PHD THESIS SUMMARY
Morphological and Functional Changes of Blood Platelets in Idiopathic Thrombocytopenic Purpura

PhD coordinator,
Professor Doctor Magda BĂDESCU

PhD student,
Mădălina MOCANU

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I.1 MOTIVATION FOR THE PHD STUDY

The study entitled “Morphological and Functional Changes of Blood Platelets in Idiopathic Thrombocytopenic Purpura” aims at determining the factors involved in the ethiopatogenesis of immune thrombocytopenic purpura and at elaborating a new (modern) diagnostic algorithm of this pathology.

The study proposes a detailed assessment of immune thrombocytopenia, using classical (clinical and paraclinical) methods of investigation, as well as a few modern techniques for exploring immunological imbalances (immunochemical assays with fluorescence or electrochemiluminescence detection). The methods of investigation cover both functional and morphological platelet disorders.

Thrombocytopenic purpura is a hematologic disease known since ancient times. It was first described in 1735 by Werlhof, who called it “Morbus maculosus hemorrhagicus”. The name of idiopathic thrombocytopenic purpura dates from 1883, when Krauss discovered a low count of platelets in ITP patients. Subsequently, the first study on the platelet changes in ITP was led by Hayem in 1890, and in 1950 Dr. William Harrington showed that a humoral factor was involved in the pathogenesis of immune thrombocytopenia (1).

The current understanding of immune thrombocytopenic purpura, which is considered “a rare disease”, especially in adults, does not accurately reflect the reality. This epidemiological error does not occur as a result of the lack of statistical data or of the means of quantifying the incidence of the disease, but rather as a result of frequent diagnostic errors. We want to emphasize the new increased frequency of immune thrombocytopenic purpura in patients of middle age. Starting from this premise, we wanted to highlight the main etiopathogenic mechanisms involved in the occurrence of ITP. In-depth knowledge of the pathogenic mechanisms of the disease which trigger morphological and functional platelet disorders will allow the determination of appropriate and individualized treatment regimens while taking into account each patient’s pathology.

It is very important to develop a modern diagnostic algorithm whose steps are responsibly followed by clinicians. Most often, diagnostic errors are due to the superficial identification of the aetiology of thrombocytopenia. The causes of thrombocytopenia can be of peripheral or central, immunological or non-immunological, primary or secondary nature. When faced with an asymptomatic patient or with a
patient who shows symptoms such as bleeding and peripheral thrombocytopenia, it is essential to analyse the mechanisms that have led to a decreased platelet count. The involvement of platelet autoantibodies in the premature massive destruction of platelets is more and more frequently encountered, which is why, in this study, we want to emphasize the importance of dosing anti-platelet antibodies in patients with peripheral thrombocytopenia. The antibodies can act only at a peripheral level, but they can also act at a central level, by inhibiting the megakaryocyte synthesis. For the differential diagnosis of peripheral immune thrombocytopenia from the central immune thrombocytopenia, the morphological disorders of platelets will be analysed by means of microscopic examination of peripheral blood smears and bone marrow smears in cases where there are no other causes of thrombocytopenia detected in the periphery.

Immune thrombocytopenic purpura remains a diagnosis of exclusion. We also focused our attention on this subject due to the related pathologies that may underlie the occurrence of thrombocytopenia. In the literature, it is known that secondary thrombocytopenia may occur in the context of autoimmune diseases (SLE, collagen diseases, Sjögren’s syndrome), viral infections with viruses such as B, C, HIV, CMV or Helicobacter pylori. The mechanisms by which the reduction of the platelet count and the appearance of anti-platelet antibodies occur in combination with other pathologies are not fully elucidated (2, 3). For this reason, we intend to identify possible cases of secondary thrombocytopenia in the patients who participated in this study and to analyze the pathological and physiological mechanisms leading to the installation of the secondary immune thrombocytopenia.

Functional disorders of platelets in ITP are often accompanied by changes in platelet morphology. We emphasise the importance of linking the information related to platelet function disorders with the platelet morphological abnormalities in determining the type of thrombocytopenia and the optimal therapeutic conduct.

The reason why we chose this topic of study has its origins in the complexity of the pathology, the diversity of manifestations and clinical contexts in which it appears, as well as the multitude of pathogenic mechanisms underlying it and whose understanding and analysis have been a challenge for the research team.
I.2. OBJECTIVES OF THE PHD STUDY

The main objectives of the study include:

- Expanding the knowledge on the etiologic factors involved in the occurrence of immune thrombocytopenic purpura.
- Establishing a clear differential diagnosis between secondary immune thrombocytopenic purpura and primary immune thrombocytopenic purpura with the purpose of establishing an appropriate therapeutic regimen.
- Identifying and defining the biological parameters that characterize the immune thrombocytopenia with the purpose of initiating an early and differentiated therapy.
- Identifying the complex mechanisms which take place at the molecular level and influence the further development of the disease.
- Establishing correlations between clinical manifestations and the values of biochemical or immunological parameters, as well as between different biological parameters.
- Obtaining current data on the morphological changes of platelets in ITP patients and correlating them with the data on platelet function disorders.
- Collecting and interpreting statistically significant data on the frequency and distribution of ITP given the demographic criterion.
- Centralizing all the collected data and drawing conclusions and correlations in order to identify potential curative therapeutic targets or to increase the disease remission period. The research conducted in this direction is up to date, thus achieving the proposed results can help to increase the quality of life of patients suffering from this condition.

II. MATERIAL AND METHOD

II.1. Evaluation of clinical parameters

Anamnestic data on patients’ age and sex, medical history, personal pathological history and family history were recorded for all patients.

The medical history of the disease guides the clinician on the onset of symptoms, the severity of bleeding, its extent and duration. It is a very useful tool that helps the clinician to determine the evolution of the disease (acute / chronic).
**Personal pathological history** is of real importance in the differential diagnosis of primary ITP from secondary ITP. Recent blood transfusions, consumption of medication known to have high risk of causing thrombocytopenia, infectious or autoimmune diseases are associated pathologies that can guide us towards the diagnosis of secondary ITP.

**The family history** is useful for determining a possible family history of thrombocytopenia; this is a situation in which we can find ourselves dealing with a patient with hereditary thrombocytopenia, a diagnosis which will have to be confirmed or excluded.

**The clinical examination** includes examination of the objective signs of the hemorrhagic cutaneous-mucous syndrome: petechiae, ecchymoses, purpuric injuries, epistaxis, gingival bleeding or spontaneous bleeding. Skin pallor can be present in patients with massive or chronic bleeding and who suffer now from post-haemorrhagic anaemia. ITP patients may be asymptomatic, and the low blood platelet count can be detected during a routine medical check. The etiopathogenic causes of asymptomatic ITP Just will be investigated in a similar way.

The patient with secondary ITP shows either the signs that are identical to those in primary immune thrombocytopenia or the symptoms that are specific to the main condition that caused the decreased platelet count. Neurological signs are in favour of the differential diagnosis of ITP from the thrombotic thrombocytopenic purpura (TTP) (4- 6).

**II.2. Haematological parameters**

**The complete blood count (CBC)** is part of the screening assessment tests. It provides essential information on a patient’s hematologic status and is a first step in the diagnosis of ITP (1). **The platelet count** is part of the blood count and is useful in the investigation of a hemorrhagic syndrome of unknown aetiology. (7 – 10).

**The mean platelet volume (MPV)** is an index which shows the uniform size of the platelet population and is calculated within the conventional CBC by means of an automatic analyzer. It is useful in the differential diagnosis of thrombocytopenia.

**The platelet distribution width (PDW)** is a parameter that is interpreted together with the MPV for distinguishing conditions
associated with poor synthesis of platelets from those associated with increased peripheral platelet destruction (11, 12).

The examination of the peripheral blood smear reflects the morphological changes of the figurative elements (13), while the microscopic examination of the bone marrow smear highlights issues concerning the size and shape of the figurative elements of the blood (red cells, white cells), as well as their precursors’ (megakaryocytes). From this point of view, the analysis of blood smears and bone marrow smears is a valuable tool in diagnosing and assessing qualitative blood disorders. We consider that the bone marrow smear needs to be done in patients suspected to have a central cause of thrombocytopenia, taking into account their age and their symptoms, the haematological picture, the associated pathologies and their medical history.

II.3. Immunoassay

Anti-platelet antibodies are present in various haematological pathologies such as immune thrombocytopenic purpura, neonatal thrombocytopenic purpura or post-transfusion purpura. The determination of anti-platelet antibodies is an essential investigation for the diagnosis of immune thrombocytopenia. The dosage of anti-platelet antibodies bound to the glycoproteins of the platelet membrane was performed by enzyme immunoassay ELISA.

Immune thrombocytopenia detected in the previous step can be considered primary only after excluding possible forms of secondary ITP. To diagnose secondary ITP cases occurring in association with other autoimmune diseases, we performed a series of immunological determinations such as: ANA – antinuclear antibodies, anti-ds DNA antibodies and anti-cardiolipin antibodies in all patients included in this study. Secondary ITP is commonly associated with SLE. Immunological markers for the diagnosis of SLE are antinuclear antibodies and anti-ds DNA antibodies.

The determination of antinuclear antibodies (ANA) was conducted by enzyme immunoassay with fluorescence detection (FEIA) (14).

Anti-double-stranded DNA antibodies (anti-ds DNA antibodies) were assessed by the ELISA immunoenzyme method, and IgG anticardiolipin antibodies were determined by the immunochemical technique with detection by chemiluminescence (CLIA) (15).
II.4. Viral infectious markers

The next phase of the study includes the analysis of the pathogenic mechanisms leading to the onset of immune thrombocytopenia associated with viral and bacterial infections. In this regard, we determined the markers of infections with viruses B, C, HIV, CMV and Helicobacter pylori. These determinations were performed for all patients, including the subjects diagnosed with an autoimmune disease responsible for triggering thrombocytopenia, as the presence of a viral or bacterial infection can be detected in this category as well, in which case the cause of thrombocytopenia is dual (autoimmune and infectious).

The immunochemical method with detection by electrochemiluminescence (ECLIA) was used for the dosage of anti-hepatitis C virus (HCV) antibodies and the determination of hepatitis B virus antigen (HBsAg).

In the current study, the anti-HIV antibodies were detected by immunochemical method with detection by electrochemiluminescence (ECLIA). The same method (ECLIA) was used to determine IgG anti-CMV antibodies.

II.5. Bacterial infectious markers

The Helicobacter pylori infection is incriminated in the onset of chronic gastritis and peptic ulcers, yet sources in the literature indicate the possible involvement of Helicobacter pylori infection in the etiopathogenesis of ITP. For these reasons, we dosed IgG anti-Helicobacter pylori antibodies for the detection of the chronic infection in the patients who took part in this study. The determination method used was the enzyme immunoassay technique with detection by chemiluminescence.

The clinical trial protocol was approved by the Medical Ethics Committee of the “Grigore T. Popa” University of Medicine and Pharmacy and was conducted in compliance with the amended Helsinki Declaration (Somerset West Amendment, 1996). Patients were informed about the study and they gave their written consent.

The clinical study was conducted in the period 01.10.2012 – 10.01.2015 in the Haematology Clinic of the “St. Spiridon” Emergency Hospital of Iaşi. Over a period of 15 consecutive months, starting with the date of 01.07.2014, the research was funded by POSDRU/159/1.5/S/133377.
The survey was carried out on a study group of 40 patients with idiopathic thrombocytopenic purpura in various stages of development. The patients were distributed into two groups:

- asymptomatic, who came in for regular checks;
- symptomatic, with bleeding: ecchymoses, petechiae, epistaxis, gingival bleeding.

**Inclusion criteria** in the study group:

- patients with peripheral thrombocytopenia (platelet counts below 100,000 / mm³ blood);
- asymptomatic patients or patients showing specific symptoms: bleeding, petechiae and ecchymotic purpura, possibly anaemia, depending on the severity of the haemorrhage;
- patients with morphologically altered platelets on the peripheral blood smear;
- patients who, prior to this study, were not diagnosed with conditions likely to induce secondary thrombocytopenic purpura;
- patients who did not recently consume drugs with high risk of causing thrombocytopenia.

**Exclusion criteria** in the study group:

- patients previously diagnosed with secondary thrombocytopenic purpura;
- cases of reversible drug-induced ITP and ITP in pregnancy were excluded.
- patients who already followed specific therapy were excluded;
- the study group does not include patients who recently underwent blood transfusions;
- noncompliant patients, whose outcomes cannot be tracked over time, were not selected.

**The criteria for a positive diagnosis** were as follows:

1. **Clinical**: aspects of importance in setting the diagnosis for the group represented only by symptomatic patients:
   - bruising especially on the legs and areas of friction and pressure;
   - petechiae and petechial purpura;
   - epistaxis;
   - bleeding gums.

2. **Paraclinical**: 

- low number of platelets in peripheral blood (less than 100,000 / mm3);
- prolonged bleeding time;
- possible anaemia due to bleeding (low haemoglobin, low hematocrit, erythrocytes below normal value);
- morphological changes of platelets on the peripheral blood smear;
- anti-platelet antibodies present in most cases.

3. radiological:
- splenomegaly detected through ultrasound in some patients.

The data from the clinical trial were processed using medical statistics software SPSS (version 13.0), EPIINFO – version 6 and Excel XP.

**III. RESULTS**

*The gender distribution* of patients in the study group highlights the predominance of females (67.5%), with a sex ratio F / M = 2/1 (Fig. 1).

![Fig. 1: Gender distribution in the study group](image)

Age ranged from 18-74 years old, with an average value that was slightly lower in males (45.08 vs. 50.04 years old; p = 0.372). We chose the age of 50 as the threshold for significance tests, as 42.5% are above this value (Table I).
Table I: Distribution according to age groups

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<th>no.</th>
<th>%</th>
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</tr>
<tr>
<td>70-79 years old</td>
<td>5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

The distribution according to the origin / background highlights the predominance of patients coming from urban background (75%); urban/rural ratio: 3/1 (Fig. 2).

![Fig. 2: Distribution according to the background](image)

III.1. Symptom assessment

Depending on the clinical evaluation of the patient when the ITP diagnosis was placed, the symptomatic / asymptomatic nature shows platelet (PLT) mean values significantly more elevated in asymptomatic patients (p = 0.002). Also, a percentage of 90.6% of patients with positive anti-PLT antibodies were asymptomatic, while 62.5% of patients with negative anti-PLT antibodies were asymptomatic (p = 0.002) (Fig. 3).
Fig. 3: Study group structure with regard to the positivity of anti-PLT antibodies and the symptomatic / asymptomatic nature

III.2. Haematological parameters

Thrombocytopenia is the common criterion for inclusion in the study group for all patients. Thrombocytopenia was defined as the low platelet count, below 100,000 platelets / μL. In the current study, the whole study group shows thrombocytopenia with a mean value for platelets (PLT) of 45.93 x 20.10 x10³/μL; all values are below the minimum cut-off value (Fig. 4).

Fig. 4: Mean values of PLT according to demographic characteristics
With a 31.9% variance, the series of values for the **mean platelet volume** (MPV) varied from 5.70 to 20.90 fL, 10% being below the minimum reference value and 60% exceeding its maximum reference value (8.5 to 12 fL). The mean value for the study group is within limits that are suggestive of ITP.

The values for the **platelet distribution width** (PDW) varied from 5.60 to 23%; 10% of the samples were below the minimum reference value and 57.5% were above the maximum reference value (10-18%). The correlation PDW – MPV revealed a strong direct correlation \( r = + 0.872; R^2 = 0.7595; p = 0.001 \), with over 87% of PDW increased values being accompanied by elevated MPV; these results can be extrapolated to the general population.

The analysis of the **peripheral blood smear** revealed a platelet series predominantly characterized by **platelet macrocytosis** (45%) and smears with **rare platelets** (27.5%). **Platelets with low density** are found in 10% of patients, and **discrete platelet anisocytosis** in 7.5% of patients (fig. 5).

![Platelet distribution](image.png)

**Fig. 5**: Distribution of patients by peripheral smear

Microscopic examination of **bone marrow smears** was done for only 6 of the 40 patients in the study group, as it is only required for patients over 65 years, with suspected thrombocytopenia of central aetiology. Given the small number of patients who required this
investigation, the statistical results obtained have mainly a descriptive value and the findings cannot be extrapolated to the general population.

Patient distribution according to the results obtained after examining the marrow smear revealed the presence of megakaryocyte series with maturation and normal morphology in 2 patients (5%), rare nuclei of megakaryocytes were present in 2 patients (5%) and a similar percentage of 5% of the assessed patients had slightly increased numbers for the megakaryocyte series.

III.3. Immunological parameters

Anti-platelet antibodies (anti-PLT antibodies) were positive in 32 patients (80%), 21 women and 18 men, aged less than 50 years. The determination of anti-platelet antibodies showed increased frequency of immune thrombocytopenic purpura, contrary to the tendency so far of being considered a “rare disease”. Thus, 80% of patients had positive anti-platelet antibodies, with platelet values of 8-98 x10^3/µL, with a mean value of PLT that was slightly lower than the mean value recorded in patients with negative anti-platelet antibodies (p = 0.130) (Fig. 6).

![Fig. 6: Distribution according to the anti-PLT antibody testing](image)

The results of antinuclear antibody dosing revealed that 10% of patients had positive ANA antibodies, 7.5% of determinations were equivocal and the rest of 82.5% of patients had negative values for
ANA antibodies. The lowest mean values for PLT were recorded in the equivocal results of ANA antibody dosing (37 x 10^3/µL).

The series of values for anti-ds DNA antibodies varied from 7 to 832 IU/mL; 20% of the results were positive, while the remaining 80% were negative. We can notice the low frequency in the study group of the subjects with positive anti-ds DNA antibodies (20%).

The series of values for the IgG anti-cardiolipin antibodies ranged from 0.30 to 55 GPL U/mL. In 20% of patients, anti-cardiolipin antibodies had positive values. All patients with positive anti-cardiolipin antibodies also had positive anti-PLT antibodies (p = 0.044). This strong correlation shows the link between the secondary immune thrombocytopenia and the antiphospholipid syndrome.

HBs antigens were determined in all patients in the study group (40). We noticed a small percentage (20%) of patients with positive HBsAg from the total number of subjects tested. We emphasize the link between HBs antigens and platelet counts with lower values in patients who carry the hepatitis B virus infection compared to those with no infection. This result suggests the influence of B virus on the number of blood platelets, causing it to decrease.

The determinations which were done within this study revealed the presence of anti-HCV antibodies in 20% of patients. The correlation of anti-HCV antibodies with anti-platelet antibodies revealed the existence of an association between the presence of anti-platelet antibodies and viral liver infection markers (p = 0.558).

A low incidence of HIV virus infection in the patients under analysis was observed, as the anti-HIV antibodies were positive in 4 patients (10%), 3 women and 3 men aged less than 50 years.

The study group was mostly positive in anti-CMV antibody testing, 12 determinations (30%) were positive and 3 (7.5%) were equivocal. Anti-PLT antibodies were significantly correlated with anti-CMV antibodies from a statistical point of view (p = 0.05).

**III.4. Bacterial infectious markers**

Individual values for IgG anti-Helicobacter pylori antibodies ranged from 0.10 to 6.50 U/mL, 22.5% of which were above the reference limit (<0.9 U / mL). PLT mean values were slightly lower (35.22 vs. 49.03 x 10^3 µL; p = 0.148) in patients with positive anti-HP antibodies.
IV. DISCUSSIONS

The study group includes 40 patients diagnosed with thrombocytic purpura; anti-platelet antibodies were present in 80% of these patients (32 patients). In line with recent epidemiological checks, which show an increasing trend in the incidence of immune thrombocytic purpura at a global level (16, 17), our findings also indicate a high frequency of ITP, as 80% of patients included in the study show a form of immune thrombocytopenia, be it primary or secondary. This high percentage shows the increased frequency of immune thrombocytopenia, which has been considered a “rare disease” so far.

Regarding our study, out of the 40 patients, a percentage of 67.5% were women, with a sex ratio F/M of 2/1. The fact that ITP prevails in women may be associated with an increased tendency for females to develop autoimmune diseases.

**Age** ranged in the study group from 18 to 74 years old. These aspects are recorded in the literature regarding the incidence of ITP from childhood age to old age. The disease is not specific to and does not predominate in a particular age group.

ITP may have an asymptomatic onset, in which case the low blood platelet count is discovered by chance in a routine check or accompanying characteristic bleeding symptomatology. The most commonly encountered symptoms in the studied patients were mucocutaneous haemorrhages such as bruising, petechiae, purpura, epistaxis and gingival bleeding; yet, the symptoms of the patients suffering from ITP are heterogeneous, as the manifestations of the hemorrhagic syndrome can vary widely. The diagnosis of ITP remains an exclusion one. There is not a “gold standard” test which can be used as a confirmation rule for ITP (18, 19).

The determination of the platelet count revealed a deficiency in their number for all the individuals included in the study group. **Thrombocytopenia** is the common inclusion criterion for all the patients in the study group.

Haematological tests that provide primary data on the existence of peripheral thrombocytopenia are complemented by platelet index values: the **mean platelet volume (MPV)** and the **platelet distribution width (PDW)**. According to the findings of our study, MPV and PDW values were slightly elevated above the normal limit, and this was correlated with low blood platelet counts. All these data show the
importance of determining the MPV and PDW for diagnosing peripheral thrombocytopenia and its characterization.

**Peripheral blood smear** analysis was performed after the detection of the low platelet counts in all patients included in the study. Thrombocytopenia needs to be confirmed after the examination of the peripheral blood smear in order to exclude the possibility of a pseudo-thrombocytopenia. A detailed analysis of the morphology of platelets by detecting platelets of normal size or pathological variances in shape and size (anisocytosis, small platelets, giant platelets) has contributed to the differential diagnosis between hereditary thrombocytopenia and acquired thrombocytopenia, as is the case of ITP. The morphological changes of platelets in this study are suggestive of ITP.

The results of the examination of **bone marrow smears** show that the marrow is functional, regenerative and easily stimulated due to the peripheral platelet deficiency. These issues lead to the conclusion that the central causes of thrombocytopenia were excluded in these patients and ITP is of primary immune type.

**The determination of anti-platelet antibodies** is an investigation which is essential for the diagnosis of immune thrombocytopenia. In our study, what prevails is the association of thrombocytopenia with the immunological disorder induced by the presence and activity of autoantibodies. Based on the results obtained until this stage of the study, we find that we are facing a lot of 40 patients with peripheral thrombocytopenia with special modifications for ITP in the peripheral blood smear examination; these arguments lead us to continue the diagnostic algorithm with the investigation of the ITP etiopathology.

The association of secondary ITP with other autoimmune diseases is well known, and **ANA antibodies** are a marker of autoimmune pathologies. The results of ANA antibody dosing confirm their limited influence on platelet counts and anti-PLT antibodies (20).

The values of anti-ds DNA antibodies were significantly correlated with the anti-PLT antibodies (p = 0.044). In those patients, we note the existence of a relationship between anti-PLT antibodies and other autoantibodies (anti-ds DNA) as well as thrombocytopenia. Thus, we note the existence of **secondary immune thrombocytopenia** of peripheral aetiology with double immune mechanism based on the presence of both anti-PLT antibodies and anti-ds DNA antibodies.

All patients with positive anti-cardiolipin antibodies also had positive anti-PLT antibodies (p = 0.044), a result which outlines the
specific picture of ITP secondary to autoimmune diseases (SLE and anti-phospholipid syndrome) (21).

The distribution of the group with regard to anti-PLT antibodies, anti-HCV antibodies and HBsAg antigens indicates the presence of the markers for liver damage of viral aetiology predominantly in patients with positive anti-PLT antibodies, compared to those with negative anti-PLT antibodies. These results are illustrative for the role of hepatis B and C viruses in the ITP etiopathogeny.

Although there was a low incidence of HIV infection within the study group, we noticed the association between this viral infection and a low platelet count in the patients examined. A similar correlation between thrombocytopenia, positive anti-platelet antibodies and viral infections has been found in subjects infected with CMV. These statistics come to support the idea of viral involvement in the ITP etiopathogeny.

The pathogenic mechanisms of the occurrence of secondary immune thrombocytopenia in association with the HP infection are not yet fully elucidated, but the statistical analyses of the current study show the clear relationship between the infection and ITP, an idea which is also confirmed by the favourable evolution of thrombocytopenia in patients where the bacteria was eradicated (22). Considering these aspects, the dosage of anti-HP antibodies in patients with thrombocytopenia is a vital step in the diagnosis protocol of ITP (236).

V. GENERAL CONCLUSIONS

1. In our study, the entire group of patients showed thrombocytopenia with a mean platelet count of 45.93 x 20.10 x10^3/µL.
2. Anti-platelet antibodies were present in 80% of patients, which translates to an increased frequency of immune thrombocytopenic purpura, contrary to the tendency so far of being considered a “rare disease”.
3. Depending on the clinical evaluation at the moment when the patients in the study group were diagnosed, most of them showed bleeding symptoms.
4. The examination of the peripheral blood smear revealed aspects specific to morphological disorders of platelets in ITP. Blood smears showing platelet macrocytosis (45%, n =
18) and smears with rare platelets (27.5%, n = 11) predominated. Positive anti-platelet antibodies were associated most frequently with platelet macrocytosis (56.3%).

5. The analysis of the **marrow smear** provided information characteristic to **morphological disorders of platelets in ITP**.

6. The association of thrombocytopenia in ITP with viral or microbial infections (Helicobacter pylori) is demonstrated by the presence of specific antibodies; thus, the best therapeutic tendency is to eradicate these infections or to block the already formed antibodies.
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I. Scientific articles published in full in journals listed ISI Thomson Reuters


II. Articles published in full in journals listed BDI


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