CLINICAL AND LABORATORY CHARACTERISTICS OF CHILDREN WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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ABSTRACT

The most common primary autoimmune thrombocytopenia in children is immune (idiopathic) thrombocytopenic purpura (ITP) with extremely variable clinical manifestations, from asymptomatic forms to life-threatening bleeding. The latest classification divides ITP into acute (ndITP), persistent (pITP) and chronic form (cITP). This research was designed as a retrospective–prospective study which analyzed basic demographic, clinical and routine laboratory parameters relevant to ITP. There was slight predominance of girls in all forms of ITP. Children of preschool age dominated in ndITP, while adolescents in cITP group. pITP and cITP patients predominantly presented as asymptomatic or with mild haemorrhagic signs, while ndITP patients had moderate or severe bleeding. Skin hematomas are the most common sites of bleeding. Mostly ndITP patients did not have other diseases, while 30% of cITP patient have other autoimmune disease. No hepatosplenomegaly was observed in ndITP patient, but almost a quarter of the cITP patients had splenomegaly. The mean value of platelet count is significantly higher in chronic groups compared to ndITP group, against mean platelet volume values that show an inverse correlation. More than half ndITP patients achieved complete remission after intravenous immunoglobulin and additional 30% experienced spontaneous remission during the persistent disease period and about one third of cITP patients required therapy. The spleen is dominant or only organ of platelet sequestration in cITP patients.

Keywords: *Idiopathic thrombocytopenic purpura, children.*

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INTRODUCTION

The most common primary autoimmune thrombocytopenia or immune (idiopathic) thrombocytopenic purpura (ITP) is an autoimmune disease characterized by binding of autoantibodies (most often IgG, less often IgM class) to specific glycoproteins on platelets (primarily GP-IIb/IIIa and GP-Ib/IX complex) which cause platelet destruction (1, 2). These antibodies are usually directed towards viral antigens, vaccine components, insect toxins, or reproduced in other autoimmune or infectious diseases. Platelets marked in this way aggregate and their extravascular destruction by the cells of the reticuloendothelial system is enhanced. Predominantly, this process occurs in the spleen, but also in the liver. In addition, platelets marked with autoantibodies trigger complement activation and consequent apoptosis is initiated (1–4).

However, regardless of these clear facts and observations, the pathophysiology of autoimmune thrombocytopenia remains unclear. For example, one group of investigators observed oligoclonality in B-lymphocytes of patients with ITP (5). A similar disorder of T-lymphocytes indicates a possible role of T-lymphocyte dysfunction (6). Studies have shown that some patients with ITP have platelet counts close to normal. This indicates the possibility that autoantibodies can also damage megakaryocytes or that only some platelets are a target for antibodies, while others are unaffected (1, 2). Many studies in recent years have shown that abnormalities of dendritic cells, natural killer (NK) cells, cytokine disorders (interleukin 2, interleukin 17 and interferon γ), programmed cell death, oxidative stress, infection, pregnancy and drugs may play a significant role in the pathogenesis of ITP (5–7).

The latest classification divides ITP into a newly diagnosed form (ndITP), which lasts up to 3 months, a persistent form (pITP) from 3 to 12 months, and a chronic form (cITP), when the disease lasts longer than 12 months (4).

Acute ITP is a disease that usually occurs in childhood, most often following a viral infection or vaccination (8, 9). The clinical manifestations of ITP are extremely variable, from asymptomatic forms to life-threatening bleeding. Usually the disease begins suddenly, mainly with skin bleeding (hematomas, petechiae and ecchymoses) and visible mucous membranes (most often epistaxis or gingival bleeding), gastrointestinal (melena or hematemesis) or urogenital tract (usually menometrorrhagia). Apart from the tendency to hemorrhagic syndrome, the other clinical findings in the majority of cases are usually normal, so any presence of lymphadenopathy or organomegaly is not consistent with the diagnosis. The most serious complication is intracranial bleeding, fortunately with a low incidence of <1% (4, 10, 11).

Acute ITP is most often a benign disease and usually resolves spontaneously without any consequences. In 60% of children with ITP, clinical manifestations may disappear spontaneously within 3 months and in an additional 20-30% within a year, when they transition to a persistent form and require more serious monitoring. It is up to the clinicians to decide, with a mandatory consultation and a detailed explanation to the parents, in accordance with the severity of the hemorrhagic syndrome, how the treatment will be carried out. Usually the degree of the hemorrhagic syndrome correlates with the platelet count (4, 11, 12).

Until now, it is not possible to predict the length of remission achieved (either clinical or laboratory), both in newly diagnosed and chronic forms. In about 10-30% of children with acute ITP, thrombocytopenia is present for more than 12 months and then chronic ITP occurs, and it is not yet possible to define the factors that can predict which patients will develop a chronic form of the disease at presentation. It is also not possible to predict which 20% of children with chronic ITP will have a spontaneous recovery (13, 14). Patients with chronic ITP and a history of hemorrhagic syndrome should be treated, especially those with platelet counts <10–20×10^9/L or moderate thrombocytopenia (20–30×10^9/L). Asymptomatic ITP with moderate thrombocytopenia (20–50×10^9/L) or patients with platelet counts greater than 50×10^9/L generally do not require treatment (4, 13, 14).

This study presents a set of the most significant clinical and laboratory characteristics of children with different forms of ITP, which can additionally help clinicians in their daily work, in terms of evaluating therapeutic and prognostic parameters for each individual patient.

MATERIAL AND METHODS

This clinical research was designed as a retrospective - prospective, cohort and observational study in children aged 6 months to 18 years with a diagnosis of various forms of idiopathic thrombocytopenic purpura. A total of 102 children divided into 4 groups were included in the research: 1.) Newly diagnosed ITP (ndITP): 27 children with some of the signs of hemorrhagic syndrome or asymptomatic, with thrombocytopenia in complete blood count; 2.) Persistent ITP (pITP): 22 children whose illness lasts longer than 3 and shorter than 12 months treated with different therapeutic modalities; 3.) Chronic ITP (cITP): 29 children in whom the disease lasts longer than 12 months, in whom a platelet kinetics test was performed with a radioactive tracer, who were treated with available treatment methods in the previous period; 4.) Control groups: a.) 12 healthy children and b.) 12 healthy children who previously suffered from ITP and were in stable complete clinical and laboratory remission for at least 12 months. The research was carried out as academic and non-profit. We used data from the medical records of children hospitalized at the Pediatric Clinic, University Clinical Centre Kragujevac over a 3-year period in retrospective part of study and in 2-year period in prospective part as newly diagnosed cases were hospitalized and in whom standard clinical and laboratory work-up related to ITP was performed.

Study inclusion criteria was: 1.) the diagnosis of newly diagnosed ITP according to the guidelines of the American Society of Hematology (4); 2.) for the persistent and chronic

form of the disease, meeting the conditions regarding the duration of the disease.

Criteria for excluding subjects from the study: 1.) pseudothrombocytopenia (20), 2.) infants younger than 6 months with a diagnosis of neonatal thrombocytopenia; 3.) children with some form of thrombasthenia; 4.) pregnant women up to the age of 18 with ITP; 5.) children with ITP and any haemostatic disorder; 6.) children with Evans syndrome; 8.) children with ITP and other diseases that require the use of chronic therapy (asthma, kidney patients, oncology patients, etc.).

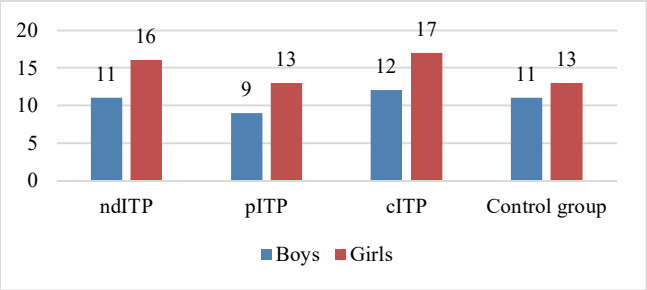
All patients were analyzed for basic demographic and clinical parameters relevant to ITP, as well as all routine laboratory and radiographic tests used in the diagnosis of ITP.

The obtained results were processed and presented using the methods of descriptive statistics. A value of $p<0.05$ was considered statistically significant. All data were analyzed using the statistical program IBM statistics SPSS 21.

RESULTS

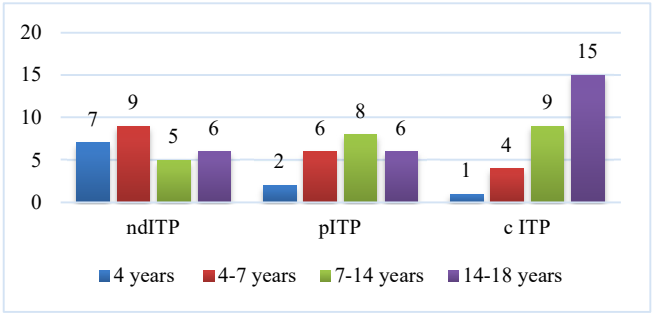
The total number of examined children was 102. The examines were divided into 4 groups: 1) 27 children with newly discovered ITP (ndITP); 2) 22 children with persistent ITP (pITP); 3) 29 children with chronic ITP (cITP) and 4) 24 children in the control group (12 healthy subjects and 12 patients cured of ITP – cITP).

Graph 1 shows gender distribution of examined children. A slight predominance of girls is observed in all forms of ITP (F:M 1.2–1.3:1).



Graph 1. Number of children with ITP according to gender

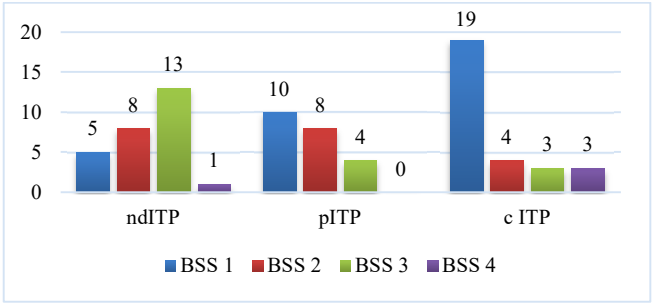
The average age of our subjects was 8.7 ± 3.8 years. However, the most of the patients in cITP group were adolescents (around 55%), while ndITP mainly affects children of preschool age (60%) (Graph 2). The average age of our examinees in the group of children with ndITP is 7.4 ± 2.5 years, while in the group with cITP the average age is 14.5 ± 3.7 years.



Graph 2. Number of children with ITP according to age

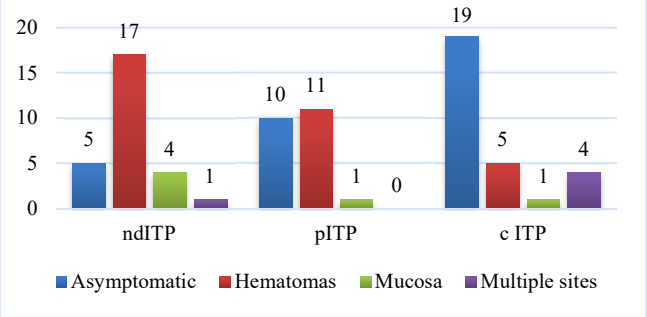
In our cohort, nearly two-thirds of patients with cITP did not have a clear causative factor that triggered the immune process (infection, vaccine, insect bite, etc.) at the time of disease diagnosis. On the other hand, in the ndITP group, almost a third of patients do not have data on a clear causative factor (8/27). In five out of six patients, who developed chronic form of disease during the follow-up period, the disease started without a clear triggering cause.

The severity of the clinical outcome in our patients was assessed according to the degree of bleeding based on the Bleeding Severity Score (BSS), dominant bleeding sites, as well as the need for transfusions of deplasmatized erythrocytes and concentrated platelets during the course of the disease. Among our subjects with ndITP and pITP, there were none one who required transfusions of blood components, while 4 chronic patients had to receive blood transfusions due to extensive bleeding. About half of patients with ndITP had moderate bleeding, on the other hand, 20% were asymptomatic at disease presentation. Persistent and chronic patients predominantly presented with mild signs of hemorrhagic syndrome or were asymptomatic, while 10% of chronic patients had moderate and severe bleeding (Graph 3).

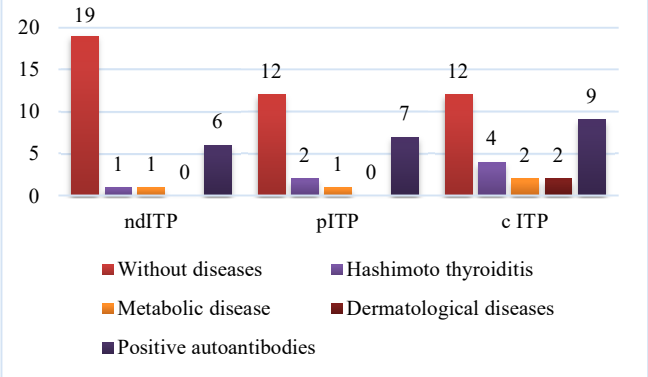


Graph 3. Degree of bleeding according to BSS in children with ITP

The most common sites of bleeding in our patients were skin hematomas, while gingival bleeding, epistaxis or other mucous membranes bleeding was generally less frequent in patients with a more severe clinical picture. There were no patients with intracranial and gastrointestinal bleeding (Graph 4).



Graph 4. Dominant sites of bleeding in children with ITP



Graph 5. Frequency of other autoimmune diseases in children with ITP

The frequency of other autoimmune diseases changes depending on the form of the disease in all examined groups, so 70% of children with ndITP did not have other diseases, while this percentage is significantly lower in pITP (55%), and around 40% in cITP group. On the other hand, there are only individual examples of patients with thyroid diseases or some metabolic disorders in the groups with ndITP and pITP, while the number is more convincing in patients with cITP. Interestingly, in each group, autoantibodies available for routine diagnostics were registered in 25-30% of patients without a clearly manifested autoimmune disease (Graph 5).

Regarding the family history, about 30% of children's family members in all groups did not have any known autoimmune diseases. Also, there were similar percentage of cases where several members of the household expressed autoimmunity, and there were individual cases with metabolic diseases and thyroid gland diseases (Table 1.).

Table 1. Frequency of autoimmune diseases in the family in children with ITP

	ndITP		pITP		cITP	
Family history	**N**	**%**	**N**	**%**	**N**	**%**
ITP in the family	0	0	1	4.5	3	10.3
Thyroid gland diseases	2	7.4	2	9.0	4	13.8
Skin diseases	0	0	1	4.5	1	3,4
Metabolic diseases	6	22.2	3	13.6	5	17.3
Negative family history	10	37.0	7	31.8	8	27.6
More members with autoimmune diseases	9	33.4	8	36.6	8	27.6

N - number

All examined patients had haemostasis screening parameters within reference values, all liver and kidney function parameters were within normal limits, and all had values of immunoglobulin subclasses also within reference values according to age.

No patient in ndITP and pITP had a positive stool antigen for *Helicobacter pylori*, but slightly more than 20% of patients (6/29) in the cITP group tested positive at some point. Three of these patients also had gastric disturbances. Esophagogastroduodenoscopy was performed on them, with definitive confirmation by urease test and PH verification. In one patient, the test was negative in every subsequent control, so eradication therapy was not carried out, while the other 5 were on quadruple therapy, according to the protocol, for a month.

Interestingly, all these patients were boys, with asymptomatic (4/6) and mild clinical presentation (2/6) and platelet count >50 x10^9/L.

Table 2 shows the frequency of hypovitaminosis D and elevated values of lactic dehydrogenase (LDH) in children with ITP. It was observed that about 65–75% of children in all groups with ITP had hypovitaminosis D. 15% of children with ndITP had vitamin D deficiency, while this number is up to 25% in pITP and cITP. Extremely high LDH values, over 750 U/L, were characteristic of children with ndITP (40%), and another half of them had elevated values over 450 U/L. On the other hand, in 65% of patients with pITP and in 40% of patients with cITP, LDH values were within normal limits.

Table 2. Values of lactic dehydrogenase and vitamin D in children with ITP

	ndITP		pITP		cITP	
Vitamin D (ng/mL)	**N**	**%**	**N**	**%**	**N**	**%**
30-40	6	22.2	4	18.2	4	13.8
20-30	3	11.2	2	9.1	4	13.8
10-20	12	44.4	13	59.1	14	48.3
<10	6	22.2	3	13.6	7	24.1
Lactic dehydrogenase (U/L)	**N**	**%**	**N**	**%**	**N**	**%**
<450	3	11.2	14	63.6	12	41.4
450–750	13	48.1	8	36.4	15	51.7
>750	11	40.7	0	0	2	6.9

N - number

No hepatosplenomegaly was observed in any patient with ndITP. On the other hand, in almost a quarter of the patients (5/22 in the pITP and 8/29 in the cITP group), splenomegaly was verified by the echosonographic examination of the abdomen at some point during the follow-up, but in the platelet kinetics test that number was lower, only 4 subjects in cITP group. Also, an accessory spleen was observed in 17% of subjects (5/29) with cITP, while it was not verified in the other groups.

The mean value of platelet count at the time of disease diagnosis in the group with ndITP was $14.7 \pm 3.6 \times 10^9$/L, significantly higher values were registered at pITP $36.4 \pm 7.8 \times 10^9$/L, and at cITP $39.8 \pm 9.1 \times 10^9$/L. This is expected because almost 70% of patients with ndITP had a platelet count $<20 \times 10^9$/L, while only about 20-25% of patients with pITP or cITP had that such a low platelet count, if we exclude clinical relapse of the disease.

Additionally, in the group with ndITP, the highest mean platelet volume (MPV) of 11.2±1.4 fL was registered, while the platelet volume was significantly lower in patients with longer disease duration, 10.3± 1.9 fL in pITP and 10.4±1.7 fL in cITP group. In all children with ITP, a cytological examination of the bone marrow aspirate was performed and no dysplastic changes were found in any of them. The interesting result was that 20-25% of the patients in all three groups with ITP had decreased megakaryocytes in the bone marrow, in contrast to others who had the expected normal bone marrow or hyper production of megakaryocytes (Table 3).

Table 3. Number of platelets, mean volume of platelets and percentage of megakaryocytes in children with ITP

	ndITP		pITP		cITP	
Platelet count ($\times 10^9$/L)	**N**	**%**	**N**	**%**	**N**	**%**
>50	2	7.4	10	45.4	9	31.1
20-50	6	22.2	8	36.4	13	44.8
10-20	9	33.3	2	9.1	5	17.2
<10	10	37.1	2	9.1	2	6.9
Mean platelet volume (fL)	**N**	**%**	**N**	**%**	**N**	**%**
7–10	6	22.2	8	36.4	10	34.5
10-12	10	37.1	14	63.6	15	51.7
>12	11	40.7	0	0	4	13.8
Percentage of megakaryocytes	**N**	**%**	**N**	**%**	**N**	**%**
Hyper production	15	55.5	14	63.6	16	55.2
Normal	5	18.5	3	13.6	7	24.1
Decreased	7	26.0	5	22.8	6	20.7

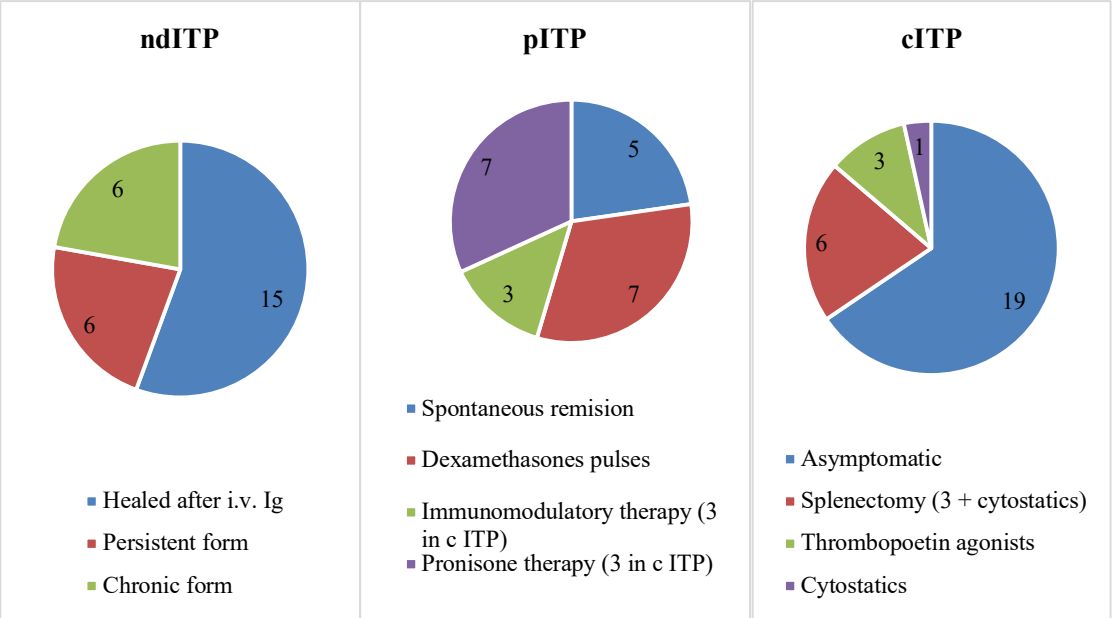
N - number

Of the 27 children with newly diagnosed ITP who were followed for at least one year, more than half (15/27) achieved complete clinical and laboratory remission after intravenous (i.v.) immunoglobulin therapy. The others mostly had a laboratory relapse of the disease, but transitioned to a persistent form (6/27), and the same number of patients had illness which lasted longer than a year, and they were in the chronic phase of ITP.

In patients with pITP, about 30% experienced spontaneous remission, and those who required the use of therapy, same number of patients treated with Prednisone and pulse doses of Dexamethasone(30% = 7/22). Three patients did not respond to Prednisone, thus they received immunomodulatory therapy with Mycophenolate mophetil. In all three patients on immunomodulatory therapy, as well as in 3 patients who were only on Prednisone therapy, the disease progressed to a chronic form. Patients treated with pulse doses of

Dexamethasone and the remaining 4 on Prednisone therapy are in complete remission.

About 35% of patients with cITP (10/29) required therapy: 3 patients were splenectomized and achieved complete remission; 3 patients were treated with thrombopoietin receptor agonists and achieved clinical remission; 4 patients were treated based on different cytostatic protocols and/or Rituximab (anti CD20 antibody) and 3 of them were also splenectomized, but without success (Graph 6.).



Graph 6. Applied therapy in children with ITP

Platelet kinetics test was done in all patients with chronic disease. The average lifespan of platelets in our study group was 0.94 ± 0.47 days (about 22h). It is striking that the largest number of patients (almost 70%) with cITP had a mean platelet lifespan of less than one day, but on the other hand, 15% of patients had an almost normal platelet lifespan. 35% of patients had normal, and another 35% very low level of platelet production in the bone marrow. In almost 60% of patients, the spleen was dominant or only organ of platelet sequestration, and in 30% there was mixed sequestration in the liver and spleen. In 10%, the spleen was not involved in the platelet sequestration process at all (Table 4).

Table 4. Presentation of parameters from the platelet kinetics test in children with cITP

Platelet life span (days)	**<0.5**	**0.5–1**	**1–1.5**	**1.5–4**	**>4**
N	6	13	5	2	3
%	20.7	44.7	17.2	6.9	10.3
Production index	**<0.5**	**0.5–1**	**1–1.5**	**1.5–2**	**>2**
N	5	6	7	6	5
%	17.2	20.7	24.3	20.7	17.2
Place (index) of sequestration	**Liver + other organs**	**Mixed (liver + spleen)**	**Predominantly the spleen**	**Spleen**	
N	3	9	3	14	
%	10.3	31.1	10.3	48.3	

N – number

DISCUSSION

In our representative sample of 90 children suffering from ITP (102 in total and 12 healthy children), it was found that the gender distribution of patients corresponds to data from the literature (F:M ~ 1.2–1.3:1) and that the highest percentage of affected children with the chronic form of ITP were adolescents, with a prevalence of about 35%, while the newly diagnosed form of ITP mostly affected children of preschool age (about 60%) (4, 8, 10). One of the most documented epidemiological differences between ITP in childhood and in adults is certainly a more pronounced predominance of women among adults with the disease in a ratio of ~2:1, while in children it is ~1.3:1, which is certainly associated with an

increased incidence of autoimmune diseases in adult women (15, 16). This fact was also confirmed in our group, because almost 70% of all patients with an associated autoimmune disease were female.

Regarding the anamnestic data, a detail that is often mentioned in the literature as one of the markers that can indicate the chronic form of the disease in patients with newly diagnosed ITP is the absence of a clear causal factor that led to the onset of the disease, such as a previous infection, vaccination, insect bite, etc. (13, 14). In our cohort, almost two-thirds of patients with chronic ITP at the time of diagnosis did not have data on a clear causative factor that triggered the immune process. On the other hand, in our ndITP group, almost a third of patients did not have information about a clear causative factor, and of the patients who transited to chronicity during the follow-up period, four out of six did not have this information. Although it is a small sample, literature data were confirmed in our group as well.

About half of patients with ndITP had moderate bleeding at diagnosis, while 20% were asymptomatic. Among persistent and chronic patients, asymptomatic clinical presentations or forms with mild signs of haemorrhagic syndrome predominate, while 10% of chronic patients had moderate and severe bleeding. By far the dominant sites of bleeding in our patients were skin hematomas, while gingival bleeding, epistaxis or bleeding from other mucous membranes was generally less frequent in patients with a more severe clinical outcome. We had no patients with intracranial and gastrointestinal bleeding. Certainly, the degree of bleeding has been left up to the individual clinicians to assess, although attempts have been made to form scales for the assessment of bleeding (17, 18), but there is still a large discrepancy among researchers in this field. Certainly, asymptomatic patients in ndITP range in most studies around 20%, in contrast to cITP where the results are in different series of 10–70% (4, 10–14, 19). It is expected that patients with a newly diagnosed disease have the most pronounced hemorrhagic syndrome, and according to the clinical experience so far, as well as according to the literature data, chronic patients with ITP have a consistent pattern of alterations of the number of platelets, and even a consistent bleeding pattern over the years, which is disturbed in infections or when using nonsteroidal anti-inflammatory drugs. We considered our chronic patients asymptomatic only if they had a clinical remission after the first acute attack of the disease, with maintenance of thrombocytopenia. After one year, almost 75% of patients were in a stable asymptomatic phase of the disease, which again correlates with literature data (4, 8, 10–14, 19). About 35–40% of patients with pITP have a milder BSS of 2, and only 10% with cITP have severe, sometimes life-threatening bleeding with a score of 4 (Graph 3).

In accordance with the previous data, among our subjects with ndITP and pITP there were none who required transfusions of blood components, while 4 of our chronic patients had to receive transfusions of concentrated platelets and/or deplasmatized erythrocytes due to extensive bleeding. And these percentages are similar to other researchers, where the number of transfused patients ranges between 5–15% (8, 10–14, 19).

Another major historical difference between paediatric and adult ITP is the incidence of comorbid medical conditions. The presence of 1 or more comorbidities was proven in only 5% of children and >30% of adults at disease presentation and in 10–15% of children and 35–50% of adults during 2 years of follow-up (15, 16). In our sample, in all examined groups with ITP, children predominantly do not have other autoimmune diseases, but the frequency changes, so 70% of children with ndITP are free of other diseases, while this percentage is lower in pITP (55%) and in cITP (about 40%). On the other hand, there are only individual examples of patients with thyroid diseases (predominantly Hashimoto's thyroiditis) or some metabolic disorders (polycystic ovary syndrome, insulin resistance, obesity) in the groups with ndITP and pITP, while the number is more convincing in patients with cITP. Interestingly, 25-30% of patients with positive autoantibodies available for diagnosis, without a clearly manifested autoimmune disease, were registered in each group (Graph 5). In most cases, that was the presence of anti-thyroglobulin, anti-nuclear and/or lupus anticoagulant antibodies, as well as a positive Coombs test (either direct or indirect) without any elements for haemolytic anemia, individually or in combination. In other studies, the presence of positive autoantibodies is often mentioned, but so far their importance has not been established(1, 2, 4, 8, 10, 20) and while some authors recommend their monitoring, others question whether they should be done at all (21, 22).

Looking at the family history, in almost the same percentage (about 30-35%) in the families of children with ITP in all groups, either there were no known autoimmune diseases or there were several affected members of the household. There were also individual cases with metabolic diseases and thyroid gland diseases (Table 1.). It must be noted, however, that patients with a chronic form of the disease have a richer family history in terms of autoimmune diseases. Only 4 subjects had relatives who had or still have ITP. Several studies have tried to determine the genetic predisposition and the importance of autoimmune diseases in the family on the occurrence of ITP itself, as well as on the development of the chronic form of the disease, and they all agree that a positive family history contributes to both occurrences, but so far there are no consistent results related specifically to the genetic predisposition to the development of ITP (4, 7, 8, 21–23).

No patient in the ndITP and pITP groups had a positive stool antigen for *Helicobacter pylori*, but slightly more than 20% of patients (6/29) in the cITP group tested positive at some point. Three of these patients also had gastric symptoms, thus esophagogastroduodenoscopy was performed on them, with definitive confirmation of infection by urease test and pathohistological verification. In one patient, the test was negative in every subsequent control, so eradication therapy was not carried out, while the other 5 were on quadruple

therapy for a month, according to the protocol. Interestingly, all these patients were boys, with asymptomatic (4/6) and mild clinical outcome (2/6) and platelet count >50 x10^9/L. So far, no mechanisms have been established that would explain how *Helicobacter pylori* can influence the pathogenesis of ITP and all results are still very contradictory. There is no clear evidence whether these are patients in whom remission would occur in any case or whether eradication therapy and elimination of the causative agent led to remission, and the exact connection between these two entities is still unknown (10, 24).

Our study showed that about 65–75% of children in all groups with ITP had hypovitaminosis D, of which 15% of children with ndITP had vitamin D insufficiency, while this number in pITP and cITP was up to 25% (Table 3). In recent years, the connection between these two entities has been in the sphere of interest. In accordance with our study, some studies have shown the connection between ITP and hypovitaminosis D in over 80%. Still, there is evidence that not only a reduced level of vitamin D contributes to the disease to such an extent, but above all vitamin D receptor gene polymorphism. However, more extensive research is still to be conducted on this topic (25, 26). The fact is that vitamin D supplementation cannot harm patients with ITP.

Extremely high LDH values over 750 U/L are characteristic of children with ndITP (40%), and another half of them had elevated values over 450 U/L. On the other hand, in 65% of patients with pITP and in 40% of patients with cITP, LDH values were within normal limits. This is expected, given that LDH is an intracellular enzyme, released by increased destruction of platelets. On the other hand, viral infections that are frequent in childhood may also cause an increase in LDH. That is why, unlike in adults, LDH is not an adequate parameter for evaluating disease activity in children (4, 8, 10).

No hepatosplenomegaly was observed in any patient with ndITP. On the other hand, splenomegaly was verified at some point during the follow-up by echosonographic examination of the abdomen in almost a quarter of chronic patients (5/22 in the pITP and 8/29 in the cITP group). Nevertheless, on the platelet kinetics test that number was lower, only 4 of respondents in the cITP group. It is true that any appearance of organomegaly automatically excludes the diagnosis of ITP. However, this is not applicable when it comes to chronic forms, given that the long-term destruction of platelets in the reticuloendothelial system of the spleen inevitably leads to their hyperplasia and causally to occasional organ enlargement depending on the activity of the immune process. A similar percentage of splenomegaly was reported by other researchers, especially those who focused on splenectomy as a therapeutic option for ITP (3, 27, 28). Also, either by ultrasound, during the performance of the kinetics test or during splenectomy, an accessory spleen was observed in 17% of subjects (5/29) with cITP, while it was not verified in the other groups. There are only individual case reports of subsequent removal of the accessory spleen, in case of worsening thrombocytopenia after splenectomy, with a good therapeutic effect (29).

The mean platelet count in the group with ndITP was 14.7 ± 3.6 ×10^9/L, significantly higher values were registered in pITP 36.4 ± 7.8 ×10^9/L, and in cITP 39.8 ± 9.1 × 10^9/L. This is expected because almost 70% of patients with ndITP had a platelet count <20 ×10^9/L, while this number is significantly lower in patients with pITP and cITP, only about 20-25% of patients and only in some controls, if we exclude the clinical relapse of the disease (Table 3). On the other hand, about 30% of patients with ndITP had platelet counts >20 ×10^9/L. A higher platelet count of 30–50×10^9/L (depending on the author) at the time of diagnosing the disease is one of the important criteria for the development of a chronic form (4, 10–14). Despite the enormous progress in clarifying the pathophysiology of ITP, both our study and other studies as well as a large number of facts and results from clinical practice still support the fact that the number of platelets is still the leading factor that determines the severity of the clinical outcome in patients with all forms of ITP (4, 8, 10–14, 19). It is relatively rare that patients with a platelet count >20–30 ×10^9/L, which we can consider a satisfactory number, especially in the chronic form, bleed more than patients with a lower platelet count, which brings us back to the beginning and the complicated pathophysiological mechanism of the disease (4, 8, 19).

In the group of patients with ndITP, the highest mean platelet volume (MPV) of 11.2 ± 1.4 fL was registered, while the platelet volume was significantly lower in patients with longer disease duration, 10.3 ± 1.9 fL in pITP and 10.4 ± 1.7 fL in cITP group. This phenomenon suggests that larger platelets are probably more active, both in the metabolic sense and in the process of haemostasis. Larger platelets try to compensate their paucity, which is dominant in the acute form of the disease, with their size. Certainly, in the later course of the disease, other factors are also involved, especially the bone marrow microenvironment (30). Over time in the chronic form, the bone marrow gets used to producing somewhat larger platelets, so this could become the subject of some future research. Numerous studies highlight the importance of MPV in patients with ITP. Some authors even proposed elevated MPV in the newly diagnosed form as one of the markers of the chronic form of the disease (4, 12, 31).

A cytological examination of the bone marrow aspirate was performed in all children with ITP and no dysplastic changes were found in any of them. The interesting result was that 20-25% of the patients with ITP had decreased megakaryocytes in the bone marrow, unlike the others who had expected normal bone marrow or hyper production of megakaryocytes (Table 4). This indicates the fact that autoantibodies, in addition to having a peripheral effect on platelets, can bind to megakaryocytes and inhibit their maturation or lead to their destruction, so that thrombopoietin cannot perform its role. This is particularly evident in patients with ITP who are bleeding and have a platelet count close to normal. This indicates the possibility that autoantibodies directly damaged

megakaryocytes or that they act on cytokines necessary for the growth and proliferation of megakaryocytes or that only some platelets are a target for antibodies, while others are not affected (1, 2, 4, 8, 22, 30, 32).

Five asymptomatic patients in the newly diagnosed group underwent a watch and wait approach, none of them experienced a complete remission, so all of them were administered IV immunoglobulins. Of the 27 children with ndITP who were monitored for at least one year, more than half (15/27) experienced a complete clinical and laboratory remission after IV immunoglobulin therapy. The rest had a mostly laboratory relapse of the disease, which is why a new watch and wait approach was tried. This approach had no effect, and after patients transitioned to a persistent form, they were given systemic corticosteroid therapy – Prednisone and pulse doses of Dexamethasone (7/22 pITP patient). A relapse 4-6 weeks after the drugs was considered a short-term response to the initial treatment with IV immunoglobulins, and a relapse after reducing the dose of Prednisone or inadequate reactivity to Dexamethasone was considered a short-term response to systemic corticosteroids. About 20% (6/27) of our ndITP patients were resistant to the applied therapy (absence of remission or relapse in a shorter period). In these patients the disease lasted longer than 12 months and after that they entered the chronic phase of the disease.

In patients with pITP, about 25% (5/22) experienced spontaneous remission. Those who required therapy, in addition to patients treated with systemic corticosteroid therapy, three patients were treated with immunomodulatory therapy with Mycophenolate mophetil due to the failure of therapy. In all three patients on immunomodulatory therapy, as well as in 3 patients who were only on Prednisone therapy, the disease progressed to a chronic form. Patients on pulse doses of Dexamethasone and the remaining 4 on Prednisone therapy are in complete remission. About 35% of patients with cITP (10/29) required the use of therapy, the rest were asymptomatic. 3 patients were splenectomized and achieved complete remission, 3 patients are on therapy with thrombopoietin receptors and achieved clinical remission, in 4 patients, in addition to other therapies, various cytostatic protocols and/or Rituximab (anti CD20 antibody) were tried, in 3 out of 4, splenectomy was also performed but without success. All of these patients have from 1-5 episodes per year of severe hemorrhagic syndrome requiring transfusions of blood products (Graphs 6.).

In addition to the mentioned standard therapeutic options for the treatment of ndITP, other approaches have appeared in recent years with the use of individual second-line therapy, but most researchers who examined the effects of standard therapy have results similar to these (4, 10 – 14, 19). The vast majority of authors previously believed that the therapy in children's acute ITP may be overdone and that whenever the degree of bleeding allows it, an observational approach should be tried (33). However, in addition to the certainly longer time to achieve remission as a downside, such an approach did not have any effect in terms of longer duration of achieved remission or less occurrence of chronicity compared to the treated group of children (4, 10 – 14, 19, 33). In our cohort, we did not use any currently available experimental drugs.

Performing a platelet kinetics test with a radioactive tracer is recommended in all patients with acute ITP who have an inadequate response to the initial therapy or in whom the disease progresses to a chronic form. A platelet kinetics test was performed in all examined patients within a period of about a year from the diagnosis of ITP, and in some after that, for the purpose of re-evaluation due to inadequate therapeutic response. The average lifespan of platelets in our study group was about 22h. It is striking that the largest number of patients (almost 70%) with cITP have a mean platelet lifespan of less than one day, but on the other hand, 15% of patients had an almost normal platelet lifespan (Table 4). This points to the already mentioned fact that the pathophysiological concept of the origin of the disease in individual patients is quite complex (1–4, 8, 10, 27).

However, earlier studies have shown that platelet lifespan, and especially the platelet production index, were the least reliable parameters for any interpretation, but these studies primarily focused on the evaluation of the therapeutic success of splenectomy (3, 27, 34, 35). In our group, almost the same percentage (35%) of patients with chronic ITP had either a normal, or reduced or very low level of platelet production in the bone marrow. On the other hand, regarding the therapeutic success evaluation, most authors agree that the most significant factor is the index (site) of platelet sequestration (34, 35). In our cohort, in more than half of the patients (60%), the spleen was the dominant or only organ of platelet destruction, and in about 30% there was mixed destruction, almost equally in the liver and spleen. In about 10%, the spleen was not involved at all in the process of platelet destruction, but the liver took over, while in only 1 patient, in addition to the liver, it was found that platelet sequestration also took place in the thymus, stomach, testicles and lungs (Table 4.). The platelet kinetics test once again confirms that the pathophysiological substrate affects megakaryocytes in the bone marrow and plateletsin the peripheral blood equally. The above results contribute to the conclusion that the place of sequestration of platelets can largely determine the prognosis of the chronic disease (3, 4, 10, 19, 27, 34, 35).

CONCLUSION

There was slight predominance of girls in all forms of ITP. Children of preschool age dominated in ndITP, while adolescents in cITP group. Persistent and chronic patients predominantly presented as asymptomatic or with mild haemorrhagic signs, while newly diagnosed patients had moderate or severe bleeding. The most common sites of bleeding are skin hematomas. Mostly ndITP patients did not have other diseases, while 30% of chronic ITP patient have other autoimmune disease. Similar number of ITP children's family members in all groups did not have any known

autoimmune diseases or expressed multiple autoimmune disorders. Supplementation of Vitamin D is strictly recommended for ITP patients. No hepatosplenomegaly was observed in ndITP patient, but almost a quarter of the chronic patients had splenomegaly. The mean value of platelet count is significantly higher in chronic groups compared to ndITP group, against MPV values that show an inverse correlation. More than half ndITP patients achieved complete remission after intravenous immunoglobulin and additional 30% experienced spontaneous remission during the persistent disease period and about one third of patients with chronic forms of ITP required therapy. The spleen is dominant or only organ of platelet sequestration in chronic form of disease.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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