

6. MRC Haemostasis and Thrombosis Database Resource Sites. [Http://europium.csc.mrc.ac.uk](http://europium.csc.mrc.ac.uk)
7. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, Abeysinghe S, Krawczak M, Cooper DN. Human Gene Mutation Database (HGMD): 2003 update. *Hum Mutat* 2003; 21:577-81.
8. Lenting PJ, van Mourik JA, Mertens K. The life cycle of coagulation factor VIII in view of its structure and function. *Blood* 1998;92:3983-96.
9. Cutler JA, Mitchell MJ, Smith MP, Savidge GF. The identification and classification of 41 novel mutations in the factor VIII gene (F8C). *Hum Mut* 2002;19:274-8.
10. Citron M, Godmilow L, Ganguly T, Ganguly A. High throughput mutation screening of the factor VIII gene (F8C) in hemophilia A: 37 novel mutation and genotype-phenotype correlation. *Hum Mut* 2002;20:267-74.

Platelets

Increased glycohalcin index and normal thrombopoietin levels in patients with idiopathic thrombocytopenic purpura with a decreased rate of platelet production

Platelet kinetic studies in idiopathic thrombocytopenic purpura (ITP) have shown that in a subgroup of patients a shortened mean platelet life (MPL) is associated with a decreased platelet production rate (PPR).¹ Other methods of studying certain aspects of thrombocytopoiesis are the plasma concentrations of thrombopoietin and glycohalcin.

haematologica 2005; 90:710-711
 (<http://www.haematologica.org/journal/2005/5/710.html>)

Clinical studies have shown elevated plasma levels of thrombopoietin in conditions with diminished megakaryocyte production.² Glycohalcin is the soluble, external part of membrane glycoprotein Ib α (GPIb α). The glycohalcin-index, normalized for individual platelet count, has been introduced as a parameter of platelet turnover.³ We investigated thrombopoietin and glycohalcin levels in ITP patients and correlated them to the platelet kinetic parameters MPL and PPR. *Platelet kinetic studies.* In order to study platelet kinetics, autologous platelets were labeled with Indium-111 tropolonate according to the recommendations of the International Committee for Standardization in Hematology.⁴ The platelet production rate (PPR) is defined as the number of platelets entering the circulation to maintain the platelet count. The normal values of PPR is 223 $\times 10^9$ /day (158-268) and the normal mean platelet life (MPL) is 9.2 \pm 1.4 days (8.9-9.4).⁵

Plasma thrombopoietin concentrations were determined with an enzyme-linked immunosorbent assay (Quantikine, R&D systems, Minneapolis, USA). The normal value in this assay is 114 pg/mL (93-146). Plasma glycohalcin concentrations were measured by enzymatic immunoassay (Takara Shuzo Co, Ltd). The glycohalcin index is derived from the glycohalcin value (mg³/mL) $\times 250 \times 10^9$ /L divided by the individual platelet count. The normal value is 0.7 (0.6-0.9).

Data are presented as the median with 25th and 75th percentiles. Statistical analysis was performed using Kruskal-Wallis non-parametric analysis of variances and the Wilcoxon two-sample test. Correlations were assessed with Spearman's rank correlation procedure. A *p*-value of <0.05 was considered statistically significant,

Table 1. Characteristics of patients.

	Production decreased	Production normal or increased	<i>p</i>
Patients, n	9	26	
Female	4	16	
Age, years	62 (30-68)	44 (32-67)	0.9
Platelet count at diagnosis, $\times 10^9$ /L	22 (13-46)	63 (43-89)	0.02
Mean platelet life, days	2.6 (1.4-3.7)	1.9 (1.1-3)	0.5
Platelet production rate, 10^9 /d	100 (88-145)	255 (188-325)	0.004
Thrombopoietin, pg/mL	109 (71-172)	111 (64-171)	0.8
Glycohalcin-index	12 (7-25)	5 (3-10)	0.03

Results are expressed as median (25th/75th percentile).

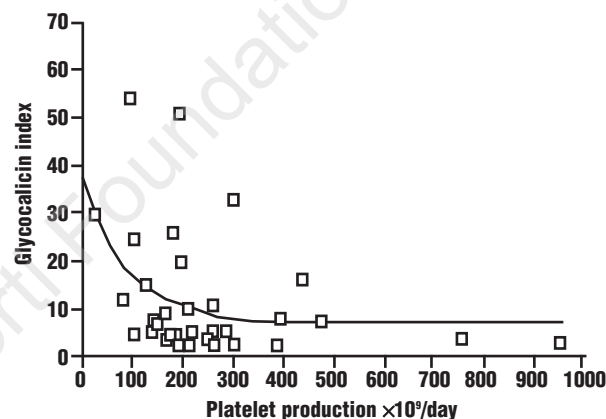


Figure 1. Correlation of glycohalcin index with platelet production rates.

and all tests were two-sided. After informed consent, 35 patients (20 women) with ITP were studied. Their mean age was 45 years (32-66) and platelet count at diagnosis was 58 $\times 10^9$ /L (22-85). MPL was 2 days (1.1-3) and PPR was 195 $\times 10^9$ /day (150-300). PPR was reduced in 9 patients whereas in 26 patients it was normal (n=17) or increased (n=9; median 395, min: 300, max: 950 $\times 10^9$ /day). The PPR were not correlated to changes in MPL. Thrombopoietin plasma levels (110 pg/mL, 68-171) were measured in all the studied patients and compared to those in controls (114 pg/mL, 93-146). No statistical difference was observed (*p*=0.7). In addition, there was no significant difference in thrombopoietin plasma levels in patients with a normal or increased PPR, (111 [64-171]) vs a reduced PPR (median 109 [71-172], *p*=0.8).

The glycohalcin index was 5 (4-13). A significant correlation was observed between this index and PPR (Figure 1; *p*=0.03). In patients with a normal or increased PPR, the glycohalcin index was 5 (3 -10), whereas it was 12 (7-25) in patients with a decreased PPR (*p*=0.03). No significant correlation was observed between the glycohalcin index and MPL (*p*=0.08). Patients with a MPL \leq 2 days demonstrated a glycohalcin index of 7 (3-26) compared to 5 (4-

10) among patients with a MPL >2 days ($p=0.3$).

Most studies point to elevated thrombopoietin levels and low levels of glycofibrinogen in the case of a hypomegakaryocytic thrombocytopenia.² However, in our study a reduced PPR was not associated with elevated thrombopoietin levels, but rather with a high glycofibrinogen-index. Comparable results were recently obtained in myelodysplastic patients.⁶ These data suggest that the reduced PPR is not due to a decline in the mass of Mpl-bearing cells in the bone marrow, but indicate an increased release of GP-Ib α complex in the circulation. This might be a consequence of shedding of the receptor complex from destroyed megakaryocytes and/or platelets in the bone marrow. This is also in concordance with the lack of significant correlation observed between the glycofibrinogen index and MPL, suggesting that the platelet antibody not only affects platelets but also megakaryocytes.^{7,8}

The finding of a lower PPR in patients with more pronounced thrombocytopenia suggests more prominent intramedullary destruction in more severe ITP. In conclusion, the present study indicates that (i) that thrombopoietin levels and the glycofibrinogen index are related to the dynamics of megakaryocyte and platelet kinetics in bone marrow and peripheral blood; (ii) in ITP patients with a decreased PPR, increased platelet and/or megakaryocyte destruction might occur in the bone marrow, as recently demonstrated by ultrastructural studies.⁹ Further research is needed to evaluate the usefulness of determining the glycofibrinogen index in ITP in clinical practice.

Ewout J. Houwerzijl,° Henk Louwes, Mariet T. Esselink,° Jan W. Smit,° Edo Vellenga,° Joost Th. M. de Wolf°*

**Department of Nuclear Medicine, Martini Hospital and
°Department of Hematology, University Hospital Groningen,
The Netherlands*

Key words: glycofibrinogen, ITP, platelet production.

Acknowledgments: we wish to thank Dr. FHJ Blok, Wilhelmina Ziekenhuis, Assen; Dr. GW Woolthuis, St Antonius Ziekenhuis, Sneek; Dr. P Joosten and Dr. J Hoving, Medisch Centrum Leeuwarden; Dr. Z Erjavic, Delfzicht Ziekenhuis, Delfzijl; and Dr. H Pothoff, Sint Lucas Ziekenhuis, Winschoten for including their patients in the study.

Correspondence: Joost Th. M. de Wolf, MD, PhD, Dept. Hematology, University Hospital, PO Box 30.001, 9700 RB Groningen, The Netherlands. Phone: international +31.50.3612354. Fax: international +31.50.3614862. E-mail: j.t.m.de.wolf@int.azg.nl

References

- Louwes H, Zeinali Lathori OA, Vellenga E, de Wolf JT. Platelet kinetic studies in patients with idiopathic thrombocytopenic purpura. *Am J Med* 1999;106:430-4.
- Porcelijn L, Folman CC, Bossers B, Huiskes E, Overbeeke MA, v d Schoot CE, et al. The diagnostic value of thrombopoietin level measurements in thrombocytopenia. *Thromb Haemost* 1998;79:1101-5.
- Beer JH, Steiner B. Glycofibrinogen: a new assay – the normal plasma levels and its potential usefulness in selected diseases. *Blood* 1994;83:691-702.
- Recommended method for indium-111 platelet survival studies. International Committee for Standardization in Hematology. Panel on Diagnostic Applications of Radionuclides. *J Nucl Med* 1988;29:564-6.
- Louwes H, van Schuur JJ. Platelet labelling with ¹¹¹Indium-tritopolonate. In: Kessler CH, Hardeman MR, Henningsen H, Petrovici JN, editors. *Clinical Application of Radiolabelled Platelets*. Dordrecht, The Netherlands: Kluwer Academic; 1990. p. 45.

- Houwerzijl EJ, Blom NR, van der Want JJJ, Louwes H, Esselink MT, Smit JW, et al. Increased peripheral platelet destruction and caspase-3-independent programmed cell death of bone marrow megakaryocytes in myelodysplastic patients. *Blood* 2004;12[Epub ahead of print].
- McMillan R, Luiken GA, Levy R, Yelenosky R, Longmire RL. Antibody against megakaryocytes in idiopathic thrombocytopenic purpura. *JAMA* 1978;239:2460-2.
- Hoffman R, Zaknoen S, Yang HH, Bruno E, LoBuglio AF, Arrowsmith JB, et al. An antibody cytotoxic to megakaryocyte progenitor cells in a patient with immune thrombocytopenic purpura. *N Engl J Med* 1985;312:1170-4.
- Houwerzijl EJ, Blom NR, Van Der Want JJ, Esselink MT, Koonstra JJ, Smit JW, et al. Ultrastructural study shows morphological features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. *Blood* 2004;103:500-6.

Thrombosis

Risk of thromboembolism in patients with idiopathic autoimmune hemolytic disease and antiphospholipid antibodies: results from a prospective, case-control study

During a period of 4 years, 21 consecutive patients with newly diagnosed idiopathic autoimmune hemolytic disease (IAHD) and 42 healthy, sex- and age-matched subjects, were tested for the presence of antiphospholipid antibodies (APA). At diagnosis, APA were detected in 10/21 (47.6%) patients and in 2/42 (4.76%) controls ($p < 0.01$). No thromboembolic events were registered during the follow-up period.

haematologica 2005; 90:711-713

(<http://www.haematologica.org/2005/05/711.html>)

From January 1996 to January 2000, 47 patients aged > 20 years were diagnosed with autoimmune hemolytic disease (AHD) at the Hematology Department of the University "La Sapienza" of Rome. Of these 47 consecutive patients, 26 had secondary AHD (SAHD) and 21 had idiopathic AHD (IAHD). The main characteristics of the IAHD patients at diagnosis are reported in Table 1. All these patients were tested for antiphospholipid antibodies (lupus anticoagulant, anticardiolipin and anti- β_2 -glycoprotein I antibodies) at diagnosis, after 8 to 10 weeks and then every six months or when clinically required. We did not test the anti-phospho-ethanolamine antibodies usually associated with IAHD. The results were then compared to those obtained in 42 healthy, sex- and age-matched subjects who tested negative to a panel of several auto-antibodies. Controls were tested at the entry into the study and when APA-positive, after 8-10 weeks for confirmation. Lupus anticoagulant (LAC) was detected according to the criteria indicated by the ISHT,¹ utilizing two procedures: the kaolin clotting time (KCT) and the diluted Russell's viper venom time (DRVVT). Tested plasmas were considered LAC positive only if at least one of the two procedures was diagnostic for LAC. Anticardiolipin antibodies (ACA) of Ig-G type (ACA-IgG) and anti- β_2 -glycoprotein I (anti- β_2 GPI) antibodies were measured with standardized ELISA assays. The differences between the variables, were calculated by the χ^2 test, using a 2x2 table. Differences were considered statistically significant when $p < 0.05$ (two tailed).

Overall, at diagnosis, 10/21 (47.6%) IAHD patients and