VALIDATION OF THE ISTH/SSC BLEEDING ASSESSMENT TOOL FOR INHERITED PLATELET DISORDERS

A study project by the SSC Platelet Physiology Subcommittee

Coordinator: Paolo Gresele. Department of Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia, Perugia, Italy

Steering Committee: Hans Deckmyn, Andrew L. Frelinger III, Paul Harrison, Diego Mezzano, Andrew D. Mumford, Patrizia Noris

Background

An accurate assessment of the presence and severity of bleeding symptoms is a critical component in the evaluation of patients with bleeding disorders (1). In particular, a well characterized mucocutaneous bleeding history is crucial, especially for the decision to embark in complex and expensive laboratory studies for the diagnosis of inherited platelet function disorders (IPFDs) and inherited platelet number disorders (IPNDs) (2). A number of bleeding assessment tools (BATs) have been developed to standardize the bleeding history in an effort to improve diagnostic accuracy and sensitivity and thus to avoid unwarranted laboratory testing, predict the future risk of bleeding, describe symptom severity and inform treatment (3).

In particular, several BATs have been developed to assess bleeding in VWD, the Vicenza-based BAT (4), the Molecular and Clinical Markers for the Diagnosis and Management of type 1 VWD (MCMDM-1 VWD) Bleeding Questionnaire (5), the Pediatric Bleeding Questionnaire (PBQ) (6), and a questionnaire specific for the Quebec Platelet Disorder has also been proposed (7).

The PBQ was applied to a small a cohort of children with IPFD to evaluate bleeding severity and revealed to be a potentially useful tool, although bleeding scores varied between different diagnostic groups and between children with the same diagnosis (8).

Very recently the bleeding score obtained with the MCMDM-1 VWD revealed to be predictive of clinical outcomes in adult patients with VWD: high bleeding scores correlated with intensive on-demand therapy and might identify cases requiring regular prophylaxis (9).

The Bleeding Assessment Tool of the International Society on Thrombosis and Haemostasis (ISTH BAT) was developed as a consensus bleeding assessment tool to record bleeding symptoms and to address diagnosis in patients with a suspected bleeding disorders in general, i.e. not only VWD patients. Moreover, this BAT considers also recurrent minor bleedings (10,11), and thus it is potentially relevant to assess bleeding also in IPFDs and IPNDs, conditions characterized by

minimal haemorrhagic symptoms that otherwise receive insufficient consideration with the use of other bleeding scores.

However, very little information is available on the utility of the ISTH-BAT for patients with IPFDs and IPNDs, on its potential ability to discriminate them from VWD patients and on its possible prognostic significance.

Recently, the utility of the ISTH-BAT to predict functional defects in platelet activation was tested in a study in 100 subjects with suspected IPFDs (79 of whom turned out to suffer from an IPFD). Although the BAT revealed to be a powerful tool to discriminate pathological excessive bleeding, it was not predictive of the presence of a platelet defect on lumiaggregometry suggesting that it may not be predictive of the likelihood of an IPFD diagnosis. It should however be considered that patients with established diagnoses of Glanzmann's thrombasthenia, Bernard–Soulier syndrome, May–Hegglin anomaly or Hermansky–Pudlak syndrome were excluded, therefore the study does not provide information on a significant fraction of IPFDs. Moreover, the number of subjects included in the study may not be sufficient to draw definitive conclusions (11).

The ISTH-BAT combines a standardized bleeding questionnaire and a well-defined interpretation grid that allows the computation of a final Bleeding Score (BS) (10). A web-based version of the ISTH BAT is freely available through Rockefeller University, with the objective of encouraging investigators to administer a bleeding history questionnaire and also to share data (https://bh.rockefeller.edu/ISTH-BATR/).

Aims

Aim of the present study is to test the diagnostic utility of the ISTH-BAT for IPFDs and IPNDs in a large cohort of patients with a definite diagnosis and to compare these with a parallel cohort of VWD patients. In particular, we aim to assess if the ISTH-BAT may be useful to discriminate between:

- IPFDs/IPNDs and healthy subjects
- different IPFDs/IPNDs
- IPFDs/IPNDs and VWD

Due to the rarity of these disorders, we will initially perform an evaluation only on patients with an established IPFD/IPND diagnosis asking to all centers involved in the management of IPFDs/IPNDs to contribute to this study by examining their patient records and to enroll in the study all cases fulfilling well defined inclusion criteria (listed below). Secondary aim is to assess the prognostic value of the ISTH-BAT bleeding score for bleeding and requirement of pro-haemostatic treatents. To this aim to all patients will be administered by a doctor of each center the

ISTH-BAT, their bleeding score will be calculated and then they will be re-evaluated after one and two years for a new evaluation of the bleeding scores and for possible bleedings occurred and the relative treatment.

In a future, prospective study we will evaluate the diagnostic utility of the ISTH-BAT in new patients presenting for the evaluation of mucocutaneous bleeding of suspected hereditary platelet origin but without a definite diagnosis.

Design of the study

This is an observational, cross-sectional/prospective cohort study carried out in patients diagnosed and followed up at various centers worldwide. Patients will be informed about the anonymous use of their data and the purpose of this study and will give written informed consent. Patients will be included in the study and the first administration of the ISTH-BAT questionnaire will be made and the bleeding score will be calculated. They will be then prospectively followed for two years with yearly follow-up visits (which may be performed by telephone interview). Every year a new ISTH-BAT questionnaire will be administered and a new bleeding score will be calculated.

INCLUSION CRITERIA

Participating patients, both pediatric and adult, will have to be living, available for direct compilation of the BAT questionnaire (telephone history taking is accepted) and give informed consent (signed by parent or legal ward for pediatric patients).

Because of the differences in bleeding risk of the different forms of IPFDs/IPNDs, it is required that patients included in the study have a definite diagnosis, as listed below. Patients with acquired IPFDs/IPNDs of any etiology (including for instance anti-GPIIb/IIIa-induced acquired Glanzmann) are excluded.

Inherited thrombocytopenias

Patients must have a definite diagnosis. Given that part of the IPNDs do not receive a diagnosis at molecular level, for these conditions minimal required diagnostic criteria are summarized in **Table 1**. For these diagnoses participants are required to report the tests that lead to the diagnosis. In particular, in biallelic (homozygous) Bernard-Soulier syndrome (BSSbi), MYH9-related disease (MYH9-RD) and Gray Platelet Syndrome (GPS), alternative methods may be used because of their high sensitivity and specificity. Moreover, clinical phenotype is considered sufficient for making diagnosis of thrombocytopenia with absent radii (TAR) and congenital thrombocytopenia with

radio-ulnar synostosis (CTRUS).

- MYH9-RD: autosomal dominant inheritance or sporadic cases, macrothrombocytopenia with Döhle-like bodies in peripheral blood neutrophils and/or positivity of the immunofluorescence screening test using antibodies against NMMHCIIA (12);
- BSSbi: autosomal recessive inheritance, macrothrombocytopenia with absent or severely defective in vitro platelet agglutination/aggregation after ristocetin stimulation in subjects without vWF deficiency and /or absent or severely reduced platelet GPIb/IX/V complex at flow cytometry or SDS-PAGE;
- TAR: autosomal recessive inheritance, thrombocytopenia in subjects with bilateral radial aplasia;
- CTRUS: autosomal dominant transmission or sporadic cases, thrombocytopenia in subjects with congenital radio-ulnar synostosis.

Inherited disorders of platelet function

As for inherited thrombocytopenias, only subjects with well-defined inherited disorders of platelet function will be enrolled. On this basis, subjects with known molecular defects are eligible for the study. A large part of the inherited disorders of platelet function do not receive a diagnosis at molecular level, because the causative gene mutations have not been described. However, these disorders (e.g. alpha and/or delta storage pool disease or primary secretion defects) are the most common platelet function disorders. Therefore, we encourage participants to include also patients with platelet function disorders that have not received diagnosis at a molecular level classifying them on the basis of the clinical picture and laboratory tests, a life-long bleeding tendency and no coagulation abnormalities (normal levels of vWF included). For some of these conditions (e.g. SPD) minimal required diagnostic criteria are summarized in **Table 2**. For these cases participants are required to indicate the results of the minimal diagnostic criteria that lead to the diagnoses. Diagnostic criteria for subjects for whom molecular diagnosis is not an absolute requirement are described below.

- Glanzmann thrombasthenia: autosomal recessive transmission, absent (or almost absent) platelet aggregometry on LTA with all agonists (except ristocetin) and/or absent or severely reduced GPIIb-IIIa complex at flow cytometry or SDS-PAGE;
- P2Y12 deficiency: autosomal recessive inheritance, failure of high dose ADP (> 10μM) to induce irreversible platelet aggregation and defective inhibition of adenylyl cycalse by ADP (VASP phosphorylation or cAMP assay);

- Delta-granule deficiency: autosomal dominant inheritance, severe reduction of platelet delta-granules (reduced platelet adenine nucleotides measured by lumiaggregometry or electron microscopy demonstrating the absence of dense granule-limiting membranes and contents);
- Hermansky-Pudlak syndrome (HPS): autosomal recessive, platelet dense granule deficiency (reduced platelet adenine nucleotides or electron microscopy demonstrating the absence of dense granule-limiting membranes and contents, or mepacrine test) in subjects with oculocutaneous albinism.
- Gray Platelet Syndrome: autosomal recessive inheritance, macrothrombocytopenia and absent or severely reduced platelet alpha-granules at electron microscopy or immunofluorescence;
- Combined alpha-delta granule deficiency: autosomal dominant transmission, severe reduction of platelet dense and alpha-granule constituents at biochemical analysis and/or electron microscopy;
- Defect of thromboxane receptor: autosomal dominant inheritance, defective platelet aggregation induced by U46619, normal TXB2 production induced by arachidonic acid.
- Defects in collagen receptors: autosomal recessive, defective platelet aggregation in the response to collagen, CRP, CVX.
- Primary secretion defect: autosomal dominant or recessive, reduced primary platelet granule secretion upon stimulation by different platelet aggregation agonists, normal TxB_2 production induced by AA (or serum TxB_2) and normal granule content.

Diagnostic criteria for VWD-type 1

- **VWF:Ag** ranging from 5% to 40%
- **VWF:RCo** decreased proportionally to VWF:Ag
- **FVIII** decreased proportionally to VWF:Ag
- **VWF:Ag/VWF:RCo** <1.5 or **VWF:RCo/VWF:Ag** >0.7
- Prolonged **RIPA** (if available)

INSTRUCTION FOR THE COMPILATION OF CRF

- Participants are requested to enroll for each patient with an inherited platelet disorder:
    a) A healthy control (no bleeding history, normal platelet count, normal light transmission aggregometry – e.g. healthy controls used in parallel to patients during platelet function testing), age- and sex- matched (±5 years).
    b) A patient with type 1 VWD, unequivocally diagnosed, possibly age- and sex-matched (±5 years).

- The ISTH-BAT questionnaires cannot be modified and all fields must be completed (Rodeghiero F et al. J Thromb Haemost 2010;8:2063 supplemental material).
- The ISTH-BAT will have to be administered to each patient by a physician experienced with the assessment of bleeding disorders (telephone administration is acceptable).

STATISTICAL ASPECTS

This is an observational, cross-sectional/prospective study aimed to test the diagnostic utility of the BAT for IPFDs and IPNDs patients. Based on these characteristics and the absence of previous data in the literature, we can not calculate the required sample size to be investigated and we simply program to collect "as much information as possible". We aim to enroll at least 300 subjects in each of the 3 cohorts. Once data of all available patients will be collected, appropriate descriptive statistics will be performed.

For the prospective arm of the study comparison between groups will be made by the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. The incidence rate of bleeding will be calculated as the number of episodes during the follow-up period divided by the total number of patient-years for that period, and 95% confidence intervals (CIs) will be calculated under the Poisson distribution assumption.

All analyses will be performed using the GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA). Statistical significance will be considered at a p of <0.05.

ETHICAL ISSUES

The University of Perugia, Perugia, Italy, has the role of coordinating the study and managing the database. Other centers will comply with the regulations of the country for the ethical aspects of the study. The database will not contain the names of the patients but only identifiers provided by the center that has enrolled the case and all information concerning patients will be anonymized.

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Table 1. Diagnostic criteria required for enrolling in the study patients with inherited forms of thrombocytopenia.

Disease (abbreviation, OMIM entry)	**Inheritance**	**Gene (chromosome localization)**	**Diagnostic criteria**
SYNDROMIC FORMS			
X-linked thrombocytopenia (XLT, 313900)	XL	*WAS* (Xp11)	Genetic analysis
MYH9-related disease (*MYH9*-RD, nd)	AD	*MYH9* (22q12-13)	Genetic analysis or positive immunofluoresce screening test or Döhle-like bodies
Paris-Trousseau thrombocytopenia (TCPT, 188025/600588), Jacobsen syndrome (JBS, 147791)	AD	Large deletion (11q23-ter)	Genetic analysis
Thrombocytopenia with absent radii (TAR, 274000)	AR	*RBM8A* (1q21.1)	Genetic analysis or typical phenotype
Congenital thrombocytopenia with radio-ulnar synostosis (CTRUS, 605432)	AD	*HOXA11* (7p15-14)	Genetic analysis or typical phenotype
Thrombocytopenia associated with sitosterolaemia (STSL, 210250)	AR	*ABCG5*, *ABCG8* (2p21)	Genetic analysis
NON-SYNDROMIC FORMS			
Bernard-Soulier syndrome (BSS, 231200) – Biallelic	AR	*GP1BA* (17p13), *GP1BB* (22q11), *GP9* (3q21)	Genetic analysis or absent GPIb/IX/V or absent RIPA
Bernard-Soulier syndrome (BSS, 231200) – Monoallelic	AD	*GP1BA* (17p13), *GP1BB* (22q11), *GP9* (3q21)	Genetic analysis
Familial platelet disorder and predisposition to acute myelogenous leukemia (FPD/AML, 601399)	AD	*RUNX1* (21q22)	Genetic analysis
ANKRD26-related thrombocytopenia (THC2, 313900)	AD	*ANKRD26* (10p2)	Genetic analysis
ITGA2B/*ITGB3*-related thrombocytopenia (*ITGA2B*/*ITGB3-RT*, 187800)	AD	*ITGA2B* (17q21.31), *ITGB3* (17q21.32)	Genetic analysis
TUBB1-related thrombocytopenia (TUBB1-RT, 613112)	AD	*TUBB1* (6p21.3)	Genetic analysis
CYCS-related thrombocytopenia (THC4, 612004)	AD	*CYCS* (7p15.3)	Genetic analysis
Congenital amegakaryocytic thrombocytopenia *(CAMT, 604498)*	AR	*MPL* (1p34)	Genetic analysis or typical phenotype
GATA1-related diseases *(GATA1-RDs,* Dyserythropoietic anemia with thrombocytopenia, 300367 – X-linked thrombocytopenia with thalassemia, 314050)	XL	*GATA1* (Xp11)	Genetic analysis
ACTN1-related thrombocytopenia *(ACTN1-RT, nd)*	AD	*ACTN1* (14q24)	Genetic analysis
FLNA-related thrombocytopenia *(FLNA-RT, nd)*	XL	*FLNA* (Xq28)	Genetic analysis

Table 2. Diagnostic criteria required for enrolling in the study patients with inherited defects of platelet function.

Disease (abbreviation, OMIM entry)	***Inheritance***	***Gene (chromosome localization)***	**Diagnostic criteria**
SYNDROMIC FORMS			
Hermansky–Pudlak syndrome (HPS, 203300)	AR	*HPS1, ADTB3A, HPS3, HPS4, HPS5,HPS6, DTNBP1, BLOC1S3*	Genetic analysis or typical phenotype + delta granule deficiency or decrease in platelet nucleotide content and increased ATP/ADP ratio (+decreased 5HT content)
Cediak-Higashi Syndrome (CHS, 214500)	AR	*CHS1 (1q42.1-42.2)*	
NON SYNDROMIC FORMS			
Glanzmann thrombasthenia (GT, 273800)	AR	*ITGA2B, ITGB3* (17q21.32)	Genetic analysis or absent platelet aggregation or absent GPIIb-IIIa
P2Y12 deficiency (nd, 609821)	AR	*P2RY12* (3q24-q25)	Genetic analysis or selective, severe defect of platelet aggregation by ADP, defect of inhibition o adenylyl cycalse by ADP (VASP phosphorylation assay)
Defect of thromboxane A2 receptor (nd, 188070)	AD	*TBXA2R* (19p13.3)	Genetic analysis or defective platelet aggregation induced by U46619, normal aggregation induced by arachidonic acid
Scott syndrome (SCTS, 262890)	AR	*TMEM16F* (12q12)	Genetic analysis
Quebec platelet disorder (QPD, 601709)	AD	*PLAU* (10q24)	Genetic analysis
Delta granule deficiency	AR/AD	*Unknown*	Absence of delta-granules (TEM) or decrease in platelet nucleotide content and increased ATP/ADP ratio (+decreased 5HT content)
Combined alpha-delta granule deficiency (nd, 185050)	AR/AD	Unknown	Severe deficiency of alpha and delta granules
Platelet-type Von Willebrand Disease (VWDP, 177820)	AD	*GP1BA (17p13.2)*	Genetic analysis
Gray platelet syndrome (GPS, 139090)	AR	*NBEAL2* (3p21.1)	Genetic analysis or absence of alpha-granules
Gray platelet syndrome with mutation in GFI1B	AD	*GFI1B* (9q34.13)	Genetic analysis
Primary secretion defect (nd, nd)	AR/AD	*Unknown*	Reduced primary platelet granule secretion upon stimulation by different platelet aggregation agonists, normal TxB2 production induced by AA (or serum TxB2) and normal granule content.

Defects in collagen receptors (nd, nd)	AR	*Unknown*	Defective platelet aggregation in the response to collagen
Stormorken syndrome (nd, 185070)	*AD*	*ORAI1 (12q24.31)* *STIM1 (11p15.5)*	*Genetic analysis*
COX-1 deficiency	*AD*	*Unknown*	*Defective aggregation in response to arachidonic acid; defective serum TXB2;*
cPLA2 deficiency	*AR*	*PLA2G4A (1q31.1)*	*Genetic analysis*
Tx synthase deficiency	*AD*	*TBXAS1 (7q34)*	*Genetic analysis*
PKC deficiency	*unknown*	*Unknown*	*Defective aggregation in response to Thrombin and PAF; defective GPIIb/IIIa activation*