Original Research Article

Predictors of Outcome in Pediatric Immune Thrombocytopenia: Relation to Thrombopoietin Levels

Abstract

Background: It is not easy to predict the course of immune thrombocytopenia (ITP) at the time of diagnosis. **Aim:** This prospective study investigated the possible predictors of ITP outcome in children and adolescents including the diagnostic potential of thrombopoietin (TPO). **Methods:** Seventy pediatric patients with ITP were studied; 45 had chronic ITP and 25 were newly diagnosed and were followed-up for 3 months. Complete blood count and TPO levels were assessed. **Results:** Chronic ITP patients were older ($p<0.001$), presented more frequently at age >5 years ($p=0.027$), had higher initial platelet count ($p=0.005$) but less likely to have platelet count >100 x 10^9/L after 4 weeks from diagnosis ($p=0.017$) and frequently used a third line of treatment ($p=0.007$). After 3 months follow-up of the 25 newly diagnosed ITP patients, 16 entered in a complete remission and they were younger than 5 years and had lower initial platelet count, higher platelet count after 4 weeks from diagnosis, and less frequently used corticosteroid as a second line of therapy compared with the 9 patients who developed persistent ITP. Increased TPO levels were found among patients with persistent ITP and those who experienced relapse. TPO levels were inversely related to platelets count. **Conclusions:** The predictors of progression to persistent ITP and chronicity among our pediatric patients were age of onset > 5 years old, high initial platelet count, low platelets count 4 weeks after diagnosis and higher baseline TPO levels which could be used as a serum biomarker of megakaryopoiesis to predict therapeutic response.

Key Word: Pediatric immune thrombocytopenia; Predictors of outcome; Thrombopoietin.

Introduction

Childhood immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by isolated thrombocytopenia (peripheral blood platelet count <100 x 10^9/L). It is one of the most common bleeding disorders in children, with an incidence of approximately four per 100,000 per year [1]. It most frequently presents with acute onset of purpura and bruising in an otherwise healthy child, often after a preceding mild viral infection. A benign and self-limited course is common, and major bleeding complications are exceptional [2]. A gold standard diagnostic test for ITP is lacking, and biomarkers to assess disease severity and guide ITP treatment are in their infancy [3**]**. The management including diagnostic investigations, treatment and follow-up is controversial [4**]**.

ITP has a strikingly different clinical course in adults and children. Spontaneous remission in adults with ITP is less frequent and the majority develops chronic ITP [5**]**. Children, on the other hand, have a much more favorable prognosis. In more than 75%, the disease resolves within 6 months irrespective of treatment [6].

In children, however, it is not possible to predict the course of the disease at the time of initial diagnosis [7,8]. Thrombopoietin (TPO) is the major regulator of platelet production. Prior studies in animal models [9] and in humans [10] have demonstrated that TPO levels vary inversely with circulating platelet mass. Additional clinical studies have suggested that TPO levels also vary inversely with the rate of megakaryopoiesis [11,12]. The regulation of megakaryopoiesis by TPO is mediated through a negative feedback loop which involves binding to and clearance of TPO by circulating platelets, megakaryocytes, and megakaryocyte precursors

[13]. Thus, measurement of serum TPO levels may help distinguish between various causes of thrombocytopenia and predict treatment response to TPO receptor agonists [12].

One prospective study involved a cohort of adult patients presenting with a newly diagnosed episode of ITP described the clinical features of adult ITP and its evolution over a 12-month period and explored the baseline predictors of chronicity [14]. However, such prospective studies have not been yet established in children with ITP and the role of TPO as a predictor of outcome has not been fully elucidated. The aim of this study was to outline the clinical features of ITP in children and adolescents and to investigate the possible predictors of outcome including the diagnostic potential of thrombopoietin levels as well as its role in predicting response to therapy.

Patients and methods

This prospective study included seventy children and adolescents with ITP recruited from the regular attendants of the Pediatric Hematology Clinic, Pediatrics Hospital, Ain Shams University. Twenty age- and sex-matched healthy children with no obvious medical disorder and with normal platelets count were recruited from the classmates or acquaintances of the case subjects and were enrolled as controls to assess reference TPO levels. An informed consent was obtained from each patient or control and their legal guardians before enrollment in the study. This study was approved from the local ethical committee of Ain Shams University and is in accordance with the Helsinki Declaration of 2008.

All pediatric patients aged less than or equal to 18 years who were diagnosed with primary ITP according to international consensus [15] were enrolled. ITP was diagnosed on the basis of the patient's medical history, physical examination and complete blood count (CBC) showing isolated thrombocytopenia without other underlying diseases with or without bone

marrow examination (normal or increased megakaryocytes with poor platelet separation and normal other hemopoietic lineages) [16]. Patients with fever or other signs of infection and those who had platelets count more than 100 x 10^9/L or causes of thrombocytopenia other than ITP were excluded.

According to the definitions of International Working Group (IWG) of both pediatric and adult experts in ITP [15], our 70 patients were classified into two groups; Group I included 25 patients with newly diagnosed ITP (those who had ITP within first 3 months from diagnosis) and Group II that included 45 patients with chronic ITP (those who had ITP lasting more than 12 months). Patients with newly diagnosed ITP (n=25) were followed-up for 3 months for identification of patients with persistent ITP (which is defined as ongoing ITP between 3 and 12 months from diagnosis).

All Patients with ITP were subjected to detailed medical history including bleeding manifestations, recent viral illness (within six weeks), recurrent infections suggesting immunodeficiency, symptoms of an autoimmune disorder, recent vaccination, history of drug intake which may cause thrombocytopenia, risk factors for HIV infection, family history of thrombocytopenia or hematologic disorder and history of any comorbid conditions, which may increase the risk of bleeding. Clinical examination was performed focusing on bleeding signs, type and severity of bleeding, presence of dysmorphic features suggestive of congenital disorder, including skeletal anomalies and auditory acuity. The ITP Bleeding Scale (IBLS) which is a bleeding assessment system comprising 11 site-specific grades [17] was used to assess bleeding severity.

Peripheral blood samples were collected on potassium-ethylene diamine tetra-acetic acid (K2-EDTA) (1.2 mg/mL) for CBC which was done using Sysmex XT-1800i (Sysmex, Kobe,

Japan), with examination of Leishman-stained smears for red blood cell (RBC) morphology and differential white blood cell (WBC) count. Serum obtained from clotted samples by centrifugation for 15 min at 1000g and stored at -80°C till subsequent use in measurement of TPO levels by enzyme linked immunosorbent assay (ELISA) using kit supplied by SinoGeneClon Biotech Co., Ltd (Hang Zhou, China). For newly diagnosed ITP patients, samples for assessment of TPO were withdrawn at diagnosis. For, patients with chronic ITP (those who had ITP lasting more than 12 months), blood samples were withdrawn at enrolment before third line of therapy.

Therapy and follow-up

According to local hospital guidelines that follows the American Society of Hematology (ASH) 2019 guidelines for immune thrombocytopenia [18], the first line of management among the studied patients included observation (no therapy), corticosteroids and/or intravenous immunoglobulins (IVIG). Those who had lack of response to the first line of therapy were offered anti D, corticosteroids or IVIG (if not given in the first line) and combined therapy while the third line included thrombopoietin analogues. The response to each line of treatment was assessed as follows; complete remission (CR) was defined as any platelet count of at least 100×10^9/L and absence of bleeding, response (R) was defined as any platelet count $\geq 30 \times 10^9$/L and at least 2-fold increase the baseline count and absence of bleeding, no response (NR) was defined as any platelet count $< 30 \times 10^9$/L or less than 2-fold increase of baseline platelet count or presence of bleeding [15].

Loss of CR or R was considered when platelet count decreased below 100×10^9/L or bleeding from CR or decreased below 30×10^9/L or less than 2-fold increase of baseline platelet count or bleeding from R. Time to response was defined as time from starting treatment to time

of achievement of CR or R. Refractory ITP was considered when patients had significant bleeding with need for treatment(s) and failure to achieve the least response (platelet count ≥ 30 x 10^9/L) while corticosteroid-dependence was defined as the need for ongoing or repeated doses administration of corticosteroids for at least 2 months to maintain a platelet count ≥ 30 x 10 9/L and/or to avoid bleeding [15]. The 25 patients with newly diagnosed ITP were followed-up every week for 3 months to prospectively assess the response after each line of treatment while chronic ITP patients were followed-up monthly.

Statistical analysis

Statistical analysis was done through SPSS software (IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA). Kolmogrov-Smirnov test was used to examine the normal distribution of variables. Quantitative variables were described in the form of mean and standard deviation or median and interquartile range (IQR: 25th-75th percentiles). Qualitative variables were described as number and percent. In order to compare parametric quantitative variables between two groups, Student t-test was applied while comparison between 3 groups was performed using Analysis of Variance (ANOVA) with post hoc test. For comparison of non-parametric quantitative variables between two groups, Mann-Whitney test was applied while Kruskal-Wallis test was used for comparison of more than two groups. Qualitative variables were compared using Chi-square (X_2) test or Fischer's exact test when frequencies were below five. Pearson correlation coefficients were used to assess the association between two normally distributed variables. When a variable was not normally distributed, a Spearman correlation test was performed. Multivariable linear regression analysis was employed to determine the relation between TPO and clinic-pathological variables. Receiver operating characteristic (ROC) curve was used to determine the best cut-off value of TPO to detect chronicity with the highest

balanced sensitivity and specificity. The area under the curve (AUC) was calculated for each plot. Logistic regression analysis was performed with estimating the odds ratio (OR) and 95% confidence interval (CI) to define independent variables for chronicity among newly diagnosed ITP patients. A p value <0.05 was considered significant in all analyses.

Results

Clinical and laboratory characteristics of the studied ITP patients and control group

The control group (n=20) included 9 males and 11 females and their median (IQR) age was 9.5 (6 – 13) years. The 70 patients with ITP included 39 males and 31 females with a median age 7.15 years (IQR, 4-12 years). Their median age of presentation was 5 (IQR, 3.2 – 10.0) years. As regard risk factors of ITP; antecedent infection was reported in 28 (40%) patients, recent vaccination in 7 (10%) patients and usage of medication predisposed to bleeding reported in one (1.4%) patient. Three (4.3%) patients had family history of thrombocytopenia. Comorbid conditions were observed in 10 (14.3%) patients in form of short stature, facial palsy, vitiligo, delayed mile stones and Down syndrome.

Nearly all patients (98.6%) had bleeding manifestation; the most common site was the skin (97.1%). None of the patients had retinal hemorrhage but one patient with chronic ITP had spontaneous intracranial hemorrhage, two patients with chronic ITP had hematuria, six chronic ITP had menorrhagia, six had gastrointestinal bleeding and required blood transfusion.

Initial platelets count $\geq 30 \times 10^9$/L was observed in six ITP patients; no treatment required in three of them while three were treated with corticosteroids (because they were not hospitalized and they live far from hospital so received treatment to ensure absence of bleeding). One ITP patient had transient lymphopenia $<1 \times 10^9$/L at the time of diagnosis and another ITP patient

had lymphopenia <1 x 10^9/L at the time of enrolment. None of the studied patients fulfilled the criteria of refractory ITP and also, none of the studied patients changed to secondary ITP.

Comparison between newly diagnosed and chronic ITP patients

Upon comparing the newly diagnosed (n=25) with chronic ITP (n=45) to outline the chronicity predicators, patients with chronic ITP were older (p<0.001) and presented more frequently at age >5 years (p=0.027) as shown in Table 1. No significant difference was found between newly diagnosed and chronic ITP as regards consanguinity (p=0.309), order of birth (p=0.781) and residence (p=0.819). Moreover, no significant difference was found with respect to the risk factors of ITP; antecedent infection (p= 0.611) and recent vaccination (p=0.617). Also, no significant difference was found as regards the site of bleeding except that patients with chronic ITP had higher incidence of epistaxis (p= 0.025). Patients with chronic ITP also frequently used a third line of treatment (p=0.007) (Table 1) and had higher initial platelet count (p=0.005) but less likely to have platelet count >100 x 10^9/L after 4 weeks from diagnosis (p=0.017) (Table 2).

Prospective follow-up of the newly diagnosed patients (n=25)

After 3 months follow-up of the 25 newly diagnosed ITP patients, 9 (36.0%) patients had persistent ITP while 16 (64.0%) entered in CR. Comparison between newly diagnosed patients who became persistent and those entered in CR (Tables 1 and 2) showed that newly diagnosed ITP patients in CR were significantly younger than 5 years and had lower initial platelet count, higher platelet count after 4 weeks from diagnosis, higher absolute lymphocytes count and less frequently used corticosteroid as a second line of therapy. All newly diagnosed patients presented by bleeding manifestations and no significant difference was found as regards risk factors of ITP, site of bleeding or bleeding score (p<0.05) between both groups after follow-up.

TPO levels

TPO levels were not significantly different between ITP patients (n=70) and healthy controls (n=20) (median [IQR], 290 [200 – 390] pg/mL versus 295 [285 – 375] pg/mL; p=0.308) or between newly diagnosed and chronic patients (median [IQR], 262.5 [220 – 375] pg/mL versus 290 [200 – 400] pg/mL; p=0.488).

No significant difference was found as regards TPO levels in relation to clinical characteristics including bleeding manifestations except for higher levels found among those who had gum or oral mucosal bleeding (Table 3).

As regards TPO levels in relation to outcome of newly diagnosed ITP patients after 3 months follow-up, a significant difference was found between persistent ITP (n=9) and those who had CR (n=11) as well as control group (n=20) where the highest TPO levels were found among those with persistent ITP (p=0.002). ROC curve analysis revealed that TPO cut-off value 310 pg/mL could detect progression to persistent ITP among newly diagnosed patients with 89% sensitivity and specificity of 81% (AUC 0.841; p<0.001).

Higher TPO levels were found among ITP patients with lack of initial response (n=23) and those who experienced relapse (loss of CR or R) (n=26) as shown in Figure 1. TPO levels were inversely related to initial platelets count (r=-0.316, p=0.009) as well as platelets count at enrollment (r=-0.392, p=0.001) among newly diagnosed and chronic ITP patients (Figure 2).

Factors contributing to progression to persistent ITP among newly diagnosed patients

The significant clinical and laboratory variables resulted from comparison between newly diagnosed patients who became persistent and those in CR entered in a logistic regression analysis to identify factors contributing to progression to persistent ITP among newly diagnosed patients noting that exposure to corticosteroids was controlled during analysis. Logistic

regression analysis revealed that age of onset > 5 years old, initial platelets count, platelets count after 4 weeks of diagnosis, platelets at sampling, absolute lymphocyte count at sampling and TPO levels were the significant independent variables related to chronicity (Table 4).

Discussion

ITP has a favorable prognosis in children and about 65-80% of new-onset ITP children recover within first year, while 20-35% has thrombocytopenia for more than 1 year after diagnosis, which is then classified as chronic ITP [19]. ITP is a treatable chronic disease. Because treatment is generally not curative and relapses can occur years later, the goal of therapy is to stabilize the platelet count in a safe range, not necessarily to normalize the count [20]. Initial clinical factors that can reliably predict a successful within-1-year resolution of childhood ITP are still unclear [7,8].

Treatment decisions should be individualized for each patient based on a combination of factors, including clinical signs, physical activity, platelet count as well as medical cost [21]. Literature provides little evidence for clinical decision to adjust the right individual treatment strategy [22]. Most epidemiology data on ITP have been based on retrospective studies and/or administrative registers [23,24], that is usually based on review of medical records and therefore, the quality of data are dependent upon the quality of the original physician documentation [21]. This creates a great need among treating physicians, patients, and parents for reliable predictors for the outcome of childhood ITP at the time of diagnosis as well as after initial therapy to minimize anxiety and the impact of the disease on daily life [8].

In this prospective study, all newly diagnosed ITP patients had bleeding manifestations and the most common was skin bleeding. Various studies also reported that clinically significant bleeding symptoms were observed in between 3% and 6% of children with ITP [25]. Serious

bleeding was rare in our studied ITP children where only one patient had spontaneous intracranial hemorrhage. This confirmed that ITP is frequently associated with cutaneous bleeding and rarely associated with life-threatening bleeding events at diagnosis [14].

We found that only 40% of ITP patients had antecedent infection with comparable percentage among newly diagnosed and chronic patients. However, a retrospective study reported that 75% of children with newly diagnosed ITP had a history of an infectious prodrome, and the majority of them had an upper respiratory tract illness. History of viral illness and a low admission mean platelet volume were found to be independent prognostic variables that predicted for the achievement of a durable CR in childhood ITP [7].

In this study, it was found that 16 out of 25 (64.0%) newly diagnosed ITP patients achieved CR while 9 (36.0%) patients had persistent ITP after 3 months follow-up. A retrospective study of children with ITP showed that CR at 1 year was achieved in 70% (n=139/199) [26]. This suggests that ITP is more likely to follow a chronic course in affected adults, compared to children in whom cure is achieved within several weeks in most patients [27].

Despotovic and Grimes [28] reported that there is evidence to define some of the more distinctive clinical qualities that characterize pediatric ITP (increased rates of acute bleeding and spontaneous remission) and adult ITP (increased rates of comorbidities, risk for intracranial hemorrhage, and likelihood of chronicity). However, closer inspection reveals what is becoming increasingly apparent about ITP in children and adults: heterogeneous treatment responses, remission rates, and clinical characteristics that result from a heterogeneous constellation of infectious, immunological, environmental, and genetic factors. Although classic spontaneously resolving childhood ITP continues to represent a significant proportion of disease, this

homogeneous variety of ITP appears to be decreasing amid the spectrum of childhood and adult ITP that is evolving from an increasingly identifiable variety of immune aberrations and shifting toward increasing similarity in ITP among older children, adolescents, and young adults [28].

In our study, none of the studied clinical characteristics were risk factors for chronicity including sex, family history of thrombocytopenia, antecedent infection or bleeding symptoms at baseline except for age of onset >5 years old where children with chronic ITP were significantly older than the newly diagnosed ones. The largest study about childhood ITP included 2540 children and found that male to female ratio of patients with acute and chronic disease was similar. They also reported that older children (> 10 years) had a lower recovery rate than younger children [29]. Similarly, the 12 months follow-up data from the prospective registry I of the Intercontinental Childhood ITP Study Group (ICIS) demonstrated that the youngest age group was significantly associated with a higher rate of recovery, and especially those aged above 3 months to 1 year, and exhibited a lower frequency of bleeding symptoms during the first 6 months after diagnosis [30]. Other reports also showed that older age is an important predictor of the chronic disease [26,31,32]. It has also been reported that females older than 10 years of age have been reported to develop a more chronic course [33].

Logistic regression analysis for factors contributing to progression to persistent ITP among our studied newly diagnosed patients revealed that age of onset > 5 years old, initial platelets count, platelets count after 4 weeks of diagnosis, platelets at enrollment, absolute lymphocyte count at enrollment and TPO levels were significant independent variables related to chronicity. It is known that lymphopenia is a common effect of treatment with systemic steroids and therefore, exposure to corticosteroids was controlled during analysis.

The Nordic Society of Pediatric Hematology and Oncology (NOPHO) ITP Working group developed a predicting score that included six clinical parameters significantly associated with short duration (<3 months) of ITP, abrupt onset less than 2 weeks, age at onset less than 10 years, preceding infection, platelet count less than 5 $\times 10^9$ /L, wet purpura, and male sex [34]. Another study published a simple prediction score based on retrospective data of 472 children. The authors identified age at onset less than 10 years and abrupt onset less than 14 days as being strong clinical predictors for disease resolution within 1 year [35].

A systematic review and meta-analysis identified the following as predictors of chronic ITP in children; female gender, older age at presentation, no preceding infection or vaccination, insidious onset, higher platelet counts at presentation, the presence of antinuclear antibodies (ANA) and treatment with a combination of methylprednisolone and IVIG [27]. A single center retrospective study of 10 years also confirmed that older age, absence of prior infection and insidious onset of symptoms were significantly associated with the development of chronic ITP [21].

In our study, high initial platelets count at diagnosis was associated with chronicity when newly diagnosed ITP patients were compared with chronic patients. Moreover, when newly diagnosed patients who entered in CR were compared with those who had persistent ITP after 3 months follow-up, it was found that low initial platelet count at diagnosis, high platelets count 4 weeks after diagnosis were associated with favorable outcome and remission. In line with our results, a prospective nationwide observational cohort study explores the clinical features of adults ITP found that the sole possible predictor of chronicity at 12 months was a higher platelet count at baseline [14]. A retrospective analysis determined factors associated with within-12-month resolution of newly diagnosed childhood ITP in a single tertiary center concluded that the

significant clinical and laboratory predictors for resolution of newly diagnosed childhood ITP within 12 months were abrupt onset less than 14 days, age less than 5 years, and platelet count at 4 weeks post-diagnosis of at least 100×10^9/L. A 4-week post-diagnosis platelet level 100×10^9 /L or higher is probably a surrogate marker of disease resolution rather than an immediate effect of treatment [8].

In this study, we assessed TPO level to investigate whether it could be a predictor of disease outcome. Early studies investigated the pathophysiological role of TPO in thrombopoiesis in normal and pathological condition and documented elevated TPO levels in severe aplastic anemia and chemotherapy-induced bone marrow hypoplasia [36], amegakaryocytic thrombocytopenia [37] and myeloproliferative disorders but normal or near normal TPO levels in patients with ITP [11]. Another study showed a lower level of TPO in acute myelogenous leukemia, hypoplastic leukemia, myelodysplastic syndrome and idiopathic thrombocytopenic purpura [38]. The TPO concentration, measured sequentially after myeloablative chemotherapy and peripheral blood progenitor cell transplantation, was inversely related to the platelet count. Therefore, it was concluded that TPO levels were significantly elevated in patients in which bone marrow megakaryocytes and platelets in circulation were markedly reduced, whereas TPO levels were normal in ITP patients, and only slightly increased in the MDS patients. This suggests that megakaryocyte mass affects the plasma TPO concentrations [36]. These data could explain the non-significant difference between all our ITP patients and controls as regards TPO level.

Although prior published studies of TPO-receptor agonists sometimes collected TPO levels, the data were not analyzed to determine whether the extent of TPO elevation was related to response [12]. In our study, no significant difference was found as regards TPO levels in

relation to clinical characteristics. However, TPO was an important predictor of clinical outcome as we found higher TPO levels among newly diagnosed ITP patients with persistent ITP after follow-up. The highest TPO levels were found among patients who had no initial response and those with loss of CR or R. There were significant negative correlations between TPO levels and initial platelets count as well as platelets count at enrollment among newly diagnosed and chronic ITP patients.

In this context, Makar et al. [12] analyzed 21 ITP patients treated with TPO receptor agonists and showed that TPO level in newly diagnosed ITP patients (n=7) did not differ substantially from those in chronic/persistent ITP patients (n=29). TPO levels in ITP patients also did not vary significantly depending upon whether these patients were receiving concurrent ITP therapy. However, when the response to TPO receptor agonists was compared across TPO levels, a significant association between elevated TPO levels and lack of response to a TPO receptor agonist was observed. A TPO level cut-off 95 pg/mL optimally discriminated between responders and non-responders. In their study, 71% (15/21) achieved a clinical response and an increased TPO level was associated with a failure to respond to a TPO receptor agonist. Thus, it was concluded that elevated TPO levels in ITP patients may predict a poor clinical response to treatment with TPO receptor agonists. This is unlikely to be confounded by patient treatment because resolution of thrombocytopenia decreased rather than increased TPO levels.

One limitation of this study is the small number of enrolled newly diagnosed patients and short follow-up period. Therefore, further larger studies with longer follow-up period are needed to verify our results. Another limitation is that we have not assessed autoimmune markers, particularly ANA; that has been reported to be a possible predictor of chronicity in childhood

ITP in some studies [39] and not by others [40]. These conflicting results explain why assays for ANA are not routinely recommended by an international panel of experts [41].

In conclusion, the predictors of progression to persistent ITP and chronicity among our pediatric patients with ITP were age of onset > 5 years old, high initial platelet count, low platelets count 4 weeks after diagnosis and higher baseline TPO levels. Increased baseline TPO levels were associated with absence of initial response as well as loss of response (relapse) denoting a poor clinical outcome. TPO could be used as a serum biomarker of megakaryopoiesis and it may predict therapeutic response and chronicity in childhood ITP and modify treatment regimen accordingly. Further prospective studies with longer follow-up including larger number of newly diagnosed ITP patients are needed to investigate predictors of chronicity in childhood ITP. As MPV may be of predictive value, its assessment and examining the relation of MPV to clinical outcome of ITP patients would provide additional information.

References

1. Labarque V, Van Geet C. Clinical practice: immune thrombocytopenia in paediatrics. Eur J Pediatr 2014;173:163-172.
2. Roganović J. Immune thrombocytopenia in children. Medical Sciences 2015;42:59-72.
3. LeVine DN, Brooks MB. Immune thrombocytopenia (ITP): Pathophysiology update and diagnostic dilemmas. Vet Clin Pathol 2019;48 Suppl 1:17-28.
4. Kühne T, Imbach P. Management of children and adolescents with primary immune thrombocytopenia: controversies and solutions. Vox Sang 2013;104:55-66.
5. Cines DB, Blanchette VS. Immune thrombocytopenic purpura. N Engl J Med 2002;346:995-1008.
6. George JN, Woolf SH, Raskob GE, Wasser JS, Aledort LM, Ballem PJ, Blanchette VS, Bussel JB, Cines DB, Kelton JG, Lichtin AE. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. Blood 1996; 88:3–40.
7. Ahmed S, Siddiqui AK, Shahid RK, Kimpo M, Sison CP, Hoffman MA. Prognostic variables in newly diagnosed childhood immune thrombocytopenia. Am J Hematol 2004;77:358-362.
8. Chotsampancharoen T, Sripornsawan P, Duangchoo S, Wongchanchailert M, McNeil E. Predictive factors for resolution of childhood immune thrombocytopenia: experience from a single tertiary center in Thailand. Pediatr Blood Cancer 2017;64:128-134.

9. Kuter DJ, Rosenberg RD. Appearance of a megakaryocyte growth-promoting activity, megapoietin, during acute thrombocytopenia in the rabbit. Blood 1994;84:1464–1472.
10. Nichol JL, Hokom MM, Hornkohl A, Sheridan WP, Ohashi H, Kato T, Li YS, Bartley TD, Choi E, Bogenberger J. Megakaryocyte growth and development factor. Analyses of in vitro effects on human megakaryopoiesis and endogenous serum levels during chemotherapy-induced thrombocytopenia. J Clin Invest 1995;95:2973–2978.
11. Yamazaki R, Kuwana M, Mori T, Okazaki Y, Kawakami Y, Ikeda Y, Okamoto S. Prolonged thrombocytopenia after allogeneic hematopoietic stem cell transplantation: Associations with impaired platelet production and increased platelet turnover. Bone Marrow Transplant 2006;38:377–384.
12. Makar RS, Zhukov OS, Sahud MA, Kuter DJ. Thrombopoietin levels in patients with disorders of platelet production: diagnostic potential and utility inpredicting response to TPO receptor agonists. Am J Hematol 2013;88:1041-1044.
13. Kuter DJ, Gernsheimer TB. Thrombopoietin and platelet production in chronic immune thrombocytopenia. Hematol Oncol Clin North Am 2009;23:1193– 1211.
14. Grimaldi-Bensouda L, Nordon C, Michel M, Viallard JF, Adoue D, Magy-Bertrand N, Durand JM, Quittet P, Fain O, Bonnotte B, Morin AS, Morel N, Costedoat-Chalumeau N, Pan-Petesch B, Khellaf M, Perlat A, Sacre K, Lefrere F, Abenhaim L, Godeau B; Group for the PGRx-ITP Study. Immune thrombocytopenia in adults: a prospective cohort study of clinical features and predictors of outcome. Haematologica 2016;101:1039-1045.
15. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, Bussel JB, Cines DB, Chong BH, Cooper N, Godeau B. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood 2009;113:2386-2393.
16. Kuwana M, Okazaki Y, Satoh T, Asahi A, Kajihara M, Ikeda Y. Initial laboratory findings useful for predicting the diagnosis of idiopathic thrombocytopenic purpura. Am J Med 2005;118:1026-1033.
17. Page LK, Psaila B, Provan D, Michael Hamilton J, Jenkins JM, Elish AS, Lesser ML, Bussel JB. The immune thrombocytopenic purpura (ITP) bleeding score: assessment of bleeding in patients with ITP. Br J Haematol 2007;138:245-248.
18. Neunert C, Terrell DR, Arnold DM, Buchanan G, Cines DB, Cooper N, Cuker A, Despotovic JM, George JN, Grace RF, Kühne T, Kuter DJ, Lim W, McCrae KR, Pruitt B, Shimanek H, Vesely SK. American Society of Hematology 2019 guidelines for immune thrombocytopenia. Blood Adv 2019;3:3829-3866.
19. Neunert C, Lim W, Crowther M, Cohen A, Solberg L, Crowther MA. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood 2011;117:4190–4207.
20. Cunningham JM. Updated Recommendations for the Treatment of Immune Thrombocytopenia. Clin Adv Hematol Oncol 2020;18:442-446.
21. Jung JY, Kim JK, Park M. Clinical course and prognostic factors of childhood immune thrombo-cytopenia: single center experience of 10 years. Korean J Pediatr 2016;59:335-340.
22. Cooper N. A review of the management of childhood immune thrombocytopenia: how can we provide an evidence-based approach? Br J Haematol 2014;165:756-767.
23. Vianelli N, Valdrè L, Fiacchini M, De Vivo A, Gugliotta L, Catani L, Lemoli RM, Poli M, Tura S. Long-term follow-up of idiopathic thrombocytopenic purpura in 310 patients. Haematologica 2001;86:504-509.

24. Moulis G, Palmaro A, Montastruc JL, Godeau B, Lapeyre-Mestre M, Sailler L. Epidemiology of incident immune throm- bocytopenia: a nationwide population- based study in France. Blood 2014;124:3308-3315.
25. Neunert CE, Buchanan GR, Imbach P, Bolton-Maggs PH, Bennett CM, Neufeld EJ, Vesely SK, Adix L, Blanchette VS, Kühne T. Severe hemorrhage in children with newly diagnosed immune thrombocytopenic purpura. Blood 2008;112:4003–4008.
26. Evim MS, Baytan B, Gunes AM. Childhood immune thrombocytopenia: long-term follow-up data evaluated by the criteria of the International Working Group on Immune Thrombocytopenic Purpura. Turk J Haematol 2014;31:32–39.
27. Heitink-Pollé KM, Nijsten J, Boonacker CW, de Haas M, Bruin MC. Clinical and laboratory predictors of chronic immune thrombocytopenia in children: a systematic review and meta-analysis. Blood 2014;124:3295-3307.
28. Despotovic JM, Grimes AB. Pediatric ITP: is it different from adult ITP?. Hematology Am Soc Hematol Educ Program. 2018;2018(1):405-411.
29. Kühne T, Buchanan GR, Zimmerman S, Michaels LA, Kohan R, Berchtold W, Imbach P. A prospective comparative study of 2540 infants and children with newly diagnosed idiopathic thrombocytopenic purpura (ITP) from the intercontinental childhood ITP study group. J Pediatr 2003;143:605-608.
30. Imbach P, Kühne T, Müller D, Berchtold W, Zimmerman S, Elalfy M, Buchanan GR. Childhood ITP: 12 months follow-up data from the prospective registry I of the Intercontinental Childhood ITP Study Group (ICIS). Pediatr Blood Cancer 2006; 6:351–356.
31. Watts RG. Idiopathic thrombocytopenic purpura: a 10-year natural history study at the Children's Hospital of Alabama. Clin Pediatr 2004;43:691–702.
32. ElAlfy M, Farid S, Abdel Maksoud A. Predictors of chronic idiopathic thrombocytopenic purpura. Pediatr Blood Cancer 2010;54:959–962.
33. Rosthøj S, Hedlund-Treutiger I, Rajantie J, Zeller B, Jonsson OG, Elinder G, Wesenberg F, Henter JI. Duration and morbidity of newly diagnosed idiopathic thrombocytopenic purpura in children: a prospective Nordic study of an unselected cohort. J Pediatr 2003;143:302–307.
34. Edslev PW, Rosthøj S, Treutiger I, Rajantie J, Zeller B, Jonsson OG; NOPHO ITP Working Group. A clinical score predicting a brief and uneventful course of newly diagnosed idiopathic thrombocytopenic purpura in children. Br J Haematol 2007;138:513-516.
35. Revel-Vilk S, Yacobovich J, Frank S, Ben-Ami T, Yechieli M, Shkalim V, Lebel A, Semo-Oz R, Tamary H. Age and duration of bleeding symptoms at diagnosis best predict resolution of childhood immune thrombocytopenia at 3, 6, and 12 months. J Pediatr 2013; 163:1335-1339.
36. Hou M, Andersson PO, Stockelberg D, Mellqvist UH, Ridell B, Wadenvik H. Plasma thrombopoietin levels in thrombocytopenic states: Implication for a regulatory role of bone marrow megakaryocytes. Br J Haematol 1998;101:420–424.
37. Porcelijn L, Folman CC, Bossers B, Huiskes E, Overbeeke MA, vd Schoot CE, de Haas M, vd Borne AE. The diagnostic value of thrombopoietin level measurements in thrombocytopenia. Thromb Haemost 1998;79:1101-1105.
38. Usuki K, Tahara T, Iki S, Endo M, Osawa M, Kitazume K, Kato T, Miyazaki H, Urabe A. Serum thrombopoietin level in various hematological diseases. Stem Cells 1996;14:558-565.

39. Altintas A, Ozel A, Okur N, Okur N, Cil T, Pasa S, Ayyildiz O. revalence and clinical significance of elevated antinuclear antibody test in children and adult patients with idiopathic thrombocytopenic purpura. J Thrombosis Thrombolysis 2007;24:163-168.
40. Vantelon JM, Godeau B, André C, Bierling P. Screening for autoimmune markers is unnecessary during follow-up of adults with autoimmune thrombocytopenic purpura and no autoimmune markers at onset. Thromb Haemost 2000;83:42-45.
41. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, Chong BH, Cines DB, Gernsheimer TB, Godeau B, Grainger J. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood 2010; 115:168-186.

Table 1: Clinical characteristics, therapeutic response and outcome of pediatric patients with immune thrombocytopenia at study entry and after 3-months follow-up for newly diagnosed patients

Variable	All patients (n=70)	Chronic (n=45)	Newly diagnosed (n=25)	Newly diagnosed after 3 months follow-up		p value	
				Persistent (n=9)	CR (n=16)	New versus chronic	Persistent versus CR
Age (years)	7.15 (4 – 12)	8 (6.4 – 13)	4 (3 – 10)	7 (4.8 – 11)	3.5 (2 – 8)	<0.001	<0.001
Males, n (%)	39 (55.7)	23 (51.1)	16 (64.0)	5 (55.6)	11 (68.8)	0.298	0.311
Age of onset > 5 years	32 (45.7)	25 (55.6)	7 (28.0)	6 (66.7)	1 (6.8)	0.027	0.004
Initial bleeding score	2.47 ± 1.22	2.47 ± 1.18	2.48 ± 1.33	2.22 ± 0.97	2.63 ± 1.5	0.966	0.478
CR after 4 weeks from diagnosis, n (%)	52 (74.3)	35 (77.8)	17 (68.0)	1 (11.1)	16 (100.0)	0.045	0.174
First line of therapy, n (%)	70 (100.0)	45 (100.0)	25 (100.0)	9 (100.0)	16 (100.0)	-	-
Details of first line therapy, n (%)						0.711	0.218
Corticosteroids	50 (71.4)	34 (75.6)	16 (64.0)	7 (77.8)	9 (56.3)		
IVIG	10 (14.3)	5 (11.1)	5 (20.0)	1 (11.1)	4 (25.0)		
Combined	2 (2.9)	1 (2.2)	1 (4.0)	1 (11.1)	0 (0.0)		
None	8 (11.4)	5 (11.1)	3 (12.0)	0 (0.0)	3 (18.8)		
Initial response, n (%)						0.783	<0.001
CR	41 (58.6)	25 (55.6)	16 (64.0)	1 (11.1)	15 (93.8)		
Response	6 (8.6)	4 (8.9)	2 (8.0)	2 (22.2)	0 (0.0)		
No response	23 (32.9)	16 (35.6)	7 (28.0)	6 (66.7)	1 (6.3)		
Second line of therapy, n (%)	39 (55.7)	29 (64.4)	10 (40.0)	8 (88.9)	2 (12.5)	0.309	0.006
Details of second line therapy, n (%)#						0.758	<0.001
Corticosteroids	29 (74.4)	21 (72.4)	8 (80.0)	8 (100.0)	0 (0.0)		
IVIG	3 (7.7)	3 (10.3)	0 (0.0)	0 (0.0)	0 (0.0)		
Anti D	2 (5.1)	2 (6.9)	0 (0.0)	0 (0.0)	0 (0.0)		
Combined	5 (12.8)	3 (10.3)	2 (20.0)	0 (0.0)	2 (100.0)		
Second response, n (%)#						0.637	<0.001
CR	14 (35.9)	11(37.9)	3 (30.0)	1 (12.5)	2 (100.0)		
Response	14 (35.9)	9 (31.0)	5 (50.0)	5 (62.5)	0 (0.0)		
No response	11 (28.2)	9 (31.0)	2 (20.0)	2 (25.0)	0 (0.0)		

Third line of therapy, n (%)	11 (15.7)	11 (24.4)	0 (0.0)	0 (0.0)	0 (0.0)	0.007	-
Condition at end of study, n (%) CR Response/No response	41 (58.6) 29 (41.4)	25 (55.6) 20 (44.4)	16 (64.0) 9 (36.0)	0 (0.0) 9 (100.0)	16 (100.0) 0 (0.0)	0.308	-
Corticosteroid-dependence, n (%)	2 (2.9)	2 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)	0.285	-
Relapse (loss of CR or response), n (%)	26 (37.1)	23 (51.1)	3 (12.0)	2 (22.2)	1 (6.3)	0.004	0.031
Time from diagnosis to relapse (months)	1 (0.5 – 3)	1 (1 – 6)	0.5 (0.5 – 0.5)	0.5 (0.5 – 0.5)	0.5 (0.5 – 0.5)	0.019	1.000

CR: complete remission; Intravenous immunoglobulin: IVIG. #: The percentage of patients was calculated out of those who needed second line of therapy. Data were expressed as median and interquartile range (IQR) where Mann-Whitney test was used for comparison unless specified as number (percentage) using $\chi 2$ test for comparison.

Table 2: Laboratory data of pediatric patients with immune thrombocytopenia at study entry and after 3-months follow-up for newly diagnosed patients

Variable	**All patients (n=70)**	**Chronic (n=45)**	**Newly diagnosed (n=25)**	**Newly diagnosed after 3 months follow-up**		**P-value**	
				Persistent (n=9)	**CR (n=16)**	**New versus chronic**	**Persistent versus CR**
Initial platelets (x 10^9/L)	10 (5.5 – 19)	12 (7 – 23)	8 (5 – 10)	14 (8 – 20)	5 (3.5 – 8)	0.005	0.007
Initial absolute lymphocytes (x 10^9/L)	3.81 ± 1.80	3.63 ± 1.68	4.11 ± 1.99	3.45 ± 2.62	4.51 ± 1.47	0.306	0.216
BM Lymphocytes (%)	16 (13 – 20)	16 (12 – 20)	15.5 (13.5 – 23)	14 (13 – 15)	18 (15 – 28)	0.819	0.121
Platelets 4 weeks after diagnosis (x 10^9/L) Median (IQR) <100 x 10^9/L	106 (45 – 252) 29 (41.4)	72 (31 – 195) 24 (53.3)	133 (76 – 257) 5 (20.0)	76 (72 – 106) 5 (55.6)	252 (136 – 340) 0 (0.0)	0.065 0.017	0.002 0.004
Platelets at enrollment (x 10^9/L)	135 (35 – 269)	116 (32 – 226)	157 (90 – 305)	54 (31 – 97)	263 (147.5 – 348)	0.162	0.002
WBC count at enrollment (x 10^9/L)	9.14 ± 4.09	8.19 ± 3.29	10.82 ± 4.84	8.93 ± 4.73	11.88 ± 4.71	0.009	0.148
Absolute neutrophil count at enrollment (x 10^9/L)	3.5 (2.6 – 5.35)	3.5 (2.5 – 4.6)	4.7 (2.8 – 10.2)	4.1 (2.2 – 8.4)	4.7 (2.8 – 10.2)	0.089	0.675
Absolute lymphocyte count at enrollment (x 10^9/L)	3.31 ± 1.37	3.11 ± 1.28	3.65 ± 1.48	2.39 ± 0.88	4.32 ± 1.29	0.135	0.001
Thrombopoietin level (pg/mL)	280 (200 – 390)	290 (200 – 400)	262.5 (220– 375)	318.5 (288 – 450)	240 (215 – 305)	0.488	0.005

BM: Bone marrow; WBC: White blood cells. Data were expressed as mean and SD where Student t test was used for comparisons or as median (IQR) using Mann-Whitney test for comparison unless specified as number (percentage) using χ^2 test for comparison.

Table 3: Thrombopoietin levels in relation to clinical characteristics and bleeding manifestations seen in > 5% of all the studied patients with ITP

Variable	Group number (%)	**Thrombopoietin** (pg/mL)	P-value
Sex			
Male	39 (55.7)	263 (200 – 390)	0.700
Female	31 (44.3)	290 (190 – 410)	
Antecedent infection			
Negative	42 (60.0)	262 (190 – 337)	0.461
Positive	28 (40.0)	240 (169 – 375)	
Recent vaccination			
Negative	63 (90.0)	251 (150 – 375)	0.293
Positive	7 (10.0)	191 (157 – 314)	
Gum or oral mucosal bleeding			
Negative	39 (55.7)	240 (190 – 375)	0.018
Positive	31 (44.3)	337 (262 – 410)	
Epistaxis			
Negative	47 (67.1)	290 (220 – 410)	0.348
Positive	23 (32.9)	290 (192.5 – 337.5)	
Gastrointestinal bleeding			
Negative	64 (91.4)	290 (191.25 – 382)	0.324
Positive	6 (8.6)	356 (230 – 417)	
Menorrhagia			
Negative	64 (91.4)	285 (191.3 – 370)	0.179
Positive	6 (8.6)	323 (290 – 387)	
Bleeding requiring blood transfusion			
Negative	64 (91.4)	290 (191 – 320)	0.817
Positive	6 (8.6)	276 (262 – 310)	
Comorbid conditions			
Negative	60 (85.7)	287 (190 – 382.5)	0.182
Positive	10 (14.3)	323 (262 – 410)	

Table 4: Logistic regression analysis for factors contributing to progression to persistent ITP among newly diagnosed patients

Independent variables	OR	95% CI for OR		p value
		Lower	Upper	
Age of onset > 5 years	15.000	1.981	113.556	0.009
Initial response	1.423	0.317	6.308	0.313
Initial platelets (x 10^9/L)	6.047	1.957	9.145	0.004
Platelets (x 10^9/L) 4 weeks after diagnosis	1.974	1.951	1.998	0.031
Platelets (x 10^9/L) at enrollment	1.990	1.981	2.919	0.024
Absolute lymphocyte count (x 10^9/L) at enrollment	1.219	1.065	1.738	0.014
Thrombopoietin level (pg/mL)	1.799	1.593	1.904	0.001

OR: odds ratio; CI: confidence interval.