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Immune Features of Complement, Immunoglobulins, and Lymphocyte Subsets in Childhood Immune Thrombocytopenia

Mengchun Chen[1], Ling Hu[2], Jing Xue[1], Biao Liu[1], and Meng Chang[1]

[1] Clinical Laboratory, Hainan Women and Children's Medical Center, Haikou, China
[2] Department of Hematology, Hainan Women and Children's Medical Center, Haikou, China

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ABSTRACT

Immune thrombocytopenia (ITP), the commonest pediatric acquired bleeding disorder, involves immune dysregulation; assessing C3/C4, Ig(G/A/M), and lymphocyte subsets has diagnostic value.

A retrospective study enrolled 40 children with ITP (0–18 years, meeting pediatric ITP criteria) admitted to our hospital (January 2023 to March 2025) as the ITP group, and 40 healthy children as controls. Serum IgG, IgA, IgM, C3, C4, lymphocyte subsets ($CD3^+$, $CD4^+$, and $CD8^+$ T cells, $CD19^+$ B cells, and $CD16^+CD56^+$ NK cells), autoimmune antibodies, TORCH, EBV, and CMV status were detected. Pearson correlation and receiver operating characteristic curves analyzed correlations and diagnostic efficacy of single/combined indicators, and multivariate logistic regression was used for statistical analysis.

Baseline data were balanced. Compared with controls, ITP patients had higher IgG, lower IgM, C3 and C4, similar IgA; lower $CD3^+$ and $CD4^+$ percentages and $CD4^+$:$CD8^+$ ratio, higher $CD8^+$ cells, similar $CD16^+/CD56^+$ and $CD19^+$; and higher epstein-barr virus/cytomegalovirus and autoantibody positivity. Pearson analysis showed reduced $CD4^+$, $CD3^+$, C3, C4 and elevated $CD8^+$, IgG correlated with lower platelet count. ROC of combined $CD4^+$, $CD8^+$, C3, C4, IgG gave AUC 0.967, sensitivity 92.5%, specificity 90.0%, outperforming single indices.

Children with ITP have abnormal immunoglobulins, inadequate complement activation, and imbalanced lymphocyte subsets; combined detection of these aids ITP diagnosis and provides clinical basis.

Keywords: Complement system proteins; Idiopathic; Immunoglobulins; Lymphocyte subsets; Purpura, Thrombocytopenic

INTRODUCTION

Immune thrombocytopenia (ITP) is the most common acquired hemorrhagic disease in the pediatric field, which is characterized by significantly lower platelet count (peripheral blood platelet count<100×10^9/L) and increased risk of bleeding.[1,2] The annual incidence of the disease is about 4–5 cases per 100 000 children, the peak age of onset is 1–5 years, and the ratio of boys to girls is equal.[3] Although the majority of pediatric ITP cases resolve spontaneously, approximately 20% to 30% progress to a chronic form,

Corresponding Author: Mengchun Chen, PhD; Clinical Laboratory, Hainan Women and Children's Medical Center, Haikou, China. Tel: (+86 138) 0765 5692, Fax: (+86 898) 3669 8931, Email: chenmengchun7001@hotmail.com

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which imposes sustained psychological strain and therapeutic difficulties on both the affected children and their families.

The pathogenesis of ITP involves complex dysregulation of humoral and cellular immunity as well as the complement system.[4–6] The traditional view is that the total serum IgG level of children with ITP is normal or slightly increased, while platelet-specific IgG autoantibodies are the core of pathogenesis.[7] Recent findings highlight the role of excessive complement activation, with reduced C3 and C4 levels suggesting pathway consumption that may accelerate platelet clearance. Concurrent immunoglobulin abnormalities, including elevated IgG and altered subclass distribution, are linked to enhanced autoantibody pathogenicity.[8–11] Lymphocyte subset imbalances, notably a decreased $CD4^+/CD8^+$ T-cell ratio and impaired regulatory T-cell function, further disrupt immune tolerance. Additionally, infections or reactivation of pathogens such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are recognized as potential triggers or aggravating factors in pediatric ITP.[12–14]

ITP diagnosis currently relies on clinical presentation, platelet count, and exclusion of secondary causes, lacking specific laboratory markers. Although antiplatelet antibody testing is included in some guidelines, its limited sensitivity, specificity, and nonstandardized methods restrict widespread clinical use. In contrast, complement (C3, C4) and immunoglobulin testing are routinely available and cost-effective, and lymphocyte subset analysis is widely accessible. A systematic evaluation of their combined changes in pediatric ITP could offer valuable diagnostic support. However, existing studies are often limited by small sample sizes and single-center designs, focusing on isolated parameters rather than assessing the diagnostic utility of multiparameter panels.

Based on this background, this retrospective study systematically evaluates the immune features of complement (C3 and C4) and immunoglobulin levels in children with thrombocytopenia. We hypothesize that, compared with healthy children, patients with ITP exhibit abnormal complement activation, immunoglobulin dysregulation, and T-cell subset imbalance. These immunological changes are expected to correlate with clinical severity markers such as platelet count and bleeding scores. Receiver operating characteristic (ROC) curve analysis will assess whether combined immune indicators outperform single parameters in distinguishing children with ITP from healthy children. The findings may provide new insights into the immune dysregulation patterns in pediatric ITP and support comprehensive clinical assessment.

MATERIALS AND METHODS

Study Design

This study employed a retrospective controlled design. Forty children with ITP admitted to our hospital from January 2023 to March 2025 and 40 healthy children during the same period were enrolled. Age was 0–18 years (the diagnostic criteria for pediatric ITP set the age range of 0–18 years).[15] Data of 56 children with ITP and 52 healthy children were extracted through the electronic medical record system. Of these, 16 subjects from the ITP group were excluded (10 did not meet inclusion criteria and 6 records were unavailable), while 12 from the control group were also excluded (6 failed to meet criteria, 5 had incomplete data, and 1 was omitted for other reasons). Ultimately, 40 participants were included in each group. The screening and allocation process is illustrated in Figure 1.

Ethical Explanation

The study was approved by the Ethics Committee of Hainan Women and Children's Medical Center (Approval No. HNWCMC2026-48) and followed the Declaration of Helsinki and international research ethics standards. Informed consent was waived for this retrospective analysis of anonymized clinical data, with all identifiers removed to ensure privacy.

Inclusion Criteria

ITP group: (1) Age 0–18 years; (2) First diagnosis of ITP, meeting the ITP diagnostic criteria in the "Revised Guidelines for the Diagnosis and Treatment of Primary ITP in Chinese Children (2021 Edition)"[15]: at least two blood routine tests show a decrease in platelet count ($<100\times10^9$/L), and there are no significant abnormalities in the morphology of blood cells in peripheral blood smears; the spleen generally does not enlarge; and bone marrow examination shows an increase in megakaryocytes or normal ones with maturation disorders; (3) No glucocorticoids, intravenous immunoglobulin (IVIG), or other immunotherapies were received within 4 weeks before enrollment.

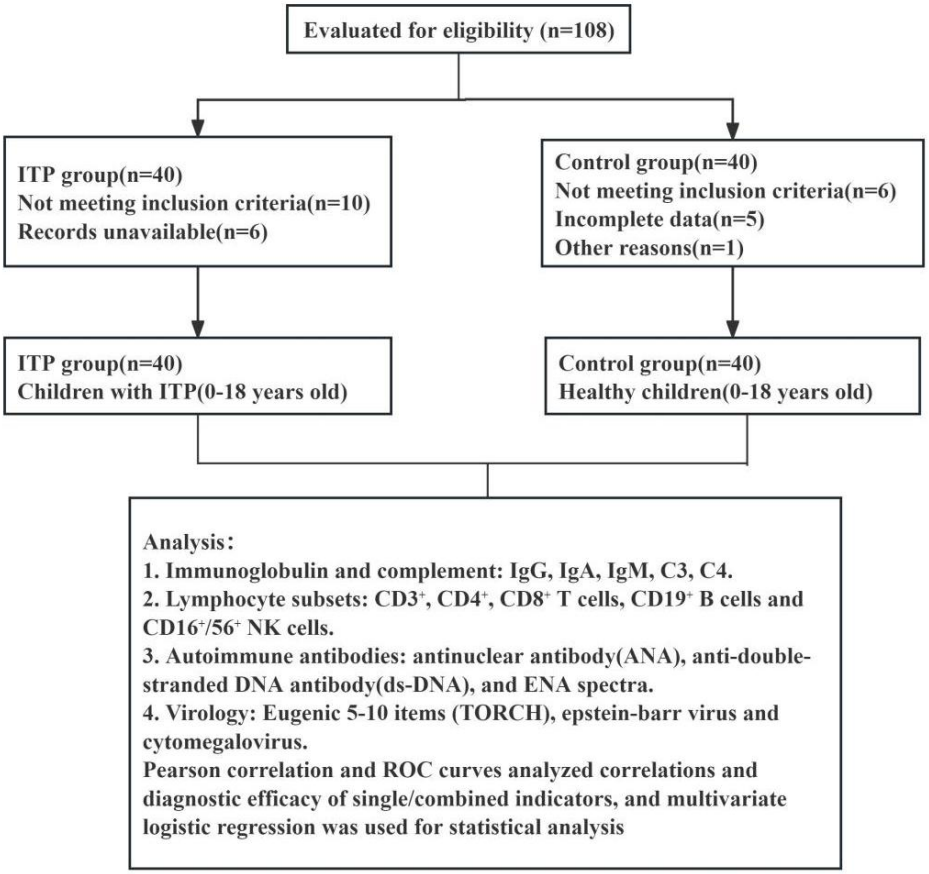

Figure 1. Design flow chart.

Control group: (1) Age 0–18 years, and the age was frequency matched with the ITP group; (2) Children undergoing health checkups at the same time; (3) There are no abnormalities in blood routine, liver, or kidney function, and no acute or chronic diseases.

Exclusion Criteria

(1) Children with chronic immune thrombocytopenia whose disease duration was more than 12 months according to the diagnosis and treatment guidelines; (2) children with overt infectious diseases; (3) children with secondary or primary immunodeficiency diseases, use of glucocorticoids or immune preparations within 1 month, and chronic underlying diseases; (4) cases diagnosed with secondary thrombocytopenia such as systemic lupus erythematosus, aplastic anemia, hematological tumors, and hereditary thrombocytopenia during the recent follow-up; (5) children with incomplete data or specimens.

Laboratory Testing and Quality Control

Specimen collection: Within 24 hours post-enrollment, 6 mL of fasting peripheral venous blood was collected and aliquoted into EDTA-K_2 anticoagulant tubes (2 mL each for lymphocyte subsets) and 2 mL of coagulation induction tube (centrifuge at 3000 rpm for 10 minutes, collect serum, store at −80 °C, for immunoglobulin, complement, autoantibody, and virology).

Quality control: (1) Specimen collection, transportation, and preservation followed the CLSI guidelines; (2) Laboratory analysts performed a blinded assessment, remaining unaware of the clinical status and group allocation of the study participants during testing; (3) Randomly insert 10% of duplicate samples in each batch. If the difference is greater than 10%, retest; (4) Double data entry, EpiData 3.1 logical verification.

Observation Indicators

(1) Baseline data of patients were collected, including demographic characteristics, clinical features, past and recent history, and routine laboratory tests.

(2) Immunoglobulin and complement: the concentrations of serum immunoglobulins (IgG, IgA, IgM) and complement components (C3, C4) were measured by immune nephelometry. The concentrations of serum immunoglobulins (IgG, IgA, IgM) and complement components (C3, C4) were measured by immunonephelometry using commercially available kits (Siemens Healthineers, Germany) (immunoturbidimetric method). The detection sensitivity was ≤0.5 mg/L, the within-run coefficient of variation (CV) of precision was ≤5%, and the between-run CV was ≤10%. The linear range covered 120% of the upper and lower limits of the clinical decision value.

(3) Lymphocyte subsets: Absolute counts and relative percentage analyses of peripheral blood lymphocyte subsets were performed using flow cytometry (BD Biosciences, BD FACS Canto II). The tests included $CD3^+$ (total T cells), $CD4^+$ (helper T cells), $CD8^+$ (cytotoxic T cells), $CD19^+$ (B cells), and $CD16^+/CD56^+$ (natural killer cells, NK cells), using antibodies of the same batch (BD Biosciences), and data were collected by flow cytometry. A minimum of 10,000 lymphocyte events was acquired per sample. Data acquisition and analysis were performed using FACS Diva software (BD Biosciences) . Lymphocyte populations were identified by $CD45^+$/SSC gating, and the percentages and absolute counts of each subpopulation expressing the respective cell-surface markers were calculated.

(4) Autoimmune antibodies: Serum levels of autoimmune antibodies were measured via enzyme-linked immunosorbent assay (ELISA), including ANA, ds-DNA, and ENA spectrum (SSA/Ro, SSB/La, Sm, RNP). The standard curve was drawn by the concentration of standard material and the absorbance value. The antibody concentration of the samples was calculated (the kits were purchased from EUROIMMUN, Germany, detection sensitivity ≤10 IU/mL).

(5) Virology: The infection of Eugenic 5–10 items (TORCH), EBV, and CMV were detected by ELISA and polymerase chain reaction (PCR), respectively. EBV and CMV DNA were detected utilizing the American ABI QuantStudio5 real-time quantitative PCR system (kit purchased from Daan Gene, detection sensitivity ≤10 IU/mL). TORCH detection: ELISA method was used to detect IgM and IgG antibodies of *Toxoplasma gondii*, rubella virus, CMV, herpes simplex virus, etc. (The kits were purchased from Zhengzhou Antu Biological Engineering Co., LTD., detection sensitivity ≤15 IU/mL). In this study, the following definitions were applied for assay results: Active viral infection: Positive viral DNA detected by real-time quantitative PCR (EBV DNA or CMV DNA positive). Recent/acute infection: Positive serum-specific IgM antibody (e.g., EBV VCA IgM or CMV IgM positive). Past infection: Positive serum-specific IgG antibody only, with negative IgM and viral DNA.

(6) The association between various immune parameters and platelet counts was assessed using Pearson correlation analysis. The diagnostic performance of the combined indicators was further assessed through the construction of ROC curves and calculation of the corresponding area under the curve (AUC).

Sample Size Calculation

Sample size estimation was performed with G*Power software. The effect size was set based on the difference of immune indicators (such as IgG) between children with ITP and healthy children in previous literature[16] and pre-analysis data (d=0.71), a 2-tailed α level of 0.05, and a statistical power of 0.8. The resulting calculation indicated a requirement of 31 participants per group, yielding a total minimum sample of 62. Ultimately, 80 subjects were enrolled, exceeding the estimated sample size and satisfying the statistical assumptions of the study.

Statistical Analysis

SPSS 25.0 software was used for statistical analysis. Normality of continuous variables was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated with Levene's test. Data conforming to normal distribution are presented as mean±standard deviation ($\bar{x}\pm s$) and were compared using independent samples t tests; otherwise, median (interquartile range) [M (IQR)] was used, and the Mann-Whitney U test was applied. Categorical data are expressed as frequency (percentage) and were compared using the χ^2 or Fisher exact test as appropriate. Serum immunoglobulins, complement levels, and lymphocyte subset percentages followed normal distribution and are reported as $\bar{x}\pm s$, with group comparisons performed using independent

samples t tests. Positivity rates of autoantibodies and virological markers are presented as number (percentage) and compared with the χ^2 or Fisher exact test. Correlations between immune indicators and platelet count were analyzed using Pearson correlation, reporting correlation coefficient (r) and *p* value. ROC curves were plotted to evaluate the ability of each indicator to distinguish children with ITP from healthy controls, with the AUC calculated. To examine whether associations between immune indicators and ITP were independent of important clinical confounders, multivariable logistic regression was conducted, with ITP status as the dependent variable (yes=1, no=0). Baseline variables showing statistical significance ($p<0.10$) in univariate analysis or of clear biological relevance—including recent infection history, presence of fever, age, and sex—were included as covariates. Immune markers with significant intergroup differences in univariate analysis (IgG, C3, $CD4^+/CD8^+$ ratio) were then separately introduced into the adjusted model to calculate the adjusted odds ratio (aOR) and its 95% confidence interval (CI) for each indicator. All tests were 2-tailed, with $p<0.05$ denoting statistical significance.

RESULTS

Comparison of Baseline Data

This study comprised 40 children with ITP in the ITP group and 40 healthy children in the control group. Baseline demographic characteristics, including age, gender, height, weight, and BMI, showed no significant differences between groups ($p>0.05$). In addition to a significantly lower platelet count, a significantly higher bleeding score, a significantly higher proportion of children with fever, fatigue, recent infection history, and upper respiratory tract infection was observed in the ITP group (all $p<0.001$). No statistically significant differences were observed between the two groups in clinical features (including hepatomegaly, splenomegaly, and lymphadenopathy), medical history (such as gastroenteritis, vaccination, drug allergy, and major illnesses), or routine laboratory parameters (including hemoglobin, white blood cell count, neutrophil count, lymphocyte count, alanine aminotransferase, aspartate aminotransferase, and creatinine) (all $p>0.05$), indicating balanced and comparable baseline characteristics. See Table 1.

Analysis of Serum Immunoglobulin and Complement Levels

Serum concentrations of immunoglobulins (IgG, IgA, IgM) and complement factors (C3, C4) were quantified and compared between the ITP and control groups. The results are shown in Table 2; the ITP group demonstrated a substantially elevated IgG level compared with controls ($p<0.001$). The serum IgM level in the ITP group was significantly lower than that in the control group ($p<0.001$). No significant difference in IgA levels was observed between the two groups ($p>0.05$). In contrast, both complement C3 and C4 levels were markedly reduced in the ITP group compared with the controls (both $p<0.001$).

Analysis of Lymphocyte Subsets

As presented in Table 3, the ITP group exhibited a marked reduction in $CD3^+$ T cells and $CD4^+$ helper T cells, accompanied by a substantial rise in $CD8^+$ cytotoxic T cells. The $CD4^+/CD8^+$ ratio was significantly lower than that of the control group ($p<0.001$). No significant differences were observed in the proportions of B cells ($CD19^+$) or natural killer cells ($CD16^+/CD56^+$) between the groups ($p>0.05$).

Positive Detection Rates of Autoimmune Antibodies and Virology

As indicated in Table 4, analysis of autoimmune antibodies and virological markers revealed significantly higher positive rates for ANA and ds-DNA antibodies in the ITP group compared with the controls ($p<0.05$). There was no significant difference in the positive rate of ENA spectrum between the two groups ($p=0.154$). Virological assessment revealed markedly elevated detection rates of EBV-DNA and CMV-DNA in the ITP group compared with controls, showing highly significant statistical differences ($p<0.01$). At the serological level, the ITP group exhibited significantly higher seropositivity rates for EBV-VCA-IgM (27.5%) and CMV-IgM (22.5%) compared with the control group, with *p* values of 0.021 and 0.022, respectively. In addition, there was no significant difference between the two groups in the five indicators of eugenic (Toxo-IgM, RV-IgM) and recent infection of herpes simplex virus (HSV1/2-IgM) ($p>0.05$).

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Correlation Analysis Between Immune Indexes and Platelet Count

Pearson correlation analysis was conducted to assess the relationship between immune parameters and platelet count in the ITP group. The results are shown in Table 5; the results revealed a positive correlation between platelet count and $CD3^+$ T cells, $CD4^+$ T cells, $CD4^+/CD8^+$ ratio, as well as complement C3 and C4 levels (all $p<0.05$). Conversely, platelet count was negatively correlated with $CD8^+$ T-cell percentage and serum IgG concentration (both $p<0.01$).

Table 1. Comparison of baseline characteristics between the two groups.

Indicator	Control group (n=40)	ITP group (n=40)	Statistic	*p*
Demographic characteristics				
Age, y	5.5±3.1	5.2±2.8	t=0.516	0.607
Gender (male/female), n	20/20	22/18	χ^2=0.202	0.653
Weight, kg	19.2±6.1	18.5±5.6	t=0.527	0.600
Height, cm	107.9±17.5	105.7±16.3	t=0.575	0.567
BMI	16.2±1.9	16.4±1.8	t=−0.476	0.635
Clinical characteristics				
Platelet count, $\times10^9$/L	245.7±52.3	32.5±18.6	Z=10.285	<0.001
Bleeding score, points	0 (0)	2 (1–3)	Z=−8.123	<0.001
With fever, n (%)	0 (0)	12 (30.0)	NA	<0.001
With fatigue, n (%)	0 (0)	15 (37.5)	NA	<0.001
Hepatomegaly, n (%)	0 (0)	2 (5.0)	NA	0.494
Splenomegaly, n (%)	0 (0)	3 (7.5)	NA	0.242
Lymphadenopathy, n (%)	0 (0)	5 (12.5)	NA	0.057
Past medical history & recent history				
Recent infection history, n (%)	6 (15.0)	28 (70.0)	χ^2=24.492	<0.001
Of which: URTI	5 (12.5)	20 (50.0)	χ^2=13.543	<0.001
Of which: Gastroenteritis	1 (2.5)	5 (12.5)	NA	0.205
Vaccination history, n (%)	40 (100)	38 (95.0)	NA	0.494
Drug allergy history, n (%)	2 (5.0)	3 (7.5)	NA	>0.99
History of major diseases, n (%)	1 (2.5)	2 (5.0)	NA	>0.99
Routine laboratory tests				
Hemoglobin, g/L	122.8±11.6	118.5±15.2	t=1.404	0.164
White blood cell count, $\times10^9$/L	7.5±2.1	7.8±2.9	t=−0.524	0.601
Neutrophil count, $\times10^9$/L	3.3±1.4	3.5±1.8	t=−0.549	0.585
Lymphocyte count, $\times10^9$/L	3.5±1.2	3.2±1.5	t=0.978	0.331
Alanine aminotransferase, U/L	18 (15–22)	20 (15–26)	Z=−1.235	0.217
Aspartate aminotransferase, U/L	24 (21–28)	25 (22–30)	Z=−1.089	0.276
Creatinine, μmol/L	43.8±10.5	45.2±12.3	t=−0.541	0.590

BMI: body mass index; ITP: immune thrombocytopenia; NA: not applicable; URTI: upper respiratory tract infection.

Table 2. Comparison of serum immunoglobulin and complement levels between the two groups of children.

Parameter	Control group (n=40)	ITP group (n=40)	Statistic	Mean difference	95% CI lower	95% CI upper	Effect size	*p*
IgG, g/L	10.76±2.64	14.82±3.15	−7.07	−4.05	−5.20	−2.91	1.42	<0.001
IgA, g/L	1.49±0.58	1.58±0.62	−0.71	−0.09	−0.32	0.15	0.15	0.476
IgM, g/L	1.32±0.41	0.86±0.31	6.67	0.46	0.32	0.59	1.26	<0.001
C3, mg/dL	105.67±22.18	78.45±18.26	6.49	27.22	18.87	35.56	1.37	<0.001
C4, mg/dL	22.45±6.89	15.23±5.67	6.97	7.22	5.15	9.28	1.17	<0.001

CI: confidence interval; ITP: immune thrombocytopenia.

Table 3. Comparison of the percentages of lymphocyte subsets in peripheral blood between the two groups.

Lymphocyte subpopulation	Control group (n=40)	ITP group (n=40)	Statistic	Mean difference	95% CI lower	95% CI upper	Effect size	*p*
$CD3^+$, %	71.89±7.56	65.34±8.27	3.65	6.55	2.97	10.12	0.83	<0.001
$CD4^+$, %	38.76±5.92	31.25±6.18	5.73	7.51	4.90	10.11	1.24	<0.001
$CD8^+$, %	25.67±4.88	32.18±5.43	−5.83	−6.51	−8.73	−4.28	1.26	<0.001
$CD4^+/CD8^+$	1.51±0.31	0.97±0.25	9.09	0.54	0.42	0.65	1.91	<0.001
$CD19^+$, %	13.87±3.95	14.56±4.12	−0.96	−0.69	−2.11	0.73	0.17	0.339
$CD16^+/CD56^+$, %	12.05±3.26	11.23±3.45	1.90	0.82	−0.03	1.67	0.25	0.060

CI: confidence interval; ITP: immune thrombocytopenia.

Table 4. Comparison of positive rates of autoimmune antibodies and virology between the two groups of children.

Parameter	Control group (n=40)	ITP group (n=40)	Odds ratio	95% CI	*p*
Positive ANA	1 (2.5)	7 (17.5)	8.08	0.94–69.65	0.038
Positive ds-DNA	0 (0)	4 (10.0)	10.03	1.18–85.20	0.035
Positive ENA spectrum	0 (0)	2 (5.0)	NA	NA	0.154
Positive EB-DNA	4 (10.0)	15 (37.5)	5.45	1.62–18.35	0.004
Positive CMV-DNA	3 (7.5)	12 (30.0)	5.33	1.36–20.92	0.009
Toxo-IgM	1 (2.5)	1 (2.5)	1	0.06–16.26	>0.99
RV-IgM	0 (0)	2 (5.0)	NA	NA	0.154
EBV-VCA-IgM	3 (7.5)	11 (27.5)	4.63	1.18–18.20	0.021
CMV-IgM	2 (5.0)	9 (22.5)	5.47	1.10–27.26	0.022
HSV1/2-IgM	1 (2.5)	3 (7.5)	3.16	0.32–31.50	0.301

ANA: antinuclear antibody; CI: confidence interval; CMV: cytomegalovirus; ds-DNA: double-stranded DNA; EBV: Epstein-Barr virus; ENA: extractable nuclear antigen; HSV: herpes simplex virus; ITP: immune thrombocytopenia; NA: not applicable; RV: rubella virus; Toxo: *Toxoplasma gondii*.

Table 5. Correlation analysis between each immune index and platelet count.

Parameter	$CD3^+$, %	$CD4^+$, %	$CD8^+$, %	$CD4^+/CD8^+$	IgG, g/L	C3, mg/dL	C4, mg/dL
r	0.412	0.523	−0.486	0.601	−0.452	0.378	0.361
p	0.009	0.001	0.002	<0.001	0.004	0.017	0.023

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Diagnostic Efficacy of Immune Indicators in ITP

The diagnostic significance of single immune indices and their combined testing in ITP was evaluated via ROC curve analysis. The results are shown in Table 6; among the single indicators, the $CD4^+/CD8^+$ ratio had the largest AUC (0.892) and the highest diagnostic value, with a sensitivity and specificity of more than 80%. IgG and complement C3 also showed good diagnostic value (AUC>0.75). A combined diagnostic predictor was constructed using logistic regression incorporating $CD4^+$, $CD8^+$, C3, C4, and IgG, yielding an AUC value of 0.967, along with a sensitivity of 92.5% and a specificity of 90.0%, respectively.

Multivariate Logistic Regression Analysis

To assess whether the association between immune indicators and ITP was independent of significant confounders, we established a multivariate logistic regression model with ITP diagnosis as the dependent variable. After adjusting for recent infection history, fever, age, and gender, the analysis results (Table 7) indicated that elevated serum IgG levels, reduced complement C3 levels, and a decreased $CD4^+/CD8^+$ ratio were independently associated with ITP (all $p<0.05$). Specifically, for every 1-g/L increase in IgG, the risk of ITP increased by 1.32-fold (aOR=1.32; 95% CI, 1.10–1.58); for every 10-mg/dL decrease in C3, the risk increased by 1.82-fold (aOR=1.82; 95% CI, 1.25–2.66); and for every 0.1 decrease in the $CD4^+/CD8^+$ ratio, the risk increased by 1.48-fold (aOR=1.48; 95% CI, 1.18–1.85). The covariates included in the model, including recent infection history, fever, age, and gender, did not show statistically significant aORs (all $p>0.05$).

Table 6. Evaluation of the efficacy of each immune index and combined detection in the diagnosis of ITP.

Parameter	AUC	Cutoff	Sensitivity, %	Specificity, %	Youden index	95% CI	*p*
$CD4^+/CD8^+$	0.892	≤1.15	85.0	82.5	0.675	0.823–0.961	<0.001
IgG, g/L	0.803	≥12.45	72.5	80.0	0.525	0.707–0.899	<0.001
C3, mg/dL	0.781	≤89.50	70.0	77.5	0.475	0.678–0.884	<0.001
$CD4^+$, %	0.842	≤35.20	80.0	75.0	0.55	0.753–0.931	<0.001
Combined testing	0.967	NA	92.5	90.0	0.825	0.928–1	<0.001

AUC: area under the curve; CI: confidence interval; ITP: immune thrombocytopenia; NA: not applicable.

Table 7. Multivariate logistic regression analysis of factors associated with ITP.

Variable	Adjusted OR (aOR)	95% CI	*p*
Recent infection (yes vs no)	1.85	0.62–5.55	0.271
Fever (yes vs no)	2.10	0.58–7.66	0.262
Age (per 1-year increase)	0.95	0.82–1.10	0.476
Sex (male vs female)	1.20	0.48–3.00	0.698
IgG (per 1-g/L increase)	1.32	1.10–1.58	0.003
C3 (per 10-mg/dL decrease)	1.82	1.25–2.66	0.002
$CD4^+/CD8^+$ ratio (per 0.1 decrease)	1.48	1.18–1.85	0.001

aOR: adjusted odds ratio; CI: confidence interval; ITP: immune thrombocytopenia.

DISCUSSION

Pediatric ITP is a multifactorial acquired autoimmune disorder, marked by accelerated platelet destruction and reduced platelet production, leading to decreased platelet number. At present, it is generally believed that the pathogenesis of ITP is closely related to the imbalance of humoral immunity and the excessive activation of complement system. This study aimed to investigate the levels of complement C3, C4, immunoglobulin levels, and lymphocyte subsets in children with ITP, in order to reveal their immunological characteristics and analyze the correlation between these indicators and clinical indicators of the disease.

Immunoglobulin and complement levels play a key role in ITP.[17,18] This study found that in children with ITP, serum IgG levels were increased, while IgM levels were decreased, which was consistent with the traditional theory of "IgG is the main autoantibody." It echoes the research results of Wang et al[19] and Li et al.[20] The role of the complement system in ITP was first identified as "the downstream effector of antibody-mediated platelet destruction." However, in the past decade, with the popularization of complement activation products detection technology, more and more evidence suggests that complement can also actively promote immune imbalance.[21,22] In this study, the serum levels of C3 and C4 were significantly decreased in children with ITP, suggesting the presence of activation and depletion of the complement system. This finding is consistent with the findings of Cheloff et al[23] and Nasr et al.[24] Serum levels of C3 and C4 were positively correlated with platelet counts, supporting the notion that the degree of complement depletion is associated with disease activity.

Changes in lymphocyte subsets were also the focus of this study. The results showed that the percentages of $CD3^+$ and $CD4^+$ cells and $CD4^+/CD8^+$ ratio in ITP group were significantly lower than those in control group, while the percentage of $CD8^+$ cells was significantly increased. The results of the change trend of T-lymphocyte subsets obtained in different studies are basically similar, such as the studies of Zahran et al[25] and Zhang et al.[26] In this study, no significant changes were observed in the percentages of B cells ($CD19^+$) and natural killer cells ($CD16^+/CD56^+$), but whether there are abnormalities in their functional status or more refined subset distribution remains to be elucidated in future studies.

Regarding virological and autoimmune serology, the ITP group demonstrated elevated rates of EBV and CMV infection, along with a significantly higher positivity for autoimmune antibodies relative to the control group. This is consistent with the results of Pratt et al[27] and Rinaldi et al,[28] suggesting that viral infection may trigger or aggravate the autoimmune response through molecular mimicry and epitope expansion. This finding emphasizes the importance of active screening and management of potential infections in the diagnosis and treatment of ITP.

In this study, Pearson correlation analysis showed that platelet count was positively correlated with $CD4^+$, $CD3^+$, C3, and C4 levels, and negatively correlated with $CD8^+$ and IgG levels. This suggests that decreased proportions of $CD4^+$ and $CD3^+$ T cells, lower complement levels, along with increased $CD8^+$ T cells and higher serum IgG, are associated with lower platelet counts in ITP. Multivariate logistic regression further demonstrated that elevated IgG, decreased C3, and a reduced $CD4^+/CD8^+$ ratio remained independently associated with ITP after adjusting for potential confounders such as recent infection and fever. ROC curve analysis was performed to evaluate the ability of these indicators to distinguish ITP from healthy children. The combination of C3, C4, IgG, $CD4^+$, and $CD8^+$ achieved an AUC of 0.967, which was significantly higher than any single indicator alone ($p<0.05$). This result aligns with previous studies supporting multiparameter approaches for ITP diagnosis.[29–31] The combined detection of multiple immune indicators can significantly improve the diagnostic accuracy of ITP in children. Depending on one immune index alone, it is easy to be misdiagnosed or missed due to the overlap of its changes among different individuals. Combined detection can comprehensively consider the changes of multiple indicators and reflect the immune status of patients more comprehensively, so as to identify the characteristic immune disorder pattern of ITP more reliably.

Study Limitations

Several important limitations must be considered when interpreting the findings of this study. First, due to its single-center retrospective design and relatively small sample size (n=80), selection bias may have affected sample representativeness, limiting the generalizability of the results to the broader pediatric ITP population. Second, a major limitation is the lack of a disease control group comprising patients with thrombocytopenia from other common causes (e.g., aplastic anemia, leukemia, or secondary immune disorders). Although the identified immune features (e.g., complement consumption, specific lymphocyte subset alterations) effectively distinguished ITP from healthy controls, their diagnostic specificity for ITP remains unverified. Prospective studies with appropriate disease controls are required to assess their utility in real-world differential diagnosis. Third, retrospective data collection is subject to potential biases in record completeness and unmeasured confounders (e.g., specific pathogen subtypes, vaccination details, environmental exposures). Consequently, observed

associations should not be interpreted as causal. Fourth, temporal relationship between viral infection and disease onset: Although we assessed viral infection status at ITP diagnosis using the defined criteria, the retrospective design could not precisely determine whether the infection occurred before, after, or concurrently with ITP onset. Consequently, the observed high positivity rate primarily indicates a significant association between viral infections and ITP in this cohort, rather than confirming a causal role for viruses as direct triggers of ITP. Prospective cohort studies or more detailed longitudinal monitoring are required to clarify the causal relationship. Fifth, this study relied on routinely available clinical immune parameters (levels, percentages, positivity rates) without functional assessments (e.g., T-cell exhaustion, B-cell antibody secretion) or quantification of complement activation fragments (e.g., C3a, C5a). Future studies integrating functional assays and multi-omics approaches are needed to elucidate the underlying mechanisms.

In conclusion, through the detection and analysis of immune indicators such as complement C3, C4, and immunoglobulin levels in children with ITP, this study revealed that children with ITP have significant immune disorder characteristics. These indicators have potential value in identifying immune characteristics of ITP. Combined detection of multiple immune indicators can more effectively reveal the immune pattern of children with ITP, which is different from that of healthy children, and provide multidimensional data for understanding the immune disorders of the disease. However, this study also has certain limitations. Future studies with larger, multicenter cohorts and appropriate disease controls are needed to validate the diagnostic performance of these immune characteristics and provide a stronger foundation for the precise management of pediatric ITP.

Significance and Innovation

This study analyzed serum complement, immunoglobulins, lymphocyte subsets, and viral infections in children with ITP, revealing distinct combined immune dysregulation. A model integrating $CD4^+$, $CD8^+$, C3, C4, and IgG effectively distinguished ITP from healthy children (AUC=0.967). The results provide multidimensional data on immune dysregulation and support the model's potential as a future laboratory tool for ITP identification.

STATEMENT OF ETHICS

The study was approved by the Ethics Committee of Hainan Women and Children's Medical Center (Approval No. HNWCMC2026-48). The principles of the Declaration of Helsinki were strictly followed and all research procedures were in accordance with international ethical standards.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Due to the retrospective nature of this study and the involvement of pediatric patient data, the data are not publicly available to protect patient privacy).

AI ASSISTANCE DISCLOSURE

None.

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