Platelet function in adult ITP patients can be either increased or decreased, compared to healthy controls, and is associated with bleeding risk

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Objectives: To test whether, together with platelet count, platelet activity could be an important predictor of bleeding risk in immune thrombocytopenia (ITP) patients.

Methods: Platelet activity was tested by flow cytometric measurement of agonist induced P-selectin expression and compared between 23 adult ITP patients and 22 healthy volunteers.

Results: Platelet activity could be either increased or decreased in ITP patients, compared to healthy volunteers. In the lowest platelet count category, normal to low platelet activity was associated with the biggest increase in bleeding risk. Risk difference 80% (95% confidence interval: 45–115%) for <32 × 10^9 platelets/L. For higher platelet counts, there was no association of platelet activity with bleeding risk.

Discussion: Increased platelet activity was associated with decreased bleeding risk, but only in patients with low platelet counts.

Conclusion: Platelet activity can be a predictor of bleeding risk in ITP patients with low platelet counts.

Keywords: Immune thrombocytopenia (ITP), Platelets, Platelet function, Bleeding

Introduction

It has been shown that platelet function is associated with bleeding risk in immune thrombocytopenia (ITP) patients. Further, agonist inducible platelet activation has been shown to be negatively associated with both platelet counts and spontaneous platelet activation.

Recently, pediatric ITP patients with lower agonist inducible platelet activity were shown to have more severe bleeding than pediatric ITP patients with higher platelet activity and this association persisted after correction for platelet counts. It is, however, unclear whether this observation also holds for adult ITP patients and how platelet activity in ITP patients compares to healthy controls.

Here we report agonist inducible platelet activation in 23 adult ITP patients compared to 22 healthy control subjects. We subsequently quantified the association of platelet activity with self-reported bleeding during the previous six months and related this to platelet counts.

Methods

Twenty-three ITP patients, consecutively visiting our out-patient clinic, of whom eight were in complete remission (i.e. ≥ 100 × 10^9 platelets/L) and of whom none had received transfusions in the previous month, all gave informed consent for participation in our study and reported any bleeding during the previous six months. Twenty-two healthy volunteers were recruited to determine the normal range of test results. All study participants had 4.5 mL of blood drawn into a 3.2% sodium citrate vacuum tube. Platelet function tests were performed by adding 5 μL of whole blood per test as previously described. In short, concentration series of collagen-related peptide (CRP) and thrombin receptor activating peptide (TRAP), in the presence of FITC-labelled mouse anti-human CD61 antibodies (Becton Dickinson, Breda, The Netherlands, diluted 1:25) and PE-labelled mouse anti-human P-selectin antibodies (Becton Dickinson, Breda, The Netherlands, diluted 1:25) and PE-labelled mouse anti-human P-selectin antibodies (Becton Dickinson, Breda, The Netherlands,
diluted 1:25) were prepared in HEPES buffered saline (HBS). Agonist concentration series were prepared by serial dilution in 7 steps of 1:4 with starting concentrations for CRP 2.5 μg/mL, and for TRAP 625 nM. CRP (2.5 μg/mL, 625 ng/mL, 156 ng/mL, 39 ng/mL, 10 ng/mL, 2.4 ng/mL, 610 pg/mL, and 153 pg/mL), and TRAP (625 nM, 156 nM, 39 nM, 10 nM, 2.4 nM, 610 pM, 153 pM, and 38 pM) in HBS were prepared Platelet responsiveness was quantified both dichotomously (i.e. percentage responsive platelets) and continuously (i.e. relative MFI in the PE channel), as previously suggested.4,5

Correction for platelet count, as a potential confounder, was performed in four categories based on the quartiles of platelet counts (<32, 32–72, 73–132, >132×10^9 platelets/L), using the Mantel–Haenszel method.6 The observed concentration response profiles for platelets from ITP patients were classified as ‘increased activity’, ‘normal activity’, or ‘decreased activity’, compared to the observed profiles in healthy volunteers.

Results

As shown in Fig. 1, some ITP patients showed markedly reduced fractions of responsive platelets in response to stimulation with CRP (panel A) or TRAP (panel C), compared to healthy volunteers, while relative MFI for P-selectin staining (panels B and D) was increased in some and decreased in other ITP patients, compared to healthy volunteers. ITP patients in complete remission never showed deceased platelet activity, neither when measured as fraction of responsive platelets nor as relative MFI for P-selecting staining. However, some ITP patients in complete remission did show increased platelet activity for the relative MFI for P-selecting staining.

Figure 2 shows the risk difference between ITP patients with high platelet activity and those with normal to low platelet activity, stratified by platelet count category. In ITP patients in the lowest count category (i.e. <32×10^9 platelets/L), the risk of bleeding was reduced by 80% (95% confidence interval (CI): 45–115%), for patients with counts of 32–72×10^9 platelets/L.
platelets/L this risk difference was 33% (CI: −4 to 71%), for counts of 73–132 × 10⁹ platelets/L this was also 33% (CI: −20 to 87%) and for ITP patients with counts >132 × 10⁹ platelets/L, platelet activity did not influence bleeding risk. The risk difference in this group was 0% because no bleeding occurred in this group, irrespective of platelet activity.

Pooling the risk difference across these different platelet count categories was not possible, since the category specific estimates were too far apart to justify the necessary assumption of one underlying effect size for the different strata.

Discussion

Our results show increased platelet activity to be associated with decreased bleeding risk, but only in ITP patients with relatively low platelet counts. This seems to suggest that high activity might partly compensate for low platelet counts, but is redundant in the presence of higher counts. However, these results cannot be interpreted causally, since other potential confounding factors could not be corrected for, due to limited study size. Limited study size could also obscure a true association, by limiting the power to detect it. However, the statistical imprecision caused by the relatively small number of patients and volunteers is adequately reflected in CIs and did not preclude the detection of differences between the different groups. This suggests study size was sufficient for this simple purpose. A further potential limitation is that bleeding occurred in the six months prior to platelet testing and could therefore have affected platelet responsiveness, instead of the other way around. Finally, self-reported bleeding is scored only as present or absent, without consideration for the severity of bleeding. This lack of detail in the type of bleeding could cause misclassification and therefore an underestimation of the observed effect. Since we still observe a difference between the bleeding and non-bleeding groups, the real difference could only be bigger than reported. Furthermore, we expect self-reported bleeding to reflect the most relevant bleeding events.

Our comparison with healthy controls shows platelet responsiveness in adult ITP patients can be either increased or decreased. ITP patients in complete remission, however, only showed increased platelet activity and never reported bleeding.

In conclusion, platelet activity can be a predictor of bleeding risk in ITP patients with low platelet counts.

Disclaimer statements

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