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The comparative immunophenotypic analysis of paediatric and adults bone marrow lymphocytes' in chronic Immune thrombocytopenic purpura (ITP) refractory to intravenous immunoglobulin and corticosteroids

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Abstract

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune disorder marked by increased platelet destruction and reduced production, primarily due to the presence of autoantibodies. While many patients respond well to therapy, a subset remains refractory, posing a significant challenge for clinicians and patients alike. This study aimed to evaluate the bone marrow lymphocyte composition in both pediatric and adult patients with refractory ITP, with the goal of uncovering possible reasons for treatment resistance. This case-control study was conducted during the period from May 2019 to December 2024, involved 30 children and 37 adults diagnosed with refractory ITP, along with 60 age- and sex-matched normal controls. All participants underwent bone marrow aspiration, followed by immunophenotyping using a standard panel. Children with refractory ITP exhibited significantly higher percentages of hematogones and mature B cells in the bone marrow (8.27% ± 4.50% and 4.52% ± 3.82%, respectively) compared to adults (1.17% ± 2.18% and 1.89% ± 1.33%). In contrast, the adult group showed elevated levels of T cells (CD3+), averaging 11.6% ± 6.25% compared to 7.80% ± 3.12% in the pediatric group (p = 0.014). Additionally, both CD4+ and CD8+ T cells were significantly more abundant in adults (6.62% ± 4.82% and 7.89% ± 7.02%). Significant differences were also observed between patient and control groups in the proportions of CD3+, CD4+, CD4/CD8 ratios, hematogones, and mature B cells. In conclusion, distinct immunophenotypic profiles in bone marrow lymphocytes were identified between pediatric and adult patients with refractory ITP. These findings highlight potential age-related differences in disease mechanisms, which could inform more targeted approaches to diagnosis and management.

Keywords: CD4+, CD8+, corticosteroids, hematogones, intravenous immunoglobulin, lymphocytes, refractory thrombocytopenic purpura..

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Introduction

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune condition marked by isolated low platelet counts (thrombocytopenia) without identifiable secondary causes. The classification of ITP is time-dependent: cases are considered newly diagnosed if within 3 months of onset, persistent if lasting between 3 and 12 months, and chronic if extending beyond 12 months. Severe ITP is typically defined by the presence of clinically significant bleeding—often from mucosal surfaces—regardless of platelet count.[1]

The development of ITP involves a complex interplay of immune mechanisms. Its pathogenesis is multifaceted, with immune system dysfunction playing a central role.[2] Key immune players include antigen-presenting cells, T lymphocytes, and B lymphocytes, which contribute to the onset and persistence of this platelet-specific autoimmune disorder.[3] A hallmark feature is the production of autoantibodies by auto-reactive B cells, primarily targeting platelet surface glycoproteins such as glycoprotein (GP)IIb/IIIa and GPIb/IX.[4] These autoantibodies can also impair or destroy megakaryocytes, the cells responsible for platelet production, both *in vitro* and *in vivo*.[5]

A previous research study indicated that platelet production in ITP is compromised, as evidenced by reduced thrombopoietin levels, disrupted megakaryocyte maturation, and abnormal apoptotic pathways. Elevated levels of circulating B cells producing anti-GPIIb/IIIa antibodies and increased B cell populations in the spleen's red pulp support the notion that B cells are crucial in the autoimmune destruction of platelets.[6]

In addition to humoral immunity, T cells are also instrumental in ITP pathophysiology. They contribute to platelet destruction and megakaryocyte suppression in the bone marrow.[7] Dysregulated T cell responses and cytokine imbalances, including decreased regulatory T cell activity and increased pro-inflammatory cytokines, exacerbate immune dysfunction during active disease phases.[8] Auto-reactive T cells exhibit impaired apoptosis and clonal expansion, further fueling the immune attack.[9]

Patients classified as having refractory ITP fail to respond to first-line treatments or develop resistance after an initial response. Diagnosis relies on ruling out other causes of thrombocytopenia.[10] Standard first-line therapies include corticosteroids and intravenous immunoglobulin (IVIG).[11] This study aimed to investigate differences in lymphocyte profiles between adult and pediatric patients with ITP to better understand the mechanisms underlying treatment resistance, potentially guiding more effective therapeutic strategies.

Subjects and Methods

This was a case–control study; included 30 children and 37 adults with chronic ITP, conducted between May 2019 and December 2024 at a tertiary Centre (Prince Faisal Cancer center) Qassim, KSA. Bone marrow aspirate/biopsy morphological evaluation as well as immunophenotyping for the bone marrow aspirate sample was evaluated. Adult and pediatric groups were compared to each other in an attempt to understand the pathogenesis of refractoriness in each group, which could help personalize the future management. A total of 60 bone marrow aspirate (BMA)/biopsy samples for morphological and immunophenotyping evaluation were collected. Apparently healthy 30 adults as well as 30 children were used as a control group. The controls age ranged 1 to 14 years for the children, while the adults were 18 to 60 years.

Inclusion criteria included chronic ITP, considered if the patient has the disease for a period of 1 year or more. These patients responded initially to IVIG and/or corticosteroids but later showed resistance to the treatment.

Exclusion criteria: Patients with <1-year presentation with the clinical and laboratory criteria for diagnosis and those who are refractory at the initial diagnosis of ITP to the standard first-line treatment were excluded.

Methods

For flow cytometry analysis BMA samples anti-coagulated with sodium heparin were used, while clotted samples were rejected. Samples were processed within 24 hrs of extraction. Samples were prepared using the following technique: briefly, antibodies for panel screening were prepared using a four-color panel fluorescein isothiocyanate (FITC), phycoerythrin (PE), Peridinin chlorophyll protein (sulpho cyanine S.S), and allophycocyanin. Then approximately one million white blood cells were added to each tube and incubated at room temperature in a dark area for 15–20 min. Red cells were lysed using diluted red blood cell lysing reagent (Becton Dickinson, USA) followed by washing cells with cell wash buffer PH between 7.0 to 7.4 (BD) with 1% bovine serum albumin (BSA) as a blocking agent to minimize nonspecific binding. If needed, cells were incubated for 10 min with 50 µl of human immunoglobulin G for blocking Fc receptors on the cells. Finally, cells were re-suspended in a cell wash buffer with 1% BSA for acquisition in the flow cytometer. The monoclonal antibody (MoAb) was used in different combinations of fluorochrome as fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-Cyanine5 (PerCP-CY5.5), and allophycocyanin. Different combinations of MoAb used were CD38, CD3, CD20, CD15, anti-kappa conjugated with FITC, CD33, CD10, CD8, CD56/CD16, anti-lambda, CD117conjugated with PE, CD71, CD19, CD34, CD4 conjugated with allophycocyanin and CD45 PerCP-CY5.5 was used as a gating marker in all the tubes. Acquisition and analysis were performed on BD FACScalibur/BD FACSCanto II, and analysis was performed using CellQuest Pro software/FACSDiva software (Becton Dickinson, USA), respectively. The cells were gated based on CD45+ and side scatter allocation of normal cells along with the expression of markers.

Statistical Analysis

Data were coded and entered using the Statistical Package for the Social Sciences (SPSS, version 25, IBM Corp., Armonk, NY, USA). Quantitative data were summarized using the mean, standard deviation, median, minimum, and maximum range, and categorical data are presented using frequency (count) and relative frequency (percentage). Comparisons between quantitative variables were done using the nonparametric Mann–Whitney U-test. For comparing categorical data, the Chi-square test was performed. Fisher's exact test was used instead when the expected frequency was <5. Correlations between quantitative variables were done using Spearman correlation coefficient.[12] A $p < 0.05$ was considered statistically significant.

Results

The adult group included 37 patients (18 males and 19 females) aged from 18 to 76 years, with a mean of 40.4 ± 15 years and a median of 39.5 years. The pediatric group was 30 patients (21 males and 9 females) with age ranged from 1.5 years to 13 years, with a mean 7.41 ± 4.24 years and a median age of 6 years [Figure 1].

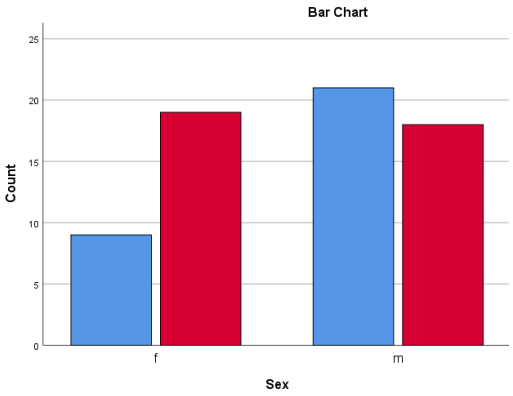

Figure 1. Sex distribution between Pediatric (blue bars) and adult (red bars) groups.

The lymphoid cells were sub-typed as mature B-cells (CD19 and CD20), hematogones (CD19 and CD10), T-cells (CD3), T-helper cells (CD4), T cytotoxic cells (CD8), nature killer (NK) cells (CD56), and plasma cells (bright CD38). The comparison between bone marrow lymphocytes in adults with ITP and controls is shown in Table 1. There was a significant difference in CD4+/CD8+ ratio with reduction of this ratio in patients in comparison to controls. The mean CD4+ count was significantly increased in controls when compared with patients.

Table 1. Difference in CD3, CD4, CD8, and CD4/CD8 percentages between adult immune thrombocytopenic purpura (ITP) patients and the adult controls.

	Adult ITP patients		Adult control		*p* value
Parameter %	Median	IQR	Median	IQR	
CD3	9.6	7.5	14.4	4.85	NS
CD4	5.0	4.6	8.45	5.12	0.01
CD8	5.5	3.4	4.9	3.02	NS
CD4/CD8	0.8	0.43	1.45	0.82	<0.001

IQR=Interquartile range $p > 0.05$ is not significant (NS).

The comparison of pediatric ITP cases and controls is shown in Table 2. It demonstrates a significant increase of hematogones and mature B-cells and a significant reduction of CD3+ and CD4+/CD8+ ratio in the pediatric patients compared to controls (Figures 2, 3). There was a significant correlation between hematogones and mature B-cells in the pediatric ITP group [Figure 4]. There was no significant correlation between age of the patient and hematogone number in pediatric and adult patients.

Table 2. Difference in CD3, CD4/CD8, hematogones (CD19/CD10 +), and mature B-cells (CD19/CD20+) between the pediatric immune thrombocytopenic purpura patients and controls.

	Pediatric ITP patients		Pediatric control		
Parameter	Median	IQR	Median	IQR	*p* value
CD3	8.3	4.3	14	13	0.02
CD4/CD8	0.8	0.38	2.0	1.1	<0.001
Hematogones	8.1	7.3	0.6	1	<0.001
CD19/CD20	3.05	4.2	0.35	0.4	<0.001

$p \leq 0.05$ is significant.

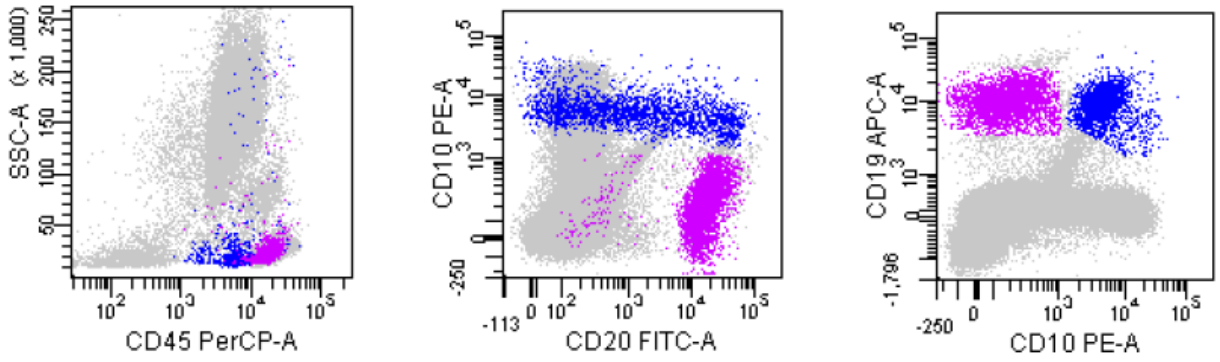

Figure 2. A pediatric case: The population in blue is Hematogones 10 %. while the magenta one is mature lymphocytes18 %.

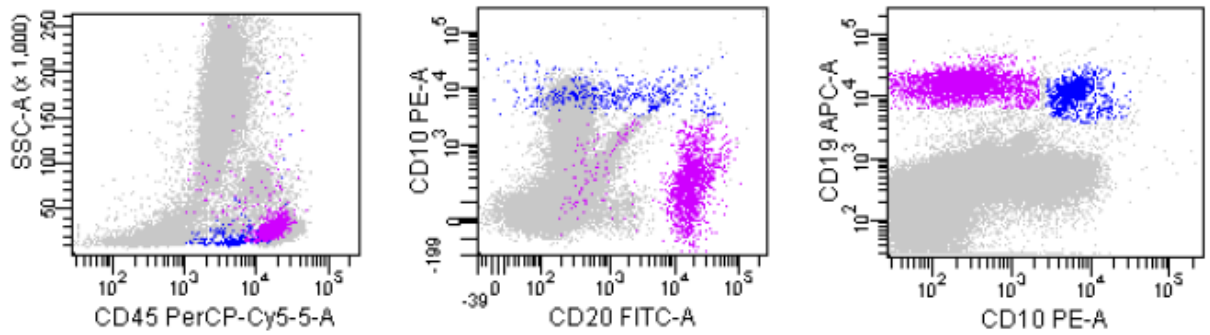

Figure 3. An adult case: The population in blue is Hematogones 0.3 %, while the magenta one is mature lymphocytes 4%.

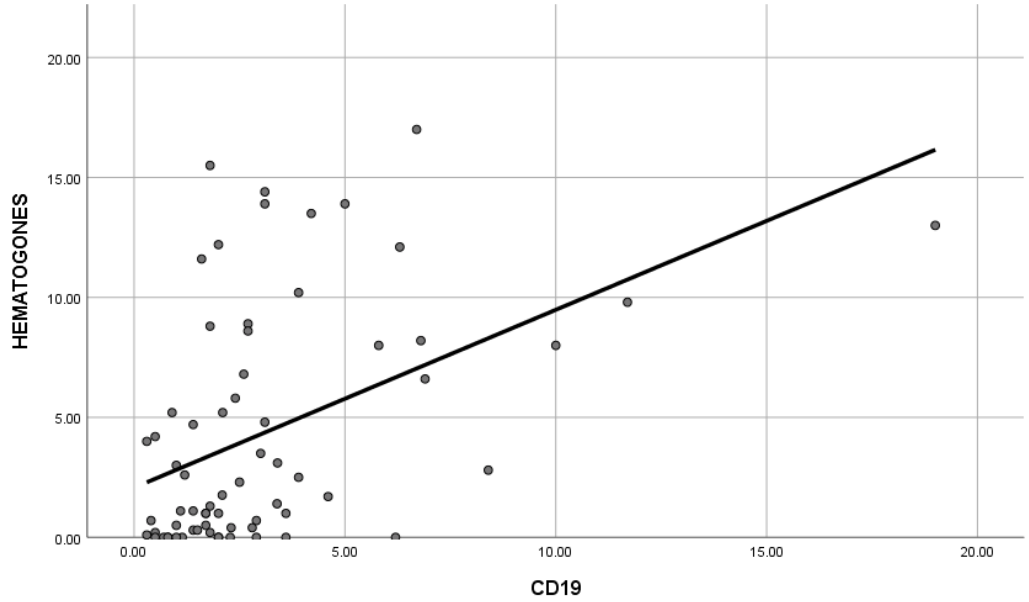

Figure 4. Correlation between hematogones and CD19 in Pediatric ITP group.(r =0.4, *p* <0.001).

The comparison of pediatric and adult ITP groups is shown in Table 3. There was a significant increase in hematogones and mature B-cells in the pediatric group compared to the adult ITP group. There was a significant increase in T-cells (CD3+) in the adult group when compared with the pediatric group. In addition, there was a significant increase in T-helper cells (CD4+) and T cytotoxic cells (CD8+) in the adult group when compared with the pediatric group. There was no significant difference between adults and pediatric patients regarding CD4+/CD8+ ratio. Furthermore, there was no significant difference between them for both natural killer (NK) cells and plasma cells detected by flow cytometry (Figure 5).

Table 3. Comparison of lymphocytic cells in the bone marrow between pediatric and adult immune thrombocytopenic purpura groups.

Parameter	Pediatric		Adult		*p* value
	Median	IQR	Median	IQR	
Age (years)	6	7.75	39.5	22.5	<0.001
CD19/CD20	3.05	4.2	1.7	1.3	<0.001
CD3	8.3	4.3	9.6	8	0.014
CD4	3.5	2.7	5.0	3.7	<0.001
CD8	4.45	1.8	5.5	3.4	0.006
CD4/CD8	0.8	0.38	0.87	0.43	NS
Hematogones (CD19/CD10)	8.10	7.3	0.4	1.1	<0.001
CD56/16	0.8	1	0.8	1.6	NS
Plasma cells (bright CD38)	0.2	0.1	0.3	0.2	NS

p > 0.05 is not significant (NS).

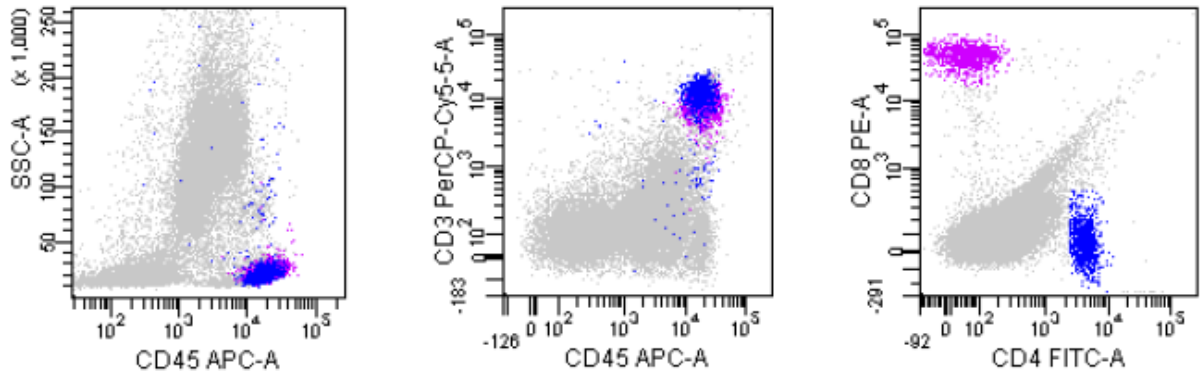

Figure 5. A pediatric case: the population in magenta is T cytotoxic cells (CD8) constitutes 6.9% while T helper (CD4) in blue represents 3.6%.

Discussion

ITP is a diverse immunopathological disorder that arises due to a breakdown in self-tolerance. Although multiple mechanisms were identified, they did not yet systematically categorized across different patient populations.

The current diagnostic guidelines for ITP do not mandate bone marrow examination during the initial evaluation. However, in cases where the disease becomes refractory following an initial treatment response, bone marrow analysis is often pursued. Comparing bone marrow findings in treatment-resistant adult and pediatric ITP cases could provide insight into the underlying immunological mechanisms that contribute to therapeutic resistance.[13] Notably, the bone marrow profile varies between children and adults. In pediatric patients, hematogones-immature B cell precursors—and mature B lymphocytes are considered normal constituents. Their numbers typically decline with age.[14] While no absolute threshold defines elevated hematogone levels, a percentage above 5% is generally considered increased.[15]

Our findings showed a marked predominance of B cell precursors in the pediatric group. There was a consistent and

homogeneous rise in both hematogones and mature B cells across children aged 1 to 16 years, in contrast to adult patients. These results align with prior studies by Samin Alavi et al., 2014,[16] who also observed an increased presence of B cell precursors compared to healthy controls. Similarly, Guiziry et al., 2005[17] confirmed elevated B cell levels in pediatric acute ITP cases when compared to age- and sex-matched controls.

Rong et al., 2014[18] reported increased levels of CD19+ B cells, particularly in non-responders compared to treatment responders. This trend was noted mainly in adult and elderly cohorts. These observations support the theory that B cells play a crucial role in ITP pathogenesis at certain ages. The proposed mechanism involves B cells producing autoantibodies in response to platelet antigens presented by antigen-presenting cells, which in turn suppress CD4+ T helper cell activity.[11]

In adults, T cells, particularly CD8+ cytotoxic cells, were the dominant lymphocyte population, with an altered CD4+/CD8+ ratio. While pediatric patients also exhibited a distorted CD4+/CD8+ balance, their CD8+ levels remained higher than these in adults. These findings are consistent with studies by Aslam et al., 2012[19] and Johnson et al., 2012,[20] both of which reported a decreased CD4+/CD8+ ratio in ITP patients.

The study by Al Rashedi et al., 2016[21] supported the observation of CD8+ cell predominance in peripheral blood. However, in contrast with our results, Zahran & El Sayeh, 2012[22] noted this pattern only in acute ITP, not in chronic forms.. This discrepancy might reflect differing underlying mechanisms between chronic and refractory ITP. The dominant presence of CD8+ cells in refractory ITP suggests a cytotoxic T-cell–driven pathology rather than one primarily mediated by antibody production. This could explain why conventional therapies such as IVIG, corticosteroids, and rituximab are often less effective in these patients.[23, 24]

CD8+ T cells are thought to play a central role in the autoimmune process through impaired regulation of CD8+ T regulatory cells. This imbalance promotes the activation of naïve CD8+ cells via antigen presentation, leading to their maturation into cytotoxic T lymphocytes that directly target platelets.[11]

Regarding NK cells, our data did not show significant differences in their numbers between pediatric and adult groups, nor between patients and healthy controls. This contrasts with findings by Taalat et al., 2014,[8] who reported a notable reduction in CD56+ and CD16+ NK cells in acute ITP cases, suggesting a more prominent role for NK cells in acute rather than chronic ITP. It is possible that NK cell dysfunction in chronic ITP may be more related to impaired function rather than decreased cell count.[25]

In conclusion, differences in lymphocyte composition exist between pediatric and adult patients with chronic refractory ITP, with both groups showing a consistent disruption in the CD4+/CD8+ ratio. These findings underscore the involvement of CD8+ cytotoxic T cells in the pathogenesis of chronic refractory ITP.

Author Contributions

NH; collected and analyzed the data. KOO; examined the patients. BB; performed the lab work. IAK and FASh; did the statistical analysis. All authors contributed to the writing and revision of the article.-

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Institutional Review Board (IRB) at Qassim health cluster, KSA (IRB approval number: 60802017). The board follow the Helsinki Declaration of ethical standards on human research.

Informed consent

A signed consent form was obtained from each study participant.

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