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

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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 ($AUC\leq0.7$) 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 (β=0.8, α=0.05).

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

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 ($\rho= 0.2$; $p= 0.0003$) and negatively with platelet count in the IT group ($\rho=-0.19$, $p=0.0009$).
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 ($0.5<AUC\leq0.7$) 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 ($0.5<AUC\leq0.7$) 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, leading 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.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

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

	Sex	Best cut-off	AUC (p)	Sensitivity	Specificity	PPV	NPV

						(95% CI)	(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
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.

Legend to the figures

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

Figure 1

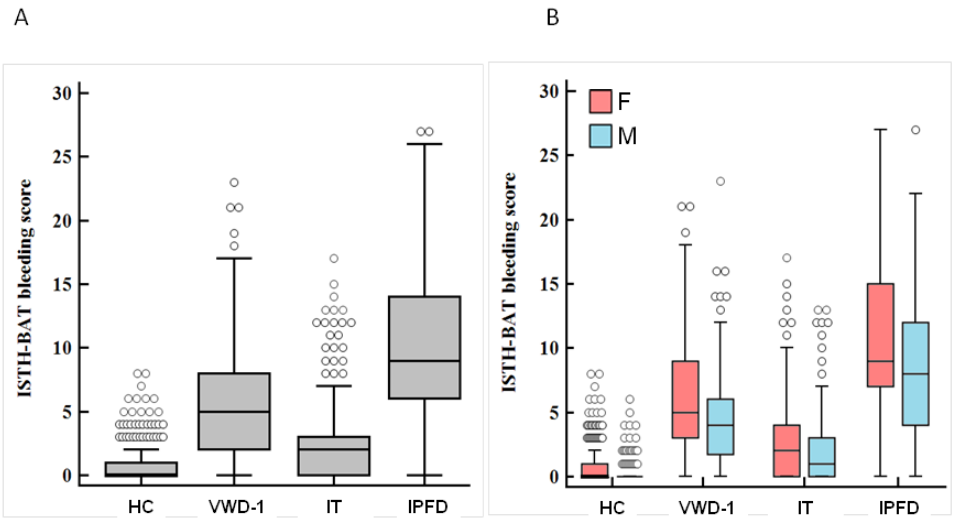

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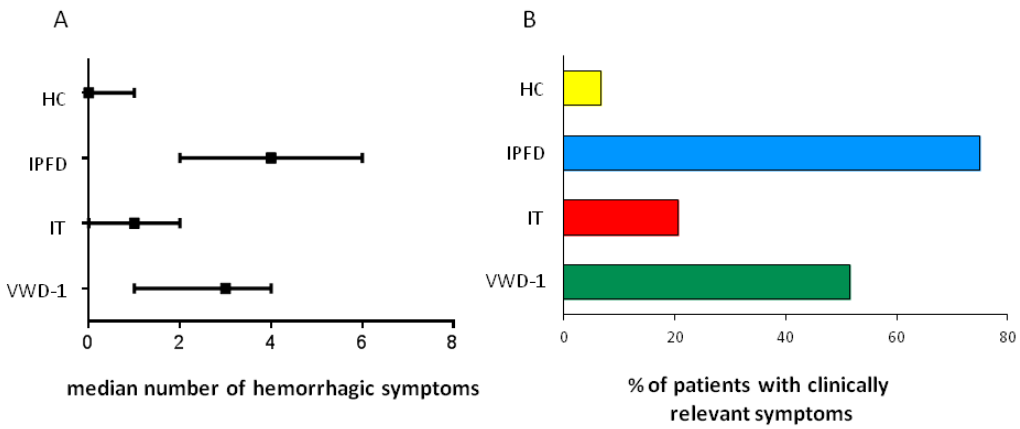
Figure 2



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