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Uncovering Type 2 inflammatory profiles in severe eosinophilic asthma with nasal polyps: the role of transcript-protein ratios and *Staphylococcus aureus* enterotoxin B-specific IgE

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Abstract

Background: Severe eosinophilic asthma with nasal polyps (SEAwNP) is a clinical phenotype characterized by elevated peripheral eosinophil counts and heightened type 2 (T2) inflammation.
Objective: In this study, we aimed to compare the transcript/protein expression ratios of factor XIII-A (F13A), B-cell activating factor (BAFF), interleukin-5 (IL-5), and thymus- and activation-regulated chemokine (TARC), as well as serum Staphylococcus aureus Enterotoxin B-Specific IgE (SEB-IgE) levels, among patients with SEAwNP, SEA without nasal polyps (NP), NP without asthma, and healthy controls groups.
Material and Methods: A total of 73 participants were enrolled and stratified into four groups: SEAwNP (n=20), SEA without NP (n=18), NP without asthma (n=15), and healthy controls (n=20). Peripheral blood samples were analyzed using real-time quantitative PCR and protein levels using ELISA for periostin, F13A, BAFF, IL-5, and TARC. Serum SEB-IgE levels were also assessed.
Results: SEAwNP patients exhibited significantly elevated transcript/protein expression ratios of periostin, F13A, BAFF, and IL-5 compared to SEA without NP (p=0.007, p=0.001, p=0.019, and p=0.017, respectively). Furthermore, periostin, F13A, BAFF, IL-5, and TARC transcript/protein ratios were significantly higher in SEAwNP patients compared to healthy controls (p=0.0001, p=0.001, p=0.003, p=0.014, and p=0.02, respectively). SEB-IgE positivity rates were 45% in SEAwNP, 38% in SEA without NP, 13% in NP without asthma, and 0% in healthy controls.
Conclusion: SEAwNP is associated with elevated transcript/protein expression ratio of key T2 inflammatory mediators and increased SEB-IgE positivity, supporting their potential role in the immunopathogenesis of this phenotype. These findings underscore the importance of

KEYWORDS
Asthma;
B-cell activating factor;
Eosinophil;
Factor 13A;
Interleukin 5;
Nasal polyp;
Periostin;
Staphylococcus aureus enterotoxin B;
Thymus-regulated chemokine

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biomarker profiling in distinguishing endotypes within the spectrum of eosinophilic airway diseases.

Introduction

Late-onset eosinophilic asthma represents a challenging phenotype within the spectrum of asthma because of its often refractory nature and suboptimal response to standard therapies. A distinct and clinically significant subgroup within this phenotype is characterized by the coexistence of nasal polyps (NP), which is frequently associated with heightened type 2 (T2) inflammatory responses. Notably, patients with severe eosinophilic asthma and nasal polyps (SEAwNP) demonstrate significantly elevated peripheral blood eosinophil counts compared to other eosinophilic asthma subtypes.[1-3]

The immunopathogenesis of SEAwNP is multifactorial and involves a complex interplay of inflammatory pathways. Proposed mechanisms include dysregulated leukotriene metabolism, persistent epithelial barrier dysfunction, and chronic activation by environmental stimuli or bacterial superantigens, such as those produced by *Staphylococcus aureus*. These stimuli promote the upregulation of epithelial-derived alarmin cytokines—thymic stromal lymphopoietin (TSLP), interleukin-25 (IL-25), and interleukin-33 (IL-33)—which in turn activate group 2 innate lymphoid cells (ILC2s), leading to enhanced interleukin-5 (IL-5) production and subsequent eosinophilic inflammation.[4-8]

In addition to IL-5, other mediators such as periostin and factor XIII-A (F13A) have been implicated in tissue remodeling and eosinophil recruitment. Furthermore, dysregulation of the fibrinolytic system and elevated levels of B-cell activating factor (BAFF) have been associated with chronic inflammation in both NP and eosinophilic asthma.[9-12]

Importantly, *S. aureus* enterotoxins (SE), particularly *S. aureus* enterotoxins B (SEB), are recognized as potent superantigens that can induce local IgE synthesis independent of systemic atopy. The presence of SEB-specific IgE (SEB-IgE), rather than allergen-specific IgE, has been frequently observed in patients with NP, especially in those with concomitant severe asthma.[7, 13-15]

While individual associations of periostin, BAFF, IL-5, TARC, F13A, and SEB-IgE with eosinophilic asthma and NP have been documented, there remains a lack of comprehensive comparative analysis across distinct patient subgroups. In this study, we aim to address this gap by evaluating the expression profiles of these key T2 biomarkers in patients with SEAwNP, SEA without NP, and NP without asthma, in comparison to healthy controls.

Methods

Participants

Adult patients who had been regularly followed for at least 1 year with a diagnosis of severe asthma at our clinic were included in this study. To investigate the impact of NP and eosinophilic inflammation, participants were categorized into four groups: SEAwNP, SEA without NP, NP without asthma, and a healthy controls group. Eosinophilic asthma was defined as blood eosinophil counts ≥ 300 cells/μL on at least two separate occasions during routine follow-up. The diagnosis of severe asthma was established in accordance with the Global Initiative for Asthma (GINA) criteria. The presence of NP was confirmed by paranasal sinus computed tomography and/or otolaryngologic (ENT) examination. All asthma patients enrolled in the study were receiving standard therapy consisting of high-dose inhaled corticosteroids (ICS)/long-acting ß2-agonists, and montelukast 10 mg tablets. People who had any additional systemic diseases other than their current diseases, who had used systemic steroids in the last 15 days, who had a history of infection in the previous month, or those who had an asthma exacerbation history in the last month were excluded from the study. Written informed consent was obtained from all the participants. The study was approved by the Research and Ethics Committee of Erciyes University (Decision no: 2020/595).

Demographic data, blood eosinophil and total IgE values, pulmonary function test results, and treatment history of the patients who were included in the study were obtained from the patient charts. Two tubes of (5 mL each,10 mL in total) blood samples were taken from the participants for ELISA and gene expression analyses.

The increased need for systemic corticosteroids in patients with SEAwNP raises the possibility that periostin, F13A, BAFF, IL-5, and TARC protein levels may be secondarily suppressed because of steroid therapy. It is important to consider that serum protein levels reflect not only production from peripheral blood mononuclear cells (PBMCs) but also contributions from endothelial and tissue sources. Therefore, in order to obtain a more accurate representation of biomarker activity and to account for potential discrepancies between gene expression and protein levels, we evaluated the transcript/protein expression ratios of periostin, F13A, BAFF, IL-5, and TARC.

Enzyme-linked immunosorbent assay (ELISA) Method

After centrifugation of the collected blood samples at 3000 g for 10 minutes, the plasma fraction was carefully extracted and transferred to separate reaction tubes. Subsequently, the levels of periostin (BTLAB, Cat No: E3226Hu), IL5 (BTLAB, Cat No: E0091Hu), TARC (BTLAB, Cat No: E0163Hu), F13A (BTLAB, Cat No: E1154Hu), and BAFF (BTLAB, Cat No: E6530Hu) cytokines were quantified utilizing the ELISA method, adhering strictly to the manufacturer's guidelines.

Total RNA Isolation

For RNA isolation from blood samples, 500 µl Trizol (Biorad, USA, CA, Cat No: 7326890) was added to 500 µl blood samples. Subsequently, total RNA isolation was carried out according to the manufacturer's instructions.

cDNA Synthesis

Isolated total RNA samples were reverse-transcribed into complementary DNA (cDNA) using an Evoscript universal cDNA master kit (Roche, Germany, Mannheim, Cat No: 07912439001) in final reaction volumes of 20 µL. All reactions were performed as specified in the manufacturer's protocol.

Determining Gene Expression Levels

In this study, gene expression levels were determined in total RNA samples isolated from blood using the Roche Light Cycler LC480 device and the real-time PCR method. Specific primer sequences targeting the *POSTN*, *IL5*, *TARC*, *F13A*, and *TNFSF13B* genes were utilized for this purpose, as detailed in Table 1. The assessment of gene expression levels was conducted in accordance with the manufacturer's instructions, employing the SYBR Green I Master Kit (Roche, Cat No: 04707516001, Germany).

SEB-IgE Measurement Method

SEB-IgE was measured with the ImmunoCAP system (Phadia, Uppsala, Sweden). Considering previous studies, the SEB-IgE cut-off value was taken as 0.1 kU/L.[16-18]

Statistical Analysis

The data recording and statistical analyses were made by using the SPSS v22.0 software (SPSS Inc., Chicago, IL, USA). The distribution of the data was determined by using the Kolmogorov–Smirnov test. The numerical data were expressed as mean ± standard deviation or median (25th–75th percentile) depending on the distribution of the variables. The categorical variables were compared by using the Chi-Square test. Comparisons between the groups were made with the Mann–Whitney U test or Kruskal–Wallis test for the parameters that were not normally distributed. For normally distributed parameters, intergroup comparisons were made with independent samples *t*-test or One-Way ANOVA, and a *p*-value of <0.05 was considered significant. For statistics applied to secondary and explorative outcomes, see the online supplement.

The suitability of the data for normal distribution was evaluated with histogram, q-q graphs, and the Shapiro–Wilk test. Depending on whether or not the data showed normal distribution, correlation analyses were made with Pearson, and Spearman Tests.

Results

A total of 73 individuals were enrolled in the study, including 20 patients with SEAwNP, 18 with SEA without NP, 15 with NP without asthma, and 20 healthy controls. The demographic and clinical characteristics of all participants are summarized in Table 2.

The number of systemic corticosteroid courses administered in the past year, the frequency of those who had received systemic corticosteroid treatment within the 1 month prior to the 15-day exclusion window, and the proportion of patients receiving high-dose intranasal corticosteroids were all significantly higher in the SEAwNP group (Table 3).

Transcript/Protein Ratios of Periostin, F13A, BAFF, IL-5, and TARC Across Groups

The comparison of Periostin, F13A, BAFF, IL5, and TARC transcript levels between the patient and healthy control groups is shown in Figure 1. No significant differences were found among the data presented in Figure 1. The comparison of Periostin, F13A, BAFF, IL5, and TARC serum levels between the patient and healthy control groups is shown in Figure 2. The transcript/protein ratios of periostin, F13A, BAFF, IL-5, and TARC were significantly elevated in the SEAwNP group compared to healthy controls (Table 4).

Table 1 Specific primer sequences for the POSTN, F13A, TNFSF13B, IL5, and TARC genes.

Gene Name	5'->3' Sequence	Base pair (bp)	Annealing temperature
POSTN	F: TGCCCAGCAGTTTTGCCCAT R: CGTTGCTCTCCAAACCTCTA	189 bp	58
IL5	F: TGGAGCTGCCTACGTGTATG R: TCGATGAGTAGAAAGCAGTGC	89 bp	54
TARC	F: TTGTAACTGTGCAGGGCAGG R: TGAACACCAACGGTGGAGGT	169 bp	60
F XIII-a	F: AGATGGGACACTAACAAGGT R: CTGCACATAGAAAGACTGCCC	90 bp	55
TNFSF13B(BAFF)	F: GGGAGCAGTCACGCCTTAC R: GATCGGACAGAGGGGCTTT	103 bp	54

Table 2 The demographic, baseline clinical, and laboratory characteristics of the patients and healthy controls.

	Total N=73	SEAwNP N=20	SEA without NP N=18	NP without asthma N=15	HC N=20	P
Age, mean±SD	43.76±13	50.8±12.1	45.6±15.02	39.4±13.32	38.5±7.66	**0.007**
Female, N (%)	49 (67.1)	11 (55)	13 (72.2)	9 (60)	16 (80)	0.335
Smoking						**0.041**
Never	44 (60.3)	9 (45)	10 (55.6)	7 (46.7)	18 (90)	
Quit	20 (27.4)	9 (45)	7 (38.9)	2 (13.3)	2 (10)	
Presence of atopy	29 (39.7)	8 (40)	12 (66.7)	9 (60)	-	**<0.001**
Presence of NERD	20 (27.4)	11 (55)	2 (11.1)	7 (46.7)	-	**<0.001**
Eosinophil percentage, median (IQR)	4.5 (2.25-8.15)	9.45 (5.85-11.48)	5.45 (3.55-8.25)	4.7 (2.8-7.3)	1.45 (1.05-2.48)	**<0.001**
Eosinophil count, median (IQR)	300 (130-640)	695 (382-980)	365 (217-670)	300 (150-560)	100 (53-160)	**<0.001**
Total IgE, median (IQR)	96.6 (26.95-241.25)	210 (114.3-406)	193 (69.63-676)	64.6 (30.9-218)	11.6 (5.89-34.8)	**<0.001**
FEV1 percent, Mean SD	-	77.22±16.54	91.54±14.12	-	-	0.051

SEAwNP: Eosinophilic severe asthma with nasal polyps, SEA without NP: Eosinophilic severe asthma without nasal polyps, NP: Nasal polyp, NERD: NSAID-exacerbated airway disease, IgE: Immunoglobulin E, FEV1: Forced expiratory volume per second, IQR: Interquartile range, HC: Healthy controls.

Table 3 The corticosteroid usage status of the patients.

	SEAwNP N=20	SEA without NP N=18	NP without asthma N=15
Use of systemic steroid in last 1 year, median (min-max)	1 (0-5)	0 (0-2)	1 (0-4)
Systemic steroid use in 1 month before being included in the study, n (%)	4 (25)	1 (6)	5 (33)
Nasal steroid use, n (%)	20 (100)	5 (28)	12 (80)
*High-dose nasal steroid use, n (%)	20 (100)	0 (0)	12 (80)

SEAwNP: Severe eosinophilic asthma with nasal polyps, SEA without NP: Severe eosinophilic asthma without nasal polyps, NP: Nasal polyp, OKS: Oral corticosteroid.
*>400 microgram/day mometasone furoate and equivalent dose of nasal steroids

In addition, serum periostin, F13A, BAFF, and IL-5 transcript/protein ratios were significantly higher in patients with SEAwNP than in those with SEA without NP (p = 0.007, 0.001, 0.019, and 0.017, respectively) (Table 4).

Intergroup Analysis of SEB-IgE Positivity

SEB-IgE positivity was observed in 9 of 20 patients (45%) within the SEAwNP group, 7 of 18 patients (38%) in the SEA without NP group, and 2 of 15 patients (13%) in the NP without asthma group. No SEB-IgE positivity was detected among the 19 healthy controls.

Discussion

In the current study, blood eosinophil levels were significantly elevated in patients with SEAwNP compared to those with SEA without NP, corroborating findings from previous research. In addition, serum transcript-to-protein ratios of periostin, BAFF, F13A, and IL-5 were markedly higher in the SEAwNP group relative to both SEA without NP patients and healthy controls. A further notable finding was the detection of SEB-IgE positivity in approximately 45% of patients with SEAwNP and 13% of NP patients without asthma, whereas none of the healthy controls exhibited SEB-IgE positivity. To our knowledge, this study is the first to comprehensively compare the transcript/protein expression ratios of key biomarkers—including periostin, F13A, BAFF, IL-5, and TARC—across distinct severe asthma phenotypes such as SEAwNP and SEA without NP.

The pathogenesis of eosinophilic asthma, particularly with regard to different phenotypes, remains complex, with multiple T2 inflammatory mediators playing a role. In this study, the biomarkers periostin, F13A, BAFF, IL-5, and TARC were examined in patients with SEA, with and without NP, and NP without asthma and compared to healthy controls.

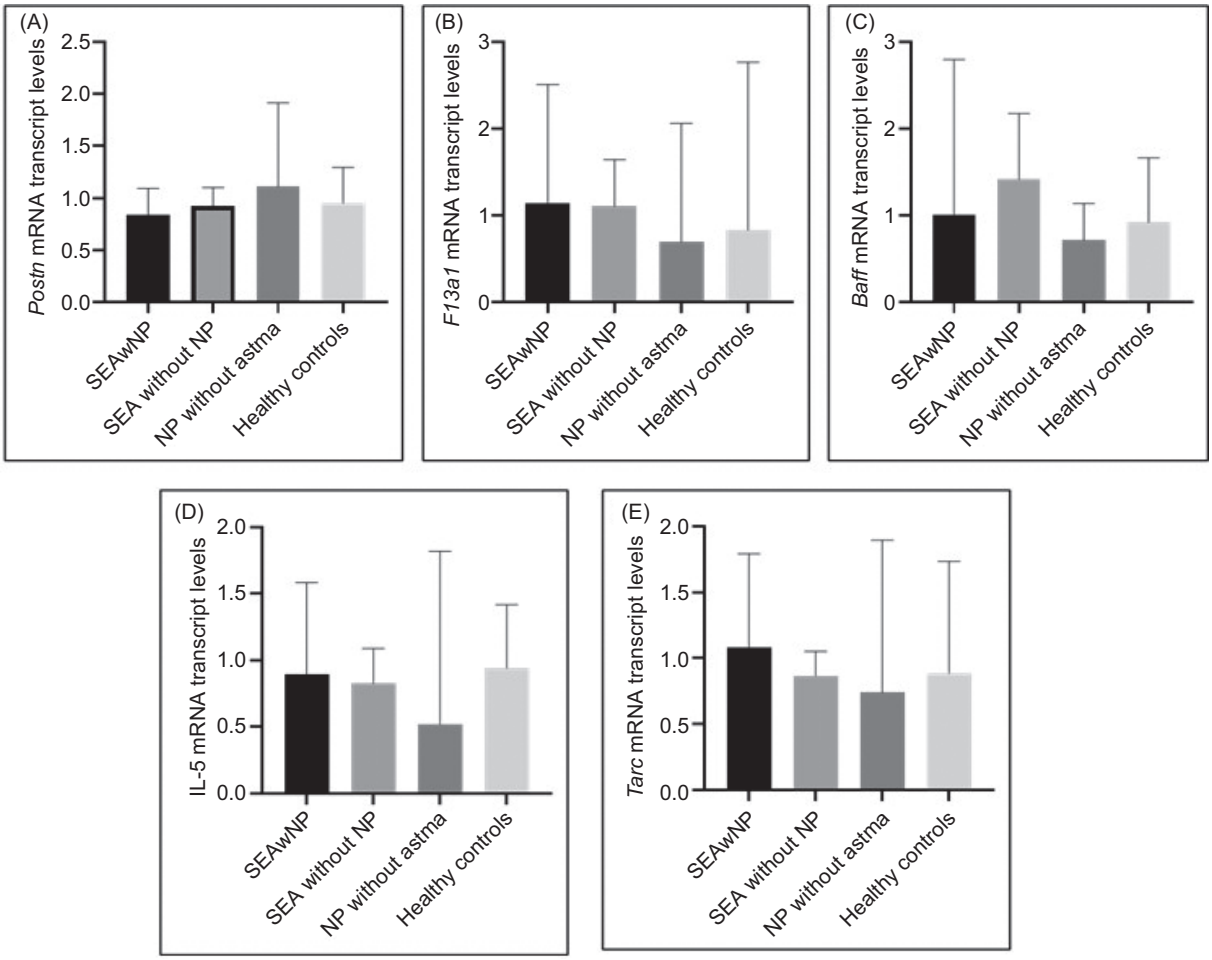

Figure 1 The comparison of (A) Periostin, (B) F13A, (C) BAFF, (D) IL5, and (E) TARC transcript levels in the patient and healthy control groups.

Periostin, which is among the T2 inflammation biomarkers, is upregulated by T2 cytokines (e.g., IL4 and IL13) and is associated with other T2 biomarkers (FeNO, sputum eosinophilia, blood eosinophilia, and IgE).[19-21] In the present study, serum periostin transcript/protein ratio were significantly higher in SEAwNP compared to SEA without NP, NP without asthma, and healthy controls. Most studies reported elevated serum periostin levels in eosinophilic asthma and showed that these levels reflected T2 inflammation and were correlated with eosinophilia.[19-22] It was also suggested that serum periostin levels were increased in SEAwNP, and serum periostin levels might even distinguish patients who had this phenotype from other asthmatic patients.[9]

Mechanisms of inhibition of the fibrinolytic system were also suggested in the immunopathogenesis of eosinophilic NP. F13A was reported to play important roles in alternatively activated macrophages (M2), causing excessive fibrin accumulation in NP and secondary tissue edema.[10-11] F13 was also shown to be expressed in monocyte/macrophage and dendritic cell lineages in patients who had asthma as a response to T2 cytokines and was related to T2 inflammation and airway obstruction.[23] In other words, F13 expression is upregulated in IL-4 and IL-13-activated macrophages.[24,25] It was also shown that there is decreased fibrinolytic activity in the peripheral blood and a significant increase in F13 plasma levels in asthma.[12] In the present study, F13A transcript/protein ratio was significantly higher in the SEAwNP group. These findings imply a potential involvement of F13A dysregulation in the immunopathogenesis of SEAwNP.

BAFF and its receptors are members of the TNF family of cytokines and were shown to play important roles in B-cell activation, differentiation, and antibody production.[26, 27] In NP, antibody production is driven in part by elevated levels of BAFF cytokines in the NP tissue.[28,29] BAFF protein expressions were found to be significantly increased in NP tissues of asthmatic patients and correlated with local IgE production and Th2 response when compared to healthy controls.[30] Also, BAFF protein levels were increased in the sputum of patients who had allergic asthma and a positive correlation was detected with B-cells, IgE+ B-cells, and eosinophils.[31, 32] Serum BAFF levels were reported to be higher in asthmatic patients and decreased after the treatment with glucocorticosteroids, which means that BAFF measurement in serum may be used as a novel diagnostic biomarker to monitor the severity of asthma symptoms.[33] In our study, the BAFF transcript-to-protein expression ratio was significantly elevated in the SEAwNP group compared

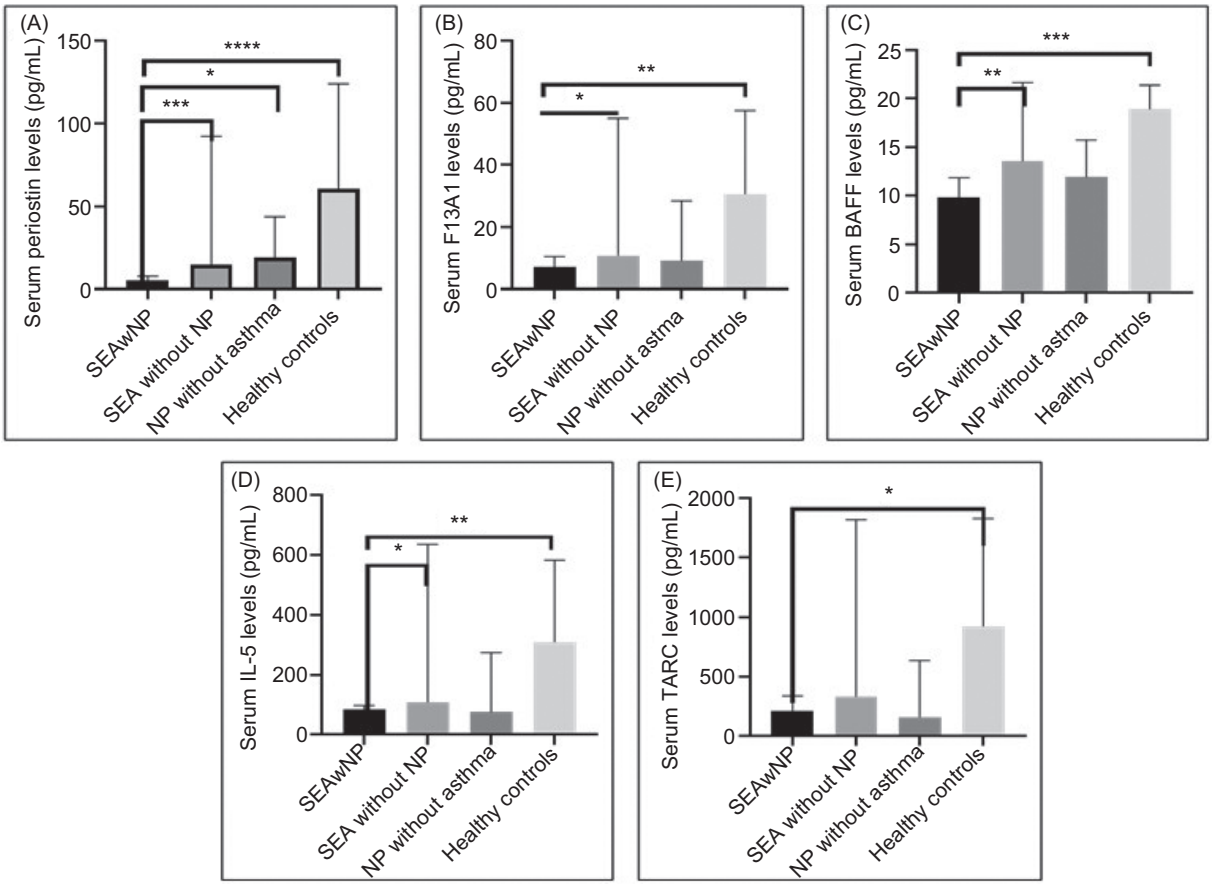

Figure 2 The comparison of (A) Periostin, (B) F13A, (C) BAFF, (D) IL5, and (E) serum levels in the patient and healthy control groups.

Table 4 Comparison of periostin, F13A, BAFF, IL5, and TARC transcript/protein ratio in the patient and healthy control groups.

	SEAwNP	SEA without NP	NP without asthma	Healthy controls	*	**	***	****
Periostin, median (IQR)	0.12 (0.05-0.33)	0.06 (0.01-0.08)	0.06 (0.03-0.14)	0.02 (0.005-0.04)	**<0.0001**	0.135	**0.002**	**0.007**
F13A, median (IQR)	0.16 (0.07-0.38)	0.04 (0.02-0.11)	0.07 (0.02-0.18)	0.04 (0.02-0.11)	**0.001**	0.605	0.335	**0.001**
BAFF, median (IQR)	0.02 (0.006-0.06)	0.005 (0.003-0.01)	0.009 (0.004-0.021)	0.005 (0.002-0.009)	**0.003**	0.605	0.179	**0.019**
IL5, median (IQR)	0.01 (0.005-0.04)	0.004 (0.001-0.01)	0.005 (0.002-0.01)	0.003 (0.00-0.01)	**0.014**	0.580	0.706	**0.017**
TARC, median (IQR)	0.005 (0.001-0.018)	0.002 (0.0004-0.006)	0.003 (0.001-0.007)	0.001 (0.0005-0.006)	**0.02**	0.532	0.302	0.063

SEAwNP: Eosinophilic severe asthma with nasal polyps, SEA without NP: Eosinophilic severe asthma without nasal polyps, NP: Nasal Polyp, F13A: Factor 13A, BAFF: B-cell activating factor, IL5: Interleukin 5, TARC: Activation by thymus "regulated" chemokine, IQR: Interquantitative Range.
*p-value is the result of SEA with NP versus healthy controls.
**p-value is the result of comparing patients who had NP without asthma with patients who had SEA without NP.
***p-value is the result of meeting SEA with NP and NP without asthma.
****p-value is the result of the comparison of SEAwNP and SEA without NP.

to both healthy controls and the SEA without NP group, suggesting that BAFF dysregulation is associated with the enhanced nasal polyp pathology observed in SEAwNP.

In the pathophysiology of eosinophilic asthma and NP, IL-5 has become among the frequently investigated cytokines.[34-38] High amounts of IL-5 were observed in NP at the mRNA and protein levels.[39-40] The highest IL-5 protein levels were observed in polyp homogenates from patients who had comorbidities (e.g., eosinophilic asthma and aspirin hypersensitivity).[41-43] In the present study, the IL-5 transcript-to-protein expression ratio was significantly elevated in the SEAwNP group compared to both healthy controls and the SEA without NP group. These findings suggest that IL-5 may play a more prominent role in the immunopathogenesis of SEAwNP relative to SEA without NP.

Another protein that was investigated in NP and asthma is TARC, which causes selective migration of Th2 cells expressing IL-4 and IL-5 but not that of Th1 cells expressing IFNγ.[44-49] Serum concentrations of TARC, which is secreted by respiratory epithelial cells and fibroblasts in the airways, are generally elevated in patients who have asthma and NP,[50-52] which were also significantly higher especially in allergic and/or eosinophilic asthma, than in healthy controls.[53, 54] It was also shown that patients with NSAID-exacerbated airway disease (NERD) had significantly higher nasal lavage TARC and periostin levels than asthmatics with accompanying allergic rhinitis.[55] In the present study, TARC transcript/protein ratio, a significantly higher ratio in SEAwNP than in healthy controls and SEA without NP suggested an ongoing increased TARC production in this asthma phenotype. A study supporting our data reported that TARC production in BAL fluid and expression of TARC transcript and TARC protein in the lung tissue were decreased in patients receiving dexamethasone treatment. This study suggested that the beneficial effects of corticosteroids in bronchial asthma might be partly because of their direct inhibitory effects on TARC production.[56] In another study, patients receiving inhaled budesonide 800 µg or placebo had bronchial biopsies taken before and after their treatments, and significant reductions were detected in the epithelial expression of TARC in the budesonide group when compared to the placebo group.[57] Therefore, in our study, we evaluated the transcript-to-protein expression ratios because of the potential impact of patients' prior systemic corticosteroid and high-dose ICS therapies on these protein levels.

IgE antibodies have been believed to contribute significantly to the local IgE production in NP.[13-15] *S. aureus* enterotoxin A and B in NP tissue homogenates of asthmatic patients who had aspirin intolerance correlated with specific IgE levels, ECP, and IL-5 levels, and therefore, eosinophilic inflammation.[58] Very high IL-5, total IgE, ECP levels, and SE-IgE positivity were observed in the NP accompanied by severe asthma.[7] A relationship between serum SE-IgE and adult-onset asthma was proposed, thus the use of SE-IgE as a biomarker was considered.[59] Serum SE-IgE was shown to be related to asthma severity and exacerbations, and it was suggested that it might have potential use in clinical practice because of its predictive value.[60-62] In our study, SEB-IgE positivity was absent in the healthy controls group and most prevalent in the SEAwNP cohort, suggesting a potential contributory role of SEB-IgE in the immunopathogenesis of SEAwNP. The fact that SEB-IgE positivity is observed much more frequently when NP is accompanied by eosinophilic severe asthma suggests that patients who have SEB-IgE positivity in nonasthmatic NP must be followed closely for asthma development. Maybe investigating not only SEB-IgE but also other SE-specific IgE in such groups will be more informative. In addition, serum total IgE and serum SE-IgE predict the presence of tissue SE-IgE in the NP with moderate sensitivity.[63] More definitive results can be obtained by investigating mucosal SE-IgE along with serum SE-IgE in these patient groups.

The present study has several limitations. First, it was not feasible to discontinue high-dose ICS and intranasal corticosteroids in the patient population included in the study. Although patients who had used oral corticosteroids within the last 15 days were excluded, this may not have been sufficient to eliminate the prolonged effects of corticosteroid therapy. As a result, the protein expression levels of the T2 inflammatory markers examined may have been underestimated. Evaluating these biomarkers in a corticosteroid-free setting could yield more accurate and reliable data. However, discontinuing ICS therapy in this patient group would not be ethically appropriate because of the severity of asthma and the potential risk of life-threatening exacerbations. Another limitation is that the biomarkers evaluated in this study were not analyzed at the tissue level. Measuring tissue-level expression could have provided a better comparison with serum data, particularly for assessing the local inflammatory environment in different eosinophilic asthma phenotypes and NP. Future studies should include both tissue and serum analyses to better reflect the local and systemic activity of these biomarkers and to more accurately understand the pathophysiological mechanisms involved. In addition, the relatively small sample size limits the generalizability of the findings. To validate and extend these results, larger-scale studies including more participants across various phenotypes of asthma and NP are needed. Such studies would enhance the statistical power and contribute to a more robust understanding of biomarker profiles in these disease subgroups.

In conclusion, our study demonstrates that the transcript-to-protein expression ratios of periostin, F13A, BAFF, and IL-5 in peripheral blood are significantly elevated in patients with SEAwNP compared to those with SEA without NP and healthy controls. These findings suggest that therapeutic strategies targeting these mediators may hold promise, particularly for SEAwNP patients with marked eosinophilia. Another notable finding is the increased SEB-IgE positivity observed in eosinophilic severe asthma, independent of the presence of NP. This raises the possibility that serum SEB-IgE could serve as a potential biomarker for diagnosis and therapeutic targeting in a subset of patients with eosinophilic severe asthma. Furthermore, the markedly higher rate of SEB-IgE positivity in patients with SEAwNP, compared to the low prevalence in nonasthmatic NP, suggests that individuals with SEB-IgE positivity in nonasthmatic NP should be closely monitored for the potential development of asthma. In addition, our study highlights the necessity of evaluating these proteins alongside their transcript levels, especially in patients receiving high-dose ICS, nasal steroids, or recent systemic corticosteroid

treatments, to obtain a more accurate assessment. Overall, these findings have important implications for clinical practice by supporting the development of more precise biomarker-guided diagnostic and therapeutic approaches in patients with SEAwNP.

Mandatory Disclosure on Use of Artificial Intelligence

The authors declare that no AI-assisted tools were used in the preparation of this manuscript. All references have been manually verified for accuracy and relevance.

Ethical Statement

The study was approved by the Research and Ethics Committee of Erciyes University (Decision no: 2020/595).

Authors Contribution

Study conception: İnsu Yılmaz, Serpil Taheri; Study design and methodology: İnsu Yılmaz, Serpil Taheri, Ahmet Eken, Şerife Erdem; Data collection and data processing: İnsu Yılmaz, Serpil Taheri, Bahar Arslan, Şerife Erdem, Ahmet Eken, Zeynep Yılmaz, Gülden Paçacı Çetin, Murat Türk; Literature review: İnsu Yılmaz, Serpil Taheri, Şerife Erdem, Zeynep Yılmaz, Ahmet Eken, Bahar Arslan, Gülden Paçacı Çetin, Murat Türk, Nuri Tutar, Zuhal Hamurcu; Manuscript writing: İnsu Yılmaz, Serpil Taheri, Şerife Erdem, Zeynep Yılmaz, Ahmet Eken, Bahar Arslan, Gülden Paçacı Çetin, Murat Türk, Nuri Tutar, Zuhal Hamurcu; Final critical review and editing: İnsu Yılmaz, Serpil Taheri, Şerife Erdem, Zeynep Yılmaz, Ahmet Eken, Bahar Arslan, Gülden Paçacı Çetin, Murat Türk, Nuri Tutar, Zuhal Hamurcu.

Conflicts of Interest

The authors declare no conflicts of interest.

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