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Association of laboratory test results with the bleeding history in patients with inherited platelet function disorders (the BAT-LAB substudy): communication from the Platelet Physiology ISTH-SSC

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function disorders (the BAT-LAB substudy): communication from the Platelet Physiology ISTH-SSC

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Background: In hemophilia and von Willebrand disease, the degree of alteration of laboratory assays correlates with bleeding manifestations. Few studies have assessed the predictive value for bleeding of laboratory assays in patients with inherited platelet function disorders (IPFD).

Objectives: To assess whether there is an association between platelet function assay results and the bleeding history, as evaluated by the ISTH BAT.

Methods: Centers participating in the international ISTH-BAT validation study were asked to provide results of the diagnostic assays employed for the patients they enrolled and the association with the individual patients' bleeding score (BS) was assessed.

Results: 68 patients with 14 different IPFDs were included. Maximal amplitude of platelet aggregation was significantly lower in patients with a pathologic BS and correlated inversely with the BS, a finding largely driven by the subgroup of Glanzmann Thrombasthenia (GT) and CalDAG–GEFI patients; after their exclusion TRAP-induced aggregation remained significantly lower in patients with a pathologic BS. The bleeding time (BT) was significantly more prolonged in patients with a high BS compared to those with a normal BS (27.1±6.2min vs 15.1±10.6min, $p<0.01$). Reduced α-granule content was significantly more common among patients with a pathologic BS compared to those with a normal BS (80% vs 20%, $p<0.05$). ROC curve analysis revealed a significant discriminative ability of all the above tests for pathologic BS ($p<0.001$), also after exclusion of GT and CalDAG–GEFI patients.

Conclusions: This study shows that altered platelet laboratory assay results are associated with an abnormal ISTH-BAT BS in IPFD.

Keywords: platelet function tests, hemorrhage, inherited platelet function disorders, blood platelets, ISTH-BAT bleeding score

Essential Bullets (lay language):

- The association of bleeding severity with platelet laboratory test results is poorly studied
- We assessed this association in patients with inherited platelet function defects
- Alterations of some platelet laboratory assays are associated with bleeding severity
- Integration of bleeding assessment tools with laboratory assays may help in patient management

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Inherited platelet function disorders (IPFD) are haemorrhagic diseases characterized by defective platelet activation and sometimes reduced platelet number, associated with mild to severe mucocutaneous bleeding. Although IPFDs make up a significant fraction of all congenital bleeding disorders [1,2], they remain poorly characterized and difficult to diagnose [3].

A diagnostic approach based on a careful clinical evaluation and a streamlined panel of appropriately selected laboratory assays has been proposed and the bleeding history represents an essential step in the diagnostic workup, and its alteration is crucial for the decision to embark in complex and expensive laboratory studies [4- 6].

The ISTH bleeding assessment tool (ISTH-BAT), initially employed only for patients with suspected von Willebrand disease (VWD), has been recently validated for the assessment of the bleeding history of patients with inherited platelet disorders (IPD) [7]. Moreover, it has been shown to be highly predictive of post-surgical bleeding in a retrospective study, with IPFD patients having an ISTH BAT ≥6 suffering a significantly higher incidence of postsurgical bleeding [8], and of spontaneous bleeding in a prospective study in a cohort of IPFD patients followed-up for 2 years, with patients showing a high baseline bleeding score (BS) having a significantly greater risk of subsequent haemorrhagic events [9].

The ISTH BAT had previously been shown to be a predictor of clinical outcome and replacement therapy in adults with VWD, and interestingly in that study the incidence of bleeding was inversely related to baseline VWF:RCo and FVIII levels: patients with type 1 VWD and low VWF:RCo (<10 U/dL) and FVIII (<20IU/dL) levels were more likely to suffer bleeding and to require replacement therapy during follow up [10].

In classical hemophilia too there is a good relationship between plasma levels of FVIII and the frequency and severity of bleeding symptoms [11] and the endogenous thrombin generation potential provides additional insight in the bleeding phenotype [12,13]. On the contrary, it is generally held that in most mild/moderate bleeding disorders laboratory abnormalities may prove inconsistent in predicting the risk of bleeding [6].

Differently from VWD or hemophilia, only few studies have assessed the predictive value of laboratory assays for bleeding manifestations in patients with IPFD, with conflicting results [1,14-19].

One study showed no relationship between the severity of bleeding and platelet dense granule content or impaired light transmission aggregometry (LTA) in 65 patients with IPFD [1]. Similarly, defective platelet function assessed by lumiaggregometry in PRP was not associated with an

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of platelet glycoprotein expression by flow cytometry, as well as decreased lumiaggregometry and α granule content, were not associated with bleeding severity in 32 patients with a primary secretion defect (PSD) [15].

On the contrary, one study in a rather large cohort of individuals with Glanzmann Thrombasthenia (GT), Bernard Soulier syndrome (BSS) and defective aggregation in response to adrenaline, reported that a higher ISTH-BAT score was predictive of the presence of a platelet defect on LTA and that, in binary logistic regression, the platelet defect had a statistically significant effect on the ISTH-BAT score model (odds ratio 3.25, 95% CI 2.13–4.37, p<0.001) [16]. Recently, a single institution study in a small cohort of patients with suspected IPD concluded that abnormal platelet transmission electron microscopy is associated with high likelihood of an abnormal ISTH-BAT [18]. Finally, a study involving 37 uncharacterized IPFD patients showed that reduced maximal platelet aggregation induced by collagen was associated with more bruises and wound healing problems and more severe dense granule deficiency with surgical bleeding [19].

The aim of the current study was to assess if there is an association between the results of the laboratory assays employed for diagnosis and the bleeding manifestations assessed by the ISTH-BAT bleeding score in a series of well-characterized patients with IPFD.

Methods

Design of the study

This was an *ad hoc* designed sub-group analysis of the BAT-VAL study, a multicentric international prospective study promoted by the Platelet Physiology SSC of the ISTH including adult and pediatric patients with a diagnosis of IPD confirmed according to well-defined laboratory and/or molecular genetic criteria [7,9] (**Supplementary Table 1**). Genetic confirmation was available for 56% of patients, but it is worth noting that 70% of patients without a genetic confirmation had a diagnosis of primary secretion defect or combined alpha/delta storage pool disorder, conditions for which a causative gene has not yet been identified. We did not collect detailed information on the reasons of initial referral of the patients to the participating centers.

The results of the laboratory platelet function assays performed for IPFD diagnosis according to the ISTH SSC guidance [20] were retrospectively retrieved from the patient files of subjects who had been enrolled in the BAT-VAL study [7]. We defined as IPFD those disorders in which platelet dysfunction was the dominant phenotypic feature independently of platelet count [7,9].

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results of the platelet function studies carried out for the diagnosis of IPFD (see **Supplementary materials**). Laboratory data included platelet count, mean platelet volume (MPV), bleeding time (BT), PFA-100® closure time, light transmission aggregometry (LTA), α- and dense-granule content and release, platelet glycoprotein surface expression by flow cytometry, clot retraction and serum thromboxane B_2 (TxB_2).

Information concerning method, type of instrument, manufacturer and concentration of agonist used for platelet activation and internally established normal reference intervals for each test was also collected (**Supplementary materials**).

Platelet function assay results were classified as abnormal when outside the internal laboratory reference interval. The ISTH-BAT BS was considered abnormal when ≥4 for males and ≥6 for females [6, 21]. For pediatric patients the ISTH-BAT BS was considered abnormal when ≥3 [21].

Laboratory assays

Details of the laboratory assays and respective normal ranges can be found in **Supplementary Table 2**. Platelet count and mean platelet volume (MPV) were assessed by different automated hematological counters. Platelet function analyzer (PFA-100®) (Siemens Healthcare Diagnostic, Marburg, Germany) was performed with the C/EPI and C/ADP cartridges and the closure time (CT) was recorded [22]. The bleeding time (BT) test was performed according to the method of Mielke [23] or Ivy [24]. Platelet aggregation was assessed in most centers by LTA [25] and in one by impedance aggregometry [26]. The concentration and types of agonists used for platelet aggregation testing differed among centers (**Supplementary Table 2**). Some centers tested more than one agonist concentration. In particular, 5 patients were tested with more than one dose for epinephrine, 14 for ADP, 3 for collagen, 6 for TRAP-6. To correlate LTA results with the BS we considered the highest agonist concentration tested.

Platelet surface glycoprotein (GP) expression was measured by flow cytometry with specific fluorescent-conjugated antibodies against GPIIb (CD41), GPIIIa (CD61), GPIbα (CD42b) and GPIX (CD42a). ADP-induced activation of the GPIIb/IIIa complex was assessed by measuring PAC-1 antibody binding by flow cytometry [27]. Platelet α-granule content was assessed by ELISA (β-TG, PAI1, fibrinogen of platelet lysates) or immunofluorescence (thrombospondin), while agonist-induced α-granule release was assessed by flow cytometry using an anti CD62P antibody to test platelet surface P-selectin expression or by ELISA (β-TG) in stimulated platelet supernatants after centrifugation [28] (**Supplementary Table 2**).

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spectrophotometry (serotonin), while release was assessed by HPLC (serotonin), lumiaggregometry (ATP) or flow cytometry (mepacrine and CD63) [28]. The concentration and types of agonists used for platelet granule secretion testing differed among centers (**Supplementary Table 2**). Finally, TxB_2 concentration in serum was measured by ELISA (**Supplementary Table 2**).

ISTH BAT bleeding score

The BS was calculated either manually, using the interpretation grid, or automatically using the web-based version (https://bh.rockefeller.edu/ISTH-BATR), at enrollment in the BAT-VAL study [7].

To evaluate associations between single items of the ISTH-BAT with platelet function laboratory assays we defined as "severe" items with score >2.

Statistical analysis

Data are reported as medians and 25th-75th percentiles (IQR) when continuous, and as counts and percentages when categorical. Correlations between laboratory variables and the ISTH BAT BS were assessed with the Pearson correlation coefficient (ρ). Receiver operating characteristic (ROC) curves for diagnostic prediction (abnormal BS) were calculated for each test and areas under the curve (AUC), with binomial exact confidence intervals for AUC, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV), were assessed. Cut-off values for the most relevant comparisons were also calculated using the Youden index.

Test results were used to estimate the likelihood of abnormal BS as ORs with 95% confidence intervals (CIs). Relationships between bleeding symptoms and laboratory test results were evaluated using the Mann-Whitney and chi-square tests.

Given that 28% of the enrolled patients had GT and CalDAG-GEFI related disorder, conditions typically associated with a high BS and severe platelet dysfunction which could introduce bias to the overall analysis, a separate analysis was performed excluding these groups.

A two-sided $p<0.05$ was considered as statistically significant. The IBM SPSS Statistics v.25 software was used for all analyses.

Results

Patient characteristics

11 centers worldwide responded to the survey and 68 patients (34.7% of the initially enrolled IPFD population) [7], 37 of whom were females (54%), with a median age of 35 years (IQR 25-50), were included in this study. Of the 68 patients included, 61 were adult (median age 37 years, IQR 26-53)

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pediatric patients was not carried out.

Fourteen different IPFD forms were represented (**Supplementary Table 3 and 4, Supplementary Figure 1**), among which there were Glanzmann Thrombasthenia (GT) (23.5%), primary secretion defect (PSD) (14.7%), δ-granule deficiency (10.3%), biallelic BSS (bBSS) (7.3%), familial platelet disorder with predisposition to myeloid leukemia (FPD/AML) (7.3%), Gray platelet syndrome (GPS) (7.3%) and others (29.6%).

Median baseline BS was 8 (IQR 2.2-12) in the overall IPFD population, 8 (IQR 2.5-12.5) in adult and 8 (IQR 2-11) in pediatric patients, and the BS was abnormal in 46 patients, 71% of pediatric patients (5/7) and 67% of adult patients (41/61). The median BS in this subpopulation did not statistically differ from the median BS of the entire BAT-VAL population (9, IQR 6-14, n=198).

Association of platelet function assays with the bleeding score

The results of 16 different laboratory assays (**Supplementary Table 2 and 5**) were recorded in the CRF and here we show only those assays for which an adequate number of patients (≥15) was available for reliable statistical analysis.

Light transmission aggregometry

Out of 67 IPFD patients assessed by LTA, a platelet aggregation defect was found in 48 (71%).

The % of patients with high BS and defective LTA in response to ADP was significantly higher compared to that of patients with normal BS and defective LTA (59.4±4.7% vs 35.5±4.8%, $p<0.05$).

Maximal amplitude of platelet aggregation induced by all tested agonists (ADP, epinephrine, collagen, TRAP-6, U46619), except arachidonic acid, was significantly lower in subjects with an abnormal BS (**Figure 1A**). Enrolled patients with biallelic BSS were only 5, all with defective response to ristocetin (*data not shown*). An abnormal LTA in response to at least two agonists was significantly associated with higher BS (**Supplementary Figure 2**).

Moreover, a significant inverse correlation between maximal amplitude of aggregation induced by ADP ($r^2=0.57$, $p<0.001$), epinephrine ($r^2=0.46$, $p<0.001$), collagen ($r^2=0.42$, $p<0.001$) and TRAP-6 ($r^2=0.52$, $p<0.001$) and the ISTH-BAT BS was found (**Supplementary Figure 3**).

Analysis performed replacing the LTA response to the highest with that to the lowest agonist dose, when available, showed that statistical significance is maintained and even strengthened (from <0.05 to <0.01 for LTA in response to ADP) (**Supplementary Figure 4**).

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with severe bleeding after surgery, while defective platelet aggregation in response to ADP and TRAP-6 with severe menorrhagia (**Supplementary Table 6**).

ROC curve analysis showed that a platelet aggregation defect predicted a pathologic BS with an area under the curve (AUC) of 0.69 for ADP, 0.68 for collagen, 0.72 for epinephrine and 0.87 for TRAP-6, in the latter case showing a good predictive ability (**Supplementary Figure 5**).

A maximal amplitude of aggregation of ≤17% for ADP (OR 6.1, 95% CI 1.7-21.1, p<0.01), <48% for collagen (OR 2.6, 95% CI 1.01-6.9, p<0.05), <14% for epinephrine (OR 2.139, 95% CI 0.744-6.151, p=ns) and <71% for TRAP-6 (OR 3.6, 95% CI 1.01-12.8, p<0.05) were significantly associated with an increased probability to have an abnormal BS.

Excluding GT and CalDAG-GEFI cases, that are characterized by absent or strongly reduced aggregation in response to all agonists, the association of enhanced BS with reduced % maximal aggregation remained only for TRAP-6 (**Figure 1B**). This is probably in part due to the reduction of the power of the statistical test after exclusion of GT and CalDAG-GEFI cases which make up around one third of the study patients. For instance, for aggregation induced by epinephrine the power of the test drops from 100% to 20.1%, by ADP from 100% to 6.3%. On the contrary, for aggregation induced by TRAP-6 the power of the test changes only from 100% to 99.8%.

Bleeding time and PFA-100®

The BT and PFA-100® closure time were altered, respectively, in 64% and 88% of tested patients.

The BT was significantly more prolonged in patients with a high BS compared to those with a normal BS (**Figure 2A**). Excluding GT and CalDAG-GEFI cases the association of pathologic BS with BT prolongation remained statistically significant (**Figure 2B**). An abnormal BT was significantly more common among patients with a pathologic compared with those with a normal BS (**Figure 2C**), even after exclusion of GT and CalDAG-GEFI cases (**Figure 2D**). On the contrary, neither C/ADP nor C/EPI PFA-100® closure times were different between patients with normal or high BS (**Figure 2E and 2F**). Moreover, a significant direct correlation between the bleeding time and the ISTH-BAT BS was found (r=0.72, p<0.01) (**Supplementary Figure 6**), while no association was found between PFA-100® closure time and the BS (**Figure 2G-H**).

ROC curve analysis revealed moderate discriminative ability of the BT for pathologic BS, with an AUC of 0.795 (p<0.001), also after exclusion of GT and CalDAG–GEFI patients. The best cut-off of BT discriminating pathologic from normal BS was >12 min, with a sensitivity of 94%, specificity of 64%,

0.87-78.01, p=ns).

ROC curve analysis showed no discriminative ability of PFA-100® for pathologic BS with an AUC of 0.642 (p=ns) (OR 0.35, 95% CI 0.05-2.24, p=ns).

Platelet surface GPIIb/IIIa expression and activation

GPIIb/IIIa surface expression by flow cytometry was abnormal in 27% of tested patients and GPIIb/IIIa activation (PAC-1 binding) in 39%.

Reduced GPIIb/IIIa expression was significantly more frequent in patients with a pathologic BS: only 9% of patients with defective GPIIb and 0% of patients with defective GPIIIa had a normal BS (**Figure 3 A,B**) (GPIIb: OR 7.2, 95% CI 1.2-40.6, p<0.05; GPIIIa: OR 7.5, 95% CI 0.759-74.157, p=ns).

Moreover, defective PAC-1 binding was more frequent in patients with a high BS (66%) compared to patients with a normal BS (33%) (**Figure 3C**) (OR 6.2, 95% CI 1.2-32.2, p<0.05, 31% were GT).

Among the single ISTH-BAT items, defective PAC-1 binding upon activation by ADP associated with severe bleeding after tooth extraction (**Supplementary Table 6**).

ROC curve analysis revealed moderate discriminative ability of PAC-1 binding for BS with an AUC of 0.778 (p<0.001). The best PAC-1 binding cut-off discriminating pathological from normal BS was <3.7 MFI (**Supplementary Figure 8**).

Excluding GT and CalDAG-GEFI cases the associations of pathologic BS with reduced GPIIb/IIIa expression and impaired PAC-1 binding were no longer significant (**Figure 3 D-F**).

α/δ granules content and release

Reduced α-granule content was found in 29% of tested patients and it was significantly more common among those with a pathologic BS (**Figure 4 A**) (OR 9, 95% CI 1.5-50.6, p<0.05). Excluding GT and CalDAG-GEFI cases the association of pathologic BS with reduced α-granule content remained statistically significant (**Figure 4 B**).

Instead, no correlation was found between α-granule release and the BS (OR 7.2, 05% CI 0.410-43.035, p=ns) (**Figure 4 C**). Among the single ISTH-BAT items, defective content of α–granules associated with severe epistaxis and bleeding after surgery (**Supplementary Table 6**).

On the contrary dense granule content or release were not correlated with the BS (content: OR 0.857, 95% CI 0.207-3.552, p=ns; release: OR 1, 95% CI 0.212-4.709).

Patients with concomitant defective α-granule content and aggregation (at least one agonist) had a higher bleeding score compared with patients with only one test defective (**Supplementary Figure**

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exclusion of the pediatric population.

Other tests

Platelet count and MPV were not different between patients with a normal or a high bleeding score, moreover we did not find any correlation between laboratory assay results and these parameters. Finally, there was no association between clot retraction or serum TxB_2 and the ISTH BS. Platelet electron microscopy (TEM) was performed in only 8 subjects therefore results were inconclusive.

Discussion

In the current study we examined whether the degree of impairment of platelet function laboratory assays is associated with the severity of bleeding in patients with IPFD. The ISTH-BAT has been previously validated cross-sectionally and prospectively to assess the severity of bleeding diathesis in patients with an IPFD showing that it reflects the severity of patient bleeding tendency [7,9].

Our data suggest that abnormal platelet function laboratory assay results are associated with a more severe bleeding tendency in IPFD. However, we could not identify a single test defective in all patients with an abnormal BS, for instance, 5 out of 46 patients (11%) with pathologic BS had completely normal LTA but an impaired granule content or release, confirming that platelet secretion studies must be performed for IPFD diagnostics even when LTA is normal [20,28]. This suggests that a single test may not be sufficient to predict an increased BS but that a panel of laboratory assays is required. This confirms previous data showing that LTA is valuable for detecting platelet function abnormalities particularly when the test shows abnormal responses to multiple agonists [29]. Indeed, in our study an abnormal LTA to at least two agonists was associated with a higher BS with particular strength.

In particular, we found that impaired LTA was associated with a high likelihood of an abnormal BS. These findings are in agreement with previously published results showing the same association in patients with GT, bBSS, patients with defective aggregation in response with adrenaline, dense granule deficiency, FPD/AML [16, 18] or with a generic diagnosis of platelet function disorder [19]. As expected, after the exclusion of GT and CalDAG-GEFI patients, who notoriously display a severe platelet aggregation defect, the association of a pathologic BS with reduced aggregation remained only for TRAP-6. This may in part be due to the relatively low statistical power remaining after the exclusion of one third of the enrolled patients. Concerning the finding of a significant association between lower TRAP-6 induced LTA and a pathologic bleeding score, this is in line with previous

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6 in patients with ITP [30].

Moreover, we showed an association between impaired GPIIb/IIIa expression and activation, as well as reduced α-granule content, with increased BS, which however was no longer significant after the exclusion of GT and CalDAG-GEFI patients.

In addition, we found that a prolongation of the bleeding time correlated with an abnormal bleeding score, confirming that, even if not recommended because of insufficient specificity, sensitivity and/or low reproducibility [20], this may be a potentially useful optional assay for suspected IPFD when performed by skilled operators [31].

BT prolongation and reduced α-granule content remained significantly associated with BS even after the exclusion of GT and CalDAG-GEFI patients.

We also found an association between some specific items of the ISTH-BAT and platelet function assays results, thus corroborating a correlation between platelet function assays and the main typical bleeding manifestations of mucocutaneous bleeding disorders.

We did not find any correlation instead between dense granule content and an abnormal BS, at variance with other authors [18,19], however they assessed dense granule content by TEM while in our study TEM was performed only in a low number of subjects making the results inconclusive. Indeed, according with our results, dense granule content assessed by lumiaggregometry did not correlate with bleeding manifestations in some previous studies [14,15]. We also did not find any correlation between PFA-100® and an abnormal BS, while in a previous study a prolonged C/EPI PFA-100® closure time was significantly associated with the bleeding severity in patients with clinical suspicion of a bleeding disorders, however in that study only 9% of the study population was represented by patients with a platelet defect [32].

Our results differ from some previous studies which did not identify a correlation between defective platelet aggregation [1,14,15], platelet glycoprotein expression [15], α-granule content [15] and the bleeding diathesis. However, one study included subjects without a conclusive diagnosis of IPFD [1], another excluded patients with GT, BSS, MYH9-related disorder and HPS [14], and the third investigated only patients with primary secretion defects [15], limiting their generalizability.

In our case-series some subjects affected by IPFD conventionally considered as severe showed a normal ISTH-BAT BS. We do not have an explanation for this, but it has been reported that among patients with some usually severe forms the ISTH-BAT BS may sometimes be only mildly altered or normal, reflecting the phenotypic heterogeneity of IPFD [3,7]. Even the spectrum of clinical bleeding manifestations of patients with GT, one amongst the most severe bleeding disorders, has been

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Strengths of our study are the inclusion of several laboratory assays, the enrollment of patients with a well-defined IPFD diagnosis and of a wide range of forms, and the worldwide representation of participating centers.

Limitations of our study are: 1) the relatively small number of enrolled patients, however these were very well characterized; 2) the inclusion of a substantial number of severe disorders, such as BSS and GT, which however were present in a similar or even higher percentage in two previous large IPFD case-series [7, 8]; 3) the relative low number of records for some tests, which however are carried out only by few specialized laboratories worldwide [34]; 4) the fact that no patients were included for whom all the tests were available; 5) the fact that laboratory tests were not centralized; 6) the post-hoc nature of the study and thus the lack of a prospective design.

Moreover, we did not collect information on race/ethnicity of participants and we acknowledge this as a limitation, because we might miss information on the socio-cultural determinants of bleeding severity of the studied population, and because a racial difference has been described for PAR4-mediated platelet aggregation [35].

Although we acknowledge the limitations of this study we believe that it suggests that a diagnostic approach integrating the assessment of the ISTH BAT bleeding score with the results of standardized laboratory platelet function assays may help to more precisely define the future bleeding risk of patients with IPFD.

In conclusion, we found that some platelet function laboratory assays may predict an enhanced bleeding tendency in patients with IPFD, with important implications for the management of these patients. A prospective validation of the results of this study by a larger, prospective, international collaborative study is highly warranted.

Authorship contributions

PG and ML conceived and designed the study; PG, EF, LBu, MCA, CF,GP, MF, TS, NB, EDC, MC, MPL, LBa and PH collected cases and provided study materials; FM, EF, LBu and GG assembled and analyzed data; EF, LBu and PG wrote the manuscript. All authors revised and gave final approval to the manuscript.

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Conflict of interest disclosure

The authors declare no conflict of interest.

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Figure 1. Percentage of maximal platelet aggregation as assessed by LTA in response to different stimuli in patients with a normal or a high BS, including all studied patients (**A**) or after the exclusion of GT and CalDAG patient (**B**) s. Data are shown as violin plots, expressing median and quartiles and the frequency distribution curve and the individual points (*p<0.05 vs normal BS, **p<0.01 vs normal BS, One-way ANOVA). EPI=epinephrine; Coll=collagen; AA= arachidonic acid.

Figure 2. Bleeding time in patients with normal or high BS, including all studied patients (**A**) or after the exclusion of GT and CalDAG patients (**B**) (*p<0.05 vs normal BS, Mann-Whitney test).
Percentage of patients with normal or high BS according to normal or prolonged BT, including all studied patients (**C**) or after the exclusion of GT and CalDAG patients (**D**) (*p<0.05 vs normal BS, Mann-Whitney test).
PFA-100® closure time with the coll/ADP (**E**) and with the coll/EPI (**F**) cartridges in patients with a normal or a high BS. Data are shown as violin plots, expressing median and quartiles, the frequency distribution curve and the individual points.
Percentage of patients with normal or high BS according to normal or prolonged PFA-100® closure time with the coll/ADP (**G**) or with the coll/EPI (**H**) cartridges.

Figure 3. Percentage of patients with normal or high BS according to normal or defective expression of GPIIb, GPIIIa or PAC-1 binding, as assessed by flow-cytometry, including all studied patients (**A-C**) or after the exclusion of GT and CalDAG patients (**D-F**) (*p<0.05 vs normal BS, χ_2 test).

Figure 4. Percentage of patients with normal or high BS according to normal or defective α-granules content, including all studied patients (**A**) or after the exclusion of GT and CalDAG patients (**B**) or normal or defective α-granules release (**C**) (*p<0.05 vs normal BS, χ_2 test).

Figure 1

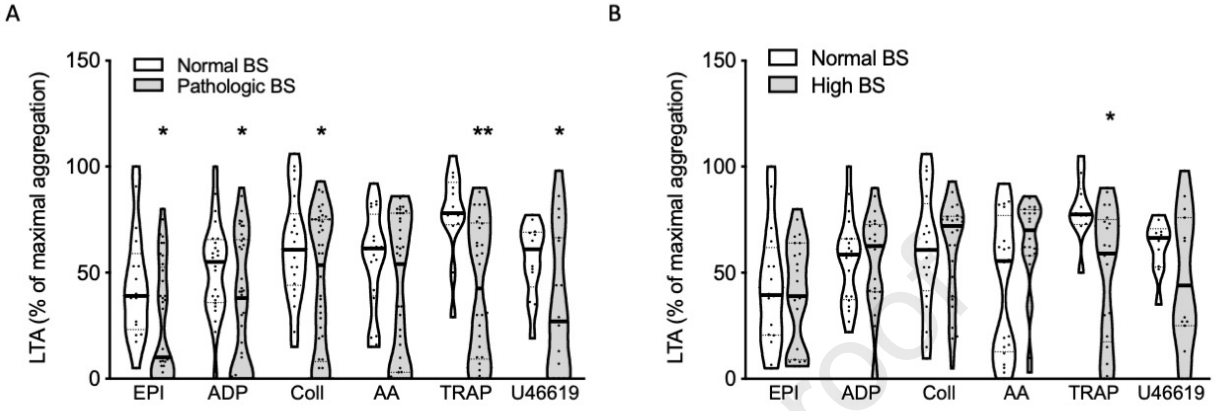

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Figure 2

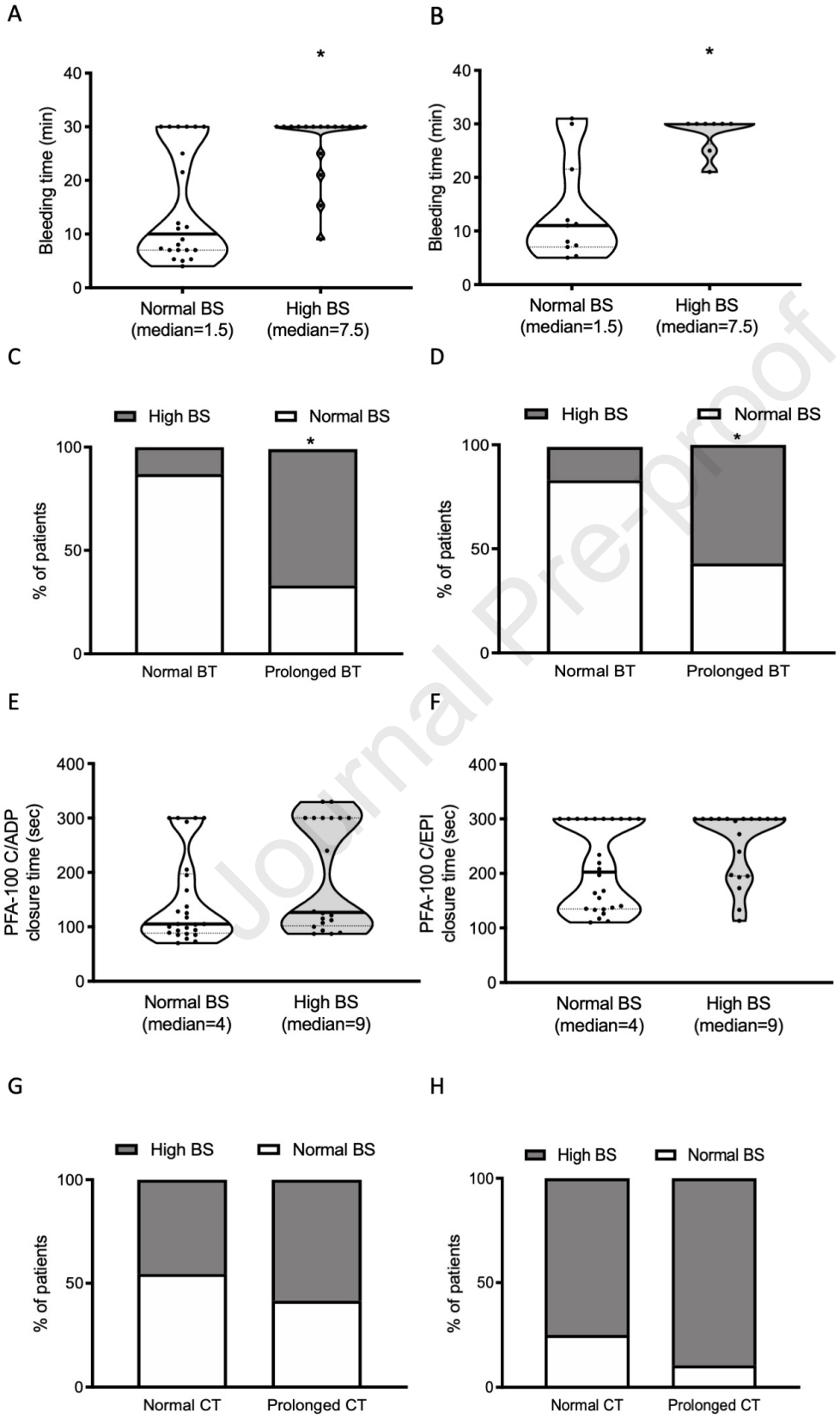

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Figure 3

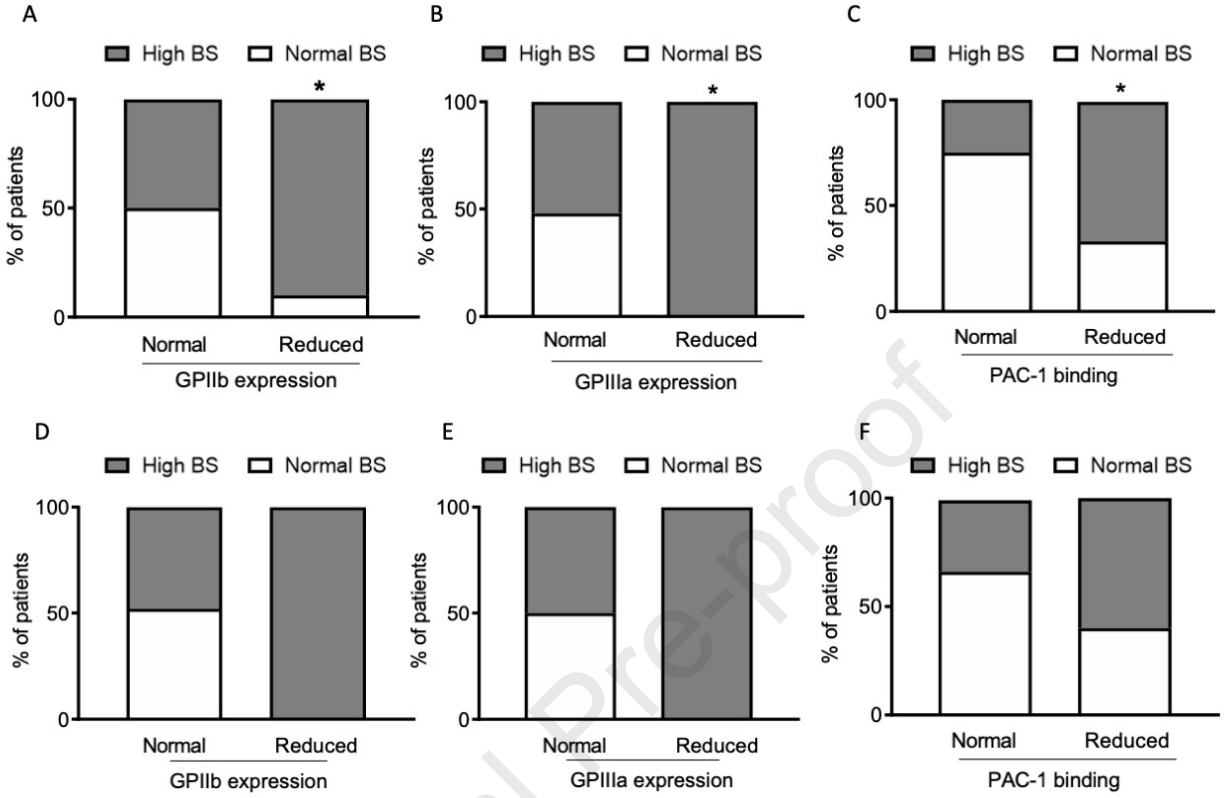

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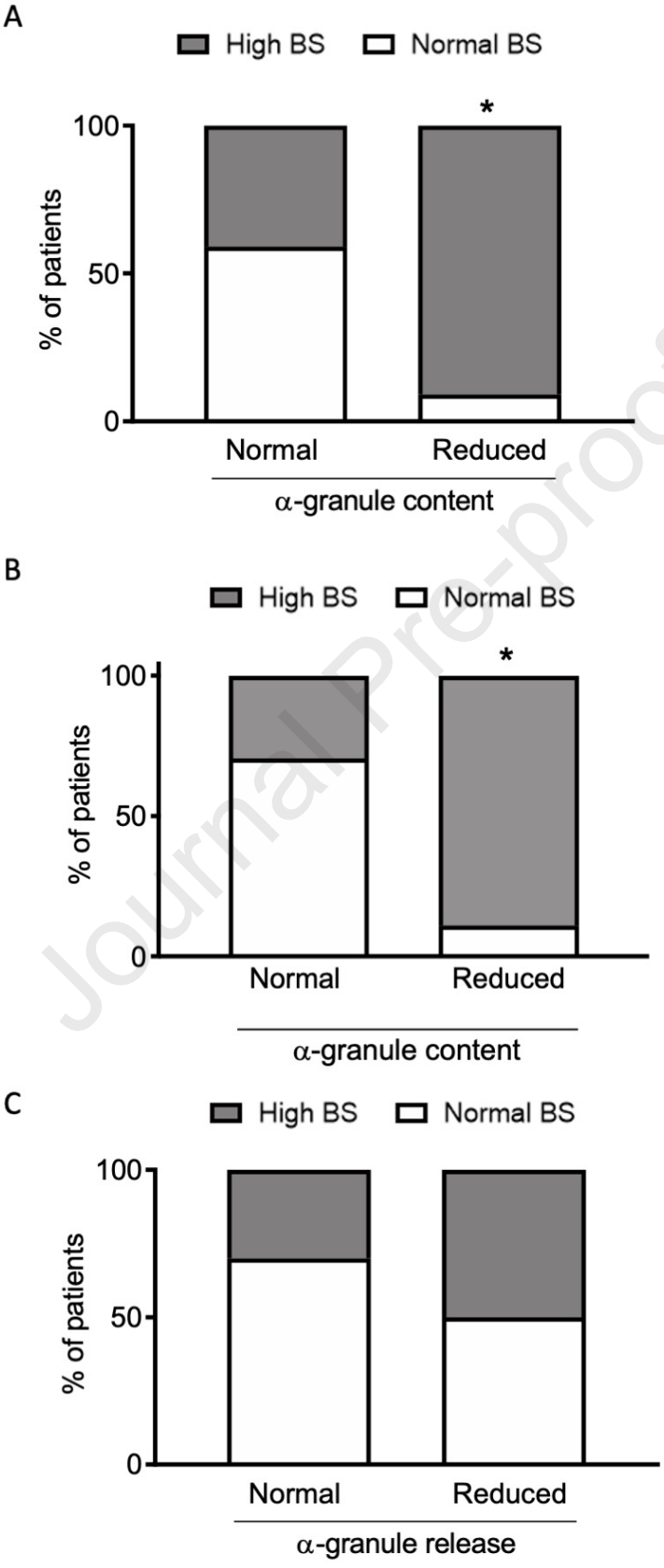
Figure 4



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