STIMULUS REPORT

blood advances

The interplay between GPIb/IX antibodies, platelet hepatic sequestration, and TPO levels in patients with chronic ITP

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Key Points

- GPIb/IX antibodies do not appear to inhibit or block TPO production in patients with ITP when stratified toward the degree of thrombocytopenia.
- GPIb/IX antibodies may be associated with higher TPO and increased platelet hepatic sequestration under severe thrombocytopenic conditions.

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder with an incompletely understood pathophysiology but includes platelet-clearance in the spleen and liver via T cells and/or platelet autoantibodies. Strikingly, thrombopoietin (TPO) levels remain low in ITP. Platelet-glycoprotein (GP)Ibα has been described to be required for hepatic TPO generation; however, the role of GPIb antibodies in relation to platelet hepatic sequestration and TPO levels, with consideration of platelet counts, remains to be elucidated. Therefore, we examined 53 patients with chronic and nonsplenectomized ITP for whom we conducted indium-labeled autologous platelet scintigraphy and measured platelet antibodies and TPO levels. Upon stratification toward the severity of thrombocytopenia, no negative association was observed between GPIb/IX antibodies and TPO levels, suggesting that GPIb/IX antibodies do not inhibit or block TPO levels. Surprisingly, we observed a positive association between GPIb/IX antibody levels and TPO levels and GPIb/IX antibodies and platelet hepatic sequestration in patients with severe, but not mild or moderate, thrombocytopenia. In addition, platelet hepatic sequestration and TPO levels were positively associated. This collectively indicates that GPIb/IX antibodies may be associated with increased platelet hepatic sequestration and elevated TPO levels in patients with severe thrombocytopenic ITP; however, further research is warranted to elucidate the pathophysiologic mechanisms.

Introduction

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by low platelet counts ($<100 \times 10^9$/L).[1] The pathophysiologic pathways of platelet clearance in ITP are not yet fully unraveled but involve T cells and/or platelet autoantibodies.[2] One of the most investigated pathways of platelet clearance include the effects of autoantibodies directed against glycoprotein (GP) complexes.[3] Autoanti-body binding to GP complexes, present on the platelet membrane, can lead to liver and/or spleen sequestration, phagocytosis, and, subsequently, to thrombocytopenia.[2] This predominantly occurs via antibody Fc–mediated recognition by Fcγ receptors on macrophages, resulting in phagocytosis in the spleen and/or liver.[4] There is, however, evidence that Fc-independent mechanisms of ITP also exist, leading to platelet hepatic sequestration.[5]

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Thrombopoietin (TPO) regulates platelet production via interaction with myeloproliferative leukemia protein receptor (Mpl; CD110), which is present on megakaryocytes and circulating platelets.[6] TPO plasma levels are mainly derived from the active and continuous TPO production by the liver and to a lesser extent by the spleen, kidney, and bone marrow.[6] In addition, TPO production is induced by the binding of desialylated aged platelets through interaction with the hepatic Ashwell-Morrell receptor (AMR).[6] Furthermore, it has been shown that certain GPIbα-antibodies trigger platelet desialylation, a process that additionally increases the clearance of these platelets via the hepatic AMR.[7] In patients with ITP, remarkably, TPO levels remain relatively low. GPIbα, independent of platelet desialylation, was demonstrated to be required for hepatic TPO generation in mice.[8] GPIbα$^{-/-}$ mice showed lower TPO levels compared with wild-type mice, and in agreement, patients with Bernard-Soulier syndrome, who lack the GPIb-IX-V complex, have low TPO levels with low to moderate platelet counts.[8] In that respect, it was suggested that GPIb/IX antibodies may interfere with hepatic TPO production in ITP, possibly explaining the relatively low TPO levels in patients with ITP.[8] A study by Porcelijn et al,[9] however, did not find an association between GPIb/IX antibodies and TPO levels in a large cohort of 3490 patients with ITP. This study did not incorporate data on platelet counts of these patients. The latter could be of importance because, in healthy subjects, it is well known that, apart from the via AMR-binding induced TPO production, the total platelet mass negatively influences the unbound and measurable TPO levels.[10] As low platelet counts in patients with ITP do not trigger high TPO levels, we aimed to shed light on the interplay among GPIb/IX platelet antibodies, the site of platelet sequestration, and TPO levels, with consideration of platelet counts in a cohort of patients with chronic and nonsplenectomized ITP.

Methods

In this study, we investigated both the association between (1) GPIb/IX antibodies and the site of platelet sequestration by indium (In)-labeled autologous platelet scintigraphy and (2) GPIb/IX antibodies and TPO levels in a cohort of 53 patients with chronic and nonsplenectomized ITP.[11] The included patients had a clinical indication for a scintigraphy scan as indicated by the treating hematologist (indication for a second/third-line therapy with splenectomy as 1 of the therapeutic options), and had a mean age at diagnosis of 36 ± 18 (standard deviation) years. Importantly, we stratified these patients by the degree of thrombocytopenia: mild (platelet counts $> 50 \times 10^9$/L) and moderate/severe ($<50 \times 10^9$/L). An additional sensitivity analysis was performed for severe thrombocytopenia (platelet count $< 25 \times 10^9$/L). Antibody levels were measured using direct (antibody bound directly on patient platelets) and indirect (antibody binding on donor platelets incubated with serum from the patient) monoclonal antibody-specific immobilization of platelet antigen (MAIPA), with a cutoff of 0.130 optical density (OD).[12] TPO levels were measured as described by Folman et al,[13] with a normal range in healthy subjects of 4 to 32 AU/mL. All patients underwent an ^{111}In-labeled autologous platelet sequestration scintigraphy; the sequestration outcome was used as a continuous variable ranging 0% to 100% sequestration in liver. In the clinical setting, the outcome is categorized in splenic, mixed, and platelet hepatic sequestration patterns based on the splenic-to-liver ratio.[14] Associations between TPO levels and anti-GP antibody levels were primarily tested using linear regression models, and multivariable models included platelet counts as a confounder. Differences were considered statistically significant at $P < .05$. The study was approved by the Dutch Medical Ethical Review Board, which was conducted in accordance with the Declaration of Helsinki.

Results and discussion

Antiplatelet antibodies were measured in 53 patients. Twenty-nine patients had mild thrombocytopenia (platelet count $> 50 \times 10^9$/L), and 24 patients had moderate to severe thrombocytopenia (platelet count $< 50 \times 10^9$/L). Of the latter group, 6 patients had severe thrombocytopenia (platelet count $< 25 \times 10^9$/L). GPIb/IX antibody OD values were found to be above the detection threshold in 13 of these patients (direct MAIPA, and 11 patients using the indirect MAIPA), of which 9 patients had mild and 4 patients had moderate/severe thrombocytopenia. The presence of other platelet antibodies, using direct and indirect MAIPA, in these 13 patients is depicted in supplemental Table 1. Upon stratification toward the severity of thrombocytopenia, no negative association was observed between GPIb/IX antibodies (direct and indirect MAIPA) and TPO levels (Table 1). This suggests that GPIb/IX antibody levels do not inhibit or block the regulation of TPO levels in patients with ITP, indicating that other factors and pathways are responsible for the relatively low

Table 1. Regression models for the association between anti-GPIb/IX antibodies, TPO levels, and platelet hepatic sequestration, under thrombocytopenic conditions, in a cohort of patients with chronic and nonsplenectomized ITP

	β (95% confidence interval), *P*		
	Linear regression for patients with ITP with platelet count $> 50 \times 10^9$/L (n = 29)	**Linear regression for patients with ITP with platelet count $< 50 \times 10^9$/L (n = 24)**	**Sensitivity analysis: Linear regression for patients with ITP with platelet count $< 25 \times 10^9$/L (n = 6)**
Association 1: anti-GPIb/IX and TPO level	Direct MAIPA: 0.000 (−0.002 to 0.002), $P = .91$ Indirect MAIPA: 0.000 (−0.0005 to 0.004); $P = .88$	Direct MAIPA: −0.045 (−0.180 to 0.089); $P = .489$ Indirect MAIPA: 0.051 (0.004-0.097) $P = .03$*	Direct MAIPA: 0.092 (0.012-0.172), $P = .03$* Indirect MAIPA: 0.116 (−0.131 to 0.364) $P = .18$
Association 2: anti-GPIb/IX and platelet hepatic sequestration	Direct MAIPA: −0.001 (−0.004 to 0.002), $P = .63$ Indirect MAIPA: −0.002 (−0.004 to 0.001) $P = .22$	Direct MAIPA: 0.025 (−0.029 to 0.079), $P = .35$ Indirect MAIPA: 0.020 (0.006-0.035) $P = .008$*	Direct MAIPA: 0.026 (0.006-0.045), $P = .02$* Indirect MAIPA: 0.045 (0.003-0.088) $P = .04$*
Association 3: TPO level and platelet hepatic sequestration	−0.076 (−0.793 to 0.641) $P = .83$	0.262 (0.124-0.400) $P = .001$*	0.228 (0.126-0.331) $P = .002$*

*Statistical significance: $P < .05$.

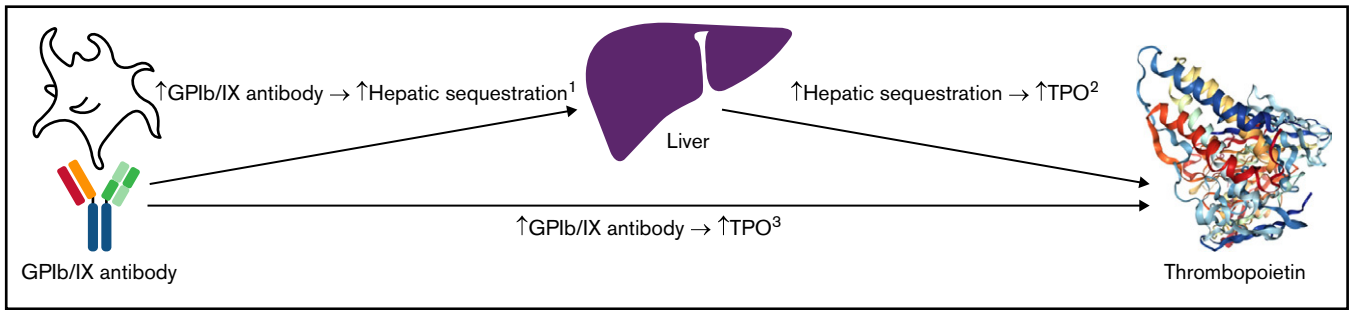

Figure 1. Interplay between GPIb/IX antibodies, platelet hepatic sequestration, and TPO levels in patients with chronic and nonsplenectomized ITP with severe thrombocytopenia. [1]Association found between GPIb/IX antibody levels and increased hepatic sequestration of platelets. [2]Association found between hepatic sequestration and increased TPO levels. [3]Direct association between GPIb/IX antibody levels and increased TPO levels.

levels of TPO in patients with ITP. Surprisingly, a significant positive association was observed between GPIb/IX antibody levels (direct MAIPA) and TPO levels and GPIb/IX antibodies (direct and indirect MAIPA) and platelet hepatic sequestration in patients with severe thrombocytopenia but not in patients with mild (direct and indirect MAIPA) or moderate thrombocytopenia (direct MAIPA; Table 1). In addition, platelet hepatic sequestration and TPO levels were positively associated (Table 1). Collectively, this may suggest that GPIb/IX antibodies could be associated with an increased platelet hepatic sequestration and elevated TPO levels in patients with severe thrombocytopenic ITP (by a direct and/or 2 sequentially indirect pathways as indicated in Figure 1). In contrast, we found no significant associations between GPIIb/IIIa or GPV antibodies (direct and indirect MAIPA) and platelet hepatic sequestration or TPO levels in patients with ITP with severe thrombocytopenia (supplemental Table 2). This indicates that the previously mentioned associations may be specific for GPIb/IX antibodies under severe thrombocytopenic conditions.

In this study, we find that GPIb/IX antibodies do not appear to inhibit or block TPO production in patients with ITP when stratified toward the degree of thrombocytopenia. In addition, we find that GPIb/IX antibodies may be associated with stimulated TPO production through the liver, but only under severe thrombocytopenic conditions. In this setting of increased clearance, there may be an additional contribution of specific GPIb/IX antibodies directed against the ligand-binding domain in an Fc-independent manner.[15] This may result in mechanomolecular signaling,[15] leading to increased platelet clearance via the liver, which may stimulate an increase in TPO levels through a feedback mechanism. Although GPIbα was suggested to be required for hepatic TPO generation independently of platelet desialylation in mice,[8] our human data suggest that, under severe thrombocytopenic conditions, there may be an additional contribution of GPIb/IX-induced platelet desialylation and consequently increased hepatic clearance via the AMR, resulting in increased TPO levels. For the first time, our results support a possible link between GPIb/IX antibodies, platelet hepatic sequestration, and increased TPO levels in patients with ITP with platelet counts below 25 x 10^9/L. Although suggestive, from observational data, we cannot conclude on causality. Possible bias may be introduced through indication of scanning relapse and refractory patients. Furthermore, it has been described that different types of GPIb antibodies can have divergent functions[16]; however, in the current study, we were unable to differentiate the different types of GPIb antibodies. Therefore, the mechanistic pathways should be further experimentally explored in in vitro and in vivo animal models. Importantly, these results will also need to be validated in larger cohorts. The current findings add to the largely unknown pathophysiology of ITP and could ultimately be applied to the development of new individualized treatment options for patients with ITP.

Authorship

Contribution: R.K., S.N.A., V.S.N., and M.R.S. conceived and designed the study; S.N.A. and R.K. performed the study and analyzed data; S.N.A. and R.K. wrote the manuscript; V.S.N., L.P., T.N., J.J.Z., M.d.H., and M.S. critically reviewed and edited the manuscript; and all authors interpreted the data and approved the final draft.

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