Validation of the ISTH/SSC bleeding assessment tool for inherited platelet disorders
BAT-VAL study investigators; Harrison, Paul

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VALIDATION OF THE ISTH/SSC BLEEDING ASSESSMENT TOOL FOR INHERITED PLATELET DISORDERS: a communication from the Platelet Physiology SSC

Paolo Gresele[1], Sara Orsini[1], Patrizia Noris[2], Emanuela Falcinelli[1], Marie Christine Alessi[3], Loredana Bury[1], Munira Borhany[4], Cristina Santoro[5], Ana C Glembotsky[6,7], Ana Rosa Cid[8], Alberto Tosetto[9], Erica De Candia[10,11], Pierre Fontana[12], Giuseppe Guglielmini[1], Alessandro Pecci[2], BAT-VAL study investigators.

BAT-VAL study investigators (online author list):
Federica Melazzini[2], Céline Falaise[3], Alessandra Casonato[13], Gianmarco Podda[14], Meganathan Kannan[15], Kerstin Jurk[16], Teresa Sevivas[17], Giancarlo Castaman[18], Elvira Grandone[19], Mathieu Fiore[20], Pamela Zuniga[21], Yvonne Henskens[22], Koji Miyazaki[23], Arnaud Dupuis[24], Catherine Hayward[25], Carlo Zaninetti[2,26], Madiha Abid[4], Grazia Ferrara[5], Maria Gabriella Mazzucconi[27], Giuseppe Tagariello[28], Paula James[29], Fabrizio Fabris[30], Alexandra Russo[16,31], Nuria Bermejo[32], Mariasanta Napolitano[33], Jennifer Curnow[34], Gkalea Vasiliki[35], Barbara Zieger[36], Marian Fedor[37], Meera Chitlur[38], Michele Lambert[39], Luca Barcella[40], Benilde Cosmi[41], Paola Giordano[42], Claudia Porri[43], Ibrahim Eker[44], Marie-Christine Morel-Kopp[45], *Hans Deckmyn[46], *Andrew L. Frelinger III[47], *Paul Harrison[48], *Diego Mezzano[49], *Andrew D. Mumford[50]

[1]Department of Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia, Italy. [2]Department of Internal Medicine, IRCCS Policlinico S. Matteo Foundation, University of Pavia, Italy. [3]Centre for CardioVascular and Nutrition research (C2VN), INSERM 1263, INRA 1260, Marseille, France. [4]Department of Hematology, Haemostasis & Thrombosis at National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan. [5]Hematology, Azienda Ospedaliera Universitaria Policlinico Umberto I, Rome, Italy. [6]Instituto de Investigaciones Médicas A. Lanari, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. [7]Departamento Hematología Investigación, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad de Buenos Aires, Instituto de Investigaciones Médicas (IDIM), Buenos Aires, Argentina. [8]Unidad de Hemostasia y Trombosis, Hospital Universitario y Politecnico La Fe, Valencia, Spain. [9]Hematology Department, S. Bortolo Hospital, Vicenza, Italy. [10]Hemostasis and Thrombosis Unit, Fondazione Policlinico Agostino Gemelli IRCCS, Roma. [11]Institute of Internal Medicine and Geriatrics, Università Cattolica del Sacro Cuore, Roma. [12]Platelet Group and Division of Angiology and Hemostasis, University Hospitals of Geneva, Geneva, Switzerland. [13]Department of Medicine, First Chair of Internal Medicine, University of Padua Medical School, Padua, Italy. [14]Medicina III, ASST Santi Paolo e Carlo, Dipartimento di Scienze della Salute, Università degli Studi di Milano. [15]Division of Blood and Vascular Biology, Department of Life Sciences, School of Life Sciences, Central University of Tamil Nadu, Thiruvarur, India. [16]Center for Thrombosis and Hemostasis, University Medical Center, Mainz, Germany. [17]Servicio de Hematologia Clinica- Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal. [18]Department of Oncology, Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy. [19]Atherosclerosis and Thrombosis Unit, IRCCS Ospedale Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy. [20]Laboratory of Hematology , University Hospital of Bordeaux, Pessac, France. [21]Department of Pediatrics, Pontificia Universidad Catolica de Chile, Santiago, Chile. [22]Hematological Laboratory, Maastricht University Medical Centre, Maastricht, the Netherlands. [23]Department of Transfusion and Cell Transplantation Kitasato University School of Medicine, Sagamihara, Japan. [24]Université de Strasbourg, INSERM, EFS Grand Est, BPPS UMR-S 949, FMTS, F-67000 Strasbourg, France. [25]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada. [26]PhD Course of Experimental Medicine, University of Pavia, Italy. [27]Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy. [28]Transfusion Service, Haemophilia Centre and Haematology, Laboratory Analysis, Castelfranco Veneto Hospital, Castelfranco Veneto, Italy. [29]Department of Medicine, Queen's University, Kingston, Ontario, Canada. [30]Clinica Medica 1 - Medicina Interna CLOPD, Dipartimento Assistenziale Integrato di Medicina, Azienda-Ospedale Università di Padova, Dipartimento di Medicina, Università di Padova. [31]Department of Pediatric Hematology/Oncology/Hemostaseology, University Medical Center Mainz, Germany. [32]Hospital San Pedro de Alcantara, Caceres, Spain. [33]Università di Palermo, Italy. [34]Westmead Hospital, Sydney, Australia. [35]Tenon University Hospital, Paris, France. [36]University Medical Center Freiburg, Freiburg, Germany. [37]Svet Zdravia a.s., Slovakia. [38]Children's Hospital of Michigan, Detroit, USA. [39]Children's Hospital of Philadelphia, Philadelphia, USA. [40]Immunohematology and Transfusion Medicine (SIMT) & Hemostasis and Thrombosis Center ASST Papa Giovanni XXIII, Bergamo, Italy. [41]S.Orsola -Malpighi University Hospital, University of Bologna, Italy. [42]University of Bari, Italy. [43]Hospital Italiano, Rosario, Argentina. [44]Gulhane Military Medical Faculty, Ankara, Turkey. [45]Royal North Shore Hospital, Sydney, Australia. [46]Laboratory for Thrombosis Research, IRF Life Sciences, KU Leuven Campus Kulak Kortrijk, Kortrijk, Belgium. [47]Center for Platelet Research Studies, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA. [48]Institute of Inflammation and Ageing, University of Birmingham Medical School , Birmingham , UK. [49]Department of Hematology-Oncology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile. [50]School of Cellular and Molecular Medicine, University of Bristol, UK, United Kingdom. * *Steering committee members*

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Corresponding Author:
Paolo Gresele, MD, PhD
Division of Internal and Cardiovascular Medicine
Department of Internal Medicine
University of Perugia,
Centro didattico - Edificio B piano 1
Strada vicinale Via delle Corse - 06132 Perugia
tel. +39/075-5783989
fax. +39/075-5858439
e-mail: paolo.gresele@unipg.it

Words: 2749

ESSENTIALS

- The ISTH-BAT has been validated for VWD-1 but not sufficiently for inherited platelet disorders
- We tested the utility of the ISTH-BAT in patients with inherited platelet disorders in comparison with VWD-1 and controls
- The ISTH-BAT clearly distinguished inherited platelet function disorders (IPFD) from controls
- Patients with a BS>6 and preliminarily excluded VWD-1 have 99% probability of having an IPFD

SUMMARY

Background: Careful assessment of the bleeding history is the first step in the evaluation of patients with mild/moderate bleeding disorders, and the use of a bleeding assessment tool (BAT) is strongly encouraged. Although a few studies have assessed the utility of the ISTH-BAT in patients with inherited platelet function disorders (IPFD) none of them was sufficiently large to draw conclusions and/or included appropriate control groups.

Objectives: Aim of the present study was to test the utility of the ISTH-BAT in a large cohort of patients with a well-defined diagnosis of inherited platelet disorder in comparison with two parallel cohorts, one of patients with type-1 VWD (VWD-1) and one of healthy controls (HC).

Patients/Methods: We enrolled 1098 subjects, 482 of whom with inherited platelet disorders [196 IPFD and 286 inherited platelet number disorders (IT)] from 17 countries.

Results: IPFD patients had significantly higher bleeding score (BS) (median 9) than VWD-1 patients (median 5), a higher number of hemorrhagic symptoms (4 vs 3) and higher percentage of patients with clinically relevant symptoms (score >2).

The ISTH-BAT showed excellent discrimination power between IPFD and HC (0.9<AUC<1), moderate (0.7<AUC<0.9) between IPFD and VWD-1 and between IPFD and inherited thrombocytopenia (IT), while it is inaccurate (AUC≤0.7) in discriminating IT from HC.

Conclusions: in conclusion, the ISTH-BAT allows to efficiently discriminate IPFD from HC, while it has lower accuracy in distinguishing IPFD from VWD-1. Therefore, the ISTH-BAT appears useful to identify subjects requiring laboratory evaluation for a suspected IPFD once VWD is preliminarily excluded.

KEYWORDS: Bleeding assessment tool; inherited platelet disorders; bleeding diathesis; platelets; bleeding disorders

Introduction

An accurate assessment of the presence and severity of bleeding symptoms is critical in the clinical evaluation of patients referred for a possible bleeding disorder, especially those with mild bleeding diatheses [1]. In particular, a well-established mucocutaneous bleeding history is crucial for the decision to embark in complex and expensive laboratory studies [2]. The high frequency of bleeding manifestations in the normal population and the wide interindividual variability in the subjective appraisal of symptoms makes the identification of a real bleeding tendency rather difficult. A number of structured bleeding history questionnaires, called bleeding assessment tools (BATs), have been developed to standardize the collection of the bleeding history in order to improve diagnostic accuracy and sensitivity, more precisely describe symptom severity, inform treatment and possibly predict the future bleeding risk [3]. In particular, several BATs have been developed for type 1 von Willebrand disease (VWD-1), like the Vicenza BAT [4], the Molecular and Clinical Markers for the Diagnosis and Management of type 1 VWD (MCMDM-1 VWD) bleeding questionnaire [5], or the Pediatric Bleeding Questionnaire (PBQ) [6], and revealed to be able to distinguish between affected and unaffected subjects with good specificity and sensitivity [4, 6-8]. More recently, a prospective study in VWD patients demonstrated that the ISTH-BAT bleeding score (BS) is a predictor of bleeding outcomes helping to identify patients requiring intensive treatment [9].

The ISTH-BAT combines a standardized questionnaire designed to capture also recurrent minor but not trivial bleeds, and a well-defined interpretation grid allowing the computation of a final BS. It was developed as a consensus tool to record bleeding symptoms in patients with any suspected bleeding disorder [10] and it has been recently proposed by an International Working Group as the main tool to discriminate bleedings deserving investigation from not relevant hemorrhages [11].

Inherited platelet disorders are a heterogeneous group of bleeding diseases of variable clinical severity associated with a reduction of platelet number (inherited thrombocytopenias, IT) and/or function (inherited platelet function disorders, IPFDs).

A study applying the ISTH-BAT to a small cohort of adult patients with suspected IPFD supported its use for documenting lifelong bleeding history, but not for predicting defective platelet function. However, the study excluded patients with Glanzmann thrombasthenia (GT), Bernard-Soulier syndrome (BSS), MYH9-related disease (MYH9-RD) or Hermansky-Pudlak syndrome (HPS), therefore it did not provide information on a significant fraction of disorders [12].

On the other hand, another study conducted in a low-income country including 261 subjects with suspected IPFD showed that the ISTH-BAT is useful in documenting bleeding symptoms and is predictive of a platelet defect on light transmission aggregometry (LTA) [13].

An additional small study enrolling only BSS and GT cases showed that the ISTH-BAT discriminates patients from normal controls with good sensitivity and specificity [14].

Finally, recently the ISTH-BAT was applied to a wide group of subjects referred for a suspected bleeding disorder, 54 of whom had a confirmed and 64 a possible IPFD, and the presence of a platelet function disorders was associated with a higher BS [15].

Although altogether these studies enrolled a substantial number of patients, none of them was sufficiently large to draw conclusions on the utility of the ISTH-BAT for IPFD and no one systematically compared a well-defined population of individuals with inherited platelet disorders with VWD patients and healthy subjects.

Aim of the present study was to test the utility of the ISTH-BAT in a large cohort of patients with an established diagnosis of inherited platelet disorder in comparison with two parallel cohorts, one of VWD-1 patients and one of healthy controls (HC). In particular, we aimed to assess if the ISTH-BAT may be useful to discriminate between: 1) inherited platelet disorders and HC, 2) inherited platelet disorders and VWD-1 and 3) different inherited platelet disorders.

Methods

Inclusion criteria

This study was promoted by the Platelet Physiology SSC of the ISTH. The Institutional Review Board of the coordinating center approved the study (CEAS Umbria, Italy, n.2473/15), each center complied with local ethical rules, and all subjects or their legal representatives signed a written informed consent.

Enrolled subjects had to be living and available for direct history taking.

The study included only patients with a diagnosis of inherited platelet disorder confirmed according to well-defined laboratory and/or molecular genetic criteria [2, 16, 17] (**Supplementary Tables 1 and 2**). Both adult and pediatric (≤ 16 years old) subjects were enrolled.

Participating centers were asked to enroll for each patient an age- (±5 years) and sex- matched HC and an unequivocally diagnosed VWD-1 patient [18]. HC were defined as subjects in ostensible good health, never referred for hemostasis evaluation for hemorrhagic symptoms, with normal platelet count and whenever available, LTA, e.g. a subject used as control for platelet function testing.

Assessment of the bleeding history

A physician or another adequately trained health professional administered the questionnaire. All hemorrhagic symptoms and related treatments occurred until diagnosis had to be considered. The BS was calculated either manually, using the interpretation grid, or automatically using the web-based

version (https://bh.rockefeller.edu/ISTH-BATR/).

Bleeding history was assessed in parallel also using the World Health Organization (WHO) bleeding assessment scale [19], as described [16].

Statistical analysis

Based on previous data on the prevalence of hemorrhagic symptoms in VWD-1, patients with IPFD or IT and HC [7, 12, 20], we expected that a significant difference in the BS would be shown by enrolling at least 300 subjects per group ($\square$=0.8, $\square\square$0.05).

Data are reported as medians and 25th-75th percentiles (IQR) when continuous, and as counts and percentages when categorical. Interaction between BS and other variables were assessed using the Pearson correlation coefficient ($\rho\square$).

Kappa statistics was used to test interrater reliability [21]. Receiver operating characteristic (ROC) curves were calculated for diagnostic prediction rule to discriminate between different groups and area under curve (AUC), with binomial exact confidence interval for AUC, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for the analyzed populations were assessed. Cut-off values using the Youden index for the most relevant comparisons were also calculated. The R software (R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org) was used for all analyses. A two-sided p<0.05 was considered as statistically significant.

Results

Participants' characteristics

1098 subjects (age 1-93 years; median 39; IQR 25-52; 58.4% females) were enrolled by 44 centers across 17 countries **(Supplementary Figure 1):** 482 with inherited platelet disorders (43.9%) (196 IPFD and 286 IT), 303 with VWD-1 (27.6%) and 313 HC (28.5%) **(Supplementary Figure 2)**. Baseline characteristics are reported in **Supplementary Table 3**. Among inherited platelet disorders, 28 different forms (17 IPFD and 11 IT) were represented **(Supplementary Table 4)**.

Among IPFD the most widely represented were GT (40.3%), δ-granule defect (10.7%), primary secretion defect (10.2%), and biallelic BSS (bBSS) (9.7%), while among IT *MYH9*-RD (40.5%), *ANKRD26*-related thrombocytopenia (RT) (21.8%) and monoallelic BSS (mBSS) (19.4%). Thrombocytopenia of IT patients was on average mild (median 57.5x10^9/L; IQR 28-85).

Bleeding scores

The average BS assessed by the ISTH-BAT was clearly increased in IPFD (median 9, IQR 6-14), moderately increased in VWD-1 (median 5, IQR 2-8) and only minimally altered in IT (median 2, IQR 0-3) as compared with HC (median 0, IQR 0-1) (p<0.05 for all comparisons) (**Figure 1A**, **Supplementary Table 5**). Some disorders, such as gray platelet syndrome (GPS), GT, primary secretion defect (PSD), Quebec platelet disorder (QPD), $P2Y_{12}$-defect, Paris-Trousseau syndrome, showed a bleeding score higher than the average of the IPFD group (**Supplementary Table 4**).

Given that around 50% of IPFD were GT and bBSS, notoriously severe forms [22, 23], we recalculated the BS of IPFD group excluding these disorders and it was still significantly higher than VWD-1 (8; IQR 4-12, p<0.0001). The BS was significantly higher in females than males in all groups (p<0.01), due to the contribution of menorrhagia and post-partum hemorrhage, especially in VWD-1 and IPFD (**Figure 1B, Supplementary Table 5**). The BS correlated positively with age in the VWD-1 group (ρ= 0.2; p= 0.0003) and negatively with platelet count in the IT group (ρ=-0.19, p=0.0009).

In IPFD the median number of hemorrhagic symptoms was 4 (IQR 2-6), in VWD-1 3 (IQR 1-4), in IT 1 (IQR 0-2), and in HC 0 (IQR 0-1) (p<0.05 for all comparison) (**Figure 2A**). The mean score of the individual symptoms was also calculated. Epistaxis and oral cavity bleeding had the highest score in IPFD, cutaneous bleeding and menorrhagia in IT and epistaxis and menorrhagia in VWD-1. Post-surgical bleeding was significantly more severe in IPFD than in all the other groups, confirming previous results [16] (**Supplementary Figure 3**).

In IPFD 75% of patients had at least one symptom requiring medical treatment (score >2), in VWD-1 51.5%, in IT 20.6% and in HC 6.7% (**Figure 2B**). The most frequent symptom in the IPFD group was epistaxis, followed by cutaneous and oral cavity bleeding; in IT cutaneous bleeding, followed by epistaxis and oral cavity bleeding, in VWD-1 cutaneous bleeding, followed by menorrhagia and epistaxis, and in HC oral cavity bleeding, followed by menorrhagia and epistaxis (**Supplementary Figure 4, Supplementary Table 6**). The frequency of symptoms and percentage requiring medical treatment in specific disorders are shown in **Supplementary Table 7**.

The average BS assessed by the WHO bleeding scale (min 0, max 4) was clearly abnormal in IPFD (median 3, IQR 2-3), mildly abnormal in VWD-1 (1, IQR 1-2) and in IT (1, IQR 0-2) and normal in HC (0, IQR 0-0) (**Supplementary Table 5**). Disorders with the highest WHO-BS were GT (3, IQR 3-3), bBSS (3, IQR 2-3), QPD (3, IQR 3-3) and $cPLA_2$ deficiency (WHO-BS=3).

Sensitivity, specificity, positive and negative predictive values
The ISTH-BAT showed high accuracy (0.9<AUC<1) in discriminating IPFD from HC, moderate accuracy (0.7<AUC<0.9) in discriminating VWD-1 from HC, IT and IPFD from VWD-1, and IPFD from IT, while it was inaccurate (0.5<AUC≤0.7) in discriminating between IT and HC

(**Supplementary Table 8A**). The best cut-off discriminating IPFD from HC was >3 (>4 for females and >3 for males), IPFD from VWD-1 was >7 (>7 for females and >5 for males), IPFD from HC was >3 (>4 for females and >3 for males), and IPFD from IT was >5 (>6 for females and >3 for males) (**Table 1, Supplementary Table 8A**). Of note, a BS >6 had a PPV of 98.0% and a NPV of 85.0% to discriminate IPFD from HC (**Supplementary Table 9**). Inter-rater agreement analysis showed that a BS >6 is highly likely to be associated with an IPFD (Cohen's kappa=0.7922, p<0.0001).

The WHO-BS showed high accuracy in discriminating IPFD from HC, moderate in discriminating VWD-1 from HC, IPFD from IT and IT from HC, while it was inaccurate ($0.5 < AUC \leq 0.7$) in distinguishing IPFD and IT from VWD-1 (**Supplementary Table 8B**). The best cut-off discriminating IPFD from HC was >1, with a PPV of 78% and a NPV of 94% (**Supplementary Table 8B**).

The comparison of the AUCs revealed that the ISTH-BAT is more sensitive than the WHO-BS in discriminating IPFD and VWD-1 from HC, IPFD from IT, and VWD-1 from IT, while the WHO-BS was better in discriminating IT from HC (**Supplementary Table 8C**). Moreover, for some specific disorders the two scores gave poorly comparable results, e.g PSD had a WHO-BS of 1 and an ISTH-BAT BS of 7.5, Paris-Trousseau syndrome a WHO-BS of 1 and an ISTH-BAT BS of 14.5, TAR a WHO-BS of 1.5 and an ISTH-BAT BS of 10.5.

Pediatric population

Pediatric subjects were 12% of total (22.4% of IPFD, 10.8% of IT, 10.5% of VWD-1 and 7.9% of HC) (**Supplementary Table 10**). WHO and ISTH-BAT BS distribution in the four groups did not differ from the adult population, the only difference being that females and males had identical BS (**Supplementary Table 11**).

The ISTH-BAT showed high accuracy in discriminating pediatric IPFD from HC and from IT, moderate in discriminating VWD-1 from HC and IPFD, while it was inaccurate in discriminating IT from VWD-1 and HC. The best cut-off discriminating IPFD from VWD-1 was >7, while that discriminating IPFD from IT was >3 (**Table 1, Supplementary table 12**).

Discussion

Our study, representing the largest systematic investigation on the diagnostic utility of the ISTH-BAT in patients with inherited platelet disorders, shows that this tool has an excellent discrimination power

between IPFD and HC, with a NPV of 85% and a PPV of 98%. Thus for the populations analyzed in this study, if a subject with a mucocutaneous bleeding diathesis has an ISTH-BAT BS >6 (in our study these were 71% of IPFD patients) and preliminary laboratory screening has excluded VWD and blood clotting defects [2] he/she has ≥99% probability of being affected by an IPFD.

Our study also shows that the bleeding history of patients with IPFD is significantly more severe than that of patients with VWD-1 and much more than that of patients with IT. Also the median number of hemorrhagic symptoms was highest for IPFD (4), followed by VWD-1 (3) and lowest in IT (1) and HC (0). Moreover, the percentage of patients with clinically relevant symptoms (score >2) was significantly higher in IPFD than in all the other groups, including VWD-1.

Many forms of IT are associated with platelet function defects and their ISTH-BAT BS was high, similar to IPFD with normal platelet count. In this case the application of the ISTH-BAT may not support the hypothesis of a IT because the bleeding tendency is too severe. Therefore, in the presence of thrombocytopenia and a high ISTH-BAT an inherited disorder should not be excluded, laeding to mis-diagnosis of acquired thrombocytopenia, and a careful assessment of the medical history and blood cell morphology should be always be made [24].

Four previous studies have shown that the ISTH-BAT is useful to discriminate IPFD from HC [12-15], but none of them included a group of patients with VWD-1, i.e. the most frequent mucocutaneous bleeding disorder, and/or IT. Moreover, all the studies included small cohorts of patients and/or patients without a well-defined diagnosis. Our study enrolled 1098 well characterized subjects, including VWD-1, IPFD, IT and HC, from 17 different countries worldwide, thus providing a thorough global representation.

Despite the preponderance of patients with GT among IPFD and with *MYH9*-RD among IT, the enrolled population was representative of a wide range of disorders and included also very rare forms, thus providing information on the whole spectrum of these heterogeneous diseases [2,11,22-26].

The BS was more severely altered in some specific conditions, like the CalDAG-related disorder, GPS, and GT among IPFD, and the TAR and X-linked thrombocytopenia among IT, although for some of these the observation requires confirmation given the low number of cases.

Some bleeding symptoms were more frequently associated with specific patient groups, such as epistaxis for IPFD, cutaneous bleeding for IT and menorrhagia for VWD-1.

The ISTH-BAT had a higher discriminative power as compared with the WHO-BS in differentiating IPFD from IT and IPFD from HC, probably because the WHO-BS is a global score based on not-structured history-taking while the ISTH-BAT BS derives from the sum of the scores of well-defined individual symptom items each one progressively graduated.

Strengths of our study are the large study population, very stringent inclusion criteria, the enrollment

of pediatric cases, the systematic comparison with another BS and the worldwide representation. Limitations are 1) the lack of a population of subjects with mucocutaneous bleeding symptoms but without a definite diagnosis, thus the sensitivity of the ISTH-BAT in differentiating IPFD from HC in bleeding of unknown cause [11,20] will require a prospective validation in a large international collaborative study, 2) the enrollment of subjects in specialized hemostasis centers, which may have led to a selection of the more symptomatic cases: however, VWD-1 and HC were enrolled by the same centers making the comparison between groups reliable; 3) the inability to provide information on the predictive value of an increased BS for subsequent bleeding, however a prospective arm of the study is ongoing to assess it prognostic capability; 4) the lack of correlative data between altered BS and the presence of a defect at diagnostic laboratory tests, however an *ad hoc* designed substudy (BAT-LAB) is ongoing and will provide this information.

In conclusion, the application of the ISTH-BAT allows to clearly discriminate IPFD from HC and, potentially also from IT, while it has limited accuracy in distinguishing IPFD from VWD-1. On the other hand, the guidance on the diagnosis of IPFD by the ISTH advices to exclude VWD before embarking in complex diagnostic tests for patients with a mucocutaneous bleeding history [2]. Our study shows that a patient with a BS>6 for whom preliminary investigations have excluded VWD or a blood clotting defect has 99% probability of being affected by an IPFD.

AUTHORSHIP CONTRIBUTIONS

PG, PN, HD, AF, PH, DM and AM conceived and designed the study; PG, PN, EF, MCA, LB, MB, CS, AG, ARC, AT, EDC, PF, AP, FM, CF, AC, GP, MK, KJ, TS, GC, EG, MF, PZ, YH, KM, AD, CH, CZ, MA, GF, MGM, GT, PJ, FF, AR, NB, MN, JC, GV, BZ, MF, MC, ML, LB, BC, PG, CP, IE and MCMK collected cases and provided study materials; SO, EF, LB and GG assembled and analyzed data; SO, EF, LB and PG wrote the manuscript. All authors revised and gave final approval of the manuscript.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1   Rodeghiero F, Tosetto A, Castaman G. How to estimate bleeding risk in mild bleeding disorders. *J Thromb Haemost*. 2007; **5 Suppl 1**: 157-66. 10.1111/j.1538-7836.2007.02520.x.
2   Gresele P, Subcommittee on Platelet Physiology of the International Society on Thrombosis and Hemostasis. Diagnosis of inherited platelet function disorders: guidance from the SSC of the ISTH. *J Thromb Haemost*. 2015; **13**: 314-22. 10.1111/jth.12792.

3   Rydz N, James PD. The evolution and value of bleeding assessment tools. *J Thromb Haemost*. 2012; **10**: 2223-9. 10.1111/j.1538-7836.2012.04923.x.

4   Rodeghiero F, Castaman G, Tosetto A, Batlle J, Baudo F, Cappelletti A, Casana P, De Bosch N, Eikenboom JC, Federici AB, Lethagen S, Linari S, Srivastava A. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. *J Thromb Haemost*. 2005; **3**: 2619-26. 10.1111/j.1538-7836.2005.01663.x.

5   Tosetto A, Rodeghiero F, Castaman G, Goodeve A, Federici AB, Batlle J, Meyer D, Fressinaud E, Mazurier C, Goudemand J, Eikenboom J, Schneppenheim R, Budde U, Ingerslev J, Vorlova Z, Habart D, Holmberg L, Lethagen S, Pasi J, Hill F, Peake I. A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). *J Thromb Haemost*. 2006; **4**: 766-73. 10.1111/j.1538-7836.2006.01847.x.

6   Bowman M, Riddel J, Rand ML, Tosetto A, Silva M, James PD. Evaluation of the diagnostic utility for von Willebrand disease of a pediatric bleeding questionnaire. *J Thromb Haemost*. 2009; **7**: 1418-21. 10.1111/j.1538-7836.2009.03499.x.

7   Bowman M, Mundell G, Grabell J, Hopman WM, Rapson D, Lillicrap D, James P. Generation and validation of the Condensed MCMDM-1VWD Bleeding Questionnaire for von Willebrand disease. *J Thromb Haemost*. 2008; **6**: 2062-6. 10.1111/j.1538-7836.2008.03182.x.

8   Biss TT, Blanchette VS, Clark DS, Wakefield CD, James PD, Rand ML. Use of a quantitative pediatric bleeding questionnaire to assess mucocutaneous bleeding symptoms in children with a platelet function disorder. *J Thromb Haemost*. 2010; **8**: 1416-9. 10.1111/j.1538-7836.2010.03846.x.

9   Federici AB, Bucciarelli P, Castaman G, Mazzucconi MG, Morfini M, Rocino A, Schiavoni M, Peyvandi F, Rodeghiero F, Mannucci PM. The bleeding score predicts clinical outcomes and replacement therapy in adults with von Willebrand disease. *Blood*. 2014; **123**: 4037-44. 10.1182/blood-2014-02-557264.

10   Rodeghiero F, Tosetto A, Abshire T, Arnold DM, Coller B, James P, Neunert C, Lillicrap D, ISTH/SSC joint VWF and Perinatal/Pediatric Hemostasis Subcommittees Working Group. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. *J Thromb Haemost*. 2010; **8**: 2063-5. 10.1111/j.1538-7836.2010.03975.x.

11   Rodeghiero F, Pabinger I, Ragni M, Abdul-Kadir R, Berntorp E, Blanchette V, I Bodó, Casini A, Gresele P, Lassila R, Leebeek F, Lillicrap D, Mezzano D, Noris P, Srivastava A, Tosetto A, Windyga J, Zieger B, Makris M, Key N. Fundamentals for a systematic approach to mild and moderate inherited bleeding disorders: a EHA consensus report. *Hemasphere*. 2019; in press.

12   Lowe GC, Lordkipanidze M, Watson SP; UK GAPP study group. Utility of the ISTH bleeding assessment tool in predicting platelet defects in participants with suspected inherited platelet function disorders. *J Thromb Haemost*. 2013; **11**: 1663-8. 10.1111/jth.12332.

13   Rashid A, Moiz B, Karim F, Shaikh MS, Mansoori H, Raheem A. Use of ISTH bleeding assessment tool to predict inherited platelet dysfunction in resource constrained settings. *Scand J Clin Lab Invest*. 2016; **76**: 373-8. 10.1080/00365513.2016.1183260.

14   Kaur H, Borhany M, Azzam H, Costa-Lima C, Ozelo M, Othman M. The utility of International Society on Thrombosis and Haemostasis-Bleeding Assessment Tool and other bleeding questionnaires in assessing the bleeding phenotype in two platelet function defects. *Blood Coagul Fibrinolysis*. 2016; **27**: 589-93. 10.1097/MBC.0000000000000496.

15   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**: 1104-12. 10.1111/jth.14454.

16   Orsini S, Noris P, Bury L, Heller PG, Santoro C, Kadir RA, Butta NC, Falcinelli E, Cid AR, Fabris F, Fouassier M, Miyazaki K, Lozano ML, Zuniga P, Flaujac C, Podda GM, Bermejo N, Favier R, Henskens Y, De Maistre E, De Candia E, Mumford AD, Ozdemir GN, Eker I, Nurden P, Bayart S, Lambert MP, Bussel J, Zieger B, Tosetto A, Melazzini F, Glembotsky AC, Pecci A, Cattaneo M, Schlegel N, Gresele P, European Hematology Association - Scientific Working Group (EHA-SWG)

on thrombocytopenias and platelet function disorders. Bleeding risk of surgery and its prevention in patients with inherited platelet disorders. *Haematologica*. 2017; **102**: 1192-203. 10.3324/haematol.2016.160754.

17   Noris P, Schlegel N, Klersy C, Heller PG, Civaschi E, Pujol-Moix N, Fabris F, Favier R, Gresele P, Latger-Cannard V, Cuker A, Nurden P, Greinacher A, Cattaneo M, De Candia E, Pecci A, Hurtaud-Roux MF, Glembotsky AC, Muniz-Diaz E, Randi ML, Trillot N, Bury L, Lecompte T, Marconi C, Savoia A, Balduini CL, Bayart S, Bauters A, Benabdallah-Guedira S, Boehlen F, Borg JY, Bottega R, Bussel J, De Rocco D, de Maistre E, Faleschini M, Falcinelli E, Ferrari S, Ferster A, Fierro T, Fleury D, Fontana P, James C, Lanza F, Le Cam Duchez V, Loffredo G, Magini P, Martin-Coignard D, Menard F, Mercier S, Mezzasoma A, Minuz P, Nichele I, Notarangelo LD, Pippucci T, Podda GM, Pouymayou C, Rigouzzo A, Royer B, Sie P, Siguret V, Trichet C, Tucci A, Saposnik B, Veneri D, European Hematology Association – Scientific Working Group on Thrombocytopenias and Platelet Function Disorders. Analysis of 339 pregnancies in 181 women with 13 different forms of inherited thrombocytopenia. *Haematologica*. 2014; **99**: 1387-94. 10.3324/haematol.2014.105924.

18   Sadler JE, Rodeghiero F, Factor ISSovW. Provisional criteria for the diagnosis of VWD type 1. *J Thromb Haemost*. 2005; **3**: 775-7. 10.1111/j.1538-7836.2005.01245.x.

19   Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer*. 1981; **47**: 207-14. 10.1002/1097-0142(19810101)47:1<207: aid-cncr2820470134>3.0.co;2-6.

20   Quiroga T, Goycoolea M, Panes O, Aranda E, Martinez C, Belmont S, Munoz B, Zuniga P, Pereira J, Mezzano D. High prevalence of bleeders of unknown cause among patients with inherited mucocutaneous bleeding. A prospective study of 280 patients and 299 controls. *Haematologica*. 2007; **92**: 357-65. 10.3324/haematol.10816.

21   Gresele P, Bury L, Falcinelli E. Inherited platelet function disorders: algorithms for phenotypic and genetic investigation. *Semin Thromb Hemost*. 2016; **42**: 292-305. 10.1055/s-0035-1570078.

22   Cohen J. A coefficient of agreement for nominal scales. *Educational and Psychological Measurement*. 1960;**20**:37-46.

23   Gresele P, Falcinelli E, Bury L. Inherited platelet function disorders. Diagnostic approach and management. *Hamostaseologie*. 2016; **36**: 265-78. 10.5482/HAMO-16-02-0002.

24   Balduini CL, Cattaneo M, Fabris F, Gresele P, Iolascon A, Pulcinelli FM, Savoia A; Italian Gruppo di Studio delle Piastrine. Inherited thrombocytopenias: a proposed diagnostic algorithm from the Italian Gruppo di Studio delle Piastrine. *Haematologica*. 2003;**88**:582-92.

25   Gresele P, Bury L, Mezzasoma AM, Falcinelli E. Platelet function assays in diagnosis: an update. *Expert Rev Hematol*. 2019; **12**: 29-46. 10.1080/17474086.2019.1562333.

26   Gresele P, Falcinelli E, Bury L. Laboratory diagnosis of clinically relevant platelet function disorders. *Int J Lab Hematol*. 2018; **40 Suppl 1**: 34-45. 10.1111/ijlh.12814.

Table 1. Sensitivity, specificity, positive and negative predictive values of the ISTH-BAT BS.

	Sex	Best cut-off	AUC (p)	Sensitivity	Specificity	PPV (95% CI)	NPV (95% CI)
IPFD vs HC	F	>4	0.962 (<0.0001)	84.87	94.68	91.0	90.8
	M	>3	0.936 (<0.0001)	82.89	96.00	92.6	90.2
VWD-1 vs HC	F	>1	0.891 (<0.0001)	87.29	76.06	77.8	86.1
	M	>0	0.876 (<0.0001)	86.40	76.80	78.8	85.0
IPFD vs IT	F	>6	0.881 (<0.0001)	75.63	89.37	84.1	83.1
	M	>3	0.846 (<0.0001)	82.89	78.95	69.2	89.0

			AUC (p)			PPV	NPV
IT vs VWD-1	F	≤3	0.753 (<0.0001)	73.12	69.61	68.0	74.6
	M	≤2	0.716 (<0.0001)	67.67	68.80	69.8	66.7
IPFD vs VWD-1	F	>7	0.722 (<0.0001)	72.27	67.96	59.7	78.8
	M	>5	0.737 (<0.0001)	72.37	72.00	61.1	81.1
IT vs HC	F	>1	0.691 (<0.0001)	57.50	76.06	67.2	67.8
	M	>0	0.694 (<0.0001)	57.89	76.80	72.6	63.2

AUC= area under curve; HC= healthy controls; IPFD= inherited platelet function disorders; IT= inherited thrombocytopenias; NPV= negative predictive value; PPV= positive predictive value; VWD-1= von Willebrand disease type 1.
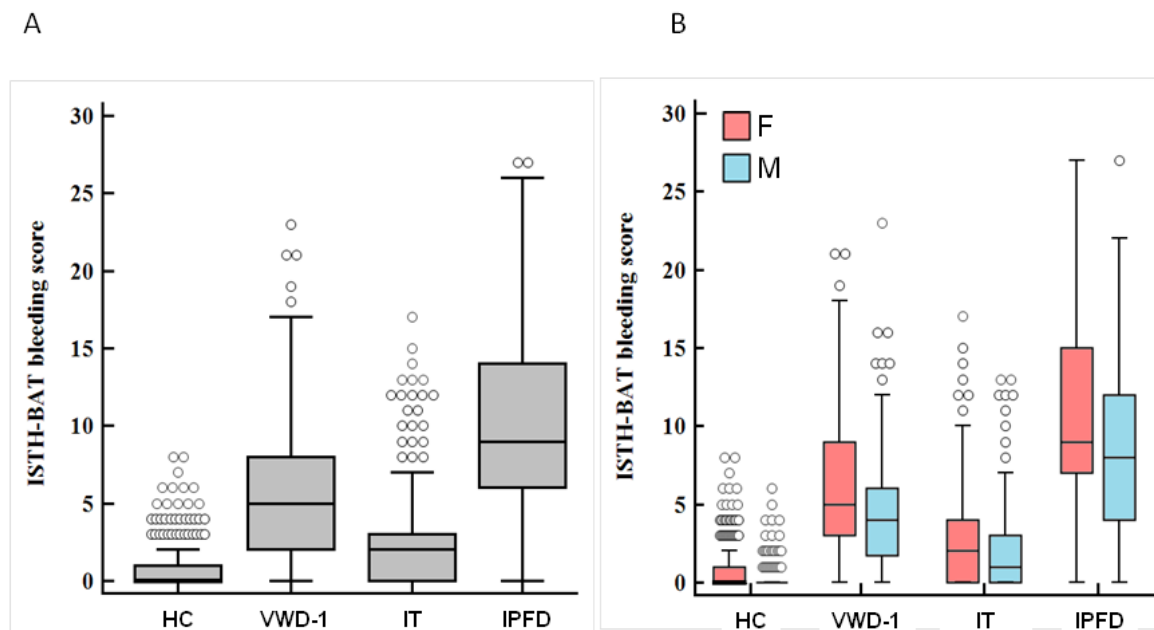
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Figure 1

Figure 1. ISTH-BAT bleeding score distribution in the global population of the different group (A) or by sex (B). Data are reported as box and whisker plots; isolated points represent outliers (outside 95% CIs). All comparisons are statistically significant (p<0.05).
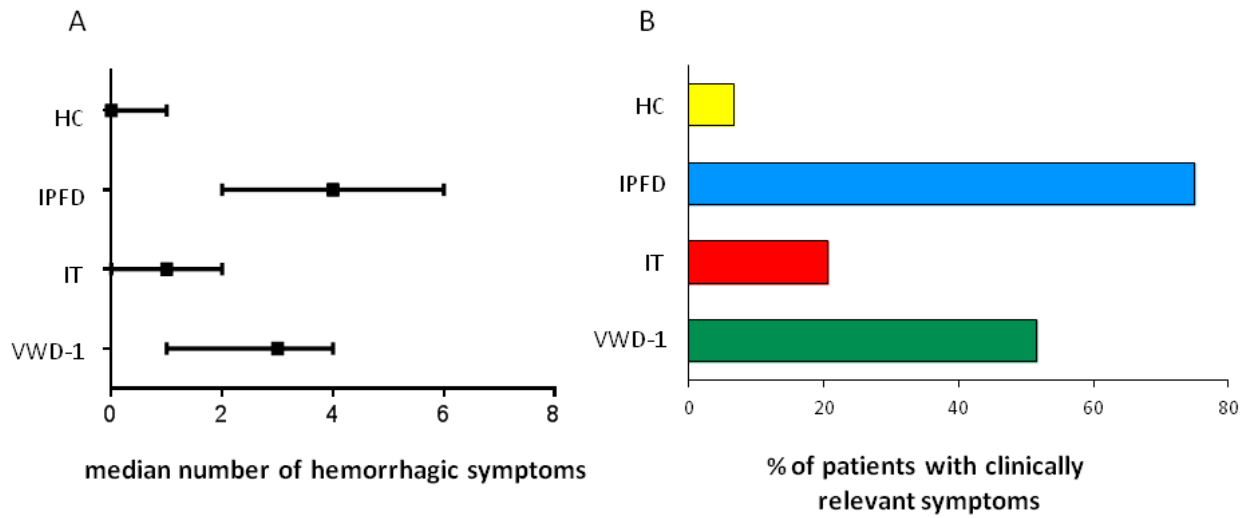
Figure 2

Figure 2. (A) Median number (IQR) of hemorrhagic symptoms and (B) percentage of clinically relevant symptoms in the different study populations. All comparisons are statistically significant (p<0.001).