17 |
| Abnormal coagulation test results | 25 (9.9) | 1 (1.6) | 0 (0) | 9 (5.4) | |
| Family history | 33 (13.0) | 0 (0) | 3 (5.6) | 6 (3.6) | |
| Reevaluation | 4 (1.6) | 0 (0) | 0 (0) | 4 (2.4) | |
| Referring physician | | | | | |
| General practitioner | 90 (35.9) | 22 (32.8) | 20 (42.6) | 68 (41.7) | 27 |
| Gynecologist | 51 (20.3) | 18 (26.9) | 18 (38.3) | 34 (20.9) | |
| Other specialist | 110 (43.8) | 27 (40.3) | 9 (19.2) | 61 (37.4) | |
| Antiaggregant treatment | 23 (9.5) | 11 (17.2) | 3 (6.8) | 17 (10.8) | 47 |
| Anticoagulant treatment | 13 (4.9) | 3 (4.3) | 2 (4.2) | 16 (9.4) | 3 |
| SSRI treatment | 16 (6.0) | 8 (11.4) | 4 (8.3) | 7 (4.1) | 57 |
| VWF:C | 110 (82, 136) | 101 (79, 136) | 98 (79, 133) | 93 (57, 134) | 72 |
| VWF activity | 107 (81, 131) | 99 (72, 126) | 100 (75, 137) | 84 (55, 126) | 73 |
| Platelet count | 235 (200, 267) | 256 (223, 307) | 235 (186, 270) | 227 (184, 273) | 4 |

Abbreviations: IQR, interquartile range; SSRI, selective serotonin reuptake inhibitors; VWF:C, von Willebrand factor antigen.
[a]Diagnosis of a platelet function disorder was made using light transmission aggregometry and platelet flow cytometry. "Confirmed platelet function disorder" was defined as abnormal results in repeated light transmission aggregometry/flow cytometry measurements in the absence of other disorders, "possible platelet function disorder" as an abnormal result in one measurement available, inconclusive results or presence of concomitant disorder.

abnormal clot lysis time) anticoagulant treatment in 5 cases (0.9%), and a systemic disorder in 38 cases (6.9%).

### 3.3 | Subgroups of PFD

Of 118 patients with a possible PFD, a confirmed subgroup could be established in 54 patients (46%). In contrast, diagnostic evaluation was not conclusive in 64 patients (54%). Distributions of PFD subgroups according to these two diagnostic groups are shown in Table 2. Gi-like defects were found most frequently (32%), followed by complex disorders (28%), and diminished procoagulant COAT platelets (8%).

### 3.4 | Results of ISTH-BAT scorings according to diagnosis

The median value of the ISTH-BAT bleeding score in patients without a bleeding disorder was 2 (IQR 1, 4). In contrast, the ISTH-BAT bleeding score was 4 (IQR 3, 7) in patients with a possible platelet function disorder and 7 (IQR, 5, 9) in patients with a confirmed platelet function disorder (Table 3; Figure 2). The score was 4 (IQR 2, 7) in patients with low VWF/VWD and 4 (IQR 1, 8) in patients with other coagulation disorders. Patients with systemic disorders associated with bleeding symptoms had a median score of 3 (IQR 1, 4).

### 3.5 | Predictive value of ISTH-BAT

The ROC curve analysis demonstrated an area under the curve (AUC) of 0.75 (95% CI 0.70, 0.80; Figure 3). In male patients only, AUC was 0.77 (0.68, 0.86); in female patients only, AUC was 0.74 (0.67, 0.80). At a threshold of 4 (males), sensitivity was 76.9% (95% CI 68.2, 84.2), and specificity was 62.4% (57.6, 67.0). At a threshold of 6 (females), sensitivity was 52.1% (42.7, 61.5), and specificity was 86.1% (82.4, 89.2).

## 4 | DISCUSSION

In our cohort of consecutive patients assessed for a suspected bleeding disorder in clinical practice, we identified 54 patients with a confirmed PFD (9.7%) and 64 patients with a possible PFD (11.5%). Results

**TABLE 2** Platelet function disorder subgroups

| Disorder | Numbers (%) | |
|---|---|---|
| | Possible PFD (n = 64) | Confirmed PFD (n = 54) |
| Glanzmann's thrombasthenia[a] | 0 | 4 (7.4) |
| Gi-like defects[b] | 20 (31.3) | 18 (33.3) |
| TxA2 pathway defects | 13 (20.3) | 1 (1.9) |
| Collagen receptor defects[d] | 6 (9.4) | 2 (3.7) |
| Dense granule disorders[e] | 1 (1.6) | 4 (7.4) |
| α-granule disorders[f] | 1 (1.6) | 5 (9.3) |
| Diminished procoagulant COAT platelets[g] | 3 (4.7) | 7 (13.0) |
| Complex disorders[h] | 20 (31.3) | 13 (24.1) |

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; LTA. Light transmission aggregometry; PFD, platelet function disorder.

[a]PFD subgroups were defined as follows: Defect in GPIIb/IIIa associated with a severely diminished aggregation of all agonists except ristocetin, reduced expression of GPIIb/IIIa, and/or markedly reduced activation of PAC1-binding.[1,3,31,32]

[b]Accentuated deficiency in aggregation to the Gi-coupled receptor antagonists ADP and adrenaline, associated with corresponding flow cytometry results.[1,3,32]

[c]Absent aggregation in response to arachidonic acid, and possibly associated with an impaired response to other agonists.[1,3,19,31,32]

[d]Isolated reduction in aggregation and secretion after stimulation with collagen and convulxin.[1,16,32]

[e]Defect in storage and/or secretion of mepacrine.[1,3,16,31,32]

[f]Reduced expression and/or secretion of P-selectin, associated with varying impaired aggregation after stimulation with collagen and epinephrine.[1,16]

[g]Impaired binding of Annexin-V after incubation with convulxin and thrombin.[16]

[h]Defects in a number of agonists (LTA) and/or several flow cytometry results that cannot be attributed to any of the disorders mentioned above.

of ISTH-BAT were substantially higher in patients with possible and confirmed PFD compared to patients without a bleeding disorder. The predictive value of ISTH-BAT for the presence of PFD was good.

Our results are essentially in line with previous investigations conducted in different settings and using other study designs. In a subgroup of patients studied in a pediatric setting, the ISTH score was higher in 5 patients with a possible PFD (median 3) compared to patients without a bleeding disorder (median 1).[30] Rashid and colleagues compared ISTH-BAT scorings between patients with suspected PFD and healthy volunteers in Pakistan and found a statistically significant difference (median 2 versus median 0).[36] Lowe at al. studied 79 patients with excessive bleeding and 21 healthy volunteers and compared ISTH-BAT according to lumiaggregometry results (adenosine triphosphate release). The ISTH-BAT was significantly higher in patients with abnormal ATP release compared to patients without (median 12 versus median 0 points).[37] Perez et al calculated ISTH-BAT retrospectively in 61 patients with thrombocytopenia and/or suspected PFD and found an association between abnormal ISTH-BAT and LTA results.[38] Kaur and colleagues compared ISTH-BAT scorings between 48 patients with Glanzmann's thrombasthenia or Bernard-Soulier syndrome and healthy controls and found a significant difference.[39]

In contrast to previous investigations, we studied the ISTH-BAT in a population that represents an unselected test target population and comprising all patients referred with a suspected bleeding disorder. Patients were consecutively included, preventing any selection bias. The number of patients studied was significantly higher than in previous investigations. We studied adult Caucasian patients and the evaluation was performed using a prespecified protocol including LTA as well as platelet flow cytometry. Our study does, however, have several limitations. First, the diagnostic evaluation was not finished appropriately in a number of patients resulting in a high proportion of patients with "suspected PFD." Such patients were reluctant to appear several times in the outpatient unit and this has been encountered in clinical practice; we analyzed patients with suspected and confirmed PFD separately and a similar association was observed in both groups. In our setting, tests beyond LTA and flow cytometry were available in some cases only (e.g. molecular diagnostics, ATP release, expanded LTA agonist panel, fluorescence

**TABLE 3** ISTH-BAT scorings according to disease in patients referred with a suspected bleeding disorder (n = 555)

| Disorder | Median score (IQR) | | |
|---|---|---|---|
| | All patients | Female patients | Male patients |
| No bleeding disorder | 2 (1, 3) | 2 (1, 4) | 2 (1, 3) |
| Possible platelet function disorder[a] | 4 (2, 7) | 4 (2, 8) | 4 (2, 6) |
| Confirmed platelet function disorder[a] | 7 (5, 9) | 7 (5, 11) | 6 (4, 8) |
| VWD/low VWF[b] | 4 (2, 6) | 4 (3, 6) | 4 (2, 6) |
| Other disorder[c] | 4 (3, 7) | 6 (3, 8) | 4 (2, 5) |

Abbreviations: IQR, interquartile range; ISTH-BAT, International Society on Thrombosis and Haemostasis bleeding assessment tool; LTA, light transmission aggregometry; VWD, von Willebrand disease; VWF, von Willebrand factor.

[a]Diagnosis of platelet function disorder was determined using light transmission aggregometry and platelet flow cytometry. "Confirmed platelet function disorder" was defined as abnormal results in repeated LTA/flow cytometry measurements in the absence of other disorders, "possible platelet function disorder" as abnormal result in one measurement available, inconclusive results or presence of concomitant disorders.

[b]Low von Willebrand values associated with blood group 0; von Willebrand disease type 1 or type 2.

[c]Systemic disorder associated with bleeding symptoms such as hereditary hemorrhagic telangiectasia, and thrombocytopenia.

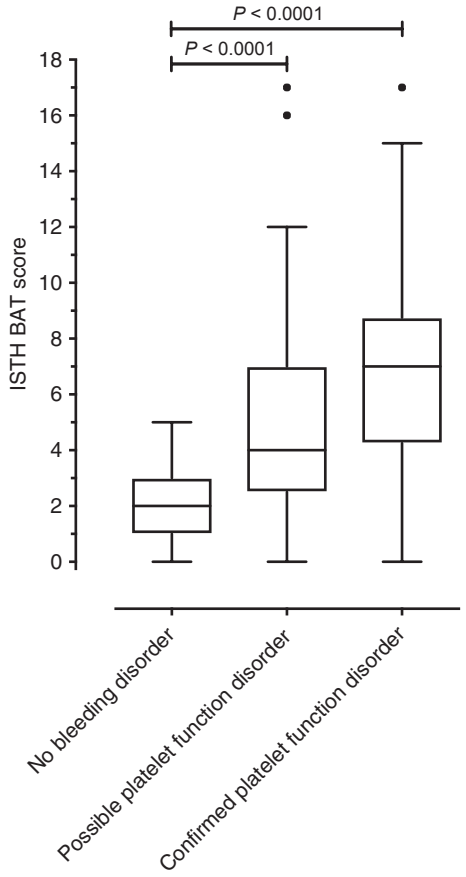


**FIGURE 2** Results of the ISTH-BAT according to the presence of a platelet function disorder. The ISTH-BAT was conducted in the evaluation of 555 consecutive patients referred with a suspected bleeding disorder. Diagnosis of platelet function disorder was made using light transmission aggregometry and platelet flow cytometry. "Confirmed platelet function disorder" was defined as abnormal results in repeated LTA/flow cytometry measurements in the absence of other disorders, "possible platelet function disorder" as abnormal result in one measurement available, inconclusive results or presence of concomitant disorders. The Mann-Whitney U test was applied. ISH-BAT, International Society on Thrombosis and Haemostasis bleeding assessment tool; LTA, light transmission aggregomtery

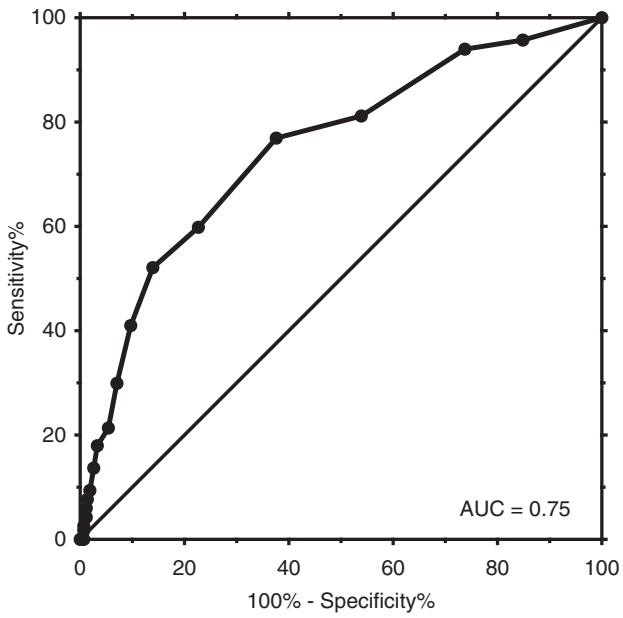


**FIGURE 3** The receiver operating characteristic (ROC) curve of ISTH-BAT for the presence of confirmed platelet function disorder. Area under the ROC curve (AUC) was 0.75 (95% CI 0.70, 0.80). At a threshold of 4, sensitivity was 76.9% (95% CI 68.2, 84.2) and specificity 62.4% (95% CI 57.6, 67.0). CI, confidence interval; IStH-BAT, International Society on Thrombosis and Haemostasis bleeding assessment tool
[Correction added on May 31, 2019 after first online publication: In Figure 3, the percentages of sensitivity at a threshold of 4 have been amended from a previous version.]

microscopy). Thus, the particular molecular defect in many patients could not be found. However, our results are in line with a previous study using ATP-release[37] and we do not anticipate that this introduced any bias.

Our results suggest that ISTH-BAT is a useful screening tool in patients with suspected PFD. This is particularly supported by the high sensitivity. The ISTH-BAT might be applied for triggering a referral of patients with any bleeding tendency at all (including patients with PFD as well as VWD), or for triggering platelet function studies in patients with normal first-line test results. We do not believe that the ISTH-BAT is able to discriminate different types of mild bleeding disorders, e.g. PFD from VWD, and we do not believe that the ISTH-BAT might replace platelet function studies. Our study was not designed to demonstrate a respective difference and two observations suggest the opposite: (a) the specificity is moderate only, suggesting a relevant number of patients with abnormal ISTH-BAT associated with other disorders than PFD and (b) a widely overlapping distribution of ISTH-BAT scorings among patients with PFD, VWD, and other disorders. This study must be replicated in other settings and employing alternative platelet function assays as well.

In conclusion, in a large study of patients referred for suspected bleeding disorders in clinical practice, patients with PFD had substantially higher ISTH-BAT scorings than patients without and the predictive value of ISTH-BAT was good. Our results suggest that ISTH is a useful screening tool for PFD in clinical practice.

## ACKNOWLEDGMENTS

We thank Prof. J. A. Kremer Hovinga for her valuable comments on the design and results of the study. No particular funding was obtained.

## CONFLICT OF INTERESTS

M. Nagler reports receiving: grants from the Swiss National Science Foundation during the conduct of the study and research grants, lecture honoraria, or consultancy fees from Bayer, Pentapharm, Daiichi Sankyo, and Roche diagnostics, outside the submitted work. The other authors state that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Marcel Adler and Jonas Kaufmann collected the data, participated in data analysis, and wrote the manuscript. Lorenzo Alberio implemented the evaluation of patients, reviewed the data analysis, and intellectually reviewed the manuscript. Michael Nagler designed the study, conducted the analysis, and wrote the manuscript. All authors approved the final version of the manuscript.

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**How to cite this article:** Adler M, Kaufmann J, Alberio L, Nagler M. **Diagnostic utility of the ISTH bleeding assessment tool in patients with suspected platelet function disorders**. *J Thromb Haemost*. 2019;17:1104–1112. https://doi.org/10.1111/jth.14454