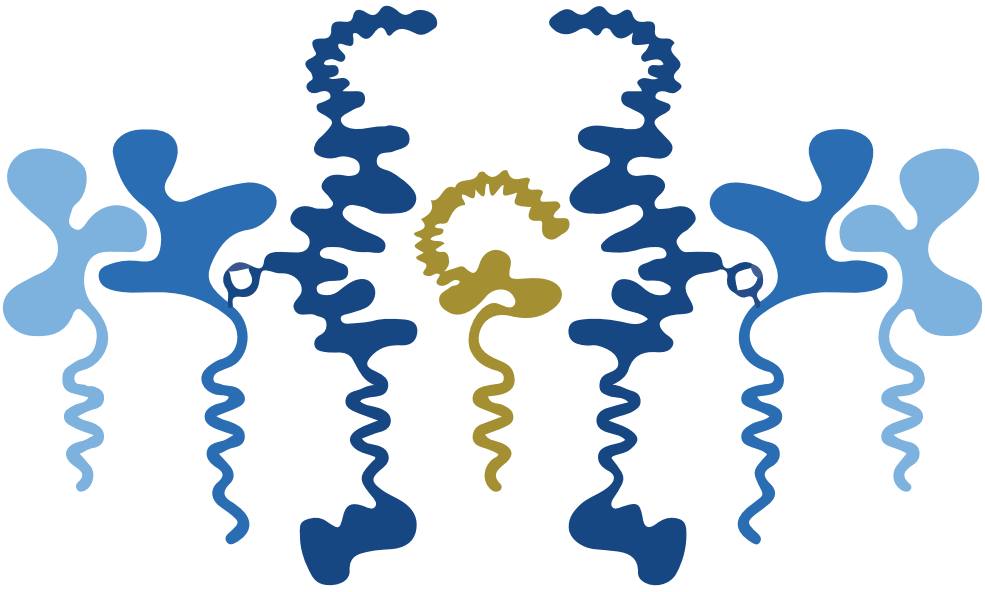
# The diagnostic value of plasma thrombopoietin levels and platelet autoantibodies

Porcelijn, L.



# 07

# CHAPTER 7

## Anti-glycoprotein Ibα autoantibodies do not impair circulating thrombopoietin levels in immune thrombocytopenia patients

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# Anti-glycoprotein Ibα autoantibodies do not impair circulating thrombopoietin levels in immune thrombocytopenia patients

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**To the editor:**

A recently discovered mechanism showed that in mice, anti-glycoprotein (GP) Ib/IX platelet antibodies interfere with hepatocyte thrombopoietin (TPO) production. This mechanism may potentially also contribute to the relatively low TPO levels observed in patients suffering from immune thrombocytopenia (ITP), an autoimmune bleeding disorder in which anti-platelet autoantibodies are able to target platelets. To investigate this we reviewed a large cohort of thrombocytopenic patients which we assessed for anti-platelet autoantibodies and TPO levels (n=3490). We show for the first time that anti-GPIb/IX antibodies, occurring alone or together with other anti-platelet autoantibodies such as anti-GPV and/or with anti-GPIIb/IIIa antibodies, do not influence circulating TPO levels in ITP patients. This suggests that anti-GPIb/IX autoantibodies do not interfere directly with TPO production in humans. Platelet production is regulated mainly by TPO, a hematopoietic growth factor that interacts with the myeloproliferative leukemia protein receptor (Mpl; CD110) on megakaryocytes and circulating platelets.[1,2] The primary site of TPO synthesis is the liver, and to a lesser extent kidney, spleen and bone marrow cells.[3] Interestingly, it was suggested that TPO production is induced by the binding of desialylated aged platelets interacting with the hepatocyte asialoglycoprotein receptor (ASGPR), also known as the hepatic Ashwell-Morrell receptor (AMR).[4] Furthermore, circulating TPO levels are influenced by binding of TPO to platelet- and megakaryocyte-Mpl.[5,6]

ITP is an autoimmune bleeding disorder with a complex pathophysiology.[7] Many ITP patients show autoantibodies to platelet GPIIb/IIIa, GPIb/IX and GPV. In ITP patients, there appears to be an ongoing platelet destruction, but with normal

or mildly elevated TPO levels.[8,9] Recently, a novel mechanism of TPO production was described, in which platelet GPIb signals in an AMR-independent manner to induce hepatocytic TPO production, and was independent of platelet desialylation.[10] In this mouse study, monoclonal antibodies to GPIb were shown to inhibit hepatic TPO production.[10] This mechanism might play an additional role in the relatively low TPO levels observed in ITP patients. However, it has not been investigated if anti-GPIb antibodies are indeed able to interfere with circulating TPO levels in ITP patients. To address this unresolved question, we evaluated TPO levels in ITP patients with anti-platelet autoantibodies including a subgroup with only anti-GPIb IgG antibodies using a large cohort of thrombocytopenic patients evaluated in our national reference laboratory
(Sanquin Diagnostic Services, Amsterdam, The Netherlands) for antigen-specific platelet autoantibodies (years 2011-2019; 3490 patients and 201 healthy controls). Data were handled under national responsible use policies (Code of Conduct for Use of Data in Health Research; https://www.federa.org/codes-conduct). All of these thrombocytopenic samples were tested for platelet autoantibodies against GPIbIX, GPV and GPIIb/IIIa using a modified monoclonal antibody-immobilization of platelet antigens (MAIPA) assay.[11] In addition, circulating TPO levels were measured in fresh EDTA plasma by an in-house ELISA, as previously described.[12,13] Control samples were obtained from non-thrombocytopenic healthy blood donors. Unfortunately, platelet counts at the time of analysis were not available in our laboratory information system. A two-sided alpha value of 0.05 was used as cut-off for statistical significance. Children below 1 year of age were excluded. The total cohort which was analyzed comprised of 3490 individual thrombocytopenic patients with
2979 and 2239 samples for direct and indirect tests, respectively, and 201 healthy controls.

Platelet-associated IgG autoantibodies (direct test) and/or circulating anti-platelet IgG (indirect test) were assessed using MAIPA. Although not all ITP patients have detectable autoantibodies by MAIPA, we have previously reported that a direct antibody test has 98% specificity for clinically diagnosed ITP.[11]
In the current study we found that, in agreement with previous studies,[8,9] TPO levels in ITP patients were significantly increased compared to healthy controls (Figure 1A; P<3.5x10-3 versus healthy controls). However, all patients with detectable antibodies to GPIIb/IIIa, GPV or GPIb/IX, as determined with a direct test, showed similar TPO levels (Figure 1A). Among the majority of ITP patients with multiple anti-platelet glycoprotein antibodies, presence of anti-GPIb antibodies did not affect TPO levels (Figure 1B). Identically to the direct test, also using the indirect test, patients with circulating antibodies against GPIb alone displayed no differences in TPO levels compared to patients with anti-GPIIb/IIIa or anti-GPV antibodies (Figure 1C). In addition, we did not observe

any differences in TPO levels when anti-GPIb/IX antibodies co-occurred with antibodies against GPV and/or GPIIb/IIIa (Figure 1D). It is conceivable that a certain antibody level is required to achieve sufficient opsonization to block GPIb-hepatocyte interactions. However, in agreement with the results above, we did not observe any significant differences between patients with low or high anti-GPIb IgG antibody levels and TPO (Figure 1E).

Our findings in human ITP samples are not in agreement with the recently proposed mechanism stating that anti-GPIb autoantibodies impair TPO production in mice.[10] Alternatively, it may be possible that platelet activation, complement activation or a mechanical feature induced by anti-GPIb antibodies[14] determines the ability to induce Fc-independent platelet clearance, which just like anti-GPIIb/IIIa Fc-mediated platelet clearance, may not induce increased hepatic TPO generation. The limitations of our study are that no additional clinical information, such as co-morbidities and platelet counts, were available. Key strengths of our study are the large number of clinical patient samples available for analysis, the ability to distinguish antibodies against multiple platelet antigens, and the standardized analysis of anti-platelet antibodies in our laboratory. To our knowledge, this is the first time such as large scale analysis is performed investigating the association between anti-platelet GPIb, GPV, and GPII b/IIIa antibodies versus circulating TPO levels in thrombocytopenic patients. Our results further support the notion that the majority of ITP patients clearly demonstrate the simultaneous presence of antibodies to multiple platelet-glycoproteins, including anti-GPV antibodies which were found to be very prevalent, as also previously reported in ITP.[11,15]

In conclusion, our data show that in ITP patients anti-GPIb/IX antibodies, alone or co-occurring with anti-GPV and/or with anti-GPIIb/III a antibodies, do not influence circulating TPO levels. It therefore appears that in humans blocking of GPIb by anti-platelet GPIb antibodies does not directly account for the reduced TPO levels observed in ITP. More research is required to understand the mechanisms which account for the slightly elevated TPO levels in ITP patients.

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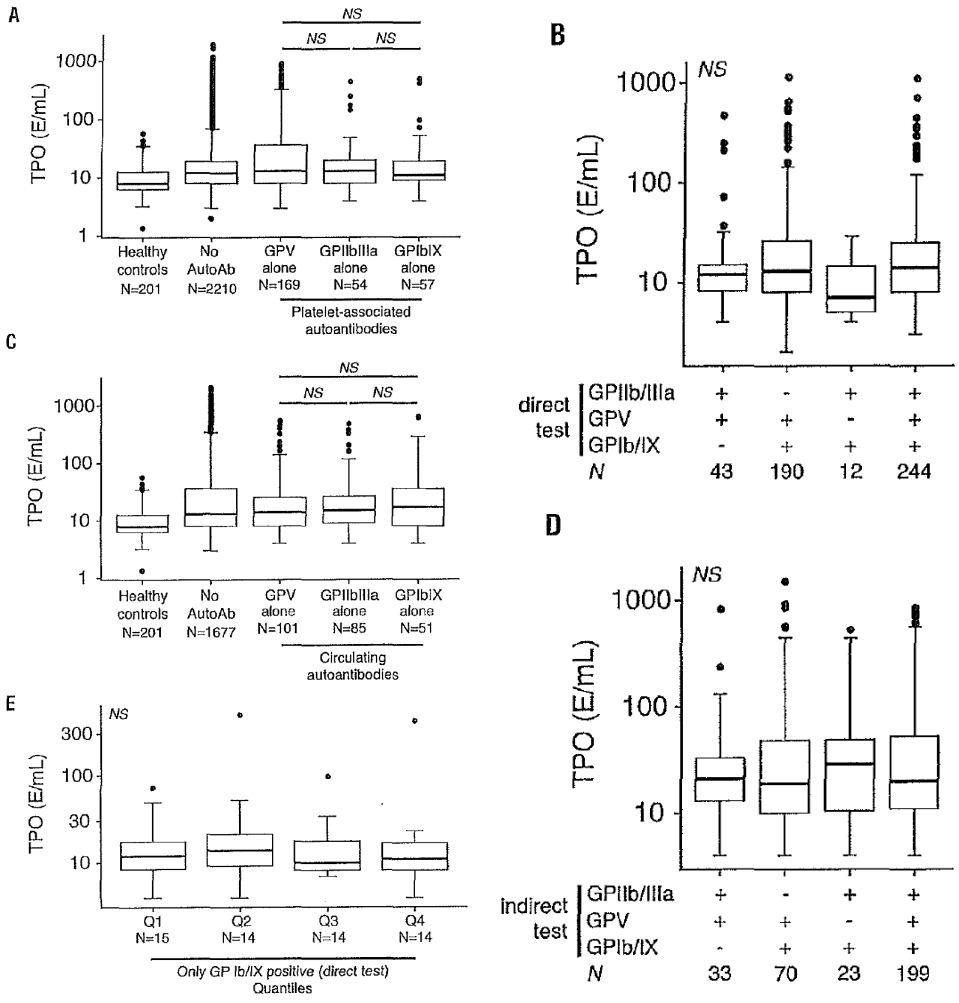


*Figure 1. Anti-GPIbIX autoantibodies do not correlate with circulating TPO levels in patients with immune thrombocytopenia. (A) TPO levels, dependent on platelet-associated antibodies (direct test) for the indicated platelet antigens. Only single-positive antibody results are displayed. Exact P-values are (Post-hoc Nemenyi test): Anti-GPV vs anti-GPIb/IX, P=0.84; anti-GPIIb/IIIa vs anti-GPIb/IX, P=0.99; anti-GPV vs anti-GPIIb/IIIa, P=0.76. (B) TPO levels for multiple specificities of anti-platelet autoantibodies in a direct test. Kruskal-Wallis test, P=0.19. (C) TPO levels for single-positive circulating autoantibodies (indirect test). Exact P-values are (Post-hoc Nemenyi test): Anti-GPV vs anti-GPI b/IX, P=0.95; anti-GPIIb/IIIa vs anti-GPIb/IX, P=0.99; anti-GPV vs anti-GPIIb/IIIa, P=0.95. (D) TPO levels by multiple specificities of anti-platelet autoantibodies in an indirect test. Kruskal-Wallis test, P=0.93. (E) No dose-dependent effect of anti-GPIbI X autoantibodies (direct test) on TPO levels in immune thrombocytopenia. Patients were categorized in 25-percent quantiles based on observed antibody levels. Kruskal-Wallis test, P=0.80.*

## References


1. Kaushansky K. The molecular mechanisms that control thrombopoiesis. J Clin Invest. 2005;115(12):3339–3347.
2. Kaushansky K. Thrombopoietin. N Engl J Med. 1998;339(11):746–754.
3. Sungaran R, Markovic B, Chong BH. Localization and regulation of thrombopoietin mRNa expression in human kidney, liver, bone marrow, and spleen using in situ hybridization. Blood. 1997;89(1):101–107.
4. Grozovsky R, Begonja AJ, Liu K, et al. The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling. Nat Med. 2015;21(1):47–54.
5. Folman CC, de Jong SM, de Haas M, Borne von dem AE. Analysis of the kinetics of TPO uptake during platelet transfusion. Transfusion. 2001;41(4):517–521.
6. Fielder PJ, Gurney AL, Stefanich E, et al. Regulation of thrombopoietin levels by c-mpl-mediated binding to platelets. Blood. 1996;87(6):2154–2161.
7. Zufferey A, Kapur R, Semple JW. Pathogenesis and Therapeutic Mechanisms in Immune Thrombocytopenia (ITP). J Clin Med. 2017;6(2):16.
8. Porcelijn L, Folman CC, Bossers B, et al. The diagnostic value of thrombopoietin level measurements in thrombocytopenia. Thromb. Haemost. 1998;79(6):1101–1105.
9. Kosugi S, Kurata Y, Tomiyama Y, et al. Circulating thrombopoietin level in chronic immune thrombocytopenic purpura. Br J Haematol. 1996;93(3):704–706.
10. Xu M, Li J, Neves MAD, et al. GPIbα is required for platelet-mediated hepatic thrombopoietin generation. Blood. 2018;132(6):622–634.
11. Porcelijn L, Huiskes E, Oldert G, et al. Detection of platelet autoantibodies to identify immune thrombocytopenia: state of the art. Br J Haematol. 2018;39(Suppl. 1):195.
12. Folman CC, Borne von dem AE, Rensink IH, et al. Sensitive measurement of thrombopoietin by a monoclonal antibody based sandwich enzyme-linked immunosorbent assay. Thromb Haemost. 1997;78(4):1262–1267.
13. Porcelijn L, Folman CC, de Haas M, et al. Fetal and neonatal thrombopoietin levels in alloimmune thrombocytopenia. Pediatr Res. 2002;52(1):105–108.
14. Quach ME, Dragovich MA, Chen W, et al. Fc-independent immune thrombocytopenia via mechanomolecular signaling in platelets. Blood. 2018;131(7):787–796.
15. Vollenberg R, Jouni R, Norris PAA, et al. Glycoprotein V is a relevant immune target in patients with immune thrombocytopenia. Haematologica. 2019;104(6):1237-1243.