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DIAGNOSTIC EFFICACY AND PROGNOSTIC RECURRENCE PREDICTION EFFECT OF TREGS COMBINED WITH TGF-β1 IN PEDIATRIC PATIENTS WITH ALLERGIC RHINITIS AND CONCURRENT ADENOID HYPERTROPHY

DIJAGNOSTIČKA EFIKASNOST I PROGNOSTIČKI EFEKAT PREDVIĐANJA RECIDIVA TREG ĆELIJA KOMBINOVANIH SA TGF-β1 KOD PEDIJATRIJSKIH PACIJENATA SA ALERGIJSKIM RINITISOM I ISTOVREMENOM ADENOIDNOM HIPERTROFIJOM

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Summary

Background: The co-occurrence of adenoid hypertrophy (AH) and allergic rhinitis (AR) is known to be linked to immune microenvironment disruptions. This research was to evaluate the performance of Tregs and transforming growth factor-β1 (TGF-β1) perform in diagnosing AH in pediatric AR cases and in predicting the likelihood of post-treatment recurrence.
Methods: From February 2023 to June 2024, a prospective cohort study was conducted, recruiting 127 children with AR alone (AR group) and 134 with AR-AH comorbidity (AR+AH group). All participants were followed prospectively for a 12-month period. Peripheral blood Treg counts were measured using flow cytometry, while serum TGF-β1 concentrations were determined via enzyme-linked immunosorbent assay (ELISA). Diagnostic effectiveness was evaluated using receiver operating characteristic (ROC) curves. A logistic regression model was developed to forecast recurrence risk, with model parameters refined by incorporating relevant clinical features.
Results: AR+AH cases exhibited statistically lower Treg counts and TGF-β1 levels than their AR counterparts (P<0.001). When Treg and TGF-β1 were combined for prediction, the area under the curve (AUC) reached 0.858, along with 91.04% sensitivity and 71.65% specificity – performance that outperformed either marker used individually (P<0.001). Following treatment, the increases in Treg and TGF-β1 levels were less pronounced in the AR+AH group than in the AR group (P<0.001). The follow-up

Kratak sadržaj

Uvod: Poznato je da je istovremena pojava adenoidne hipertrofije (AH) i alergijskog rinitisa (AR) povezana sa poremećajima imunog mikrookruženja. Ovo istraživanje je imalo za cilj da proceni efikasnost Treg ćelija i transformišućeg faktora rasta-b1 (TGF-β1) u dijagnostikovanju AH kod pedijatrijskih slučajeva AR i u predviđanju verovatnoće recidiva nakon lečenja.
Metode: Od februara 2023. do juna 2024. godine sprovedena je prospektivna kohortna studija, u kojoj je obuhvaćeno 127 dece samo sa AR (AR grupa) i 134 sa komorbiditetom AR-AH (AR+AH grupa). Svi učesnici su praćeni prospektivno tokom perioda od 12 meseci. Broj Treg ćelija u perifernoj krvi meren je protočnom citometrijom, dok su koncentracije TGF-β1 u serumu određene imunosorbentnim testom (ELISA). Dijagnostička efikasnost je procenjena korišćenjem ROC krivih. Razvijen je logistički regresioni model za predviđanje rizika od recidiva, sa parametrima modela usavršenim uključivanjem relevantnih kliničkih karakteristika.
Rezultati: Slučajevi AR+AH pokazali su niži broj Treg ćelija i nivoe TGF-β1 nego njihovi AR partneri (P<0,001). Kada su Treg i TGF-β1 kombinovani za predviđanje, površina ispod krive (AUC) dostigla je 0,858, zajedno sa osetljivošću od 91,04% i specifičnošću od 71,65% – performanse koje su nadmašile bilo koji marker korišćen pojedinačno (P<0,001). Nakon tretmana, povećanje nivoa Treg ćelija i TGF-β1 bilo je manje izraženo u AR+AH grupi nego u AR grupi (P<0,001). Podaci praćenja ukazali su na 42 slučaja

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data indicated 42 cases of recurrence, whose post-treatment Treg and TGF-β1 levels remained lower relative to those who did not experience a recurrence ($P<0.05$). A composite predictive model, integrating age, adenoid-to-nasopharynx (A/N) ratio, Treg, and TGF-β1, yielded an AUC of 0.869, with sensitivity and specificity being 95.24% and 68.48%, respectively.

Conclusions: A synergistic effect is observed when Tregs and TGF-β1 are measured together, enhancing the diagnostic accuracy for AR-AH co-occurrence. Furthermore, their combination with clinical characteristics optimizes stratification of recurrence risk.

Keywords: tregs, TGF-β1, allergic rhinitis, adenoid hypertrophy, diagnostic marker, prognostic model

recidiva, čiji su nivoi Treg ćelija i TGF-β1 nakon tretmana ostali niži u odnosu na one koji nisu doživeli recidiv ($P<0,05$). Kompozitni prediktivni model, koji integriše starost, odnos adenoida i nazofarinksa (A/N), Treg i TGF-β1, dao je AUC od 0,869, sa osetljivošću i specifičnošću od 95,24% i 68,48%, respektivno.

Zaključak: Sinergistički efekat se primećuje kada se Tregs i TGF-β1 mere zajedno, što poboljšava dijagnostičku tačnost za ko-pojavu AR-AH. Štaviše, njihova kombinacija sa kliničkim karakteristikama optimizuje stratifikaciju rizika od recidiva.

Ključne reči: tregs, TGF-β1, alergijski rinitis, adenoidna hipertrofija, dijagnostički marker, prognostički model

Introduction

Allergic rhinitis (AR) ranks among the most prevalent allergic conditions among children, affecting roughly 20% of the pediatric population worldwide (1). Prolonged exposure to allergic inflammation leads to continuous flow and irritation of inflammatory secretions on the adenoids, ultimately resulting in adenoid hypertrophy (AH) (2). Clinical data indicate the co-occurrence of AH in around 23.4% of pediatric AR cases (3). AR-AH comorbidity can lead to serious complications, including sleep-disordered breathing, abnormalities in maxillofacial development, and reduced learning capacity—all of which have a substantial negative impact on children's quality of life (4). Current clinical management strategies primarily involve nasal glucocorticoids, antihistamines, and adenoidectomy. Nevertheless, a subset of children still faces a high risk of recurrence post-treatment: the one-year postoperative recurrence rate can range from 8% to 10%, and around 80% of children on maintenance medication require more intensive intervention due to repeated symptom flare-ups (5). The ability to accurately predict recurrence risk and develop personalized intervention plans has thus become a critical unmet need in clinical practice.

Currently, the clinical assessment of AR with comorbid AH mainly relies on clinical symptom scoring or adenoid size measurements. However, these indicators only reflect the phenotypic aspects of the disease and fail to uncover the underlying immunopathological mechanisms (6). In recent years, immune microenvironment imbalances have been identified as a key driver of AH onset and progression in AR (7). Tregs, a key immunosuppressive cell subset, participate in maintaining immune tolerance by secreting substances like interleukin-10 (IL-10) and transforming growth factor-β1 (TGF-β1), which in turn inhibit the activation of effector T cells (8). TGF-β1 possesses both immunomodulatory and tissue remodeling properties, and its abnormal expression may contribute to adenoid tissue fibrosis or persisting inflammation (9). Existing research has established an intimate association between Treg count reductions or Treg function impairments with acute episodes and recurrence of allergy-related conditions like asthma and atopic dermatitis (10). Additionally, elevated TGF-β1 levels have been linked to the persistence of nasal mucosal inflammation and airway hyperresponsiveness in pediatric AR (11). However, the collaborative role of Treg and TGF-β1, as well as their value in predicting prognostic recurrence, remains unclear in the specific patient group of children with both AR and AH.

This study seeks to move beyond the limitations of traditional phenotypic assessment by, for the first time, measuring both Treg proportion and TGF-β1 level in combination. It aims to systematically analyze the synergistic immune effects of these two markers in AR with comorbid AH and develop a composite prediction model that integrates "clinical characteristics + immune indicators" to enhance the accuracy of recurrence prediction. The findings not only facilitate the early identification of children at high risk of recurrence for formulating preemptive strategies, but also enrich the immunological theory underlying pediatric upper airway chronic inflammation. This study promotes a paradigm shift from symptomatic treatment to targeted etiology-based interventions, ultimately enhancing long-term patient outcomes.

Materials and Methods

Study Design

This research was carried out at our institution from February 2023 to June 2024. Referring to previous studies (12), the overall prevalence was assumed to be 50%, with 70% positive cases and 30% positive controls. 5% type I error rate ($Z\alpha/2=1.96$), and 80% power ($Z\beta=0.84$), the initial calculation indicated a need for 114 subjects per group (by G-power 3.1). This number was increased to 125 per group, anticipating a 10% rate of attrition or non-evaluable data (due to loss to follow-up, incomplete data collection, etc.).

Inclusion and Exclusion Criteria

Pediatric patients aged 3 to 12 years, who fulfilled AR (2022) (13) or AH (2024) (14) diagnostic guidelines, and whose legal guardians had provided written informed consent were enrolled in this study. The exclusion criteria encompassed: a concurrent diagnosis of nasal polyps, sinusitis, or benign/malignant adenoid tumors; the presence of congenital immunodeficiency or autoimmune disorders; systemic or high-potency topical corticosteroid or immunosuppressive therapy within the preceding three-month period; and mortality occurring during the treatment or follow-up phases.

Study Subjects and Follow-up

We finally enrolled 127 children with AR (AR group) and 134 children with AR combined with AH (AR+AH group). After being hospitalized, each child received a standardized therapeutic regimen in conformity with the given medical instructions. The AR+AH group underwent a one-year prognostic follow-up (once per month, via outpatient clinic or telephone) to record the AH recurrence rate. Recurrence criteria: re-emergence of nasal congestion/snoring ≥2 episodes/week for ≥4 consecutive weeks (excludinglaboratory-confirmed upper respiratory tract infections); or polysomnography indicating an obstructive apnea-hypopnea index (OAHI) ≥5 events/hour. Recurrence assessment was performed by two independent otolaryngologists blinded to biomarker results. The study has been approved by the ethics committee of our hospital, and all guardians of the study subjects signed an informed consent form.

Sample Collection and Testing

Before treatment initiation and upon treatment completion, a 3 mL sample of morning fasting venous blood was collected from each enrolled child for subsequent analysis. The samples were divided into two portions. One was subjected to density gradient centrifugation to isolate peripheral blood mononuclear cells (PBMCs) for Treg detection. The isolated PBMCs were resuspended to 1×10 cells/mL and stained with anti-CD4-FITC (clone RPA-T4), anti-CD25-PE-Cy7 (clone M-A251), and anti-FoxP3-APC (clone 236A/E7), with isotype controls for 30 minutes at 4 °C protected from light. This was followed by PBS washing, addition of fixation/permeabilization working solution, and incubation for 45 minutes at 4 °C. Intracellular staining was then performed using a FoxP3-APC antibody. Data acquisition was carried out on a BD FACSCanto II flow cytometer. Cell gating strategy: whole blood (SSC/FSC) → CD4+ T cells → Tregs ($CD25^+FoxP3^+$: Treg subset was characterized as those cells co-expressing CD25 and FoxP3). Laser intensity and photomultiplier tube voltage were calibrated daily using FlowCheck Pro microspheres. A coefficient of variation (CV) exceeding 5% necessitated mandatory recalibration.

For serum separation, another set of samples was collected in coagulation-promoting tubes. These tubes were allowed to stand at room temperature for 30 minutes before being centrifuged at 1505 × g for 15 minutes. The resultant serum was then analyzed for TGF-β1 concentration via ELISA. The process began with the activation of TGF-β1 in blood samples by mixing with an equal volume of 1N HCl (1:1 volume) and a 10-minute room temperature incubation. Subsequent to activation, a neutralizing buffer (1M HEPES, pH 7.4) was added. A centrifugation step (10,000×g, 10 minutes) was then employed to clarify the solution by sedimenting any precipitate. In the coated plate, 100 μL of standard and sample solutions were added per well and subjected to a 2-hour room-temperature incubation with orbital shaking. The plate was washed thrice, followed by the sequential addition of an antibody and a substrate solution. Protected from light, the plate was developed for 30 minutes prior to the reaction being terminated with a stop solution. Sample concentrations were derived from the standard curve following the measurement of optical density (OD450) using a microplate reader. Quality control samples (low: ~50 pg/mL; high: ~800 pg/mL) were run on each plate, and a CV of less than 15% was deemed acceptable.

Statistical Analysis

GraphPad Prism 9.3 software was used for graphing and statistical analysis. Categorical variables [n (%)] were compared using Chi-square tests. Continuous data were confirmed to follow a normal distribution by the Shapiro-Wilk test ($P>0.05$); between-group comparisons employed independent t-tests and within-group analyses utilized paired t-tests. Receiver operator characteristic (ROC) curve analysis (optimal cutoff by Youden index) was adopted for diagnostic performance assessment. Combined detection was modeled via logistic regression. No multiple comparison adjustment was applied due to the exploratory nature of biomarker combinations. $P<0.05$ indicated the presence of statistical significance.

Results

Comparison of Baseline Characteristics between AR and AR+AH Groups

No statistically significant differences were observed in age, sex, or AR duration between AR and AR+AH groups ($P>0.05$). This ensures a low impact from confounding factors and establishes comparability between the two cohorts. However, the A/N (measured by nasal endoscopy, expressed as a ratio with-

Table I Clinical data of the AR and AR+AH groups.

	AR group (n=127)	AR+AH group (n=134)	t or χ^2 values	P values
Age	6.65±2.56	6.49±2.68	0.496	0.620
Sex			0.630	0.427
boys	62 (48.82)	72 (53.73)		
girls	65 (51.18)	62 (46.27)		
Family History of AR			1.433	0.231
have	34 (26.77)	45 (33.58)		
no	93 (73.23)	89 (66.42)		
Family History of AH			1.818	0.178
have	12 (9.45)	20 (14.93)		
no	115 (90.55)	114 (85.07)		
Course of AR (months)	4.06±1.50	3.91±1.21	0.860	0.390
Smoking parents			0.389	0.533
yes	88 (69.29)	88 (65.67)		
no	39 (30.71)	46 (34.33)		
A/N	0.46±0.11	0.84±0.07	33.104	<0.001

Table II Diagnostic effect of Treg and TGF-β on AR complicated with AH.

	Cut-off	Sensitivity (%)	Specificity (%)	AUC (95%CI)	P values
Treg (%)	<5.940	85.07	64.57	0.806 (0.752–0.860)	<0.001
TGF-β1 (ng/mL)	<4.835	70.90	69.29	0.723 (0.659–0.787)	<0.001
Treg+TGF-β1	>0.407	91.04	71.65	0.858 (0.811–0.906)	<0.001

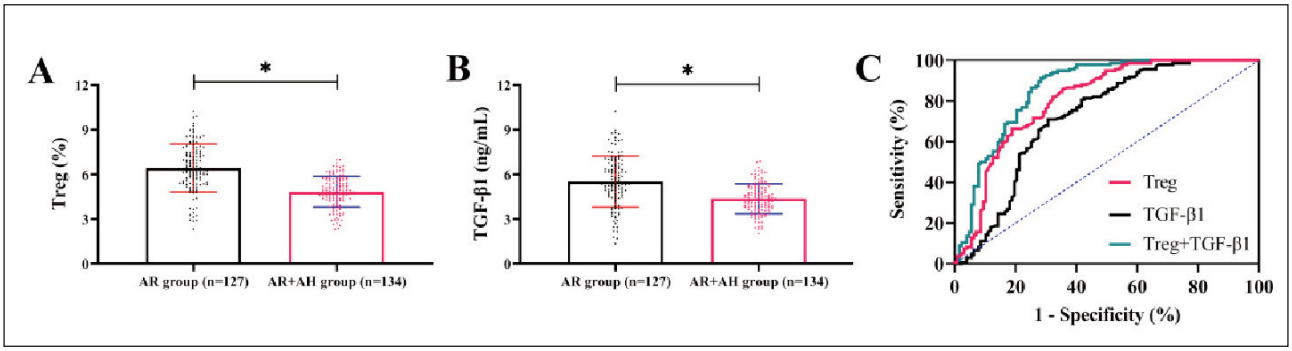

Figure 1 Comparison of Treg, TGF-β1 between AR and AR+AH groups (Error bars: SD; n=127 for AR, n=134 for AR+AH). (A) Comparison of Tregs. (B) Comparison of TGF-β1. (C) Diagnosing AH complications in AR pediatric patients through Treg and TGF-β detection.

Table III Changes in Treg and TGF-β1 before and after treatment.

Groups		Before treatment	After treatment	t values	P values
AR group (n=127)	Treg (%)	6.43±1.61	7.55±1.64	5.503	<0.001
	TGF-β1 (ng/mL)	5.51±1.72	7.03±1.33*	7.867	<0.001
AR+AH group (n=134)	Treg (%)	4.83±1.04	5.50±1.45	4.361	<0.001
	TGF-β1 (ng/mL)	4.37±1.00	5.42±1.47*	6.827	<0.001

Note: vs. AR group *P<0.05.

Table IV Clinical data of children with and without recurrence of AH.

	Non-recurrence (n=92)	Recurrence (n=42)	t or χ^2 values	P values
Age	7.02±2.85	5.33±1.80	3.529	<0.001
Sex			0.286	0.593
boys	48 (52.17)	24 (57.14)		
girls	44 (47.83)	18 (42.86)		
Family History of AR			0.724	0.125
have	30 (32.61)	15 (35.71)		
no	62 (67.39)	27 (64.29)		
Family History of AH			0.819	0.366
have	12 (13.04)	8 (19.05)		
no	80 (86.96)	34 (80.95)		
Course of AR (months)	3.83±1.20	4.10±1.23	1.196	0.234
Smoking parents			0.899	0.343
yes	58 (63.04)	30 (71.43)		
no	34 (36.96)	12 (28.57)		
A/N	0.61±0.08	0.66±0.08	2.870	0.005

out units) ratio of the AR+AH group was higher than that of the AR group (P<0.001), because A/N is a direct manifestation of AH (*Table I*).

Diagnostic Value of Treg Cells and TGF-β1 for AH in AR Patients

Relative to the AR group, patients with AR complicated by AH showed decreased Treg and TGF-β1 levels—24.88% and 20.69% lower, respectively (P<0.001). The diagnostic performance of each marker alone for identifying AH among AR children, as assessed by ROC curve analysis, produced AUC values of 0.806 for Treg and 0.723 for TGF-β1 (P<0.001). When combined into an integrated diagnostic index, the AUC increased to 0.858, accompanied by 91.04% sensitivity and 71.65% specificity (*Figure 1* and *Table II*).

Changes in Treg and TGF-β1

Both groups exhibited significant post-treatment increases in Treg and TGF-β1 levels (P<0.001). The AR group, however, demonstrated greater increases in Treg (17.42%) and TGF-β1 (27.59%) compared to the AR+AH group (P<0.001) (*Table III*).

Follow-up Outcomes

All children in the AR+AH group completed the one-year follow-up. AH recurrence occurred in 42 cases. While no differences were found in sex or family history between children with and without recurrence (P>0.05), those who experienced recurrence were younger and had higher A/N ratios (P<0.05) (*Table IV*).

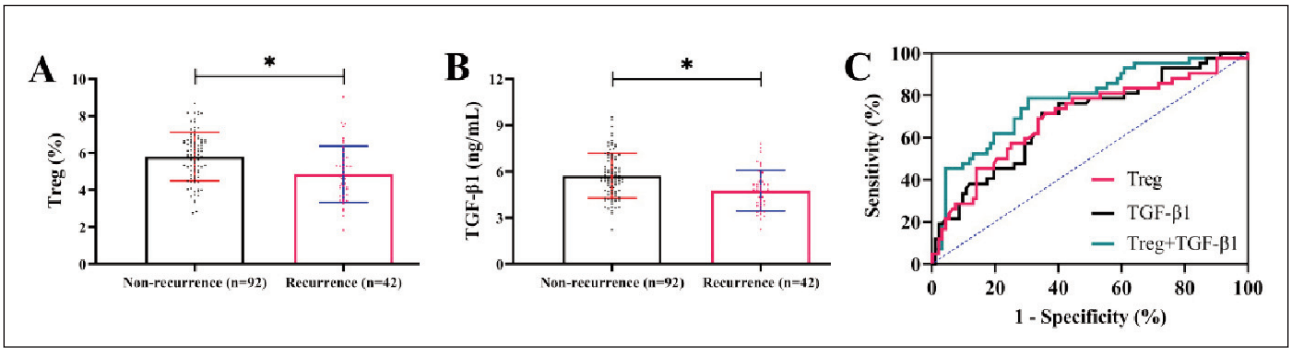

Figure 2 Comparison of Treg, TGF-β1 between recurrence and non-recurrence groups (Error bars: SD; n=92 for non-recurrence, n=42 for recurrence). (A) Comparison of Tregs. (B) Comparison of TGF-β1. (C) Diagnosing AH recurrence through Treg and TGF-β detection.

Table V Diagnostic efficacy of Treg and TGF-β in diagnosing AH recurrence.

	Cut-off	Sensitivity (%)	Specificity (%)	AUC (95%CI)	P values
Treg	<5.940	85.07	64.57	0.806 (0.752–0.860)	<0.001
TGF-β1	<4.835	70.90	69.29	0.723 (0.659–0.787)	<0.001
Treg+TGF-β1	>0.407	91.04	71.65	0.858 (0.811–0.906)	<0.001

Table VI Multivariate analysis of factors affecting recurrence in ah prognosis.

	B	SE	Wald χ^2	P values	OR	95%CI
Treg	-0.585	0.174	11.28	0.001	0.557	0.396–0.784
TGF-β1	-0.615	0.179	11.804	0.001	0.541	0.381–0.768
Age	-0.34	0.101	11.392	0.001	0.712	0.584–0.867
A/N	8.144	2.901	7.88	0.005	1.326	1.178–4.647

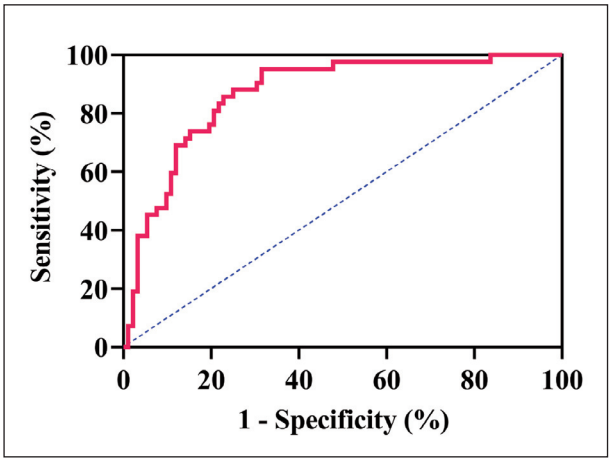

Figure 3 Risk prediction model based on multivariate prognostic recurrence of AH. When the cut-off value was >0.213, the sensitivity and specificity of the model for predicting the prognosis recurrence of AH were 95.24% and 68.48% (AUC=0.869).

Prognostic Value of Treg and TGF-β1 for AH Recurrence

Post-treatment Treg and TGF-β1 levels were lower in recurrence patients compared to non-recurrence cases (P<0.05). The AUC values for predicting recurrence using Treg and TGF-β1 individually were 0.806 and 0.858, respectively. The combined use of both biomarkers yielded a sensitivity of 91.04%, specificity of 71.65%, and an AUC of 0.858 (*Figure 2* and *Table V*).

Development of a Predictive Model for AH Recurrence

To create a prognostic recurrence model for AH, a multivariate regression analysis was conducted utilizing the age and A/N ratios of children, categorized by recurrence status, alongside the biomarkers Treg and TGF-β1. This integrated model outperformed the

combined Treg-TGF-β1 assay in ROC analysis, achieving an AUC of 0.869 (95%CI=0.806–0.933). It also exhibited high sensitivity (95.24%) and specificity (68.48%) for identifying patients at risk of recurrence (cut-off>0.213) (*Table VI* and *Figure 3*).

Discussion

The comorbid mechanism of AR and AH is jointly fueled by disorders in the immune microenvironment and anatomical anomalies of the upper airway (15). Treg, serving as key modulators of immune tolerance, hinders inflammatory reactions through the secretion of cytokines like transforming growth factor-β1 (TGF-β1). Aberrant expression of TGF-β1, however, may contribute to fibrotic changes in adenoid tissue (16). In the present study, our initial observation revealed a marked reduction in both Treg and TGF-β1 levels among children with concurrent AR and AH. This finding implies their possible involvement in the onset of AH in AR-affected children. As immunosuppressive cells, a decline in Treg count may trigger excessive activation of effector T cells (e.g., Th2 cells), thereby intensifying the inflammatory response in the nasopharyngeal region; while a drop in TGF-β1 levels may diminish its ability to suppress adenoid hyperplasia (17, 18). It is important to highlight that while individual detection of Treg or TGF-β1 offers some diagnostic value for AH in children with AR, combining these two markers yields a more enhanced diagnostic performance. This outcome aligns with findings from asthma-related research, where the combined detection of the Treg-TGF-β1 axis was shown to boost the accuracy of predicting immune thrombocytopenia (19). A novel aspect of this work is the first-ever application of this specific set of immune markers in diagnosing AR combined with AH. This approach moves beyond conventional imaging, offering new insight into immune-mediated diseases.

Through further analysis, we found that Treg and TGF-β1 levels underwent changes as treatment progressed. Furthermore, these levels exhibit a strong predictive capacity for the recurrence of AH in children with AR and AH. This suggests that Treg + TGF-β1 detection may more thoroughly reflect the local immune balance in the adenoid, as it integrates information on immunosuppressive function and tissue remodeling activity. Besides, Treg and TGF-β1 levels differences between recurrent and non-recurrent cases highlight early immune imbalance as a potential trigger for recurrence. Previous work by Zhou Y et al. on allergic rhinitis also linked Th2-type inflammation persisting for more than three months to nasal mucosal remodeling (20), which provides support for our viewpoint.

To enhance prediction accuracy, we integrated clinical parameters (age, A/N ratio) with immune markers. This composite model achieved an AUC of 0.869, with a sensitivity of 95.24% and specificity of 68.48%. This model increased the sensitivity by 21% on the basis of ensuring the specificity of the combined detection of Treg and TGF-β1, which had a better reference value. This model boasts two key advantages: ① Multi-dimensional information integration: Age reflects biological characteristics, while the A/N ratio represents anatomical risks. When combined with immune indicators, these parameters enable effective risk stratification. Younger age correlated with higher recurrence risk, potentially due to immature immune regulation in preschool children and accelerated adenoid regrowth. ② Dynamic prediction capability: By including post-treatment Treg and TGF-β1 levels, the model addresses the shortcomings of traditional static evaluation methods. Clinically, this model serves as a practical decision-making tool: reducing overtreatment in low-risk cases, while guiding enhanced immunomodulation (like prolonging the course of nasal glucocorticoid treatment) or earlier surgical intervention in high-risk patients.

Based on the study results, the following translational strategies are put forward: ① incorporating Treg/TGF-β1 testing in standard assessments for AR with AH, especially when imaging is inconclusive. This can help clinical better develop the treatment strategy for children with AR combined with AH, and provide a more reliable guarantee for the prognosis of children; ② adjusting treatment based on Treg dynamics; ③ and developing machine-learning-assisted follow-up systems for real-time risk monitoring. Importantly, the cytokine TGF-β1 demonstrates biphasic activity—attenuating inflammation under low concentrations but accelerating fibrosis when highly expressed (21). Future studies should define thresholds separating protective versus pathological effects.

Undoubtedly, some constraints in this investigation deserve attention. For example, while the sample size was determined to maintain statistical power, the single-center origin of the data may introduce selection bias. Also, recurrence was monitored only over a one-year period, leaving longer-term outcomes uncertain and in need of further validation. And the lack of mechanistic studies means molecular pathways linking Tregs, TGF-β1, and adenoid fibrosis remain unclear. These limitations highlight the need for more thorough and extended research. In the future, we will add more cases and extend the follow-up time to verify the results of this study, so as to provide more reliable reference for clinical practice.

Conclusion

Treg and TGF-β1 co-measurements can enhance the diagnostic effectiveness for AR combined with AH. Moreover, by developing a composite

model, the prediction of recurrence risk can be optimized. Dysregulation of the Treg-TGF-β1 axis is likely the core immune mechanism underlying AR-AH comorbidity. Implementing combined testing and ongoing monitoring could offer valuable guidance for precise diagnosis and treatment. Future work should include larger, longer-term studies and explore therapeutic strategies targeting the Treg-TGF-β1 pathway.

Competing Interests

The authors report no conflict of interest.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

Not applicable.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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