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Characterization of Serum Cytokine Patterns in Frequent-Exacerbation Asthma: Implications for Phenotyping and Management

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Highlights:

Main findings

- Asthma patients with frequent exacerbations show significantly elevated serum concentrations of IL-4 and IL-13, along with higher rates of allergic history and specific comorbidities.
- Significant positive correlations exist between inflammatory cytokines (IL-17 and IL-1β) and IgE levels and both IFN-α and TNF-α, as well as between FeNO levels.

Implications

- The distinct cytokine signature in frequent exacerbators suggests potential targets for personalized therapeutic approaches in this high-risk population.
- Integration of cytokine profiling with clinical characteristics may improve patient stratification and guide targeted treatment selection in severe asthma.

Abstract: (1) Background: Asthma exacerbations represent significant clinical events, yet the underlying inflammatory mechanisms and cytokine profiles in patients with frequent exacerbations remain incompletely understood; (2) Methods: In this prospective, cross-sectional study of 120 stable asthma patients, we compared serum concentrations of eight key cytokines (IL-4, IL-12, IL-13, IL-17, IFN-α, IFN-γ, TNF-α, and IL-1β) between two groups: 60 patients with frequent exacerbations (≥2/year) and 60 matched controls with few exacerbations (1/year); (3) Results: Patients with frequent exacerbations showed significantly higher serum concentrations of IL-4 and IL-13 ($p < 0.05$), along with increased prevalence of allergic history and comorbidities (chronic rhinosinusitis, GERD, OSA; all $p < 0.05$). IgE levels correlated positively with IFN-α (rh = 0.26) and TNF-α (rh = 0.29), while FeNO levels correlated with IL-17 (rh = 0.26) and IL-1β (rh = 0.33) (all $p < 0.05$); (4) Conclusions: Our findings identify a distinct cytokine signature in frequent exacerbators characterized by elevated IL-4 and IL-13 levels. The correlations between specific cytokines and established biomarkers suggest potential mechanisms underlying exacerbation susceptibility, which may inform targeted therapeutic strategies for this high-risk population.

Keywords: serum cytokine; frequent asthma exacerbations; asthma phenotype

1. Introduction

The global burden of asthma continues to rise significantly despite therapeutic advances. Recent epidemiological data indicate an increase in cases from 226.9 million in 1990 to 262.41 million in 2019, with an estimated 461,000 deaths globally in 2019 [1,2]. This trend is particularly concerning in developing nations, where urbanization and environmental changes substantially contribute to disease burden [3,4]. Asthma's impact extends beyond mortality, significantly affecting healthcare systems and socioeconomic structures worldwide [5].

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Despite recent advances in biological agents and targeted therapies, optimal disease control remains challenging. Approximately 17% of asthma patients have difficult-to-treat disease, while 3.7% meet severe asthma criteria [6,7]. These patients frequently experience exacerbations, leading to accelerated lung function decline and increased healthcare utilization [8,9]. The economic burden is particularly substantial in this population, with frequent exacerbators accounting for a disproportionate share of asthma-related healthcare costs [10,11].

The pathogenesis of frequent exacerbations involves complex inflammatory pathways. Recent evidence highlights the interplay between Th2 and non-Th2 inflammatory responses [12,13], characterized by enhanced airway inflammation and remodeling [14], dysregulated immune responses [15], and variable therapeutic responses [16]. Cytokines serve as crucial mediators in these processes [12,17], with specific patterns potentially defining inflammatory phenotypes [18], predicting exacerbation risk [19], and guiding targeted therapies [16,20].

While numerous studies have investigated cytokine profiles during acute exacerbations [12,18], limited data exist regarding stable asthma patients who experience frequent exacerbations. This study aims to characterize serum cytokine concentrations (IL-4, IL-12, IL-13, IL-17, IFN-α, IFN-γ, TNF-α, and IL-1β) in such patients, potentially providing insights into underlying inflammatory mechanisms and therapeutic targets.

2. Materials and Methods

2.1. Study Design

We conducted a prospective, cross-sectional study at the Asthma Management Department of Hai Phong International General Hospital, Vietnam, between January 2020 and May 2023. The study protocol was designed in accordance with STROBE guidelines for observational studies.

2.2. Study Population

The study population consisted of 120 stable asthma patients who were not experiencing acute exacerbations at the time of investigation, with sample size determined using the formula

$$n = [(Z_1\text{-}\alpha/2)^2 \times \sigma^2] / \varepsilon^2\mu^2 \quad (1)$$

where $Z_1\text{-}\alpha/2 = 1.96$, $\sigma = 17.57\%$ (standard deviation of FVC from Denlinger et al. [8]), $\mu = 77.45\%$, and $\varepsilon = 0.06$, yielding a minimum requirement of 55 patients per group. We enrolled 60 patients per group to account for potential data loss, dividing participants into two equal cohorts: Group 1 (n=60) with frequent exacerbations (≥2 events/year) and Group 2 (n=60) with few exacerbations (1 event/year) in the most recent year. Eligible participants were required to be ≥16 years old, have a confirmed diagnosis of asthma according to GINA 2019 guidelines [21], be receiving standardized control treatment, and provide written informed consent. We excluded patients with acute respiratory infections, coexisting respiratory conditions (e.g., COPD overlap, bronchiectasis), significant comorbidities (as defined by Pavord et al. [20], or those receiving systemic corticosteroid or immunosuppressive therapy.

2.3. Clinical Assessment

All participants underwent a comprehensive clinical evaluation following standardized protocols. This assessment encompassed detailed medical and allergic history documentation, thorough family history review, systematic comorbidity screening, and careful documentation of exacerbation frequency over the previous 12 months. Disease control was evaluated using the validated Asthma Control Test (ACT), and treatment intensity was classified according to the GINA step approach [21]. This standardized assessment protocol ensured consistent data collection across all study participants and aligned with current clinical practice guidelines.

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2.4. Laboratory Methods

Comprehensive laboratory analysis encompassed both cytokine quantification and standard biomarker assessment. Serum cytokine levels were measured using flow cytometry-assisted immunoassay (Bio-Plex system, Bio-Rad, USA) with reagents supplied by Bender Medsystems GmbH (Austria) and Thermo Fisher Scientific. The analysis focused on three distinct cytokine groups: non-Th2-dependent inflammatory cytokines (IL-17, IFN-α, IFN-γ), Th2-dependent inflammatory cytokines (IL-4, IL-13), and inflammatory/regulatory cytokines (IL-12, TNF-α, IL-1β). Additionally, we measured serum IgE levels, and fractional exhaled nitric oxide (FeNO), and conducted standard hematological assessments to provide a comprehensive inflammatory profile for each patient.

2.5. Statistical Analysis

Statistical analyses were conducted using SPSS 20.0 and STATA 14.0 software packages, employing a comprehensive analytical approach. Descriptive statistics were used to characterize demographic and clinical parameters. For continuous variables, we applied either Student's t-test or Mann-Whitney U test, depending on data distribution normality, while categorical variables were analyzed using Chi-square tests or Fisher's exact test when observation frequencies were less than 5. Associations between variables were assessed using Spearman's correlation coefficient (rh), with positive values indicating direct correlations and negative values suggesting inverse relationships. Throughout all analyses, statistical significance was defined as $p < 0.05$.

2.6. Ethical Considerations

The study protocol was approved by the Ethics Committee of Hai Phong International General Hospital (No. 09/2020/HIH-IRB, January 6, 2020) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to study enrollment.

3. Results

3.1. Subsection

3.1.1. Patient Demographics and Clinical Characteristics

Analysis of 120 asthma patients revealed comparable baseline demographics between frequent exacerbation (Group 1, n=60) and few exacerbation groups (Group 2, n=60), with similar mean ages (50.73 ± 15.05 vs. 50.43 ± 16.56 years, p = 0.92), disease duration (26.57 ± 18.32 vs. 29.55 ± 17.62 years, p = 0.38), and BMI (22.59 ± 2.86 vs. 22.46 ± 2.45 kg/m², p = 0.79) (Table 1). However, Group 1 demonstrated significantly higher rates of personal allergic history (73.3% vs. 36.7%, p < 0.001) and key comorbidities including chronic rhinosinusitis (70.0% vs. 45.0%, p = 0.006), GERD (38.33% vs. 18.33%, p = 0.02), and OSA (33.33% vs. 13.33%, p = 0.01). Additionally, Group 1 exhibited poorer disease control with lower mean ACT scores (21.02 ± 3.36 vs. 22.27 ± 3.10, p = 0.04) and a higher proportion of patients requiring step 4-5 treatment (86.67% vs. 76.67%, p = 0.16), suggesting a distinct clinical phenotype characterized by increased allergic burden and comorbidity prevalence.

Table 1. Characteristics of the study population.

Characteristics		Group 1 ($n_1 = 60$)	Group 2 ($n_2 = 60$)	p
Age ($\bar{X}$ ± SD) (years)		50.73 ± 15.05	50.43 ± 16.56	0.92
Gender:	- Male: n (%)	34 (56.67)	26 (43.33)	0.09
	- Female: n (%)	43 (71.67)	17 (28.33)	
Allergic history	Personal; n (%)	44 (73.3)	22 (36.7)	< 0.001*
	Family; n (%)	14 (23.3)	15 (25.0)	0.83
Asthma onset	≤ 12; n (%)	34 (56.67)	40 (66.67)	0.26

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(years old)	> 12; n (%)	26 (43.33)	20 (33.33)	
Smoking: n (%)		10 (16.7)	18 (30.0)	0.084
Step of asthma	Step (2-3); n (%)	8 (13.33)	14 (23.33)	0.16
	Step (4-5); n (%)	52 (86.67)	46 (76.67)	
Duration of asthma (years) ($\overline{X}$ ± SD)		26.57 ± 18.32	29.55 ± 17.62	0.38
Comorbidities:	- Sinusitis: n (%)	42 (70.0)	27 (45.0)	0.006*
	- GERD: n (%)	23 (38.33)	11 (18.33)	0.02*
	- OSA: n (%)	20 (33.33)	8 (13.33)	0.01*
	- Diabetes: n (%)	6 (10.0)	6 (10.0)	1
	- Hypertension: n (%)	9 (15.0)	6 (10.0)	0.41
ACT score	< 20	26 (43.33)	17 (28.33)	0.16
	20 - 24	16 (26.67)	16 (26.67)	
	≥ 25	18 (30.0)	27 (45.0)	
	($\overline{X}$ ± SD)	21.02 ± 3.36	22.27 ± 3.10	0.04*
BMI ($\overline{X}$ ± SD) (kg/m²)		22.59 ± 2.86	22.46 ± 2.45	0.79

[1] Baseline demographic and clinical characteristics of asthma patients with frequent exacerbations (Group 1, n=60) compared to those with few exacerbations (Group 2, n=60). Data are presented as mean ± SD or number (percentage). *p<0.05 indicates statistical significance. BMI: Body Mass Index, GERD: Gastroesophageal Reflux Disease, OSA: Obstructive Sleep Apnea, ACT: Asthma Control Test.

3.1.2. Comparison of Serum Cytokine Levels

A comprehensive analysis of serum cytokine profiles revealed distinctive patterns between asthma patients with frequent versus few exacerbations. Notably, IL-4 levels were significantly elevated in the frequent exacerbation group (median 21.10 pg/mL, IQR 15.99-33.67) compared to those with few exacerbations (median 16.48 pg/mL, IQR 6.75-25.54; p = 0.007). Similarly, IL-13 concentrations were markedly higher in frequent exacerbators (median 9.93 pg/mL, IQR 1.73-13.83 vs. 3.95 pg/mL, IQR 1.73-10.52; p = 0.01). While other inflammatory mediators, including IL-17 (8.62 vs. 4.86 pg/mL), IL-12 (55.11 vs. 32.24 pg/mL), TNF-α (10.25 vs. 7.30 pg/mL), and IL-1β (1.61 vs. 1.39 pg/mL), showed trends toward elevation in the frequent exacerbation group, these differences did not reach statistical significance (all p > 0.05) (Table 2). Levels of IFN-α and IFN-γ remained comparable between groups, suggesting specificity in the Th2-associated cytokine elevation pattern.

Table 2. Serum cytokine levels of study groups.

Cytokine level Median (p_{25}-p_{75}) (pg/mL)	**Group 1 (n_1 = 60)**	**Group 2 (n_2 = 60)**	**p**
IL-17	8.62 (2.88 - 14.92)	4.86 (1.47 - 13.95)	0.11
INF-α	0.57 (0.40 – 1.43)	0.50 (0.40 – 1.43)	0.67
INF-γ	6,54 (5.05 - 11.95)	5.57 (5.05 - 9.96)	0.63
IL-4	21.10 (15.99 - 33.67)	16.48 (6.75 - 25.54)	0.007*
IL-13	9,93 (1.73 - 13.83)	3.95 (1.73 - 10.52)	0.01*
IL-12	55.11 (28.17 - 269.03)	32.24 (17.22 - 271.04)	0.06
TNF-α	10.25 (4.76 - 18.92)	7.30 (3,53 - 17,88)	0.18
IL-1β	1,61	1.39	0.78

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	(0.81 - 2.68)	(0.81 - 3.76)

[2] Comparison of serum cytokine concentrations between asthma patients with frequent exacerbations (Group 1, n=60) and those with few exacerbations (Group 2, n=60). Values are presented as median with interquartile range (25th-75th percentiles). *p<0.05 indicates statistical significance.

3.1.3. Association Between Cytokines and Allergic History

Within the frequent exacerbation group (n=60), analysis of cytokine profiles revealed distinct patterns between patients with (n=44) and without (n=16) allergic history. IL-4 demonstrated the most significant difference, with substantially higher levels in allergic patients (22.96 pg/mL, IQR 17.68-35.81) compared to non-allergic patients (18.27 pg/mL, IQR 6.80-23.48; p = 0.02) (Table 3). This finding aligns with our earlier observation of elevated IL-4 in the frequent exacerbation group and supports the Th2-predominant inflammation pattern described in our introduction [10,11]. Other Th2-associated cytokines, notably IL-13, showed a trend toward elevation in allergic patients (10.52 pg/mL vs. 4.37 pg/mL, p = 0.09), though not reaching statistical significance. Interestingly, while IL-17 levels were higher in allergic patients (8.78 pg/mL vs. 5.99 pg/mL, p = 0.51) and TNF-α showed similar trends (11.59 pg/mL vs. 6.62 pg/mL, p = 0.17), these differences did not achieve statistical significance, suggesting a more specific role for IL-4 in the allergic phenotype of frequent exacerbators.

Table 3. Relation between serum cytokine concentrations and allergic history.

Cytokine level Median **(p_{25} - p_{75})** (pg/mL)	**Asthma patients with frequent exacerbations (n_1 = 60)**		
	No allergy (n = 16)	**Allergy (n = 44)**	**P**
IL-17	5.99 (2.13 - 14.24)	8.78 (3.55 - 15.21)	0.51
IFN-α	0.40 (0.29 - 0.89)	0.59 (0.40 - 1.47)	0.11
IFN-γ	5.81 (4.83 - 8.52)	7.51 (5.05 - 16.02)	0.11
IL-4	18.27 (6.80 - 23.48)	22,96 (17.68 - 35.81)	0.02*
IL-13	4.37 (1.73 - 10.52)	10.52 (3.48 - 14.94)	0.09
IL-12	174.96 (32.47 - 265.83)	45.09 (27.44 - 269.03)	0.48
TNF-α	6.62 (3.53 - 12.61)	11.59 (5.26 - 19.05)	0.17
IL-1β	1.61 (0.69 - 2.16)	1.63 (0.81 - 2.68)	0.41

[3] Comparison of serum cytokine levels between asthma patients with frequent exacerbations with (n=44) and without (n=16) allergic history. Values are presented as median with interquartile range (25th-75th percentiles). *p<0.05 indicates statistical significance.

3.1.4. Relationship with Disease Severity

The analysis of cytokine profiles in relation to asthma severity within the frequent exacerbation group revealed distinctive patterns between patients with steps 4-5 disease (n=52) versus steps 2-3 (n=8). Patients with more severe disease (steps 4-5) showed higher concentrations of several key cytokines, notably IL-4 (22.58 pg/mL, IQR 16.53-33.88 vs. 17.30 pg/mL, IQR 10.05-27.45) and IL-13 (10.39 pg/mL, IQR 1.96-14.38 vs. 2.61 pg/mL, IQR 1.73-12.46). TNF-α levels were also elevated in the more severe group (10.93 pg/mL vs. 6.62 pg/mL), while IL-17 showed an inverse trend (8.32 pg/mL vs. 10.37 pg/mL) (Table 4). However, it's important to note that none of these differences reached

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statistical significance (all $p > 0.05$), which may be attributed to the relatively small number of patients in the steps 2-3 group. While suggestive of a relationship between disease severity and inflammatory patterns, these findings warrant further investigation in larger cohorts.

Table 4. Relationship between serum cytokine concentrations and asthma steps.

Cytokine level Median (p_{25} - p_{75}) (pg/mL)	Asthma patients with frequent exacerbations ($n_1 = 60$)		
	Asthma steps (2,3) (n = 8)	Asthma steps (4,5) (n = 52)	P
IL-17	10.37 (6.42 - 14.92)	8.32 (2.13 - 15.12)	0.63
IFN-α	0.68 (0.4 - 0.98)	0.57 (0.4 - 1.47)	0.88
IFN-γ	5.57 (4.83 - 15.58)	7.02 (5.05 - 11.95)	0.63
IL-4	17.30 (10.05 - 27.45)	22.58 (16.53 - 33.88)	0.20
IL-13	2.61 (1.73 - 12.46)	10.39 (1.96 - 14.38)	0.17
IL-1β	1.61 (0.99 - 2.16)	1.61 (0.81 - 2.68)	0.68
IL-12	242.21 (33.52 - 333.52)	249.53 (28.17 - 262.23)	0.37
TNF-α	6.62 (4.40 - 14.39)	10.93 (4.76 - 19.05)	0.29

[4] Serum cytokine concentrations in asthma patients with frequent exacerbations according to asthma severity steps: steps 2-3 (n=8) versus steps 4-5 (n=52). Values are presented as median with interquartile range (25th-75th percentiles).

3.1.5. Association with Asthma Control

In the frequent exacerbation group, analysis of cytokine profiles stratified by ACT scores showed that patients with poor control (ACT < 20, n=26) exhibited higher median concentrations of several pro-inflammatory cytokines compared to those with better control (ACT ≥ 20, n=34): IL-17 (9.74 pg/mL, IQR 4.22-17.70 vs. 8.16 pg/mL, IQR 1.47-14.43, p = 0.36), IFN-α (0.80 pg/mL, IQR 0.34-1.54 vs. 0.56 pg/mL, IQR 0.40-1.0, p = 0.37), TNF-α (11.59 pg/mL vs. 7.75 pg/mL, p = 0.14), IL-1β (2.04 pg/mL vs. 1.16 pg/mL, p = 0.14), and notably IL-12 (140.96 pg/mL, IQR 29.14-332.72 vs. 45.67 pg/mL, IQR 27.68-257.42, p = 0.32). Th2-associated cytokines showed similar levels between groups: IL-4 (21.10 pg/mL vs. 22.58 pg/mL, p = 0.93) and IL-13 (9.93 pg/mL vs. 10.06 pg/mL, p = 0.59). IFN-γ levels remained consistent across both groups (6.54 pg/mL vs. 6.54 pg/mL, p = 0.96) (Table 5).

Table 5. Relationship between serum cytokine concentrations and ACT score.

Cytokine level Median (p_{25} - p_{75}) (pg/mL)	Asthma patients with frequent exacerbations ($n_1 = 60$)		
	ACT < 20 (n = 26)	ACT ≥ 20 (n = 34)	P
IL-17	9.74 (4.22 -17.70)	8.16 (1.47 - 14.43)	0.36
IFN-α	0.80 (0.34 - 1.54)	0.56 (0.40 - 1.0)	0.37
IFN-γ	6.54 (5.05 - 11.95)	6.54 (5.05 - 14.82)	0.96

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IL-4	21.10 (15.44 - 33.52)	22.58 (16.53 - 33.81)	0.93
IL-13	9.93 (1.73 - 10.87)	10.06 (2.44 - 14.39)	0.59
IL-12	140.96 (29.14 - 332.72)	45.67 (27.68 - 257.42)	0.32
TNF-α	11.59 (7.98 - 19.72)	7.75 (3.53 - 18.78)	0.14
IL-1β	2.04 (0.81 - 2.68)	1.16 (0.81 - 2.68)	0.14

[5] Serum cytokine levels in asthma patients with frequent exacerbations stratified by ACT (Asthma Control Test) scores: ACT<20 (n=26) versus ACT≥20 (n=34). Values are presented as median with interquartile range (25th-75th percentiles).

3.1.6. Biomarker Analysis

Analysis of inflammatory biomarkers in the frequent exacerbation group (n=60) revealed several significant correlations. Serum IgE levels demonstrated weak but significant positive correlations with IFN-α (rh = 0.26, p = 0.04) and TNF-α (rh = 0.29, p = 0.02). FeNO concentrations showed significant positive correlations with IL-17 (rh = 0.26, p = 0.04) and IL-1β (rh = 0.33, p = 0.009), and a trend towards positive correlation with IL-12 (rh = 0.23, p = 0.08). Notably, eosinophil counts showed no significant correlations with any measured cytokines: IL-17 (rh = 0.22, p = 0.82), IFN-α (rh = 0.006, p = 0.96), IFN-γ (rh = 0.17, p = 0.18), IL-4 (rh = 0.04, p = 0.71), IL-13 (rh = 0.05, p = 0.65), IL-1β (rh = 0.03, p = 0.80), IL-12 (rh = -0.05, p = 0.65), and TNF-α (rh = 0.17, p = 0.20) (Table 6).

Table 6. Correlation between serum cytokine concentrations and eosinophil count, IgE, FeNO.

Indices			**IL-17**	**IFN-α**	**IFN-γ**	**IL-4**	**IL-13**	**IL-1β**	**IL-12**	**TNF-α**
Asthma patients with frequent exacerba-tions (n_1 = 60)	**Eosinophil**	r_h	0.22	0.006	0.17	0.04	0.05	0.03	- 0.05	0.17
		p	0.82	0.96	0.18	0.71	0.65	0.80	0.65	0.20
	IgE	r_h	0.14	0.26	0.17	0.15	0.21	0.14	- 0.054	0.29
		p	0.28	0.04	0.19	0.25	0.10	0.27	0.68	0.02*
	FeNO	r_h	0.26	0.06	0.14	0.004	0.07	0.33	0.23	0.05*
		p	0.04	0.64	0.30	0.97	0.59	0.009	0.08	0.72

[6] Correlations between serum cytokine concentrations and eosinophil count, IgE levels, and FeNO in asthma patients with frequent exacerbations (n=60). rh represents Spearman's correlation coefficient. *p<0.05 indicates statistical significance. FeNO: Fractional Exhaled Nitric Oxide.

4. Discussion

4.1. General Characteristics of Study Population

Our findings regarding the relationship between clinical characteristics and inflammatory patterns in frequent exacerbators align with and extend recent literature. In terms of demographic patterns, our observation of gender distribution and comorbidity profiles supports the recent systematic review by Wang et al. [1], who identified similar risk factors in their analysis of global asthma burden. The high prevalence of allergic history in our frequent exacerbators (73.3%) closely corresponds with Gerday et al.'s [11] findings, where they demonstrated that age-specific inflammatory profiles significantly influence the atopic-nonatopic asthma paradigm.

The cytokine patterns we observed, particularly elevated IL-4 and IL-13, correlate with Sun et al.'s [22] comprehensive analysis of serum cytokine profiles across different asthma severity groups. Their study similarly identified distinct inflammatory signatures in severe asthma, though our research extends these findings specifically to frequent exacerbators. Our observation of significant

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correlations between FeNO levels and inflammatory cytokines supports recent work by Irina et al. [23], who demonstrated the importance of biomarker integration in understanding asthma phenotypes.

The comorbidity patterns in our study, particularly the high prevalence of chronic rhinosinusitis (70.0%) and GERD (38.33%), align with Kaasgaard et al.'s [24]findings on the impact of multiple comorbidities on asthma control. Furthermore, our observation of complex cytokine interactions beyond the traditional Th2 pathway supports the recent work by Salama et al. [25], who identified similar inflammatory complexity in their assessment of respiratory impairment in chronic airway diseases.

4.2. Characteristics of Inflammatory Markers

Our study reveals several significant findings in the inflammatory profile and clinical characteristics of asthma patients with frequent exacerbations. Most notably, we identified a distinct cytokine signature characterized by significantly elevated serum concentrations of IL-4 (21.10 pg/mL vs. 16.48 pg/mL, $p = 0.007$) and IL-13 (9.93 pg/mL vs. 3.95 pg/mL, $p = 0.01$) in frequent exacerbators, aligning with recent observations by Muñoz-Cofré et al. [26]. These patients also demonstrated a significantly higher proportion of allergic history (73.3% vs. 36.7%, $p < 0.001$) and specific comorbidities - chronic rhinosinusitis (70.0%), GERD (38.33%), and OSA (33.33%), supporting findings by Gerday et al. (11) regarding the impact of atopic status on airway inflammation. Furthermore, our biomarker analysis revealed significant positive correlations between IgE levels and both IFN-α ($rh = 0.26$, $p = 0.04$) and TNF-α ($rh = 0.29$, $p = 0.02$), as well as between FeNO levels and IL-17 ($rh = 0.26$, $p = 0.04$) and IL-1β ($rh = 0.33$, $p = 0.009$), consistent with recent work by Sun et al. [22], suggesting complex inflammatory interactions beyond the traditional Th2 pathway in frequent exacerbators, as highlighted in Wang et al.'s (2023) [1] comprehensive review of asthma's inflammatory mechanisms.

5. Conclusions

Our study demonstrates several key findings regarding cytokine profiles in stable asthma patients with frequent exacerbations. Most notably, IL-4 and IL-13 concentrations were significantly elevated in frequent exacerbators and showed specific associations with allergic phenotypes. Furthermore, we identified significant correlations between inflammatory markers, with IgE levels correlating positively with both IFN-α and TNF-α, and FeNO levels showing positive associations with IL-17 and IL-1β. These findings suggest a distinct inflammatory signature in frequent exacerbators that could inform targeted therapeutic approaches [9,13,20].

Limitations

The single-center design and sample size that was just sufficient according to power calculations may limit the generalizability of our findings. The study's cross-sectional nature prevented the evaluation of temporal changes in cytokine patterns and treatment responses. Technical constraints included one-time cytokine measurements and the inability to assess changes following treatment modifications. Additionally, our focus on stable patients excluded insights from acute exacerbations, and the regional nature of our population may affect the broader applicability of our findings.

Future Directions

Several key areas warrant further investigation based on our findings. Longitudinal studies with larger, multi-center cohorts are needed to validate our observations and evaluate cytokine pattern changes over time, particularly during and between exacerbations. Future research should focus on integrating additional biomarkers for comprehensive inflammatory profiling and developing predictive models to guide personalized treatment approaches. Investigating targeted therapies based on specific cytokine profiles and assessing preventive strategies for frequent exacerbators could advance precision medicine approaches in severe asthma [6,7,19]. These directions align with

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recent developments in precision medicine and could significantly improve our understanding and management of exacerbation-prone phenotypes.

Author Contributions: D.N.B. and N.T.D. conceptualized and designed the study with guidance from T.B.T.; D.N.B. and N.T.D. conducted the clinical investigation and data collection; T.B.T. supervised the project and provided resources alongside P.D.T.; D.N.B., N.T.D., and P.T.K.N. performed data analysis; B.Q.T. handled statistical analysis; V.M.D. and L.T.D.H. assisted with clinical assessments; D.N.B. wrote the original draft with significant input from N.T.D.; T.B.T. provided critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript. T.B.T. was responsible for project administration.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of Hai Phong International General Hospital (No. 09//2020/HIH-IRB dated January 6, 2020). The study was also conducted using good clinical practice following the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data supporting this research are available from the authors on reasonable request.

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