Inborn Errors of Immunity in Children and Adolescents with Immune Thrombocytopenia

Julia M. Beatrice
University of Sao Paulo Children Institute: Universidade de Sao Paulo Instituto da Crianca
Bernadete L Liphaus (bernadete.liphaus@hc.fm.usp.br)
University of Sao Paulo Children Institute: Universidade de Sao Paulo Instituto da Crianca
https://orcid.org/0000-0002-2624-3233
Priscila E. Kamioka
University of Sao Paulo Children Institute: Universidade de Sao Paulo Instituto da Crianca
Lucy C. Matsumoto
University of Sao Paulo Children Institute: Universidade de Sao Paulo Instituto da Crianca
Simone Correa-Silva
University of Sao Paulo Children Institute: Universidade de Sao Paulo Instituto da Crianca
Magda M. S. Carneiro-Sampaio
University of Sao Paulo Children Institute: Universidade de Sao Paulo Instituto da Crianca
Jorge D. A. Carneiro
University of Sao Paulo Children Institute: Universidade de Sao Paulo Instituto da Crianca

Research Article

Keywords: complement, immune thrombocytopenia (ITP), inborn errors of immunity (IEI), immunoglobulin, lymphocyte subsets

Posted Date: May 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1625733/v1

Abstract

Immune thrombocytopenia (ITP) is a frequent autoimmune manifestation among Inborn Errors of Immunity (IEI). This study analyzed immunoglobulin and complement levels and lymphocyte subsets proportions in children and adolescents with primary ITP to reassess underlying IEI and establish relations with disease features and outcomes. Sixty ITP patients had IgA, IgM, IgG, IgE, C3, and C4 levels measured by standard methods used by the hospital. CD3+, CD4+, CD8 + and CD19 + lymphocyte proportions were determined by flow cytometry. Overall, 23.3% of the reassessed ITP patients could be considered to have underlying IEI namely, IgA deficiency (1/60), IgM deficiency (2/60), C4 deficiency (1/60), low B cells (3/60), low T cells (2/60) and unclassified IEI (4/60). In addition, as a whole, 86.7% of ITP patients had at least one altered immunoglobulin or complement level or lymphocyte subset proportion specifically, low IgG (23.3%), low IgM (8.3%), high IgG (1.7%), high IgM (11.7%), and high IgE (18.3%). Moreover, 6.7% of ITP patients had an additional autoimmune disease namely, type 1 diabetes and thyroiditis; 56.7% had an allergy diagnosis; 20.0% had a positive family history of autoimmune disease, and 70.0% had a family history of allergy. Patients with low IgM levels underwent significantly more splenectomy (87.5% vs. 25.0%, p = 0.04). In conclusion, underlying IEI could be diagnosed in children and adolescents previously presumed as primary ITP, reinforcing the idea that immunoglobulin and complement levels and lymphocyte subset proportions should periodically be reassessed during follow-up. Further genetic analyses will be a cornerstone for unveiling additional IEI related to ITP.

Introduction

Immune thrombocytopenia (ITP) is a complex and multifactorial autoimmune disorder characterized by low platelet counts resulting from enhanced destruction and/or impaired production, mediated by autoantibodies and/or autoreactive CD8 + T lymphocyte cytotoxicity that recognize platelets and/or megakaryocytes [1–3].

ITP may occur as primary when isolated and not associated with any other condition or as secondary when in the setting of other autoimmune cytopenias, associated with an autoimmune disease or an infectious condition, or as the manifestation of inborn errors of immunity (IEI) [1, 2, 4]. However, ITP patients have not been systematically studied for secondary causes once the consensus is not further investigated unless there are specific indications [5]. Otherwise, as knowledge of pathogenesis develops, the line between primary and secondary ITP blurs [1, 3].

IEI may manifest as increased susceptibility to infections and diversity of autoimmune, autoinflammatory, allergic, and/or malignant phenotypes [6–9]. There are few IEI in which an autoimmune manifestation had never been observed [6, 9]. In this regard, thrombocytopenia is a frequent autoimmune manifestation, and studies report IEI occur in up to 50% of patients with cytopenias [6, 10, 11]. Likewise, low, high, and skewed immunoglobulin levels and lymphocyte subsets counts are frequent among IEI features [6, 10]. ITP is described in IEI that affect both cellular and humoral immunity, such as IgA deficiency; common variable immunodeficiency (CVID); autoimmune lymphoproliferative syndrome

(ALPS); Hyper-IgM syndrome; immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome; Lipopolysaccharide-responsive and beige-like anchor protein (LRBA) deficiency; Cytotoxic T-lymphocyte antigen 4 (CTLA4) haploinsufficiency; and Signal transducer and activator of transcription 3 gain-of-function (STAT3-GOF) mutation [6, 9, 10, 12–17]. In fact, ITP appears in 69 IEI in a developed smartphone application to help clinicians' diagnosis [12]. In addition, ITP guidelines discuss the need to periodically reassess the etiological hypothesis as disease causes may become evident over time [2, 5, 18].

Therefore, this study analyzed immunoglobulin and complement levels and lymphocyte subsets proportions in children and adolescents with primary ITP to reassess underlying IEI and establish relations with age at disease onset, disease classification, disease outcome, and treatments.

Methods

Patients

This cross-sectional study consecutively enrolled 60 ITP children and adolescents during their follow-up at our tertiary referral pediatric hematology unit. After the ethics committee approval, informed consent/assent was achieved from parents and participants. All procedures were under the Helsinki Declaration.

ITP patients included in the study were followed up for at least six months and were already confirmed as primary ITP. In detail, ITP patients during the follow-up were classified according to current guidelines as persistent (thrombocytopenia from 3 to 12 months), chronic (thrombocytopenia longer than 12 months), or those who presented dysmorphic features have had bone marrow aspiration, immunoglobulin levels, and lymphocyte subsets analyzed and were confirmed as primary ITP [2,5]. ITP patients classified as newly diagnosed (thrombocytopenia up to 3 months) were assumed as primary ITP [5]. The enrolled patients' median period for ITP diagnosis was 3.9 months. Most have had a purpuric rash, no major bleeding event or thrombosis, and the peripheral blood smear done at diagnosis did not suggest other etiology. ITP patients were treated with prednisone and/or IVIG except for four that sustained platelet numbers $\geq$ 50.0x103/mm3. For patients with platelet numbers < 20.0x103/mm3 and/or had bruising and/or epistaxis, rescue treatments included prednisone, IVIG, azathioprine, cyclophosphamide, cyclosporine, rituximab, and splenectomy. None of the ITP patients had autoimmune hemolytic anemia (AHA).

Otherwise, ITP patients excluded from the study were suspected of an active infection, received intravenous immunoglobulin (IVIG) within the last three months, or had incomplete data.

Taking into account the predictive value of infections, allergic phenotypes, early disease onset, positive autoantibodies, other autoimmune disease, family history of autoimmune disease and/or IEI and/or allergy, presence of altered immunoglobulin and complement levels, and low lymphocyte subset count for diagnosing underlying IEI [6-12] these features were assessed at study enrollment by: 1- asking parents

for patient's previous diagnosis of infections (recurrent pyogenic infections, mycobacteriosis, fungal infections, herpes zoster and/or sepsis) and/or allergy (atopic dermatitis, acute or chronic urticaria, rhinitis, bronchitis, asthma, drug and/or food allergic reactions) and/or family history of autoimmune disease and/or IEI and/or allergy; 2- revising medical records for patient's demographic data, treatments, clinical and laboratory features, including diagnosis of other autoimmune disease and/or positive organ-specific and/or non-organ-specific autoantibodies not directly related to ITP, collected upon diagnosis or during the follow-up and 3- reevaluating immunoglobulin and complement levels, and lymphocyte subsets proportions.

Moreover, platelet number ≥ 150.0x103/mm3, at study enrollment, was used to classify ITP patients, according to disease outcome, as in remission.

Immunoglobulins Analysis

IgA, IgM, and IgG levels were measured by immunoturbidimetry (Roche Diagnostics, Indianapolis, USA) and IgE levels by nephelometry (Dade Behring/Siemens, Deerfield, USA) as hospital standard methods. ITP patients were considered to have altered immunoglobulin levels, e.g., high or low levels, when values were out of the hospital's reference range according to age, and whether there was at least one other altered measure on a different occasion.

Complement Analysis

Serum C3 and C4 levels were determined by nephelometry (Dade Behring/Siemens, Deerfield, USA). ITP patients were considered to have high or low complement values when out of the reference ranges, 79 to 152 mg/dL for C3 and 10 to 40 mg/dL for C4, and whether there was at least one other altered measure on a different occasion.

Lymphocyte Subsets Analysis

The flow cytometry assay was performed on samples from 25 IPT patients. The following anti-human monoclonal antibodies were used: PE-Cy7 anti-CD3 (BD mouse IgG1-347344), APC anti-CD4 (BD mouse IgG1-341095), V500 anti-CD8 (BD mouse IgG1-560774) and V500 anti-CD19 (BD mouse IgG1-561121). Briefly, 0.5 mL of peripheral venous blood was placed in each tube containing lysis buffer (BD Lysing buffer) for 30 min at room temperature. Cells were then washed two times and resuspended in staining buffer to obtain 2x106 cells in 100 µL. The cell suspension was placed in 96 wells plate, and membrane antibodies were added and incubated for 30 min at 4°C in the dark. Cells were washed, the supernatant discarded by inversion, and resuspended in 300 µL of staining buffer. The acquisition was performed on the eight-color LRS II Fortessa flow cytometer (BD Biosciences), and analysis was done on FlowJo software (ThreeStar, Ashland, OR, USA). Lymphocytes were gated based on physical and immunological forward and sideward scatter light properties. Lymphocyte subsets percentages are shown within the lymphocyte gate. Double-negative T lymphocyte subset proportion was not analyzed. Four non-related children with no personal history of autoimmune disease and/or IEI and/or allergy and/or positive

autoantibodies and/or chronic diseases (healthy controls) had CD3+ (median 58.7%, range 54.0 to 62.7%), CD4+ (median 52.3%, range 49.8 to 59.0%), CD8+ (median 36.6, range 30.6 to 38.0%) and CD19+ (median 12.3%, range 6.6 to 26.0%) lymphocyte subsets proportions similar to those reported in literature, confirming flow cytometric analyzes were properly performed. ITP patients were considered to have a low lymphocyte subset proportion when reduced compared with age reference values established in the literature [19] and healthy controls' values.

IEI adopted criteria

ITP patients with at least one altered immunoglobulin or complement level or lymphocyte subset proportion were evaluated for underlying IEI by the criteria below.

1- IgA deficiency when IgA level was < 10 mg/dL, with normal IgM and IgG levels, in children > 4 years of age [8,15]; 2- IgM deficiency when IgM value was < 35 mg/dL prior to the patient undergoing splenectomy [8,20]; 3- Hyper-IgM syndrome when high IgM was associated with low IgG, IgA or IgE levels [10,14]; 4- IgG deficiency when serum level was < 400 mg/dL [13,17]; 5- CVID when low IgG level occurred along with low IgA and/or IgM level associated with recurrent infections [13]; 6- High IgE when the value was > 100 IU/mL [21]; 7- C3 or C4 deficiency when the low level was in the presence of other components normal level [8]; 8- Low T or B cells when the subset proportion was considered reduced by the criteria established in this study; 9- unclassified IEI when there was at least one altered immunoglobulin or complement level or lymphocyte subset proportion associated with other cytopenias (leukopenia <4,000/mm3, neutropenia <1,500/mm3, lymphopenia <1,500/mm3) and/or splenomegaly and/or lymphadenopathy [6,10].

Statistical Analysis

The descriptive analysis is shown by number and percentage or median and range when appropriate. Group frequencies were compared by Fisher's exact test and continuous variables by the Mann-Whitney test. P values ≤ 0.05 were considered significant.

Results

Of the 60 ITP patients, 34 (56.7%) were female, the median age at enrollment was 10.6 years (range 2.8 to 17.9 years), the median age at disease onset was 5.6 years (range 15 days to 15.7 years), and median disease duration was 4.4 years (range 7 months to 14.3 years). Eight ITP patients had been classified as a newly diagnosed disease, seven as a persistent disease, and 45 as a chronic disease. At study enrollment, 25 ITP patients were in remission, and four were still taking prednisone.

IEI were diagnosed in 14 (23.3%) patients previously presumed as primary ITP. In detail, one patient was diagnosed with IgA deficiency. She was a newly diagnosed 5.9-year-old girl with positive antinuclear antibody (ANA) and anti-cardiolipin, personal history of infections (tonsillitis, diarrhea) and allergy

(bronchitis), positive family history of allergy, and also high IgG levels, ruling out CVID diagnosis (Supplementary Table).

Two 12-year-old boys were diagnosed with IgM deficiency. They had early disease onset at 15 days and seven months of life, personal history of infections and allergies, and high IgE levels with normal IgG and IgA levels, ruling out CVID. Although they underwent splenectomy, their diseases were not in remission (Supplementary Table).

One ITP patient was diagnosed with C4 deficiency. She also had low IgG levels, and although her autoantibodies were negative, she had two family members, an aunt, and a cousin, with the diagnosis of systemic lupus erythematosus (SLE) (Supplementary Table).

Four ITP patients were diagnosed with low B cells. Most had positive autoantibodies including one positive for anti-tyrosine phosphatase-related islet antigen 2 antibody (anti-IA2), another positive for anti-dsDNA, and other diagnosed with thyroiditis (Supplementary Table).

Two ITP patients were diagnosed with low T cells. One patient also had low IgG and high IgE levels (Supplemental Table).

Four ITP patients had unclassified IEI. Two patients had neutropenia, and one of these was diagnosed with ITP at 5.6 years of age, had low IgM levels, and infections, and underwent splenectomy, but his disease was not in remission. The other patient had high IgE levels, positive ANA, infections, and a family history of vitiligo. Two other patients had lymphopenia, and one of these was diagnosed with ITP at 4.3 years of age, had an IgE level of 1,810 IU/mL, a high C4 level, and his disease was in remission. The other patient also had high IgE, and C4 levels, positive anti-GAD, and his disease was not in remission (Supplementary Table).

In addition, other 38 (63.3%) ITP patients had skewed immunoglobulin levels. Specifically, 14 (23.3%) ITP patients had low IgG levels, but none could be considered to have IgG deficiency or CVID. One of these patients with positive anti-thyroglobulin antibody (anti-TG) and anti-thyroid peroxidase antibody (anti-TPO) was also diagnosed with thyroiditis, and another had the highest IgE level (2,250 IU/mL), positive anti-double-stranded DNA antibody (anti-dsDNA) and lupus anticoagulant (LAC) (Supplementary Table). ITP patients with low IgG levels had similar age at disease onset, disease classification, disease outcome, and treatments as those with non-altered immunoglobulin or complement level or lymphocyte subset proportion (Table 1).

Table 1
Demographic and laboratory features, disease classification and outcome, and submission to splenectomy of the 60 patients with immune thrombocytopenia (ITP) and altered or unaltered immunoglobulin or complement levels or lymphocyte subset proportion.

Characteristic	Altered parameter n = 52	Low IgM levels n = 8	Low IgG levels n = 17	Unaltered parameter n = 8
Age at enrollment (years)	10.5 (2.8–17.5)	11.5 (6.3–17.1)	7.5 (2.8–17.5)	11.5 (2.8–17.9)
Age at disease onset (years)	5.5 (15 days-13.1)	5.5 (15 days-10.4)	4.9 (1.1–13.1)	6.4 (1.7–15.7)
Gender (female / male)	31 / 21	4 / 4	8 / 9	3 / 5
Disease classified as chronic	37 (71.2)	7 (87.5)	10 (58.8)	8 (100.0)
Disease outcome as remission	22 (42.3)	2 (25.0)	7 (41.2)	3 (37.5)
Platelet number at enrolment ($10^3/mm^3$)	140.5 (1.0-639.0)	910.0 (10.0-552.0)	134.0 (1.0-400.0)	116.5 (35.0-408.0)
Positive autoantibodies	23 (44.2)	3 (37.5)	6 (35.3)	4 (50.0)
Submission to splenectomy	15 (28.9)	7 (87.5)*	2 (11.7)	2 (25.0)

The results are presented by the median (range) or number (%), and the statistical analysis by the Mann-Whitney test or two-tailed Fisher's exact test. * $p \leq 0.05$, refers to comparison with patients with unaltered immunoglobulin or complement levels or lymphocyte subset proportion.

Five (8.3%) ITP patients had low IgM levels. One patient also had the highest IgA level (456.0 mg/dL), the other had low IgA (98.0 mg/dL), high IgE levels, and positive anti-smooth muscle antibody (SMA) and another had high IgE levels, positive anti-TG, and SMA (Supplementary Table). Interestingly, ITP patients with low IgM levels underwent significantly more splenectomy (87.5% vs. 25.0%, p = 0.04, Table 1).

One (1.7%) ITP patient had high IgG levels but no recurrent infections, splenomegaly, and/or lymphadenopathy, ruling out ALPS diagnosis.

Seven (11.7%) ITP patients had high IgM levels, most associated with high IgE levels, but all had normal IgG and IgA levels, ruling out Hyper-IgM syndrome diagnosis. Interestingly, none of the ITP patients with high IgM levels underwent splenectomy (Supplementary Table).

Eleven (18.3%) ITP patients had high IgE levels. Most had positive autoantibodies, one patient was diagnosed with type 1 diabetes (T1D), and another with thyroiditis. Although some patients had a previous allergy diagnosis, none had severe dermatitis, ruling out Hyper-IgE syndrome diagnosis (Supplementary Table).

Therefore, as a whole, 52 (86.7%) ITP patients had at least one altered immunoglobulin or complement level or lymphocyte subset proportion. These patients had similar age at disease onset, disease classification, disease outcome, and treatments as the eight ITP patients with non-altered immunoglobulin or complement level or lymphocyte subset proportion (Table 1).

Moreover, four (6.7%) ITP patients had an additional autoimmune disease namely, thyroiditis and T1D. Thirty-four (56.7%) patients had at least one allergic disease, and 12 (20.0%) had a positive family history of autoimmune disease, namely vitiligo, T1D, thyroiditis, rheumatoid arthritis, SLE, rheumatic fever, and ITP. None reported a family history of IEI, and 42 (70.0%) patients had a positive family history of allergy, namely dermatitis, rhinitis, bronchitis, and/or asthma (Supplementary Table).

Discussion

This study showed in a cohort of children and adolescents previously presumed as primary ITP that 23.3% could be reassessed as having underlying IEI namely, IgA deficiency, IgM deficiency, C4 deficiency, low B cells, low T cells, and unclassified IEI. In addition, 86.7% of the ITP patients presented at least one altered immunoglobulin or complement level or lymphocyte subset proportion. Moreover, 6.7% of ITP patients had an additional autoimmune disease, 56.7% had an allergy diagnosis, 20.0% had a positive family history of autoimmune disease, and 70.0% had a positive family history of allergy all considered main clues to underlying IEI.

As suggested by current guidelines, it is essential to point out that the enrolled ITP patients had already been evaluated for IEI at diagnosis and when they evolved to persistent or chronic disease. Even so, during this study, we were able to diagnose additional IEI, reinforcing the need for a systematic periodical investigation of the etiological disease cause [2, 5, 18].

IEI diagnosis may be important clues to unveil ITP pathogenesis since 1- both loss and gain of function disorders may present with thrombocytopenia, 2- not only complete but also partial lesions of genes related to immune functions may give rise to autoimmune diseases, and 3- substantial clinical heterogeneity exists within patients with the same mutation [2, 3, 6, 12, 13, 22, 23].

IgA has anti-inflammatory roles, participates in the tolerance, and its levels go increasing through life. Thereby, its lack or maintained or decreasing values associated or not with other autoimmune diseases and/or positive autoantibodies in ITP patients may suggest for clinicians underlying IEI [8, 13, 15]. In addition, since the lack of IgA may impair mucosal antigens' clearance, it has also been linked to abnormalities in the gastrointestinal-associated mucosal immune system [15]. Interestingly, both the ITP patient diagnosed with IgA deficiency and the patient with a low IgA level had personal histories of diarrhea. ITP associated with low IgA appears in 19 IEI in the application developed to help clinicians' diagnosis [12].

IgM modulates both innate and adaptive immune responses, and its lack may impair apoptotic cell clearance and consequently lead to autoantibodies production, including those related to ITP [20, 24, 25].

Of note, IgM deficient patients frequently have high IgE levels, as observed in our patients [6, 13, 20]. Moreover, IgM deficiency has been observed in other autoimmune diseases including SLE [8, 16]. Notably, ITP patients with low IgM levels underwent significantly more splenectomy, suggesting worse treatment response and longer disease persistence as previously observed for SLE [16].

Complement system activation is important for platelet destruction [3]. Otherwise, complement deficient patients have an increased risk for autoimmune diseases, and it is described that 30% of ITP patients may evolve to SLE [4, 8, 9, 24–26]. Noteworthy, although the patient with C4 deficiency had negative autoantibodies, she had two family members with SLE. This finding puts in check the current perception that complement levels assessment has an uncertain benefit on ITP diagnosis, and reinforces the idea of reassessing not only immunoglobulin but also complement levels during the follow-up [5].

IEI that affect T and B lymphocytes also develop autoimmune manifestations, and low B cell number appears in at least 32 IEI in the developed application to help clinicians' diagnosis [3, 6, 9, 12]. Of note, most ITP patients with low B cells had positive autoantibodies, including one diagnosed with thyroiditis. Low B cell number is a peculiar feature of CVID [6, 13]. Genetic analysis will be fundamental to revealing underlying IEI like CTLA4 haploinsufficiency and LRBA deficiency [6]. Yet, although this study did not analyze regulatory or double-negative T cells or T lymphocyte functions, a previous study regarding low T-cell receptor excision circles (TREC) in ITP [27] and the current findings reiterate the need to periodically reassess T and B lymphocytes during ITP follow-up [2, 5, 18].

Low IgG is characteristic of CVID but also appears in STAT3-GOF and LRBA deficiency in which diverse genetic defects lead to various forms of non-organ and organ-specific autoimmunity, including ITP [10, 12, 13, 17, 23]. Interestingly, of the ITP patients with low IgG, 35.7% (5/14) had positive autoantibodies, including a patient diagnosed with thyroiditis. Yet, five ITP patients, including one with a low C4 level, had a positive family history of autoimmune diseases namely, vitiligo, SLE, rheumatoid arthritis, and ITP. Moreover, CVID is usually diagnosed over 20 years of age, and the ITP patients with low IgG may have this diagnosis in the future [13].

High IgG and IgM levels associated with ITP appear in a series of IEI including ALPS and Hyper-IgM syndrome [12, 14]. ALPS diagnosis was impaired in this study since double-negative T cell proportion was not analyzed. However, none of the enrolled ITP patients had splenomegaly and/or lymphadenopathy, making ALPS diagnosis unlikely [6, 7, 10, 12]. Hyper-IgM syndrome diagnosis was ruled out since the patients with high IgM had normal IgA and IgG levels. Again, genetic analysis will be important to reveal additional underlying IEI.

High IgE levels, allergic phenotypes, family history of allergy, and thrombocytopenia are frequently observed in partial and regulatory T cell defects, including IPEX [9, 10, 21]. High IgE, even without allergic or parasitic disease, was also observed in SLE [21]. Notably, SLE-like disease has been described in patients with Hyper-IgE syndromes [9, 10]. Interestingly, among ITP patients with high IgE levels, eight had disease onset before 6 years of age, and almost all had positive autoantibodies, including anti-dsDNA. Yet, one patient was diagnosed with T1D and another with thyroiditis. These findings corroborate with the

idea that high IgE may not be directly related to disease pathogenesis, but it is a biomarker of immune dysregulation [21], and when associated with other altered immunoglobulin and/or an autoimmune disease and/or positive autoantibodies may be a warning sign for clinicians for underlying IEI.

Other limitations of this study were its cross-sectional design, the sample size, not measuring IgG subclasses, and not performing genetic analyses. Therefore, some IEI may have been missed. Otherwise, taking into account that genetic analysis is still expensive for most centers our findings may be clues for sorting ITP patients to be screened for molecular testing.

IEI diagnosis increases with the presence of positive family history [6, 10, 11]. However, no ITP patient had family members with IEI. In addition, this study showed that ITP patients may have IEI diagnosis regardless of the age at disease onset, disease classification, and disease outcome. Moreover, various ITP patients had a positive family history of autoimmune disease, including ITP and/or additional autoimmune disease and/or positive autoantibodies, strengthening the idea of a genetic cause [1, 3, 18, 22, 25, 28]. Furthermore, growing relevance has been given to combined biomarkers since bear better positive and negative predicted values for IEI diagnosis than genotype-phenotype correlation [6]. Beyond, studies point out thrombocytopenia improvement with the treatment of the primary disease [5].

Finally, as a whole, 86.7% of ITP patients had altered immunoglobulin or complement levels or lymphocyte subset proportion. This data is relevant and may have direct implications for physicians' care of children with ITP, especially for those with chronic disease, and particularly when splenectomy is considered to avoid additional infectious risk [5, 6].

In conclusion, underlying IEI could be diagnosed in children and adolescents previously presumed as primary ITP, reinforcing the idea that immunoglobulin and complement levels and lymphocyte subset proportions should periodically be reassessed during follow-up. Further genetic analyses will be a cornerstone for unveiling additional IEI related to ITP.

Declarations

Acknowledgments The authors would like to thank all patients and parents for their kind participation.

Author contributions JMB, PEK, and LCM performed data acquisition and interpretation. BLL contributed to study conception and design, data interpretation, manuscript writing, and intellectual content. SC-S performed flow cytometry assays and analysis. MMSC-S critically revised the manuscript. JDAC contributed to study conception and design, data interpretation, manuscript intellectual content, and critically revised the manuscript. All authors approved the final manuscript as submitted.

Funding The work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grants nº 2008/58238-4 and 2014/50489-9.

Conflict of interest The authors declare no conflict of interest.

References

1. Swinkels M, Rijkers M, Voorberg J, Vidarsson G, Leebeek FWG, Jansen AJG. Emerging Concepts in Immune Thrombocytopenia. Front Immunol. 2018;9:880.
2. Neunert C, Terrell DR, Arnold DM, et al. American Society of Hematology 2019 guidelines for immune thrombocytopenia. Blood Adv. 2019;3:3829–66.
3. Marini I, Backchoul T. Pathophysiology of Autoimmune Thrombocytopenia: Current Insight with a Focus on Thrombopoiesis. 39: Hämostaseologie; 2019. pp. 277–37.
4. Kim JK, Facó MMM, Lotito APN, Liphaus BL, Carneiro JDA, Silva CAA. Thrombocytopenic Purpura and Autoimmune Hemolytic Anemia In Hospitalized Patients with Juvenile Systemic Lupus Erythematosus. Rev Bras Reumatol. 2007;47:10–5.
5. Provan D, Arnold DM, Bussel JB, Chong BH, Cooper N, Gernsheimer T, Ghanima W, Godeau B, González-López TJ, Grainger J, Hou M, Kruse C, McDonald V, Michel M, Newland AC, Pavord S, Rodeghiero F, Scully M, Tomiyama Y, Wong RS, Zaja F, Kuter DJ. Updated international consensus report on the investigation and management of primary immune thrombocytopenia. Blood Adv. 2019;3(22):3780–817.
6. Bousfiha A, Jeddane L, Picard C, et al. Human Inborn Errors of Immunity: 2019 Update of the USIS Phenotypical Classification. J Clin Immunol. 2020;40:66–81.
7. Jesus AA, Diniz JC, Liphaus BL, Jacob CMA, Carneiro-Sampaio M, Silva CA. Primary Immunodeficiencies and Autoimmune Diseases Association in Childhood. Rev Bras Reumato. 2007;47:418–23.
8. Jesus AA, Liphaus BL, Silva CA, Bando SY, Andrade LEC, Coutinho A, Carneiro-Sampaio M. Complement and antibody primary immunodeficiency in juvenile systemic lupus erythematosus patients. Lupus. 2011;20:1275–84.
9. Carneiro-Sampaio M, Coutinho A. Early-onset autoimmune disease as a manifestation of primary immunodeficiency. Front Immunol. 2015;6:7.
10. Notarangelo LD. Primary Immunodeficiencies (PIDs) presenting with cytopenias. Hematology 2009;139–143.
11. Delmonte OM, Castagnoli R, Calzoni E, Notarangelo LD. Inborn Errors of Immunity with Immune Dysregulation: From Bench to Bedside. Front Pediatr. 2019;7:353.
12. Jeddane L, Ouair H, Benhsaien I, Bakkouri JE, Bousfiha AA. Primary Immunodeficiency Classification on Smartphone. J Clin Immunol. 2017;37:1–2.
13. Podjasek JC, Abraham RS. Autoimmune cytopenias in common variable immunodeficiency. Front Immunol. 2012;3(189):1–7.
14. Jesus AA, Duarte AJS, Oliveira JB. Autoimmunity in Hyper-IgM syndrome. J Clin Immunol. 2008;28:62–6.
15. Jacob CMA, Pastorino AC, Fahl K, Carneiro-Sampaio M, Monteiro RC. Autoimmunity in IgA deficiency: Revisiting the role of IgA as a silent housekeeper. J Clin Immunol. 2008;28:56–61.

16. Perazzio SF, Granados A, Salomão R, Silva NP, Carneiro-Sampaio M, Andrade LE. High frequency of immunodeficiency-like states in systemic lupus erythematosus: a cross-sectional study in 300 consecutive patients. Rheumatology. 2016;55(9):1647–55.
17. Agarwal S, Cunningham-Rundles C. Autoimmunity in common variable immunodeficiency. Ann Allergy Asthma Immunol. 2019;123:454–60.
18. Del Vecchio GC, De Santis A, Accettura L, De Mattia D, Giordano P. Chronic immune thrombocytopenia in childhood. Blood Coagul Fibrinolysis. 2014;25(4):297–9.
19. Shearer WT, Rosenblatt HM, Gelman RS, Oymopito R, Plaeger S, Stiehm ER, Wara DW, Douglas SD, Luzuriaga K, McFarland EJ, Yogev R, Rathore MH, Levy W, Graham BL, Spector SA. Lymphocyte subsets in healthy children from birth through 18 years of age: The pediatric AIDS clinical trials group P1009 study. J Allergy Clin Immunol. 2003;112:973–80.
20. Louis AG, Gupta S. Primary Selective IgM Deficiency: An Ignored Immunodeficiency. Clin Rev Allerg Immunol. 2014;46:104–11.
21. Liphaus BL, Jesus AA, Silva CA, Coutinho A, Carneiro-Sampaio M. Increased IgE serum levels are unrelated to allergic and parasitic diseases in patients with juvenile systemic lupus erythematosus. Clinics. 2012;67:1275–80.
22. Kamioka PE, Liphaus BL, Beatrice JM, Matsumoto LC, Reis JMA, Lima L, Carneiro-Sampaio MMS, Carneiro JDA. Latent and Overt Polyautoimmunity in Children and Adolescents with Immune Thrombocytopenia. J Pediatr Hematol Oncol. 2020;42:e606–9.
23. Liphaus BL, Caramalho I, Rangel-Santos A, Silva CA, Demengeout J, Carneiro-Sampaio MMS. LRBA deficiency: a new genetic cause of monogenic lupus. Ann Rheum Dis. 2020;79(3):427–8.
24. Liphaus BL, Bittencourt Kiss MH. The role of apoptosis proteins and complement components in the etiopathogenesis of systemic lupus erythematosus. Clinics. 2010;65:327–33.
25. Zhang W, Dang SY, Wang JH, Nardi MA, Zan H, Casali P, Li ZD. Specific cross-reaction of anti-dsDNA antibody with platelet integrin GPIIIa49-66. Autoimmunity. 2010;43:682–9.
26. Carneiro-Sampaio M, Liphaus BL, Jesus AA, Silva CAA, Oliveira JB, Kiss MH. Understanding systemic lupus erythematosus physiopathology in the light of primary immunodeficiencies. J Clin Immunol. 2008;28:34–41.
27. Santana-Santos D, Liphaus BL, Beatrice JM, Carneiro JDA, Carneiro-Sampaio MMS, Rangel-Santos A. Low T-cell receptor excision circles (TREC) in children and adolescents with immune thrombocytopenia. Br J Haematol. 2020. doi:10.1111/bjh.17289.
28. Rischewski JR, Imbach P, Paulussen M, Kühne T. Idiopathic Thrombocytopenia Purpura (ITP): Is there a Genetic Predisposition? Pediatr Blood Cancer. 2006;47:678–80.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableIEIinITPMEDO.doc