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Machine learning in lymphocyte and immune biomarker analysis for childhood thyroid diseases in China

Ruizhe Yang[1†], Wei Li[2†], Qing Niu[3†], WenTao Yang[3], Wei Gu[4*] and Xu Wang[2*]

Abstract

Objective This study aims to characterize and analyze the expression of representative biomarkers like lymphocytes and immune subsets in children with thyroid disorders. It also intends to develop and evaluate a machine learning model to predict if patients have thyroid disorders based on their clinical characteristics, ultimately providing insights to enhance the clinical guidelines for the pathogenesis of childhood thyroid disorders.

Method This cross-sectional study conducted in China examined diagnosed cases to describe the characteristics and expression of lymphocyte and immune subsets as predicted by the model. The study included two groups of children: 139 who were hospitalized in the Department of Endocrinology and a control group consisting of 283 children who underwent routine health checks at the Department of Children Healthcare. Cases were classified into three groups based on diagnoses: Graves' disease (GD), Hashimoto's thyroiditis (HT), and hypothyroidism. By employing 11 readily obtainable serum biochemical indicators within three days of admission, the median concentrations and percentages of subset measurements were analyzed. Additionally, nine machine learning (ML) algorithms were utilized to construct prediction models. Various evaluation metrics, including the area under the receiver operating characteristic curve (AUC), were employed to compare predictive performance.

Results GD cases had increased levels of CD3-CD19 + and CD3 + CD4 + T lymphocytes, and a higher CD4+/CD8 + ratio. In both GD and HT, the levels of complement C3c, IgA, and IgG were higher than those in the control group. HT cases also had an increasing percentage of CD3-CD16 + 56 + T lymphocytes. Most immune markers increased in hypothyroidism, except for some T lymphocyte percentages and the CD4+/CD8 + ratio. To reduce age-related bias, propensity score matching was used, yielding consistent results. Among the nine machine learning models evaluated, logistic regression showed the best performance, being useful in clinical practice.

Conclusions Specific lymphocytes with different biomarkers are positively correlated with autoimmune thyroid disease (AITD) in children. Complement proteins C3c and C4, along with IgG, IgA, IgM, and T/B cells, are significant

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in childhood thyroid diseases. Our best model can effectively distinguish these conditions, but to enhance accuracy, more detailed information such as clinical images might be needed.

Keywords Childhood AITD, Immune biomarker, Propensity score match, Machine learning, Explainable prediction model

Introduction

Thyroid diseases (TD) are widespread, affecting approximately 5% of the general population, and they are often diagnosed in children [1–3]. One example is autoimmune thyroid disease (AITD), which includes Graves' disease (GD) and Hashimoto's thyroiditis (HT). AITD is characterized by the self-destruction of thyroid antigens, resulting in the circulation of antibodies and infiltration by lymphocytes [4]. Although HT is mainly associated with cell-mediated autoimmunity and GD with humoral autoimmunity [5, 6], both disorders share a highly similar pathogenesis [7]. In AITD, it is considered that the immune mechanisms of the two conditions are interrelated and closely associated [8]. Epidemiologically, the most common age of onset for GD in the pediatric population is adolescence, while HT can manifest at any stage throughout the life cycle [9, 10]. While autoimmune thyroid disease (AITD) is rarely life-threatening, it can result in a range of serious complications, including abnormal growth and development in children, cardiovascular diseases, mental health issues, polycystic ovary syndrome (PCOS) in women, and an increased risk of thyroid cancer [11–15]. Furthermore, hypothyroidism is recognized as the second most common endocrine disease in the pediatric population, after type one diabetes [16]. Most studies suggest that the levels of thyroid-stimulating hormone (TSH) are crucial for diagnosing and managing treatment in children [16], but few have focused on the immunological characteristics of hypothyroidism in children [16]. Consequently, considering the prevalence and notable complications of AITD and general hypothyroidism in children, there is a pressing need for more research to explore their underlying pathogenesis [17]. It is widely acknowledged that AITD is affected by multiple underlying factors, involving a complex interplay of genetic, hormonal, and environmental elements that trigger improper immune responses against the thyroid at multiple levels, leading to a chronic autoimmune reaction [18–20]. Genetic susceptibility appears to be one of the most crucial factors influencing the development of AITD [21].

A wide range of immune cells and biomarkers in the human body have been recognized as risk factors related to AITD and general hypothyroidism. Approximately 70% of the genes known to contribute to the risk of AITD are related to T-cell function, such as CTLA4, PTPN22, and IL2RA. Furthermore, the anti-thyroid antibodies generated by B cells not only act as markers of the disease but also aggravate thyroid damage in HT through mediating complement fixation and antibody-dependent cytotoxicity (ADCC), as well as stimulating the TSH receptor (TSH-R) in GD [22]. Likewise, natural killer (NK) cells and additional immune biomarkers have been recognized as playing a role in autoimmune thyroid disease [23]. Although differences in genetic risk alleles and environmental exposures have been examined to a certain extent in children and adults with TD, currently, there are no machine learning (ML) models specifically designed to characterize the expression of lymphocyte and immune biomarkers in thyroid disease within a specific age group. This deficiency limits the potential for enhancing guidance and laying a foundation for preventive interventions and changes in health behavior.

At present, a multitude of machine learning techniques have been utilized in the establishment of disease prediction models, a considerable number of which have exhibited considerable predictive value, including applications in forecasting outcomes for pediatric patients [24–26]. Notwithstanding the powerful capabilities of machine learning methods, which arise from the complexity of their models, the outcomes are typically more abstract compared to clinical conclusions, thereby making direct interpretation and application challenging [27]. To tackle these challenges, we adopted the SHapley Additive explain (SHAP) method to interpret the model and visualize individual variable predictions in the pediatric TD cases we gathered. This study entailed the characterization of serum biomarkers and the utilization of machine learning to analyze variations in the expression of lymphocytes, complements, and immunoglobulins, along with their relationships to healthy controls, for predicting the strength of these associations. Our aim is to obtain a more comprehensive understanding of biomarker expression in thyroid disease, which might result in enhanced screening and prevention strategies for high-risk children and superior treatment options for AITD and other autoimmune conditions.

Material and method

Participants and samples

The research was conducted at the Children's Hospital of Nanjing Medical University, a premier public pediatric hospital in Jiangsu, China, renowned for its comprehensive testing and treatment services for childhood AITD and other thyroid disorders. We encompassed 139 cases of children admitted to the Department of

Table 1 The healthy control and TD cases

Types	Cases, No. (%)
Control	
Health Examination	283 (100)
Cases	
Graves' disease	114 (82.01)
Hashimoto's thyroiditis	18 (12.95)
Hypothyroidism	7 (5.04)

TD represents thyroid disease

Endocrinology, among which 132 suffered from AITD and 7 had hypothyroidism. In the cases of AITD, 114 children were diagnosed with GD, and 18 with HT. These diagnoses were clinically confirmed by endocrinologists based on clinical symptoms, abnormal thyroid hormone levels, the presence of autoantibodies, and ultrasound examinations. The diagnostic criteria included: (1) elevated serum levels of thyroid hormones fT4 and fT3; (2) suppressed serum TSH levels; (3) positive serum TRAb titer. Those with Plummer's adenoma, which causes toxic nodular goiter, were excluded from participation in the study. The control group, consisting of 283 healthy children, was established according to the general health examination records from the Department of Child Healthcare. To qualify, participants had to be free of any diagnosed autoimmune diseases or complications associated with AITD and had no prior medical history of thyroid or other autoimmune disorders (Table 1). The clinical notes were manually reviewed by two physicians (WTY and XW), who extracted the pertinent information. To verify accuracy and consistency, two researchers (RZY and WL) randomly selected and cross-checked 20% of the dataset during the extraction process. The flow chart detailing the inclusion criteria is illustrated in Fig. 1.

Immunoassay method

Lymphocyte sub-populations were evaluated in freshly collected peripheral blood through flow cytometry with a FACSCanto II flow cytometer. Samples were aseptically obtained by venipuncture into BD Vacutainer blood collection tubes, demanding a minimum of 100 µl of whole blood for measurement. Anticoagulated blood was stored at room temperature (20 to 25 °C) and stained within 24 h of collection, and the analysis was conducted within 6 h. Scatter plots were produced with CD45 and side scatter (SSC) as coordinates, enabling the visualization of stained cells. The lymphocyte subpopulations were

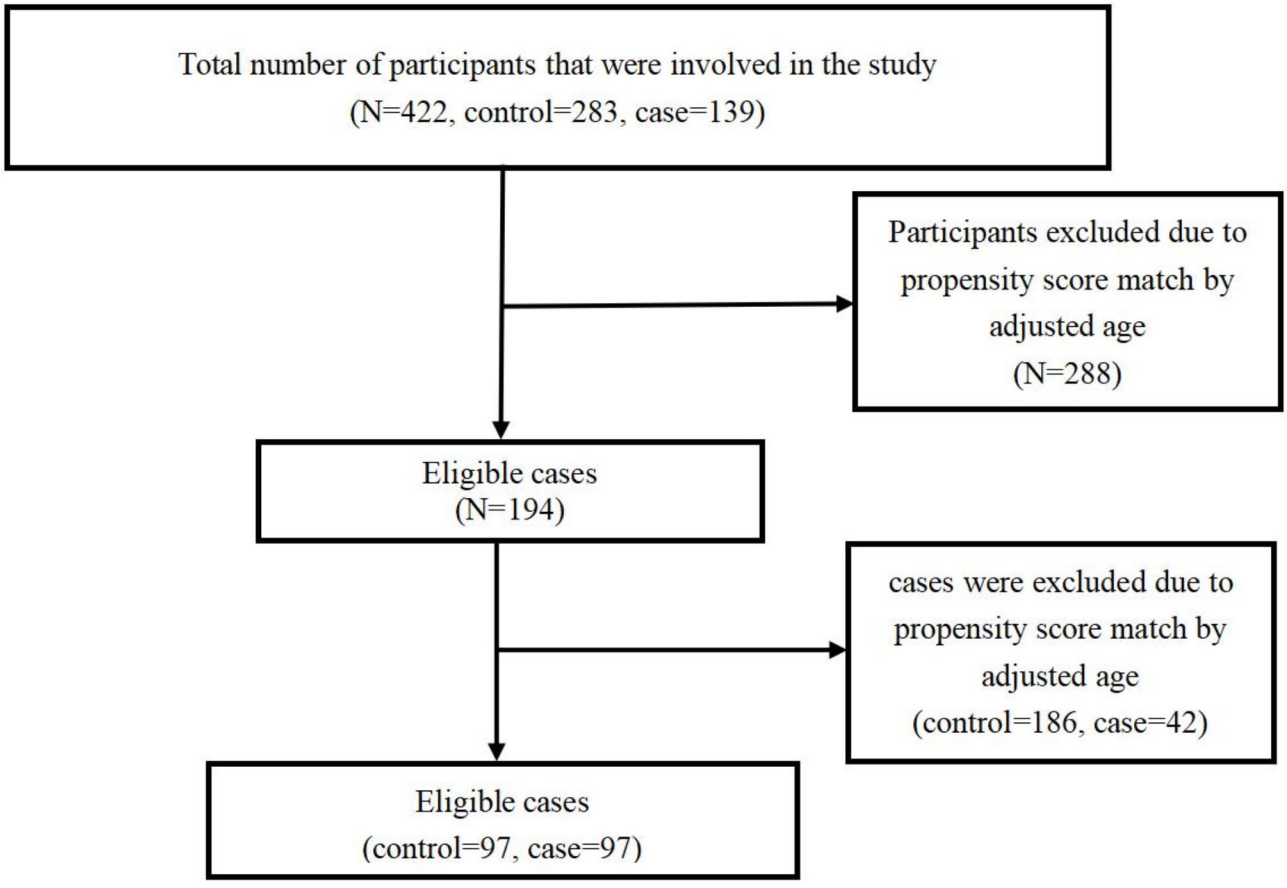

Fig. 1 The flow chart of inclusion criteria

depicted as bright, compact communities in the low SSC region, and the results were presented as the percentage of positive cells in relation to the total lymphocyte count.

The BN™ II System from Siemens, a fully automated nephelometric analyzer, was employed in this study to evaluate a wide range of protein assays, specifically for determining the concentrations of complements and immunoglobulins in serum samples. Specimens were stored at +2 to +8 °C for no longer than 8 days or deep-frozen, and diluted to a 1:400 ratio with diluent prior to measurement within four hours. Quality control was upheld by measuring low, medium, and high protein levels after each establishment of the reference curve, following the initial use of the antiserum vial, and after each run of serum samples. If quality control results were outside the confidence range, repeat measurements were carried out. Additionally, if discrepancies were observed in duplicate assay results, a new reference curve was established.

Data preprocessing

The integrity and quality of our dataset were independently verified by two authors (WL and WG). The average age of the healthy group was 4.45 ± 2.82 years, in contrast to an average age of 8.63 ± 3.19 years in the case group (Table 2). Recognizing that the laboratory characteristics of immune biomarkers can be influenced by various growth phases, we adjusted for age through propensity score matching. After data preprocessing, our final dataset encompassed 194 cases. As presented in Table S1, both the healthy and case groups included 97 participants respectively. The case group was further subdivided into 78 cases of GD, 15 cases of HT and 4 case of hypothyroidism. After implementing propensity score matching, the healthy group consisted of 61 males and 36 females, with an average age of 6.90 ± 2.90 years. In contrast, the case group comprised 23 males and 74 females, with an average age of 7.14 ± 2.50 years (Table S2). Additionally, according to the Clinical Laboratory at the Children's Hospital of Nanjing Medical University, the normal reference ranges for IgA, IgG, and IgM are detailed in Tables S6, S7, and S8, respectively.

Table 2 Baseline characteristics of healthy control and TD cases

Characteristics	Control (*N* = 283)	Cases (*N* = 139)	*P*
Age(years)	4.45 ± 2.82	8.63 ± 3.19	**< 0.001**
Gender			**< 0.001**
Male	187 (66.08)	33 (23.74)	
Female	96 (33.92)	106 (76.26)	

P < 0.05 was considered statistically significant

Data partition for training and testing

To investigate the primary distribution of the case and control groups, we divided the raw data prior to propensity score matching into two segments at a 7:3 ratio, yielding 295 cases for training and validation and 127 cases for testing. Stratified random sampling was adopted to ensure that the proportions of cases and healthy controls in both the training and testing datasets precisely reflected their proportions in the overall dataset. This study utilized Python software for data analysis.

Model development and comparison

Thirteen features were employed to develop the prediction models. Nine machine learning (ML) models were utilized to predict biomarkers in thyroid disease cases, specifically Naive Bayes (NB), decision tree (DT), extra tree (ET), gradient boosting machine (GBM), neural networks (NN), logistic regression (LR), random forest (RF), support vector machine (SVM), and eXtreme gradient Boosting (XGBoost). Furthermore, although interpreting ML models can be intricate, the SHAP method provides a means to rank the significance of input features and clarify the outcomes of the prediction model [27]. The SHAP method provides global and local explanations for model interpretation. The global explanation generates consistent and accurate attribution values for each feature in the model, highlighting the relationships between input features and thyroid diseases.

Statistical analysis

Data related to immune molecules and lymphocytes, both before and after propensity score matching, were presented as concentrations and percentages of total lymphocytes. The Independent Samples T-test was employed to determine statistically significant differences between the two groups. Statistics are shown as medians with interquartile ranges (IQR). All statistical analyses were conducted using SPSS software version 26, with a p-value of < 0.05 (two-tailed) considered statistically significant.

Ethical considerations

Our study have received ethics approval by IEC of Children's Hospital of Nanjing Medical University (Ethics Approval Number: 202403014-1). As this was a retrospective study and all participant data were obtained exclusively from pre-existing electronic medical records, we applied for a waiver of informed consent. This application was approved by the Institutional Ethics Committee (IEC) of Nanjing Medical University Children's Hospital.

Results

Immune biomarkers in TD cases

In the GD group ($n = 114$), C4 levels were significantly different from those in the control group ($p = 0.014$), whereas C3c levels were slightly elevated, although not significantly ($p = 0.268$). In HT ($n = 18$), levels of above complements were both significantly lower than those of controls ($p = 0.001$ and $p = 0.017$, respectively). For hypothyroidism ($n = 7$), they were higher than in controls, but these differences were not statistically significant ($p = 0.386$ and $p = 0.104$, respectively). Flow cytometric analysis disclosed that the percentages of three T-cell subsets were significantly lower in children with GD compared to controls ($p < 0.001$), indicating a significant correlation between the variables. When comparing the ratios between cases and controls, all three T-cell subsets were higher in the cases; however, significant differences were only observed in two of the subsets. In HT cases, the percentages of the three biomarkers were higher than in controls, but these differences did not reach statistical significance. Furthermore, the study revealed a higher percentage of three T-cell subsets in the healthy control group compared to the HT cases. Notably, the difference in a specific T-cell subset was the only one that achieved statistical significance ($p = 0.007$). In hypothyroidism ($n = 7$), the percentages of three biomarkers were higher in the HT cases compared to the controls. In contrast, the percentages of the other three T-cell subsets showed a decrease, with only the reduction in one being statistically significant ($p = 0.007$).

In all three case groups, the levels of IgA were higher than those in healthy controls. Nevertheless, significant differences were noted only in GD ($p = 0.001$) and hypothyroidism ($p = 0.005$), while no significant difference was detected in HT cases ($p = 0.260$). The median concentrations of IgG were determined to be statistically significantly higher in the cases of GD ($p = 0.001$) and HT ($p = 0.008$) compared with the control group. Nevertheless, the IgG concentration in the hypothyroidism cases was significantly lower than that in the control group, although this difference did not reach statistical significance ($p = 0.716$). Although the median concentration of IgM was higher in HT and lower in GD and hypothyroidism in comparison with healthy controls, none of these differences were statistically significant in any of the three case groups (Table 3).

Lymphocyte subsets in control and TD cases

The frequencies of three lymphocyte subests were higher in the total cases ($n = 139$) compared to healthy controls, with significant differences ($p < 0.001$), except one of them ($p = 0.526$). We also noted significantly lower frequencies of three T-cells in the total cases in contrast to healthy controls (Table 4).

Immune molecules in control and TD cases

Table 5 shows that the median concentrations of complement C3c and C4 in total cases. The results were largely in line with those of the control group, except for a statistically significant difference in C4 ($p = 0.012$). The median concentrations of IgA, IgG, and IgM were respectively determined. Among the three immunoglobulins, the levels of IgA and IgG in the total cases were significantly higher than those in the control ($p < 0.001$). Furthermore, the median IgM concentration was marginally lower in the total cases than in the control group, although this difference was not statistically significant ($p = 0.241$).

Propensity score matching

Tables S3, S4, and S5 detail the laboratory characteristics of total immune biomarkers, lymphocyte subsets, and immune molecules in both the control and case groups following Propensity Score Matching. These findings are largely consistent with the results obtained prior to matching. Propensity Score Matching achieves a post-randomization effect, effectively mitigating the impact of selection bias and confounding factors, thereby ensuring a valid and comparable analysis between the observation group and the control group.

Model development and explanation

By using the case data collected in this study, we developed nine machine learning models to evaluate the correlation of various immune marker variables in childhood thyroid diseases. Among these models, the logistic regression (LR) model exhibited the highest predictive performance, attaining an area under the curve (AUC) of 0.88. This was closely followed by the XGB model and the GMB model, both of which achieved an AUC of 0.87.

To clarify the results of the final model, we employed the SHAP method to quantify the contribution of each variable to the predictions. Figures 2 and 3 illustrate the assessment of individual variable contributions through average SHAP values, ranked in descending order. A higher SHAP value indicates a greater probability of thyroid disease. Moreover, we represented each patient's SHAP value for each variable with a dot, where the color indicates the actual value of the variable – red for higher values and blue for lower values. The vertical arrangement of the points shows their density. In conclusion, SHAP dependence plots help clinicians understand the influence of individual features on the outcomes of the prediction model (Fig. 4).

Discussion

To our best knowledge, few studies have hitherto examined the expression of immune biomarkers in children with thyroid diseases [28]. Notwithstanding the predominant focus of previous research on adult patients,

Table 3 Laboratory characteristics of immune biomarkers in control and cases

		Median (IQR)		
	Normal Range	**Control**	**Cases**	***P***
Grave's disease (*n* = 114)				
C3c	0.88 ~ 2.01 g/L	1.06 (0.95 ~ 1.19)	1.09 (0.98–1.20)	0.268
C4	0.16 ~ 0.47 g/L	0.21 (0.16 ~ 0.27)	0.20 (0.16–0.23)	**0.014**
CD3-CD16 + 56+	6 ~ 27%	9.63 (6.69 ~ 13.19)	7.36 (5.22–10.33)	**< 0.001**
CD3-CD19+	7 ~ 22%	16.88 (13.55 ~ 21.67)	18.75 (14.52–23.84)	**0.018**
CD3 + CD4+	31 ~ 60%	36.02 (31.88 ~ 40.61)	39.89 (34.45–44.10)	**< 0.001**
CD3 + CD8+	13 ~ 38%	27.34 (23.21 ~ 31.17)	24.08 (20.50-27.36)	**< 0.001**
CD4+/CD8+	0.9 ~ 3.6	1.32 (1.07 ~ 1.68)	1.60 (1.35–2.08)	**< 0.001**
CD4 + CD8+	0 ~ 3%	1.05 (0.68 ~ 1.51)	0.75 (0.47–1.16)	**< 0.001**
IgA	See Table S6	0.97 (0.55 ~ 1.57)	1.28 (0.91–1.74)	**0.001**
IgG	See Table S7	8.80 (6.99 ~ 10.80)	10.04 (8.15–11.63)	**0.001**
IgM	See Table S8	1.06 (0.78 ~ 1.37)	1.00 (0.74–1.29)	0.086
Hashimoto's thyroiditis (*n* = 18)				
C3c	0.88 ~ 2.01 g/L	1.06 (0.95 ~ 1.19)	0.91 (0.72–1.09)	**0.001**
C4	0.16 ~ 0.47 g/L	0.21 (0.16 ~ 0.27)	0.18 (0.13–2.01)	**0.017**
CD3-CD16 + 56+	6 ~ 27%	9.63 (6.69 ~ 13.19)	10.60 (8.02–16.69)	0.364
CD3-CD19+	7 ~ 22%	16.88 (13.55 ~ 21.67)	12.63 (11.15–16.73)	**0.007**
CD3 + CD4+	31 ~ 60%	36.02 (31.88 ~ 40.61)	39.92 (34.19–43.25)	0.275
CD3 + CD8+	13 ~ 38%	27.34 (23.21 ~ 31.17)	26.49 (22.98–31.43)	0.981
CD4+/CD8+	0.9 ~ 3.6%	1.32 (1.07 ~ 1.68)	1.39 (1.12–1.62)	0.962
CD4 + CD8+	0 ~ 3%	1.05 (0.68 ~ 1.51)	0.91 (0.44–1.34)	0.236
IgA	See Table S6	0.97 (0.55 ~ 1.57)	1.27 (0.76–1.67)	0.260
IgG	See Table S7	8.80 (6.99 ~ 10.80)	10.29 (8.49–12.93)	**0.008**
IgM	See Table S8	1.06 (0.78 ~ 1.37)	1.24 (0.83–1.54)	0.229
Hypothyroidism (*n* = 7)				
C3c	0.88 ~ 2.01 g/L	1.06 (0.95 ~ 1.19)	1.19 (1.04–1.31)	0.386
C4	0.16 ~ 0.47 g/L	0.21 (0.16 ~ 0.27)	0.25 (0.16–0.33)	0.104
CD3-CD16 + 56+	6 ~ 27%	9.63 (6.69 ~ 13.19)	14.74 (12.02–20.30)	**0.006**
CD3-CD19+	7 ~ 22%	16.88 (13.55 ~ 21.67)	7.93 (6.45–12.84)	**< 0.001**
CD3 + CD4+	31 ~ 60%	36.02 (31.88 ~ 40.61)	33.40 (31.32–35.98)	**0.046**
CD3 + CD8+	13 ~ 38%	27.34 (23.21 ~ 31.17)	36.44 (22.13–47.87)	0.221
CD4+/CD8+	0.9 ~ 3.6	1.32 (1.07 ~ 1.68)	0.91 (0.63–1.71)	0.182
CD4 + CD8+	0 ~ 3%	1.05 (0.68 ~ 1.51)	1.93 (1.01–2.31)	**0.016**
IgA	See Table S6	0.97 (0.55 ~ 1.57)	1.84 (1.54–2.45)	**0.005**
IgG	See Table S7	8.80 (6.99 ~ 10.80)	7.28 (6.62–11.10)	0.716
IgM	See Table S8	1.06 (0.78 ~ 1.37)	0.79 (0.71–1.19)	0.325

P < 0.05 was considered statistically significant

numerous immune biomarkers in AITD and hypothyroidism have been investigated and correlated with disease diagnosis, serving as a valuable reference for our study [29]. We witnessed an ascending trend in the percentage and ratio of T lymphocytes among cases of GD. The levels of complement were significantly heightened in both GD and HT cases as compared to healthy children. Nevertheless, in cases of hypothyroidism, all immune biomarkers demonstrated increasing trends, with the exception of the percentages of specific T lymphocytes.

Recognizing the key role of T lymphocytes in the pathogenesis of AITD, we conducted an analysis of the expression of their subpopulations, each defined by distinct surface biomarkers [4]. In light of previous findings, T lymphocytes are implicated in both the prevention and the pathogenesis of autoimmune diseases, inclusive of AITD [30–32]. Nevertheless, T lymphocytes counts in peripheral blood can fluctuate significantly among patients with AITD, as these cells frequently demonstrate dysfunction and are incapable of fulfilling their immunosuppressive roles in affected individuals [33–35]. It is probable that this phenomenon is related to the potential conversion of T lymphocytes into pro-inflammatory cells, particularly Th17 and Th1 lymphocytes [36, 37]. Multiple studies have reported reduced levels of T regulatory cells (CD4 + Foxp3 + or CD4 + CD25 + FoxP3+) in the peripheral blood of

Table 4 Laboratory characteristics of lymphocyte subests in control and total TD cases

		Median (IQR)		*P*
	Normal Range	Control (*N* = 283)	Cases (*N* = 139)	
CD3-CD16 + 56+	6 ~ 27%	9.63 (6.69 ~ 13.19)	8.08 (5.42 ~ 11.69)	**0.006**
CD3-CD19+	7 ~ 22%	16.88 (13.55 ~ 21.67)	17.06 (13.40 ~ 22.13)	0.526
CD3 + CD4+	31 ~ 60%	36.02 (31.88 ~ 40.61)	39.37 (33.83 ~ 43.74)	**< 0.001**
CD3 + CD8+	13 ~ 38%	27.34 (23.21 ~ 31.17)	24.64 (21.10 ~ 27.88)	**0.001**
CD4+/CD8+	0.9 ~ 3.6	1.32 (1.07 ~ 1.68)	1.57 (1.28 ~ 1.96)	**< 0.001**
CD4 + CD8+	0 ~ 3%	1.05 (0.68 ~ 1.51)	0.78 (0.48 ~ 1.23)	**0.001**

P < 0.05 was considered statistically significant

Table 5 Laboratory characteristics of immune molecules in control and total TD cases

		Median (IQR)		*P*
	Normal Range	Control (*N* = 283)	Cases (*N* = 139)	
C3c	0.88 ~ 2.01 g/L	1.06 (0.95 ~ 1.19)	1.07 (0.97 ~ 1.19)	0.904
C4	0.16 ~ 0.47 g/L	0.21 (0.16 ~ 0.27)	0.20 (0.16 ~ 0.23)	**0.012**
IgA	See Table S5	0.97 (0.55 ~ 1.57)	1.31 (0.92 ~ 1.78)	**< 0.001**
IgG	See Table S6	8.80 (6.99 ~ 10.80)	9.97 (7.97 ~ 11.60)	**< 0.001**
IgM	See Table S7	1.06 (0.78 ~ 1.37)	1.00 (0.74 ~ 1.32)	0.241

P < 0.05 was considered statistically significant. 16 samples in healthy group can not measure the IgA which use lower limits of measurement divided by 1.414

patients with GD [38–40]. The research conducted by Abigail et al. revealed a dysfunction of Treg cells in both HT and GD, notwithstanding the distinct pathophysiology of each disease, suggesting a common immunoregulatory defect [2]. B lymphocytes were observed to express only CD19 and not CD3 in our measurements. This indicates that they contribute not only to humoral immunity but also function as activated cells in patients with AITD [41]. Moreover, T helper (Th) lymphocytes are known to reciprocally enhance the activation of B lymphocytes in adults [4], which is in agreement with the findings observed in children [4]. In the case of GD, B lymphocytes are crucial for generating specific activating autoantibodies (TRAb) targeting thyroid-stimulating hormone receptors (TSHR) [8]. Although their significance in HT might not be as prominent as in GD, it is essential to note that they also produce autoantibodies directed against thyroglobulin (Tg) and thyroid peroxidase (TPO) [8].

In the realm of AITD, current research has generally produced inconsistent findings regarding disease status and the activity of NK cells [42]. , and only one study reported no difference in NK cell activity between patients with HT, GD or general hypothyroidism and normal controls [43]. Given that NK cell activity is affected by age, a study which compared patients with GD and HT to age-and sex-matched healthy controls discovered significantly decreased NK cell activity in both patient groups in comparison with the normal controls [44, 45], which is consistent with our results. Our findings revealed significant differences in the median serum concentrations of IgG, IgA, and IgM between healthy

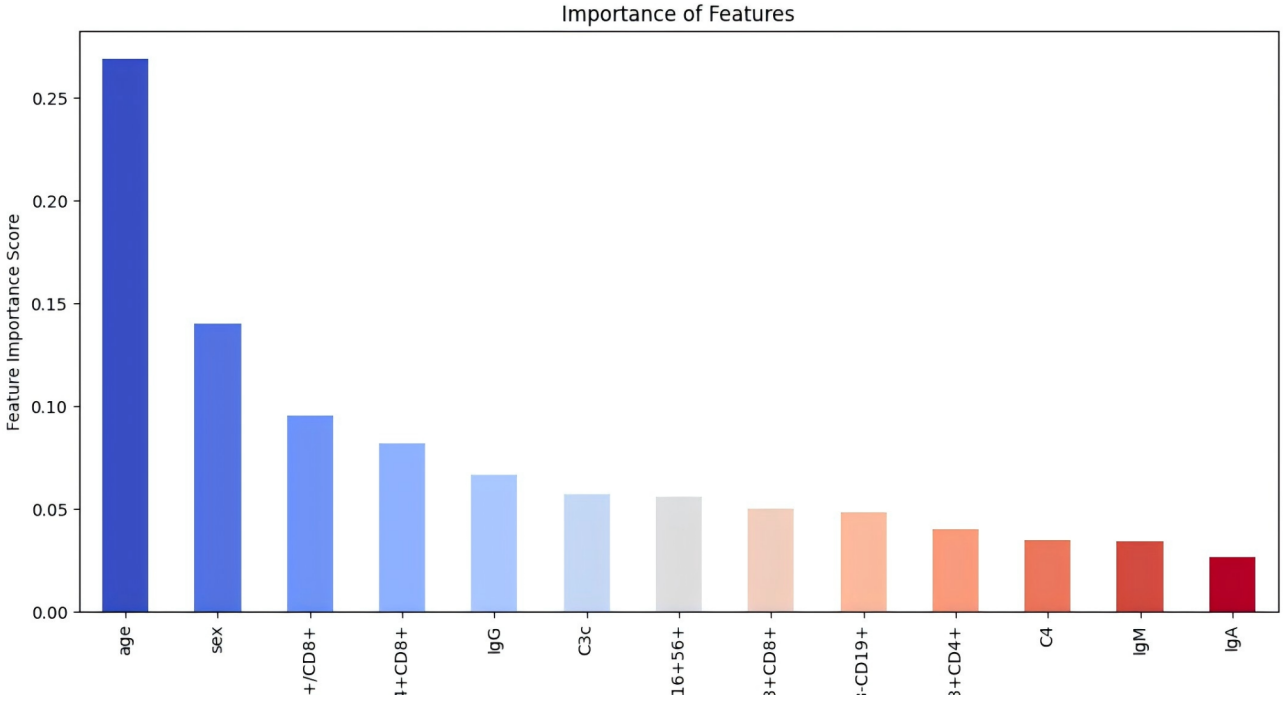

Fig. 2 Individual variable contributions of Importance of Features

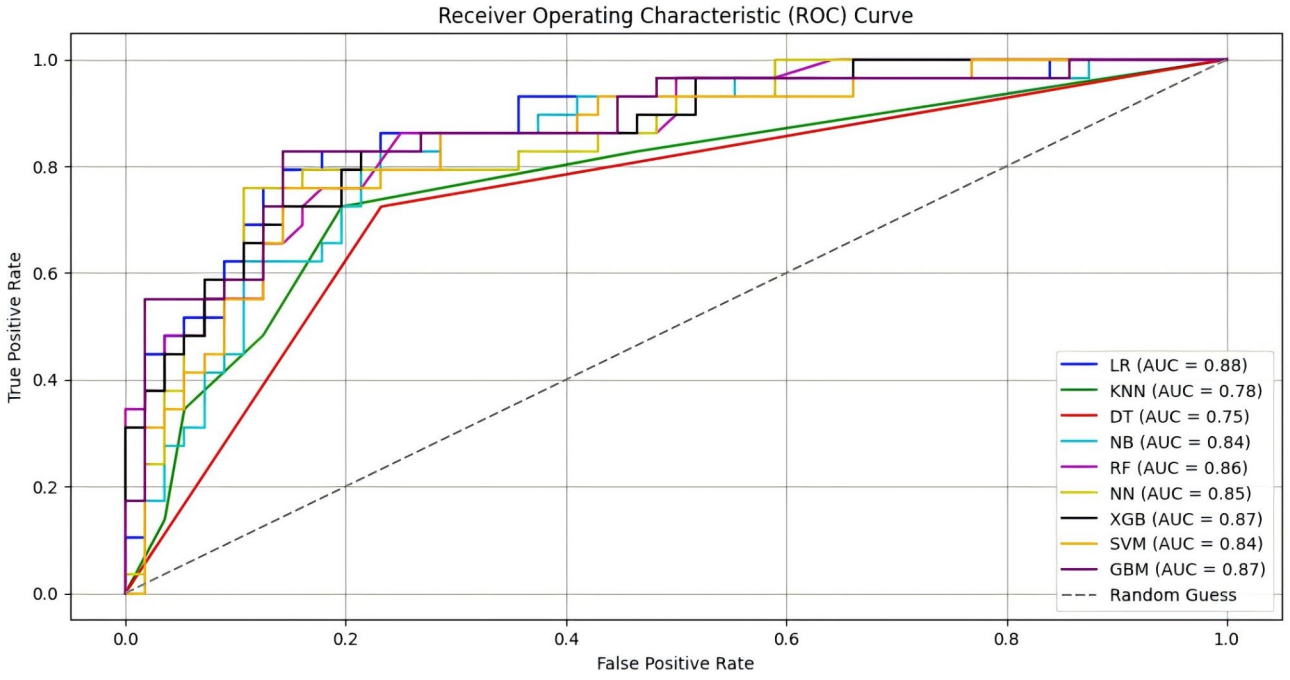

Fig. 3 The ROC AUC curves of nine ML models

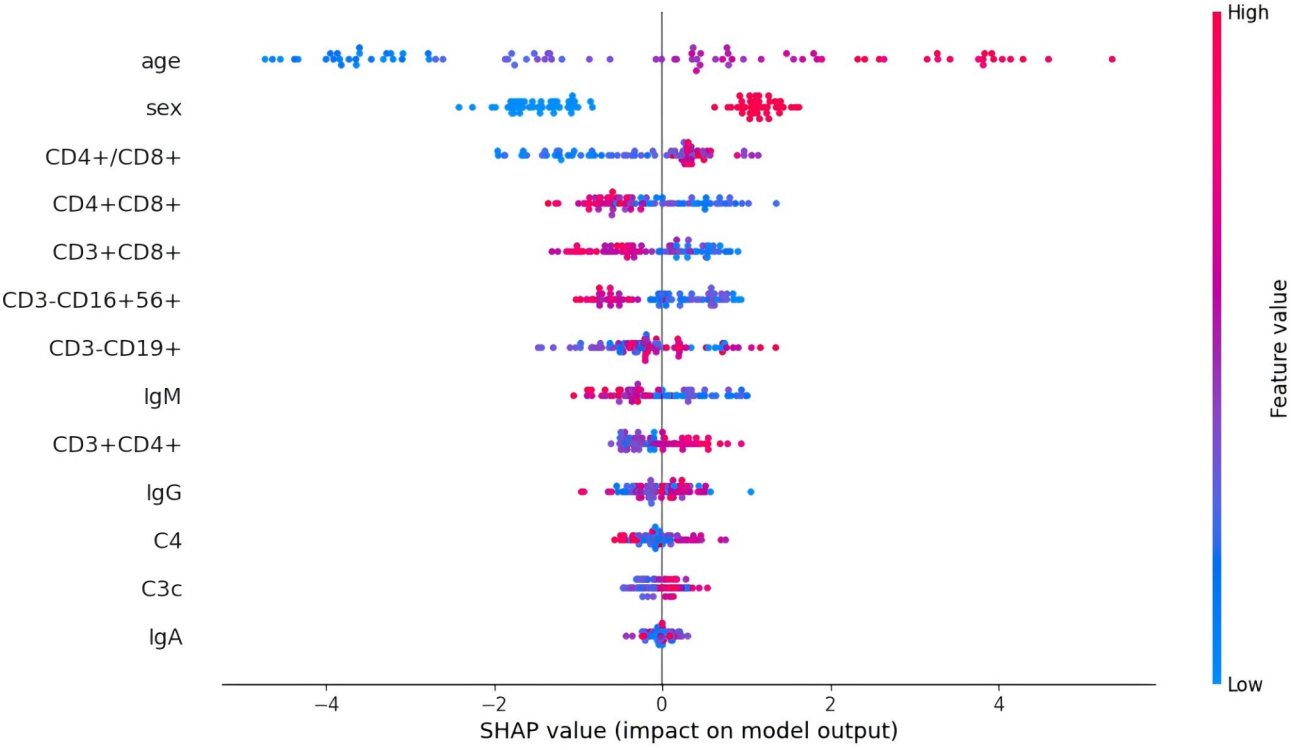

Fig. 4 Visualization of the features importance in the CatBoost Classifier model

individuals and patients with thyroid disease, particularly in cases of AITD. In their research, Kawashima et al. [46] strengthened our findings by evaluating serum IgG levels in adults with HT, indicating that adults might have outcomes similar to those in pediatric cases. They also discovered that a small number of HT patients with high levels of anti-thyroid antibodies could potentially have IgG4-related thyroiditis. Furthermore, the complement

system plays a crucial role in the innate immune system and is often protective in systemic autoimmune diseases [47]. In contrast, the complement actively attacks thyroid cells in AITD because the cells express multiple complement components and complement suppressor molecules [47, 48].

From the gathered data, we identified several predictable risk factors, such as gender, age, and biochemical immune marker parameters in each serum, and constructed a predictive model. The machine learning (ML) and SHAP-based prediction model exhibited strong predictive accuracy for thyroid disease, performing satisfactorily when the majority of significant variables were incorporated. The findings disclosed that the XGB model had the optimal ROC value and a preferable net benefit. However, the dearth of established guidelines or consensus regarding feature selection for predictive models renders it arduous to determine the ideal number of features to incorporate. While a larger number of features can provide more information for the prediction model, incorporating an excessive number of features may constrain its clinical practicability. Furthermore, the existence of non-causal features could adversely affect the accuracy of the predictions [49]. The SHAP method was employed in this study to facilitate feature selection, and our final model could be readily utilized for clinical decision-making in the target population [27].

Our study possesses several notable strengths. Firstly, all the participants were hospitalized children, guaranteeing full compliance and measurement accuracy. Additionally, although anti-thyroid medications, particularly propylthiouracil, are known to exert immunosuppressive effects [50], their utilization in patients can result in an increase in circulating suppressor T cells, while reducing the numbers of helper T cells, NK cells, and activated T cells [50, 51]. Nonetheless, all participants had not taken any therapeutic medications, which allowed us to exclude potential influencing factors. As a cross sectional study, our study could be used to measure the prevalence of health outcomes, understand determinants of health, and describe features of a population as well as inexpensive and easy to conduct [52]. In addition, we considered the effect of age by adjusting and matching the samples artificially. The results after adjustment could ensure the accuracy of the original data.

However, the study presented certain limitations. Firstly, being a single-center study with a total sample size of merely 139 subjects, the sample size was relatively small, making it challenging to extend the findings to populations with notable differences in characteristics such as ethnicity, socioeconomic status, and geographical location. As a result, the results might not be applicable to other regions of the country. Moreover, cross-sectional studies are a kind of observational research that examines the data from a population at a specific moment in time [52]. The lack of follow-up constitutes a significant weakness in our study. Consequently, it was difficult to monitor the dynamic alterations in the percentage or concentration levels of specific immune biomarkers between cases and controls. Furthermore, this study could offer insights into the future efficacy of treatments, highlighting the need for additional follow-up. In the future, we will carry out relevant prospective cohort studies. We will consider increasing the number of cases and using more similar and comparable groups of healthy children to avoid the influence and error of gender and age on the variables in the study.

Conclusion

In this study, we manifested that the expression of certain lymphocytes with specific surface markers positively correlates with autoimmune thyroid disease (AITD) in children. Complement proteins C3c and C4, along with immunoglobulins IgG, IgA, and IgM, as well as T and B cells, exert significant roles in pediatric thyroid diseases. The interactions among immune cells in the field of pediatrics have crucial implications for comprehending the etiology and treatment of various thyroid disorders. While our study indicates that serum immunological markers significantly differ in children with thyroid disease compared to healthy controls, further research is requisite to validate the roles of different immune cells in thyroid disease and to clarify conflicting findings in the existing literature. Additionally, more studies are needed to appraise the practical significance of immune cell imbalances and to determine whether these imbalances are causative factors or consequences of the disease.

Supplementary Information

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Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8

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Author contributions
X. W. and W. L.; Data curation, R. Y. and W. L.; Formal analysis, X. W.; Investigation, R. Y.; Methodology, WT. Y., R. Y.; Resources, W. L. and X. W; Software, R. Y.; Su-prevision, WT. Y.; Validation, X. W.; Visualization, R. Y., and X. W.;

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Data availability
The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Our study have received ethics approval by IEC of Children's Hospital of Nanjing Medical University (Ethics Approval Number: 202403014-1). As this was a retrospective study and all participant data were obtained exclusively from pre-existing electronic medical records, we applied for a waiver of informed consent. This application was approved by the Institutional Ethics Committee (IEC) of Nanjing Medical University Children's Hospital.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
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