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Simultaneous assessment of TNF-α, TIM-1, and TLR4 plasma levels for predicting the severity of allergic rhinitis

Istovremena procena nivoa TNF-α, TIM-1 i TLR4 u plazmi kao prediktora težine alergijskog rinitisa

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Abstract

Background/Aim. Allergic rhinitis (AR) is a chronic inflammation of the nasal mucosa caused by allergens. To date, some individual biomarkers, such as total immunoglobulin E (IgE), have been shown to be possible factors for the assessment of the severity of AR. The aim of the study was to determine the value of simultaneously measuring the levels of tumor necrosis factor (TNF)-α, T-cell immunoglobulin and mucin domain 1 (TIM-1), and toll-like receptor 4 (TLR4) as predictors of AR severity. **Methods.** The study included two groups of respondents: the AR group – ARG (n = 96), which consisted of patients with AR treated between March 2021 and May 2023, and the control group – CG (n = 60), which consisted of healthy individuals undergoing physical examinations during the same period. Levels of TNF-*a*, TIM-1, and TLR4 were compared between the ARG and CG, and their associations with disease severity were analyzed. **Results.** Logistic regression analysis revealed that elevated eosinophil counts, IgE, TNF-α, TIM-1, and TLR4 levels were independent risk factors for the occurrence of moderate-to-severe AR (odds ratio > 1, $p < 0.05$). The areas under the receiver operating characteristic curves for plasma TNF-α, TIM-1, and TLR4 levels in predicting disease severity were 0.889, 0.831, and 0.842, respectively, while the combined predictive value reached 0.932, indicating excellent diagnostic performance. **Conclusion.** The simultaneous measurement of plasma TNF-α, TIM-1, and TLR4 levels provides a novel and reliable approach for predicting the severity of AR. Their combined assessment demonstrates higher predictive accuracy than that of individual markers, offering potential value for disease stratification and clinical decision-making.

Keywords:
immunoglobulins; prognosis; rhinitis, allergic; severity of illness index; tumor necrosis factor-alpha.

Apstrakt

Uvod/Cilj. Alergijski rinitis (AR) je hronično zapaljenje nosne sluznice izazvano dejstvom alergena. Do sada se pokazalo da su pojedini biomarkeri, kao što je ukupni imunoglobulin E (IgE), mogući pokazatelji za procenu težine AR. Cilj rada bio je da se proceni vrednost istovremenog određivanja nivoa faktora nekroze tumora (*tumor necrosis factor*-TNF)-α, *T-cell immunoglobulin and mucin domain* (TIM)-1 i *toll-like* receptora 4 (TLR4) kao prediktora težine AR. **Metode.** U studiju su uključene dve grupe ispitanika: AR grupa – ARG (n = 96), koju su činili bolesnici lečeni od marta 2021. do maja 2023. godine, i kontrolna grupa – KG (n = 60), koju su činile zdrave osobe koje su tokom istog perioda bile pregledane od strane lekara. Nivoi TNF-*a*, TIM-1 i TLR4 upoređivani su između ARG i KG i analizirana je njihova povezanost sa težinom bolesti. **Rezultati.** Logistička regresiona analiza pokazala je da su povišen broj eozinofila i povišeni nivoi IgE, TNF-α, TIM-1 i TLR4 nezavisni faktori rizika od pojave umerenog do teškog AR (*odds ratio* > 1, $p < 0,05$). Površine ispod *receiver operating characteristic* krive za nivoe TNF-α, TIM-1 i TLR4 u plazmi, u predviđanju težine bolesti, iznosile su 0,889, 0,831 i 0,842, redom, dok je prediktivna vrednost kombinacije markera dostigla 0,932, što ukazuje na odličan dijagnostički učinak.

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Zaključak. Istovremeno merenje nivoa TNF-α, TIM-1 i TLR4 u plazmi pruža nov i pouzdan pristup za predviđanje težine AR. Procena njihove kombinacije pokazuje veću prognostičku tačnost od procene pojedinačnih markera, nudeći potencijalnu vrednost za stratifikaciju bolesti i donošenje kliničkih odluka.

Ključne reči:
immunoglobulini; prognoza; rinitis, alergijski; bolest, indeks težine; faktor nekroze tumora-alfa.

Introduction

Allergic rhinitis (AR) is a common non-infectious immune disorder characterized by variable inflammatory lesions of the nasal mucosa, which arises from immune cell infiltration and the release of various inflammatory cytokines upon exposure to allergens. Its prevalence has been steadily increasing worldwide, and environmental as well as lifestyle factors are believed to contribute to this trend [1]. In mild AR, patients often present with nasal obstruction, itching, sneezing, and rhinorrhea. In moderate-to-severe cases, additional symptoms such as dizziness and impaired memory and cognitive function may occur, which severely compromise both physical and mental health and substantially diminish quality of life [2]. Thoroughly discovering the factors related to the development and progression of AR and accurately assessing the severity of the disease are of great significance for implementing effective treatments and improving patients' quality of life. Tumor necrosis factor (TNF)-α, a systemic inflammatory cytokine, is a key player in immunity and inflammatory responses [3, 4]. T-cell immunoglobulin and mucin domain 1 (TIM-1), linked to atopic diseases, including AR and asthma, has been shown to play a role in regulating T helper (Th) type 2 (Th2) cell responses. Numerous studies have shown that Th type 1 (Th1) cell /Th2 cell immune imbalance acts as a vital immune basis for the development of AR [5, 6]. It is therefore inferred that there is a relationship between TIM-1 and the severity of AR. Toll-like receptor (TLR)4 – TLR4, a member of the TLR family, is a transmembrane receptor in the innate immune system that is involved in the pathogenesis of allergic diseases. Allergic reactions are induced or aggravated by allergen-induced endogenous stimuli that activate target cells through TLR-related signaling pathways [7].

In this study, the differences in the plasma TNF-α, TIM-1, and TLR4 levels were examined in patients with AR, and their associations with disease severity were analyzed to provide a reference for disease assessment and treatment guidance.

Methods

General data

The present study was reviewed and approved by the Hospital Research Ethics Committee of the Zhangjiagang Third People's Hospital (approval from March 5, 2021). A total of 96 patients with AR undergoing treatment in that hospital from March 2021 to May 2023 were selected as a case group (AR group – ARG).

The inclusion criteria were set as follows: patients who met relevant diagnostic criteria for AR according to the Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update and the Chinese Guidelines for the Diagnosis and Treatment of Allergic Rhinitis (2022 edition) [8, 9], which define AR as an immunoglobulin (Ig) E -mediated inflammatory disorder of the nasal mucosa characterized by nasal obstruction, itching, sneezing, and rhinorrhea. In this study, symptoms were required to be triggered by exposure to relevant allergens, including both inhalant allergens (dust mites, pollens, molds, animal dander) and food allergens known to induce IgE-mediated nasal symptoms [10], and to have persisted at least two years. Eligible participants also included those diagnosed with AR for the first time, those who or whose family members had signed the informed consent form, and those with normal cardiac, hepatic, and renal function.

The exclusion criteria involved: patients with a history of trauma surgery or respiratory infection in the last month, those taking anti-allergic drugs or other related drugs within one month before enrollment, those with connective tissue diseases, pregnant or lactating women, those complicated with severe deviation of nasal septum, nasal polyps or sinonasal inflammation, those with atopic dermatitis or asthma, those with respiratory system tumor or other serious diseases, those with systemic acute or chronic infectious diseases, or those with polycythemia, severe anemia, leukocytosis, or other hematologic diseases.

Moreover, 60 healthy people undergoing physical examinations in the same period were incorporated into a control group (CG). The inclusion criteria were set as follows: patients who or whose family members had signed the informed consent form, and those with normal cardiac, hepatic, and renal function. The exclusion criteria involved: patients with a history of trauma surgery or respiratory infection in the last month, those taking anti-allergic drugs or other related drugs within one month before enrollment, those with connective tissue diseases, pregnant or lactating women, those complicated with severe deviation of nasal septum, nasal polyps, or sinonasal inflammation, those with atopic dermatitis or asthma, those with respiratory system tumor or other serious diseases, those with systemic acute or chronic infectious diseases, or those with polycythemia, severe anemia, leukocytosis, or other hematologic diseases.

Measurements

To determine allergen sensitization, all subjects in ARG underwent standardized allergen testing upon enrollment.

Sensitization was evaluated using serum specific IgE (sIgE) testing performed on an automated immunoassay analyzer (ImmunoCAP®, Thermo Fisher Scientific), following the manufacturer's instructions. The allergen panel included the following major inhalant and food allergens: house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*); pollen allergens (grass pollen, tree pollen, and weed pollen mix); food allergens (egg, milk, peanut, shellfish); other allergens (defined as mold spores, animal dander, and miscellaneous food allergens not included in the primary inhalant panel). A sIgE level ≥ 0.35 kU/L was considered positive and indicative of sensitization.

After overnight fasting for 7–8 hr, 4–5 mL of venous blood was collected from the cubital vein of each subject in the morning. The samples were placed into a special anticoagulation tube containing sodium citrate and centrifuged at 3,000 revolutions *per* min (r/min) for 10 min with a centrifugal radius of 10 cm. The plasma was then separated and stored at -70 °C until further analysis. The levels of TNF-*α*, TIM-1, and TLR4 were measured using an enzyme-linked immunosorbent assay (ELISA) in strict accordance with the instructions provided in the corresponding assay kits (Beijing Solarbio Science & Technology Co., Ltd., China).

Eosinophil counts (ECs) were determined from blood samples using an automatic blood cell analyzer (i2000, Sysmex). IgE levels were measured using an automatic protein analyzer (BN™, II Siemens) and corresponding reagents.

Assessment of the severity of allergic rhinitis

AR was classified into mild and moderate-to-severe cases. Mild AR was defined according to the ARIA guidelines as the presence of symptoms such as nasal obstruction, itching, watery rhinorrhea, and paroxysmal sneezing, without sleep disturbance, impairment of daily activities, work, or school performance, and with symptoms not considered troublesome. Moderate-to-severe AR was defined as the presence of one or more of the following: sleep disturbance, impairment of daily activities, leisure, sport, work, or school performance, or symptoms considered troublesome by the patient.

Evaluation of outcomes

Plasma TNF-α, TIM-1, and TLR4 levels were compared between ARG and CG.

According to the severity of AR, ARG was subdivided into a mild group and a moderate-to-severe group. Comparisons were made between the clinical data and plasma TNF-α, TIM-1, and TLR4 levels of the mild and moderate-to-severe groups. Clinical data included gender (male or female), age, family history (yes or no, defined as a documented history of AR, asthma, or atopic dermatitis in first-degree relatives), smoking history (yes or no), drinking history (yes or no), allergen sensitivity (pollen, dust mites, food, or other), EC, and IgE level.

Statistical analysis

Statistical data processing was conducted in the SPSS software, version 23. The measurement data were expressed as mean ± standard deviation (SD) and analyzed using the *t*-test, whereas the count data were presented as number (percentage) – n (%) and analyzed using the χ^2 test. The correlations of plasma TNF-α, TIM-1, and TLR4 levels with the severity of AR were explored using point-biserial correlation analysis. Logistic regression analysis was conducted to discover the factors influencing the severity of AR. The receiver operating characteristic (ROC) curves were plotted to analyze the values of plasma TNF-α, TIM-1, and TLR4 levels for predicting the severity of AR. Differences were considered statistically significant at $p < 0.05$, and highly significant at $p < 0.01$.

Results

The plasma TNF-α, TIM-1, and TLR4 levels were higher in ARG than those in CG ($p < 0.01$) (Table 1, Figure 1).

Among the ARG (n = 96), 37 patients were classified as mild AR (mild group) and 59 patients as moderate-to-severe AR (moderate-to-severe group). Compared with the mild group, the moderate-to-severe group showed significantly higher EC, IgE, TNF-α, TIM-1, and TLR4 levels ($p < 0.01$). No significant differences were observed between the two subgroups in terms of gender distribution, family history, smoking history, drinking history, and allergen sensitivity ($p > 0.05$) (Table 2).

The results of point-biserial correlation analysis showed that the plasma TNF-α, TIM-1, and TLR4 levels were positively related to the severity of AR ($r > 0.5$, $p < 0.01$) (Table 3).

Table 1

Plasma TNF-α, TIM-1, and TLR4 levels in allergic rhinitis and control groups of respondents

Biomarker	Group		*t*	*p*
	allergic rhinitis (n = 96)	control (n = 60)		
TNF-α (ng/L)	24.63 ± 5.81	13.95 ± 2.03	13.711	< 0.001
TIM-1 (ng/mL)	296.53 ± 21.61	98.64 ± 13.75	63.331	< 0.001
TLR4 (μg/L)	64.52 ± 16.34	21.58 ± 10.72	18.060	< 0.001

TNF – tumor necrosis factor; TIM-1 – T-cell immunoglobulin and mucin domain 1; TLR4 – toll-like receptor 4; n– number.

All values are given as mean values ± standard deviation.

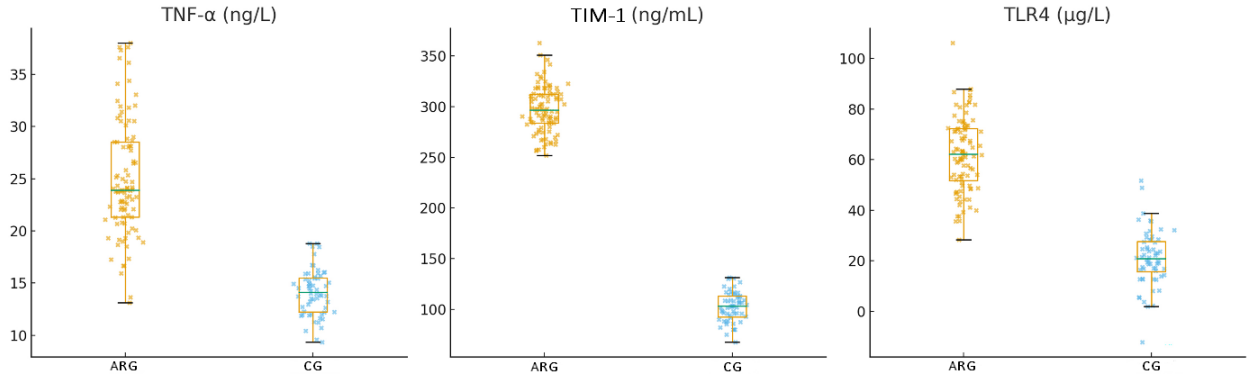

Fig. 1– Plasma TNF-α, TIM-1, and TLR4 levels.
Data are shown as boxplots with overlaid scatter points. The box indicates the interquartile range, the horizontal line within the box marks the median, and whiskers show variability outside the upper and lower quartiles.
TNF– tumor necrosis factor; TIM-1 – T-cell immunoglobulin and mucin domain 1; TLR4 – toll-like receptor 4; ARG – allergic rhinitis group; CG – control group.

Table 2

Clinical data and plasma TNF-α, TIM-1, and TLR4 levels in mild and moderate-to-severe groups of patients with allergic rhinitis

Parameter	Allergic rhinitis group		Statistical value	*p*
	mild (n = 37)	moderate-to-severe (n = 59)		
Gender				
male	19 (51.35)	31 (52.54)	$\chi^2 = 0.013$	0.910
female	18 (48.65)	28 (47.46)		
Age, years	40.79 ± 8.73	39.97 ± 9.54	$t = 0.423$	0.673
Family history				
yes	16 (43.24)	21 (35.59)	$\chi^2 = 0.562$	0.454
no	21 (56.76)	38 (64.41)		
Smoking history				
yes	9 (24.32)	12 (20.34)	$\chi^2 = 0.211$	0.646
no	28 (75.68)	47 (79.66)		
Drinking history				
yes	11 (29.73)	16 (27.12)	$\chi^2 = 0.077$	0.782
no	26 (70.27)	43 (72.88)		
Allergen sensitivity				
pollen	11 (29.73)	20 (33.90)	$\chi^2 = 0.270$	0.966
dust mite	18 (48.65)	28 (47.46)		
food	4 (10.81)	5 (8.47)		
others	4 (10.81)	6 (10.17)		
Eosinophil count ($\times 10^9$/L)	0.12 ± 0.03	0.17 ± 0.04	$t = 6.533$	< 0.001
IgE (IU/mL)	81.56 ± 23.39	116.28 ± 34.59	$t = 5.378$	< 0.001
TNF-α (ng/L)	20.39 ± 4.69	27.28 ± 4.81	$t = 6.896$	< 0.001
TIM-1 (ng/mL)	282.13 ± 15.60	305.55 ± 19.96	$t = 6.065$	< 0.001
TLR4 (μg/L)	52.76 ± 11.57	71.91 ± 14.52	$t = 6.781$	< 0.001

IgE – immunoglobulin E. For other abbreviations, see Table 1.
All values are given as numbers (percentages) or mean values ± standard deviation.

Table 3

Correlations of plasma TNF-α, TIM-1, and TLR4 levels with the severity of allergic rhinitis

Biomarker	*r*	*p*
TNF-α	0.580	< 0.001
TIM-1	0.530	< 0.001
TLR4	0.573	< 0.001

For abbreviations, see Table 1.

Logistic regression analysis was conducted with the indicators presenting significant differences as independent variables and the severity of AR in patients as dependent variables (1 = moderate-to-severe, 0 = mild). It was discovered that high levels of EC [odds ratio (OR): 1.303, 95% confidence interval (CI): 1.021–1.814], IgE (OR: 1.042, 95%

CI: 1.022–1.062), TNF-α (OR: 1.411, 95% CI: 1.146–1.736), TIM-1 (OR: 1.083, 95% CI: 1.021–1.148), and TLR4 (OR: 1.169, 95% CI: 1.076–1.270) were risk factors for moderate-to-severe AR (OR > 1, $p < 0.01$) (Table 4, Figure 2).

The ROC curves were plotted with plasma TNF-α, TIM-1, and TLR4 levels as the test variables and the severity of AR as the state variable (1 = moderate-to-severe, 0 = mild) (Figure 3). It was uncovered that the areas under the ROC curves (AUCs) of plasma TNF-α, TIM-1, and TLR4 levels for predicting the severity of AR were 0.889, 0.831, and 0.842, respectively, and the AUC of the combination of the three indicators reached 0.932, demonstrating certain predictive value (Table 5).

Table 4

Results of multivariate logistic regression analysis on influencing factors for the severity of allergic rhinitis

Independent variable	β	SE	Wald χ^2	*p*	OR	95% CI
Eosinophil count	27.896	6.809	16.787	< 0.001	1.303	1.021–1.814
IgE	0.041	0.010	17.411	< 0.001	1.042	1.022–1.062
TNF-α	0.344	0.106	10.522	0.001	1.411	1.146–1.736
TIM-1	0.079	0.030	6.990	0.008	1.083	1.021–1.148
TLR4	0.156	0.042	13.557	< 0.001	1.169	1.076–1.270
Constant	-40.113	11.465	12.242	< 0.001	< 0.001	-

SE – standard error; OR – odds ratio; CI – confidence interval.
For other abbreviations, see Tables 1 and 2.

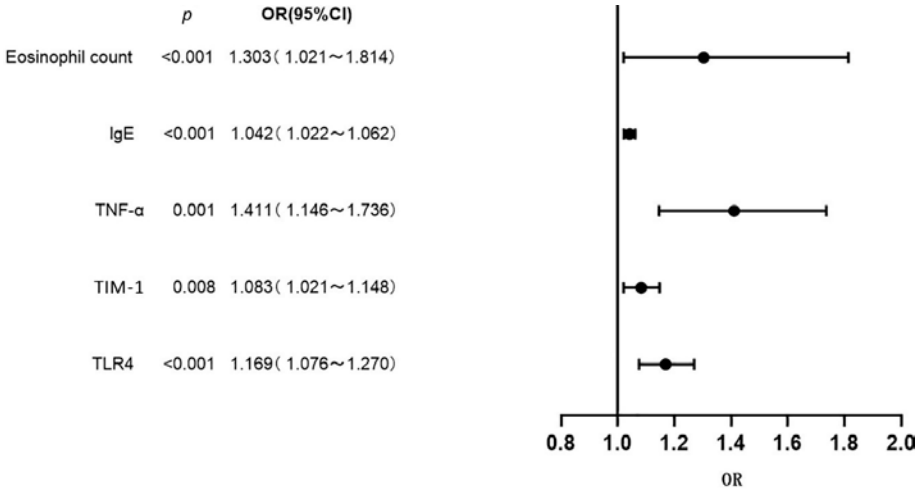

Fig. 2 – Forest plot of clinical characteristics based on multivariate logistic regression analysis.
IgE – immunoglobulin E; OR – odds ratio; CI – confidence interval. For other abbreviations, see Figure 1.

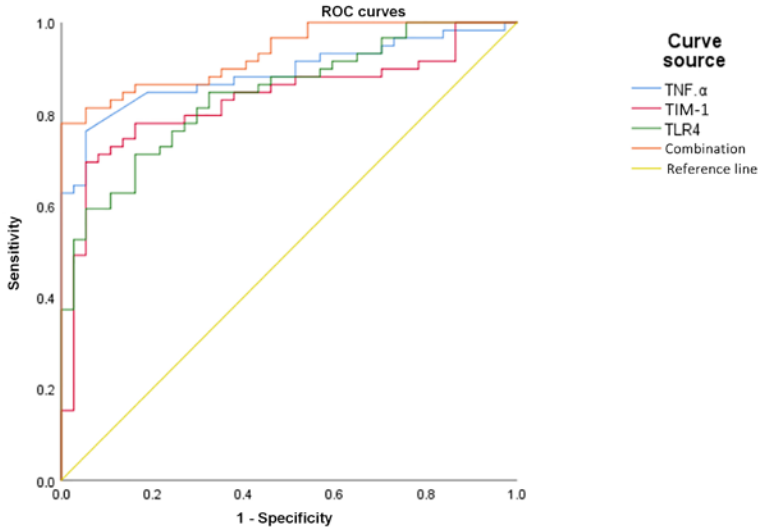

Fig. 3 – ROC curves of plasma TNF-α, TIM-1, and TLR4 levels for predicting the severity of allergic rhinitis.
ROC – receiver operating characteristic. For other abbreviations, see Figure 1.

Table 5

Predictive values of plasma TNF-α, TIM-1, and TLR4 levels for the severity of allergic rhinitis

Variable	AUC	SE	*p*	95% CI	Cut-off value	Sensitivity	Specificity	Youden index
TNF-α	0.889	0.033	< 0.001	0.824–0.955	23.930 ng/L	0.864	0.703	0.567
TIM-1	0.831	0.043	< 0.001	0.747–0.915	286.460 ng/mL	0.847	0.622	0.469
TLR4	0.842	0.039	< 0.001	0.765–0.918	55.705 μg/L	0.847	0.676	0.523
Combination	0.932	0.024	< 0.001	0.885–0.978	-	0.932	0.649	0.581

AUC – area under the curve; SE – standard error; CI – confidence interval.
For other abbreviations, see Table 1.

Discussion

Significant progress has been made in understanding the pathogenesis of AR over the past few years. The pathogenesis of AR involves many inflammatory mediators, cytokines, and other related molecules, and the disease is a result of the combined action of multiple factors and pathways [11, 12]. In this study, we demonstrated that plasma TNF-α, TIM-1, and TLR4 levels were significantly elevated in patients with AR compared with healthy controls, and that higher levels of these markers were independently associated with an increased risk of moderate-to-severe disease. Importantly, ROC curve analyses revealed that each biomarker individually had good predictive accuracy, while their combined assessment yielded an excellent discriminative ability (AUC = 0.932), underscoring the value of a multi-marker strategy in stratifying disease severity.

TNF-α is a well-established pro-inflammatory cytokine that mediates multiple aspects of allergic responses, including recruitment of eosinophils and amplification of Th2-driven inflammation [13, 14]. Recent studies have shown that TNF-α is abundantly expressed in mast cells and epithelial cells of the nasal mucosa in AR [15]. Likewise, elevated levels of TNF receptor-related cytokines can be detected in AR patients [16]. *In vivo* experiments further demonstrated that TNF-α inhibition can slow the progression of AR [17]. Collectively, these findings suggest a critical role of TNF-α in the pathogenesis of AR. Consistent with previous evidence, our results revealed that plasma TNF-α levels were significantly elevated in patients with AR, particularly in those with moderate-to-severe disease, suggesting that TNF-α may function not only as a key inflammatory mediator but also as a reliable biomarker indicative of disease severity.

TIM-1 has been recognized as a susceptibility gene in atopic diseases, acting as a modulator of Th2 cell activation and cytokine production [18, 19]. In AR, the Th1/Th2 imbalance, characterized by enhanced Th2 responses, plays a central role in disease pathogenesis [20]. Notably, the discovery of TIM-1 expression in Th2 cells sheds new light on the pathogenic mechanism of allergic diseases [21, 22]. Our data showing elevated TIM-1 levels in moderate-to-severe patients support the hypothesis that TIM-1 contributes to disease exacerbation by promoting Th2 dominance. These results align with previous animal and clinical studies linking the *TIM-1* gene family to airway hyperresponsiveness and IgE production [23, 24], thereby reinforcing its mechanistic involvement in allergic inflammation.

TLR4, a pattern recognition receptor, bridges innate and adaptive immunity by recognizing pathogen- and damage-associated molecular patterns [25, 26]. Recent evidence indicates that TLR4 signaling participates in allergic airway inflammation by activating downstream nuclear factor *kappa* B (NF-*κ*B) and mitogen-activated protein kinase (MAPK) pathways, thereby enhancing cytokine release [27, 28]. The elevated TLR4 levels observed in our cohort, particularly among patients with more severe disease, support the view that innate immune dysregulation contributes to disease progression in AR.

Although each of the three tested markers demonstrated substantial predictive power, their combination significantly enhanced diagnostic performance. This finding emphasizes the multifactorial nature of AR pathogenesis, where both innate and adaptive immune mechanisms converge to drive disease progression. A multi-marker panel may therefore provide a more accurate reflection of the complex immunopathology and enable better risk stratification compared with single biomarkers. Clinically, such a combined assessment could facilitate early identification of patients at risk for severe disease and guide timely initiation of targeted therapies. From a practical perspective, simultaneous measurement of TNF-α, TIM-1, and TLR4 in plasma is minimally invasive and feasible in routine clinical practice. Their combined predictive value could complement traditional clinical assessments, such as symptom scores and eosinophil/IgE levels, providing clinicians with an objective tool for disease monitoring. Moreover, these markers may also serve as potential therapeutic targets. For instance, anti-TNF-α has been evaluated in allergic airway diseases, and modulation of TIM-1 or TLR4 pathways could represent novel strategies for preventing disease progression [29–31].

Limitations of the study

Several limitations of this study should be acknowledged. First, this was a single-center study with a relatively small sample size, which may limit the generalizability of the findings. Second, only baseline biomarker levels were assessed; dynamic changes during disease progression or treatment were not evaluated. Third, mechanistic experiments to directly verify the causal role of these molecules in AR were not conducted. Future multicenter studies with larger cohorts are needed to validate the predictive value of these biomarkers. Additionally, longitudinal investigations and mechanistic studies could

provide further insights into their roles in disease pathogenesis and therapeutic responsiveness.

Conclusion

This study highlights the significant associations of TNF-α, TIM-1, and TLR4 with allergic rhinitis severity. The study also demonstrates the superior predictive accuracy of their combined assessment. These findings provide a novel framework for biomarker-based stratification in allergic rhinitis and may inform both clinical decision-making and the development of targeted therapeutic strategies.

Conflict of interest

The authors declare no conflict of interest.

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