

IMMUNE THROMBOCYTOPENIC PURPURA: CORRELATIONS BETWEEN THROMBOCYTOPENIA SEVERITY AND ITS CLINICAL SYMPTOMS

Oana Bădulescu¹, Magda Bădescu¹, Manuela Ciocoiu¹, Florentina Dudu²,
Mădălina Mocanu¹

¹ Pathophysiology Department, University of Medicine and Pharmacy „Grigore T. Popa“, Iasi, Romania

² Department of Internal Medicine, “C.I.Parhon” Hospital, Iasi, Romania

ABSTRACT

Introduction. Immune thrombocytopenic purpura is an autoimmune hematological condition. The decrease of the number of platelets in the peripheral blood and the occurrence of anti-platelet antibodies are the pathognomonic elements of the disease. The clinical symptoms in immune thrombocytopenic purpura are heterogeneous and vary especially from the viewpoint of the intensity of the symptoms. Some cases are asymptomatic or show only slight cutaneous-mucous hemorrhages, yet in other cases the hemorrhagic syndrome may be severe and impair the mucosa, the tegument and also the viscera (retina, brain, kidneys).

The aim of the study was to point out the connection between the autoimmune thrombocytopenia severity and the intensity of the clinical symptoms of the disease.

Material and methods. The research was performed on 40 patients divided into two groups: the first group included asymptomatic patients who came in for routine blood tests, whereas the second group comprised patients with hemorrhagic symptoms: petechiae, bruises, epistaxis, gingivorrhagia.

Results. All the patients included in the study had thrombocytopenia, having mean platelet count values

RÉSUMÉ

Le purpura thrombopénique immunologique: corrélations entre la gravité de la thrombocytopénie et les signes cliniques

Introduction. Le purpura thrombopénique immunologique est une maladie hématologique à caractère auto-immun. Les éléments pathognomoniques de cette maladie sont la baisse du nombre des thrombocytes dans le sang périphérique et la présence des anticorps antiplaquetaires. La symptomatologie clinique du purpura thrombopénique immunologique est hétérogène et varie surtout du point de vue de l'intensité des symptômes. Certains cas sont asymptomatiques ou présentent uniquement quelques légères hémorragies cutanéomuqueuses, mais des fois le syndrome hémorragique peut avoir des formes graves et provoquer l'atteinte des muqueuses, du tégument et des viscères (rétine, cerveau, reins).

Le but de l'étude a été de mettre en évidence le rapport entre la gravité de la thrombocytopénie auto-immune et l'intensité des signes cliniques de la maladie.

Méthodes. L'étude s'est déroulée sur 40 patients répartis en deux groupes : le premier groupe comprenant

Correspondence address:

Oana Viola Bădulescu, MD, PhD
Department of Pathophysiology, „Grigore T. Popa“ University of Medicine and
Pharmacy, Universității street, no.16, 700115, Iași, Romania.
Cell phone: 0722.242.397 E-mail: violabadulescu@yahoo.com

of $45.93 \times 20.10 \times 10^3/\mu\text{L}$, whereas most of the patients (80%) exhibited positive anti-platelet antibodies. This demonstrates the increased frequency of immune thrombocytopenic purpura contrary to the fact that till nowadays it was considered to be a rare disease. The mean value for patients with positive anti-platelet antibodies was $42,91 \pm 24,10 \times 10^3/\mu\text{L}$, which is slightly lower than the average value of patients with negative anti-platelet antibodies ($p = 0.130$). These results prove that the platelet functions in immune thrombocytopenic purpura are influenced by anti-platelet antibodies.

In conclusion, the intensity of the clinical hemorrhagic symptoms in immune thrombocytopenic purpura is in correlation with the decreased number of platelets.

Keywords: immune thrombocytopenic purpura, hemorrhagic syndrome, thrombocytopenia, anti-platelet antibodies.

Abbreviations list:

EDTA = ethylenediaminetetraacetic acid
ELISA = enzyme-linked immunosorbent assay
PLT = platelet
EU = European Union

INTRODUCTION

Immune thrombocytopenic purpura is a hematological condition characterized by the decrease of the number of platelets in the peripheral blood below the limit of $100,000$ elements/ mm^3 blood. Premature blood platelet hyperdestruction may have central origin (poor synthesis at the medullar level) or it may be due to the activity of the anti-platelet antibodies found on the platelet membrane.^{1,2,3,4} The occurrence of anti-platelet antibodies is the distinctive sign for the immune thrombocytopenic purpura diagnosis.^{5,6}

The onset of the disease may be asymptomatic, in which case the low platelet count is detected by accident during routine tests, or it may be accompanied by specific hemorrhagic symptoms. The cutaneous-mucous hemorrhagic syndrome specific to immune thrombocytopenic purpura may have different forms: petechiae, bruises, epistaxis, gingivorragia. The term „purpura“ defines a tegument color change due to the spontaneous extravasation of the erythrocytes inside the capillary vessels in the skin and mucosa. Purpura lesions are macular, cannot be felt on palpation and are red-violet. From the morphological point of view, purpura is divided into two categories: petechiae and bruises. Petechia lesions are smaller than 3 mm in diameter, whereas the bruises

des patients asymptomatiques qui viennent pour des analyses de routine et le second formé de patients présentant une symptomatologie hémorragique : pétéchies, ecchymoses, épistaxis, gingivorragies.

Résultats. Tous les patients présentent une thrombocytopenie, ayant une valeur moyenne du nombre de thrombocytes de $45,93 \times 20,10 \times 10^3/\mu\text{L}$, tandis que la plupart (80%) d'entre eux ont présenté des anticorps antiplaquetaires positifs. Cela démontre l'augmentation de la fréquence du purpura thrombocytopenique immunitaire, contrairement au fait que jusqu'à nos jours il était considéré comme une maladie rare. La valeur moyenne pour les patients présentant des anticorps antiplaquetaires positifs était de $42,91 \pm 24,10 \times 10^3 / \mu\text{L}$, ce qui est nettement inférieur à la valeur moyenne pour les patients avec des anticorps antiplaquetaires négatifs ($p = 0,130$). Ces résultats prouvent que les fonctions plaquetaires dans un purpura thrombocytopenique immun sont influencées par des anticorps antiplaquetaires.

En conclusion, l'intensité des symptômes cliniques hémorragiques dans le purpura thrombocytopenique immun est en corrélation avec le nombre diminué de plaquettes.

Mots-clés: purpura thrombocytopenique immunologique, syndrome hémorragique, thrombocytopenie, anticorps antiplaquetaires.

are more consistent and measure up to 5 mm in diameter. Petechiae do not disappear when pressure is applied.^{7,8,9,10}

Hemorrhagic symptoms have variable intensity, from mild to severe, depending on thrombocytopenia severity. The cutaneous signs which raise the suspicion of a hemorrhagic syndrome (purpura, petechiae) are located especially in the friction and pressure areas, on the body and lower limbs. Prolonged bleeding after minor injuries (blows, stings) or surgical procedures is also suggestive of a condition of the immune thrombocytopenia type. Cases of severe life-threatening hemorrhages associated with immune thrombocytopenia, such as brain or retina hemorrhages, are also found in literature.^{6,8,11,12}

In our research, we aimed at pointing out the connection between the auto-immune nature of the disease (anti-platelet antibodies), thrombocytopenia severity and extent of clinical signs.

MATERIAL AND METHOD

This is an observational study, as its goal is to point out the relation between the high platelet number, the occurrence of anti-platelet antibodies and the symptomatic or asymptomatic nature of immune

thrombocytopenia. The study group included 40 patients on record with the Hematology Clinic of „Sf. Spiridon“ Teaching Hospital of Iași Romania. The study was conducted during a period of 3 years, between October 2012 and October 2015. The patients included in the study were aged 18 to 74 years (mean age $48,43 \pm 16,22$ years), of whom 27 were female and 13 male. 30 patients were living in urban areas and 10 patients were living in rural areas. The patients were divided into two groups:

- asymptomatic, who came in for routine tests;
- with hemorrhagic symptoms: bruises, petechiae, epistaxis, gingivorragia.

The common study inclusion criteria applying to all the patients was peripheral thrombocytopenia. None of the patients had taken thrombocytopenia-inducing medicines before being diagnosed with thrombocytopenia and none of them had other active pathologies involving the decrease of the number of platelets. Pregnant women and patients who had recently had blood transfusions were not included in the study.

The patients included in the study group signed an informed consent form where they agreed with being included in the research. The study was conducted in compliance with the Law 677/2001 on personal data collection, use and processing. The research was carried out in strict compliance with the bioethical requirements of the EU legislation.

The following diagnosis protocol stages were used for both groups:

- The platelets were counted by an automatic analyzer while they passed in a single line through a hole, using the hydrodynamic focus method.^{13,14,15} The determination was done by a test on venous blood sampled in vacutainers containing tripotassium/dipotassium/disodium-K3 EDTA as anticoagulant);

the samples were analyzed within 6 hours from blood sampling.

- Dosing of the anti-platelet antibodies bound to the glycoproteins on the platelet membrane using the ELISA method. Venous blood was sampled in anticoagulant-free vacutainers, which were filled to the brim.^{15,16}
- The clinical assessment consisted of the examination of the objective signs of the hemorrhagic cutaneous-mucous syndrome: bruises, petechiae, gingivorragia, spontaneous bleeding, and the quantification of their occurrences, extent and severity. Patient, past medical history, offered information about the onset of the symptoms, about hemorrhage evolution (intermittent/permanent) and duration (acute/chronic).

RESULTS AND DISCUSSIONS

Platelet count

In our study, the whole group of patients exhibited thrombocytopenia, a platelet count mean (PLT) of $45.93 \times 20.10 \times 10^3/\mu\text{L}$ and a 43.7% variance, i.e. within the $8 - 98 \times 10^3/\mu\text{L}$ range, whereas all the values were below the minimum reference level. The platelet reference range is 150-450,000 platelets/ μL , and thrombocytopenia is defined in this case as a platelet count below 100,000 platelets/ μL . (table 1)

Anti-platelet antibody dosage

Anti-platelet antibodies were positive in most patients, i.e. in 32 (80%) of the 40 patients included in the study, 21 female and 18 male under 50 years of age. From the demographic repartition viewpoint, positive results were reported in 83.3% of the patients living in urban areas, and in 70% of the patients living in rural areas. (figure 1)

Table 1. PLT* $10^3/\mu\text{L}$ descriptive indicators

Parameter	N	Mean	Std. deviation	Std. error	Confidence interval 95%		Min	Max	p
					-95%CI	+95%CI			
Total	40	45.93	25.10	3.97	37.90	53.95	8	98	-
Gender									
Male	13	36.08	23.91	6.63	21.63	50.52	8	87	
Female	27	50.67	24.68	4.75	40.90	60.43	12	98	0.048
Age group									
< 50 years	23	50.22	27.26	5.69	38.43	62.01	11	98	
≥ 50 years	17	40.12	21.23	5.15	29.20	51.03	8	82	0.212
Origins									
Urban	30	45.07	26.60	4.86	35.13	55.00	8	98	
Rural	10	48.50	20.97	6.63	33.50	63.50	18	87	0.713

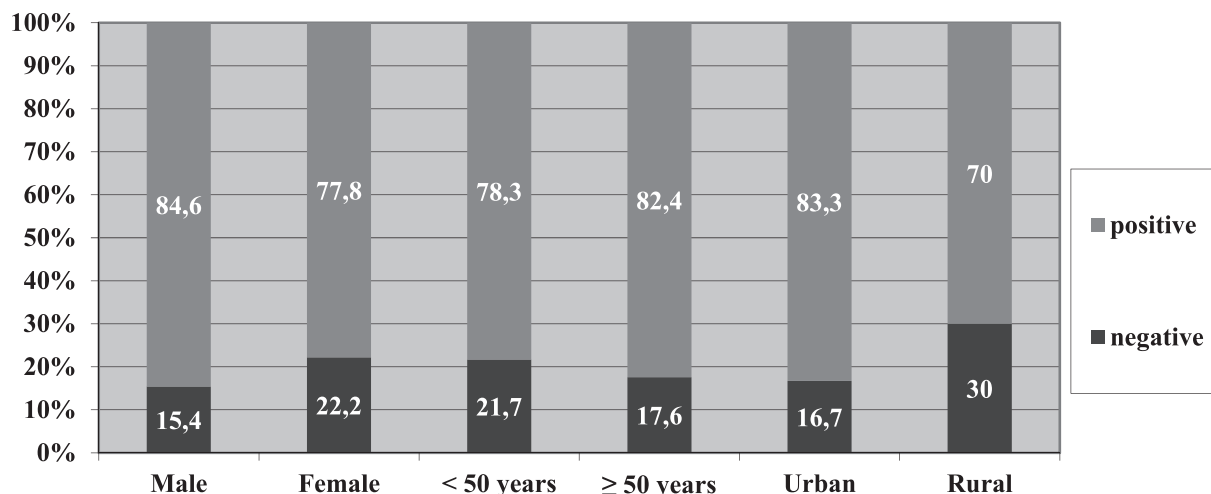


Figure 1. Group distribution depending on anti-platelet antibodies testing

Anti-platelet antibodies determination emphasized the high occurrence of immune thrombocytopenic purpura, which contradicts the current trend of considering it „a rare disease“. Thus, positive anti-platelet antibodies were detected in 80% of the patients, with platelet values within the $8-98 \times 10^3/\mu\text{L}$ range and a mean value of $42.91 \pm 24.10 \times 10^3/\mu\text{L}$, slightly lower than the mean value detected in the patients with negative anti-platelet antibodies ($p=0.130$). (figure 1)

The mean platelet values were slightly lower in the patients with positive anti-platelet antibodies than in the patients with negative antibodies (42.91 vs. $58 \times 10^3/\mu\text{L}$; $p=0.130$). We noticed that influence of anti-platelet antibodies on the number of peripheral blood platelets, as the platelet count was lower in most patients with autoimmune thrombocytopenia than in subjects with negative anti-platelet antibodies. (table 2)

Symptom assessment

Depending on the patient's clinical assessment at the time of thrombocytopenia diagnosis setting, the symptomatic/asymptomatic character reveals the following aspects (figure 2):

- 15.4% of the men and 22.2% of the women were asymptomatic ($p=0.606$);
- 26.1% of the asymptomatic subjects were under 50 years of age and 11.8% of the patients aged 50 or older were asymptomatic ($p=0.252$);
- 20% of the patients living in urban areas and the same percentage of patients living in rural areas were asymptomatic ($p=1.0$).

The results we achieved enabled us to notice the relatively low occurrence of asymptomatic patients in the study group.

The mean platelet values were significantly higher in asymptomatic patients ($p=0.002$), which means

that symptoms are correlated with the decrease of the number of platelets, and the intensity of symptoms depends on the severity of thrombocytopenia.

The symptoms of the patients with immune thrombocytopenic purpura are heterogeneous and the hemorrhagic syndrome symptoms vary within a wide range. Few of the patients included in the study were asymptomatic and diagnosed after routine tests, while most of them had hemorrhagic symptoms. The symptoms and signs varied greatly, from asymptomatic patients or patients with small lesions in the mouth or nose mucosa, to patients with massive hemorrhage. These symptoms usually occur in cases of severe thrombocytopenia, associated with the decrease of the number of platelets below $30,000/\text{mm}^3$ of blood. The intensity of the hemorrhagic syndrome mainly depends on the number of platelets, which means that the patients with platelet counts below $10,000/\text{mm}^3$ of blood have a higher risk of massive hemorrhage, including intracranial or retinal hemorrhage. (table 3)^{14,15,16,17,18}

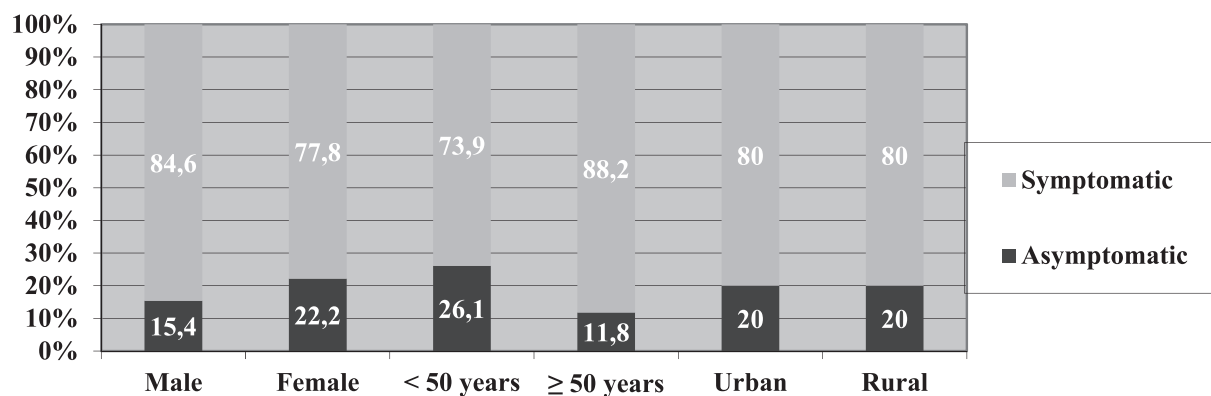
90.6% of the patients with positive anti-platelet antibodies were asymptomatic, whereas 62.5% of the patients with negative anti-platelet antibodies were asymptomatic ($p=0.002$) (Figure 3). We infer from here that there is no direct correlation between the anti-platelet antibodies occurrence and the symptoms specific to immune thrombocytopenic purpura.

CONCLUSIONS

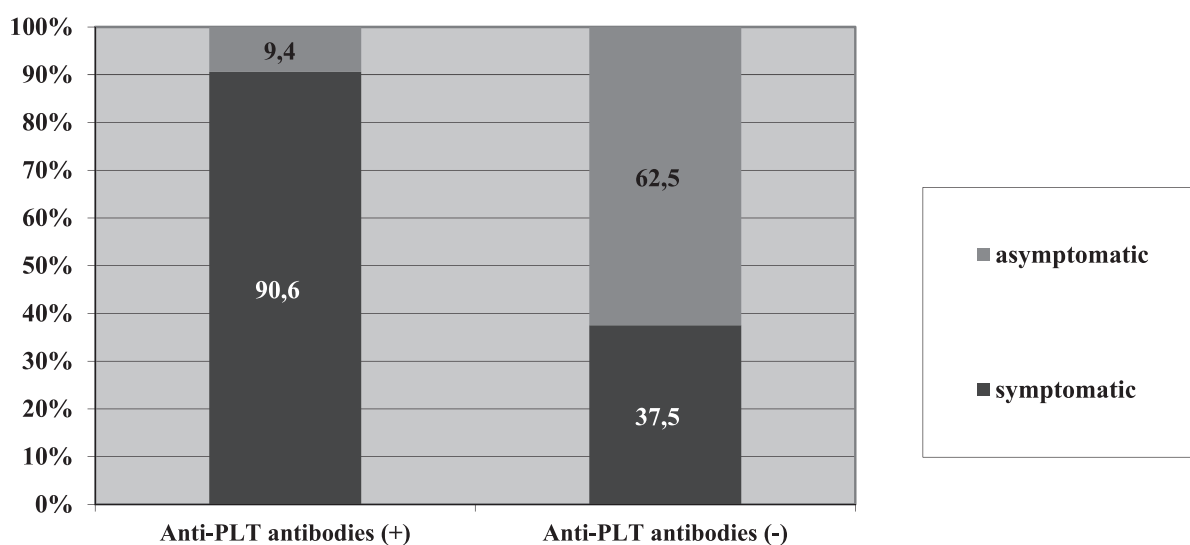
Immune thrombocytopenic purpura is a hematological condition with a variety of clinical forms. It may initially be asymptomatic, with insidious evolution, or, on the contrary, with sudden and violent

Table 2. PLT *10³/μL descriptive indicators depending on anti-platelet antibodies

Anti-PLT antibodies	N	Mean	Std. deviation	Std. error	Confidence interval		Min	Max	F (ANOVA) p test
					95%				
					-95%CI	+95%CI			
Negative	8	58.00	26.98	9.54	35.45	80.55	18	98	0.130
Positive	32	42.91	24.10	4.26	34.22	51.60	8	98	
Total	40	45.93	25.10	3.97	37.90	53.95	8	98	

**Figure 2.** Group distribution depending on the symptomatic/asymptomatic character**Table 3.** PLT x 10³/μL descriptive indicators depending on the symptomatic/asymptomatic character

Character	N	Mean	Std. deviation	Std. error	Confidence interval		Min	Max	p
					95%				
					-95%CI	+95%CI			
Asymptomatic	8	78.75	16.52	5.84	64.94	92.56	55	98	0.001
Symptomatic	32	37.72	19.57	3.46	30.66	44.78	8	79	
Total	40	45.93	25.10	3.97	37.90	53.95	8	98	

**Figure 3.** Group structure depending on anti-platelet antibodies positivity and the symptomatic/asymptomatic character

onset, revealed in most cases by a cutaneous-mucous hemorrhagic syndrome.

The hemorrhagic manifestations induced by the drop in the number of blood platelets determine platelet pathology-specific symptoms, regardless of the mechanisms involved in thrombocytopenia occurrence and regardless whether it is a primary or secondary, central or peripheral thrombocytopenia. The presence of anti-platelet antibodies is commonly associated with the absence of symptoms; in symptomatic subjects, the clinical picture is not altered by the presence of antibodies. Classical hemorrhagic symptoms may be absent or accompanied by additional clinical signs in secondary immune thrombocytopenia. This form occurs when other related diseases are present and the clinical thrombocytopenia signs may be accompanied by signs of the primary disease, such as other autoimmune or infectious diseases. In our current research, it is undeniable that the intensity of the clinical hemorrhagic symptoms specific to immune thrombocytopenic purpura was related to the severity of the decrease of the number of platelets.

REFERENCES

- Gaurav K, McCrae KR. Immune Thrombocytopenia. *Hematol Oncol Clin North Am* 2013; 27(3): 495-520.
- Stasi R. Immune thrombocytopenia: pathophysiologic and clinical update. *Semin Thromb Hemost* 2012; 38(5): 454-462.
- Neunert C, Lim W, Crowther M et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 2011; 117: 4190-4207.
- Kistangari G, McCrae KR. Immune thrombocytopenia. *Hematol Oncol Clin North Am*. 2013; 27(3): 495-520.
- Shahid Iqbal, Syed Z A Zaidi, Ibraheem H Motabi et al. Thrombotic thrombocytopenic purpura - analysis of clinical features, laboratory characteristics and therapeutic outcome of 24 patients treated at a tertiary care center in Saudi Arabia. *Pak J Med Sci* 2016; 32(6): 1494-1499.
- Johnsen J. Pathogenesis in immune thrombocytopenia: new insights. *Hematology* 2012; 306-312.
- Tarantino MD. Treatment options for chronic immune (idiopathic) thrombocytopenia purpura in children. *Semin Hematol* 2000; 37(1): 35-41.
- Beardsley DS, Warkentin TE, Sadler JE. Thrombocytopenia. *Hematology* 2006; 402-407.
- L. Kayal, S. Jayachandran, Khushboo. Singh Idiopathic thrombocytopenic purpura. *Contemp Clin Dent* 2014; 5(3): 410-414.
- <http://www.uptodate.com/contents/immune-thrombocytopenia-ITP-in-adults-clinical-manifestations-and-diagnosis>.
- Levine S. Thrombocytopenia: Pathophysiology and Classification. In: Wintrobe's Clinical Hematology, Ed. Lippincott Williams & Wilkins Publishers, Philadelphia 2004; 1529-1531.
- Nomura S. Advances in Diagnosis and Treatments for Immune Thrombocytopenia. *Clin Med Insights Blood Disord*. 2016; 9: 15-22.
- Mikias Negash, Aster Tsegaye and Amha G. Diagnostic predictive value of platelet indices for discriminating hypoproductive versus immune thrombocytopenia purpura in patients attending a tertiary care teaching hospital in Addis Ababa, Ethiopia. *BMC Hematology* 2016; 16:18.
- Frelinger AL, Grace RF, Gerrits AJ et al. Platelet function tests, independent of platelet count, are associated with bleeding severity in ITP. *Blood* 2015; 126(7): 873-879.
- Laborator Synevo. (2010) Referințele specifice tehnologiei de lucru utilizate. <https://www.synevo.ro/anticorpi-antitrombocitari-liberi-in-ser/>. (accessed January 2017).
- Kuwana M, Kurata Y, Fujimura K et al. Preliminary laboratory based diagnostic criteria for immune thrombocytopenic purpura: evaluation by multi-center prospective study. *J Thromb Haemost*. 2006;4(9): 1936-1943.
- Frelinger AL, Grace RF, Gerrits AJ et al. Platelet function tests, independent of platelet count, are associated with bleeding severity in ITP. *Blood* 2015; 126(7): 873-879.
- Zimmer J, Andres E, Noel E et al. Current management of adult idiopathic thrombocytopenic purpura in practice: a cohort study of 201 patients from a single center. *Clin Lab Haematol* 2004; 26(2): 137-142.