

Inflammatory signatures in asthma–COPD overlap: cytokine profiling across asthma subtypes

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Abstract

English:

Objective: This study aimed to compare clinical characteristics and plasma cytokine profiles among patients with asthma, asthma combined with smoking history, asthma with fixed airflow obstruction (FAO) and asthma–COPD overlap (ACO), to identify distinct inflammatory signatures.

Methods: A prospective, cross-sectional study was conducted involving 120 clinically stable asthma patients stratified into four groups: asthma ($n = 56$), asthma + smoking ($n = 15$), asthma + FAO ($n = 34$) and ACO ($n = 15$). Clinical assessments, spirometry and multiplex cytokine analysis were performed to evaluate inflammatory and functional parameters.

Results: Significant elevations in IL-1 β , IL-17 and tumour necrosis factor alpha (TNF- α) were observed in the ACO group compared to asthma and asthma + FAO cohorts ($P < 0.01$). Specifically, IL-1 β levels in ACO reached 3.8 ng/L [2.7–4.8], nearly threefold higher than in asthma (1.4 ng/L) and asthma + FAO (1.0 ng/L). IL-17 and TNF- α concentrations were similarly elevated in ACO (16.4 ng/L and 18.8 ng/L, respectively). No significant differences were found in IFN- α , IFN- γ , IL-4, IL-12 or IL-13.

Conclusion: ACO exhibits distinct immunopathological characteristics, notably elevated concentrations of IL-1 β , IL-17 and TNF- α relative to asthma and asthma + FAO cohorts. The combined use of spirometric indices and biomarker profiling may enhance phenotypic stratification and inform the development of targeted biologic therapies for obstructive airway diseases.

Keywords

asthma • asthma–COPD overlap • fixed airflow obstruction • cytokine

Profilu inflamator în suprapunerea astm–BPOC: analiza citokinelor între subtipurile de astm

Rezumat

Romanian:

Obiectiv: Scopul acestui studiu a fost de a compara caracteristicile clinice și profilurile plasmatice ale citokinelor la pacienți cu astm, astm asociat cu fumatul, astm cu obstrucție fixă a fluxului de aer (FAO) și suprapunere astm–BPOC (ACO), pentru a identifica semnături inflamatorii distincte.

Metode: S-a realizat un studiu prospectiv, transversal, care a inclus 120 de pacienți cu astm clinic stabil, împărțiți în patru grupuri: astm ($n = 56$), astm + fumat ($n = 15$), astm + FAO ($n = 34$) și ACO ($n = 15$). Au fost efectuate evaluări clinice, spirometrie și analize multiplex ale citokinelor pentru a evalua parametri inflamatori și funcționali.

Rezultate: S-au înregistrat creșteri semnificative ale nivelurilor plasmatice de IL-1 β , IL-17 și TNF- α în grupul ACO comparativ cu grupurile astm și astm + FAO ($P < 0,01$). Nivelurile de IL-1 β la pacienții cu ACO au fost de 3,8 ng/L [2,7–4,8], aproape de trei ori mai mari decât la pacienții cu astm (1,4 ng/L) și astm + FAO (1,0 ng/L). Concentrațiile de IL-17 și TNF- α au fost de asemenea crescute la pacienții cu ACO (16,4 ng/L și, respectiv, 18,8 ng/L). Nu s-au observat diferențe semnificative în valorile IFN- α , IFN- γ , IL-4, IL-12 sau IL-13.

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Concluzie: ACO prezintă caracteristici imunopatologice distincte, marcate prin creșteri semnificative ale IL-1 β , IL-17 și TNF- α în comparație cu astmul și astmul cu FAO. Combinarea indicilor spirometrici cu analiza biomarkerilor poate îmbunătăți stratificarea fenotipică și poate ghida dezvoltarea terapiei biologice țintite în bolile obstructive ale căilor respiratorii.

Cuvinte-cheie

astm • suprapunere astm-BPOC • obstrucție fixă a fluxului de aer • citokine

Introduction

Traditionally, asthma has been regarded as distinct from chronic obstructive pulmonary disease (COPD). However, exposure to cigarette smoke in individuals with asthma may exacerbate airway inflammation, alter immune responses and promote irreversible airway remodelling. Such patients may demonstrate overlapping clinical and pathophysiological features of both conditions, a phenomenon commonly referred to as asthma-COPD overlap (ACO) (1). In a subset of individuals with asthma who have not been significantly exposed to conventional COPD risk factors, persistent fixed airflow obstruction (FAO) may still develop. This phenotype is often characterised by neutrophil-predominant airway inflammation and reduced responsiveness to corticosteroid therapy (2). These overlapping clinical and pathophysiological features obscure the distinction between asthma and COPD, thereby complicating disease classification and presenting significant challenges to the development of personalised treatment strategies.

Accurate differentiation between asthma-associated FAO and COPD-associated FAO is of critical clinical importance, as these conditions differ in long-term prognosis, underlying inflammatory mechanisms and therapeutic responses.

This study aims to compare plasma levels of key inflammatory cytokines among four patient groups: Asthma, Asthma with Smoking history, Asthma with FAO and ACO. By delineating the cytokine profiles across these phenotypes, we seek to better understand the immunopathological differences and potential biomarkers that may aid in diagnosis and personalised treatment strategies.

Materials and methods

Study design and population

A prospective, cross-sectional study was conducted at the Asthma Management Department of Hai Phong International General Hospital, Vietnam, from January 2020 to May 2023. Inclusion criteria were: age ≥ 16 years, a confirmed diagnosis of asthma according to the Global Initiative for Asthma (GINA) (3) guidelines, ongoing standardised controller therapy and provision of written informed consent. All participants were clinically stable,

having experienced no asthma exacerbations for at least 1 month before enrolment.

Patients receiving biologic therapies were excluded. Additional exclusion criteria included the presence of other pulmonary diseases (e.g., bronchiectasis, lung cancer, interstitial lung disease and tuberculosis), severe systemic comorbidities involving cardiovascular, hepatic, or renal systems and parasitic infections confirmed by stool examination.

A total of 120 patients met the inclusion criteria and were enrolled. Participants were stratified into subgroups based on smoking history, spirometric parameters and established diagnostic criteria (Figure 1):

- **Asthma + Smoking Group:** Included patients with a smoking history of ≥ 10 pack-years, without clinical or spirometric evidence of chronic airflow obstruction. This subgroup comprised 15 patients.
- **Asthma + FAO Group:** Comprised asthmatic patients with a post-bronchodilator forced expiratory volume in 1 s (FEV₁)/forced vital capacity (FVC) ratio $< 70\%$, in the absence of known exposure to COPD risk factors (4). A total of 34 patients met these criteria.
- **ACO Group:** Diagnosed according to the criteria established by the Spanish Society of Pulmonology and Thoracic Surgery, which incorporate clinical, functional and laboratory parameters (5). This group included 15 patients.
- **Asthma Group:** Comprised 56 patients with asthma who did not meet criteria for the above subgroups.

Clinical assessment

All participants underwent a comprehensive clinical evaluation following standardized protocols. This assessment included detailed documentation of medical and allergic history, review of family history, systematic screening for comorbid conditions and quantification of exacerbation frequency. Comorbidities – such as diabetes mellitus, hypertension, gastroesophageal reflux disease (GERD) and obstructive sleep apnoea (OSA) – were diagnosed and managed by relevant specialists to ensure clinical stability at the time of enrolment.

Allergic history was assessed primarily through clinical evaluation. Diagnoses of drug hypersensitivity, vaccine reactions, allergic rhinitis, allergic conjunctivitis and atopic

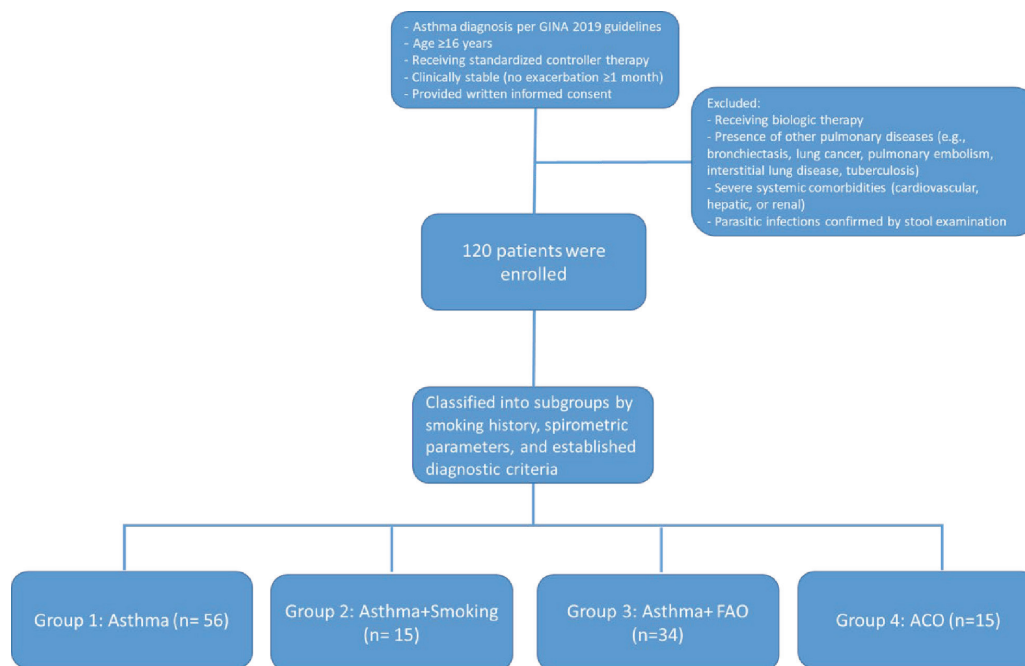


Figure 1. Study design. ACO, asthma–COPD overlap; FAO, fixed airflow obstruction.

dermatitis were confirmed by specialists. Additional allergic manifestations, including urticaria and angioedema triggered by seafood, insect stings or other environmental exposures, were recorded based on patient history. Allergen-specific testing (e.g., skin prick test, radioallergosorbent test) was not performed as part of this study protocol.

Laboratory methods

Peripheral blood eosinophil counts were measured using the DxH 800 automated haematology analyzer (Beckman Coulter, Brea, California, USA). Serum total immunoglobulin E (IgE) concentrations were quantified on the Cobas E411 immunoassay analyzer (Roche Diagnostics, Rotkreuz, Switzerland) employing the immune nephelometry method, in strict accordance with the manufacturer's protocols. Pulmonary function testing and bronchodilator reversibility assessments were performed using the Koko spirometry system (nSpire Health, Longmont, Colorado, USA), following standardised spirometric procedures as outlined in the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (6). Fractional exhaled nitric oxide (FeNO) levels were determined with the Medisoft FeNO analyzer (Medisoft S.A., Sorinnes, Belgium).

Serum cytokine concentrations were determined via a multiplex bead-based immunoassay utilizing flow cytometry technology (Bio-Plex platform, Bio-Rad Laboratories, Hercules, California, USA), with assay reagents procured from Bender MedSystems GmbH (Vienna, Austria) and Thermo Fisher Scientific (Waltham, Massachusetts, USA).

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics software, version 27 (IBM Corp., Armonk, NY, USA). Categorical variables were described as percentages, and continuous variables as means $\pm$ standard deviation or median with interquartile range, depending on distribution. For continuous variables, one-way analysis of variance (ANOVA) was applied when the assumptions of normality and homogeneity of variance were satisfied, with Tukey's honestly significant difference test performed for post hoc pairwise comparisons. When these assumptions were not met, non-parametric methods were used, including the Kruskal–Wallis test followed by Mann–Whitney *U* tests for pairwise comparisons. The Bonferroni adjustment was employed to account for multiple testing and control the family-wise error rate.

Categorical variables were examined using Pearson's chi-square test, and when expected cell counts were below five, Fisher's Exact Test with Monte Carlo simulation techniques was applied to obtain more reliable *P*-value estimates. For all statistical procedures, a two-sided *P*-value <0.05 was considered statistically significant.

Ethical considerations

The research protocol was approved by the Ethics Committee of Hai Phong International General Hospital (Approval No. 09/2020/HH-IRB, dated January 6, 2020) and was conducted in full compliance with the principles of the Declaration of Helsinki. All participants provided written informed consent after receiving comprehensive information about the

Table 1. Clinical and laboratory characteristics in asthma, asthma + smoking, asthma + FAO, and ACO groups.

Variable	Asthma (n = 56)	Asthma + Smoking (n = 15)	Asthma + FAO (n = 34)	ACO (n = 15)	P-value
Age (years)	45.0 ± 15 ^{§, &}	53.0 ± 16	54 ± 15 [*]	64.0 ± 8.0 [*]	<0.01^y
Male, n (%)	11 (19.6) ^{#, &}	14 (93.3) ^{*, §}	4 (11.8) ^{#, &}	14 (93.3) ^{*, §}	<0.01^z
BMI (kg/m ²)	22.3 ± 2.6	22.7 ± 2.1	23.0 ± 2.7	22.0 ± 3.3	0.57 ^y
Smoking, n (%)	0 ^{#, &}	15 (100.0) ^{*, §}	0 ^{#, &}	15 (100.0) ^{*, §}	<0.01^z
Allergic history, n (%)	32 (57.1)	7 (46.7)	20 (58.8)	7 (10.6)	0.78 ^t
Diabetes, n (%)	6 (10.7)	1 (6.7)	5 (14.7)	0	0.43 ^z
Hypertension, n (%)	8 (14.4)	1 (6.7)	4 (11.8)	2	0.89 ^z
OSA, n (%)	11 (19.6)	6 (40.0)	10 (29.4)	1 (6.7)	0.12 ^z
GERD, n (%)	15 (26.8)	2 (13.3)	10 (29.4)	7 (46.7)	0.24 ^z
Serum total IgE (IU/mL)	158.1 [130.0–356.4]	251.4 [70.0–460.6]	154.5 [54.3–464.3]	216.7 [66.3–462.5]	0.88 ^x
FeNO (ppb)	34 [19–52]	25 [17–39]	39 [29–61]	29 [22–48]	0.22 ^x
Blood eosinophil (cells/μL)	220.4 [130.0–356.4]	310.5 [134.2–461.1]	205.5 [121.8–427.8]	477.9 [170.1–92.0]	0.06 ^x

ACO, asthma–COPD overlap; ANOVA, analysis of variance; BMI, body mass index; FAO, fixed airflow obstruction; FeNO, fractional exhaled nitric oxide; OSA, obstructive sleep apnoea; GERD, gastroesophageal reflux disease; IgE, immunoglobulin E.

*Kruskal-Wallis *H* test.

^yANOVA.

^zFisher's exact test.

^tPearson's chi-square test.

P-values indicate statistical comparisons among groups.

The bold values indicate statistically significant results.

-*P* < 0.05 versus asthma group (*).

-*P* < 0.05 versus asthma + smoking group (#).

-*P* < 0.05 versus asthma + FAO group (\$).

-*P* < 0.05 versus ACO group (&).

study's objectives, procedures, potential risks, and benefits. The consent process was conducted in accordance with institutional and ethical guidelines, and the study was approved by the Ethics Committee of Hai Phong International General Hospital.

Results

The demographic, clinical, and laboratory characteristics of patients in asthma (*n* = 56), asthma + smoking (*n* = 15), asthma + FAO (*n* = 34), and ACO (*n* = 15) groups are summarized in Table 1.

A significant difference in age was observed among the groups (*P* < 0.01), with patients in the ACO (64.0 ± 8.0 years) and Asthma + FAO (54.0 ± 15 years) groups being significantly older than those in the Asthma group (45.0 ± 15 years).

Sex distribution also varied significantly across groups (*P* < 0.01), with a markedly higher proportion of male patients in the asthma + smoking and ACO groups (93.3% each) compared to the Asthma (19.6%) and Asthma + FAO (11.8%) groups.

No statistically significant differences were found in body mass index (BMI) among the four groups (*P* = 0.57). Similarly, the prevalence of allergic history, diabetes mellitus, hypertension, OSA and GERD did not differ significantly between groups (*P* > 0.05).

With respect to laboratory parameters, serum total IgE levels, FeNO and peripheral blood eosinophil counts were comparable across groups.

Pulmonary function indices among the Asthma, Asthma + Smoking, Asthma + FAO and ACO groups are detailed in Table 2. Statistically significant differences were observed across several spirometric parameters.

Significant differences were observed in several spirometric parameters. FEV₁% predicted differed significantly among groups (*P* < 0.01). Patients in the Asthma + FAO group exhibited the lowest median FEV₁ (58% [45–66]), significantly lower than those in the Asthma (79% [67–88]) and Asthma + Smoking (88% [75–95]) groups.

FEV₁ improvement (%) following bronchodilator administration was significantly different across groups (*P* < 0.01). The ACO (9% [4–16]) and Asthma + FAO (8% [5–11]) groups demonstrated greater reversibility compared to the Asthma (4% [0–8]) and Asthma + Smoking (3% [0–6]) groups. Although FEV₁ improvement in absolute volume (mL) did not reach statistical significance (*P* = 0.06), the ACO group tended to show the greatest improvement (170 mL [70–270]).

FVC% predicted was significantly lower in the Asthma + FAO group (72% [63–81]) compared to the Asthma group (82% [71–93]) (*P* = 0.02), while values in the ACO group were comparable to those in other group.

Table 2. Pulmonary function indices in asthma, asthma + smoking, asthma + FAO and ACO groups.

Parameter	Asthma (n = 56)	Asthma + Smoking (n = 15)	Asthma + FAO (n = 34)	ACO (n = 15)	P-value
FEV ₁ % predicted (%)	79 [67–88] [§]	88 [75–95] [§]	58 [45–66] ^{*,#}	72 [58–79]	<0.01
FEV ₁ improvement (mL)	80 [0–150]	80 [0–130]	100 [70–160]	170 [70–270]	0.06
FEV ₁ improvement (%)	4 [0–8] ^{§,&}	3 [0–6] [§]	8 [5–11] ^{*,#}	9 [4–16] [*]	<0.01
FVC% predicted (%)	82 [71–93] [§]	86 [75–91]	72 [63–81] [*]	81 [72–85]	0.02
FEV ₁ /FVC ratio (%)	76 [73–80] ^{§,&}	78 [74–79] ^{§,&}	64 [58–67] ^{*,#}	64 [60–68] ^{*,#}	<0.01
PEF% predicted (%)	62 [51–71] [§]	66 [62–76] [§]	43 [35–52] ^{*,#}	56 [33–71]	0.09
FEF _{25%–75%} predicted (%)	66 [52–74] [§]	80 [64–102] [§]	33 [22–40] ^{*,#}	49 [37–65]	<0.01

ACO, asthma–chronic obstructive pulmonary disease overlap; FAO, fixed airflow obstruction; FEF_{25%–75%}, forced expiratory flow at 25%–75% of FVC; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow.

Overall group comparisons were performed using the Kruskal–Wallis *H* test. Post hoc pairwise comparisons were conducted using the Mann–Whitney *U* test, with Bonferroni correction applied for multiple testing.

P-values indicate statistical comparisons among groups.

The bold values indicate statistically significant results.

–*P* < 0.05 versus asthma group (*).

–*P* < 0.05 versus asthma + smoking group (#).

–*P* < 0.05 versus asthma + FAO group (§).

–*P* < 0.05 versus ACO group (&).

Table 3. Plasma inflammatory cytokine levels in asthma, asthma + smoking, asthma + FAO, and ACO groups.

	Asthma (n = 56)	Asthma + Smoking (n = 15)	Asthma + FAO (n = 34)	ACO (n = 15)	P-value
IFN-α (ng/L)	0.53 [0.4–1.0]	0.9 [0.4–1.5]	0.4 [0.4–1.4]	1.1 [0.6–2.3]	0.12
IFN-γ (ng/L)	5.3 [5.1–10.0]	6.1 [5.1–12.0]	5.1 [5.1–10.0]	9.0 [7.0–12.4]	0.26
IL-1β (ng/L)	1.4 [0.8–2.7] [§]	1.6 [0.8–2.7]	1.0 [0.81–2.7] [§]	3.8 [2.7–4.8] ^{*,§}	<0.01
IL-4 (ng/L)	19.7 [6.9–29.8]	20.3 [15.0–32.8]	18.1 [10.3–33.8]	21.1 [11.8–29.9]	0.66
IL-12 (ng/L)	37.5 [19.5–269.0]	38.5 [22.6–283.1]	33.2 [20.0–195.0]	284.7 [52.8–373.6]	0.054
IL-13 (ng/L)	7.2 [1.7–10.5]	5.3 [1.7–12.1]	6.1 [1.7–10.5]	10.5 [1.7–48.4]	0.27
IL-17 (ng/L)	6.8 [1.5–12.8] [§]	8.5 [1.5–17.7]	5.1 [1.5–10.0] [§]	16.4 [6.9–26.5] ^{*,§}	<0.01
TNF-α (ng/L)	7.6 [3.5–16.0] [§]	12.9 [3.5–21.5]	5.3 [3.5–17.2] [§]	18.8 [10.2–23.0] ^{*,§}	0.01

ACO, asthma–chronic obstructive pulmonary disease overlap; FAO, fixed airflow obstruction; IFN-α/IFN-γ, interferon alpha/gamma, IL, interleukin, TNF-α, tumour necrosis factor alpha.

Overall group comparisons were performed using the Kruskal–Wallis *H* test. Post hoc pairwise comparisons were conducted using the Mann–Whitney *U* test, with Bonferroni correction applied for multiple testing.

P-values indicate statistical comparisons among groups.

The bold values indicate statistically significant results.

–*P* < 0.05 versus asthma group (*).

–*P* < 0.05 versus asthma + FAO group (§).

–*P* < 0.05 versus ACO group (&).

Although PEF% predicted did not differ significantly among groups (*P* = 0.09), the Asthma + FAO group had the lowest values (43% [35–52]).

FEF_{25%–75%} predicted, an indicator of small airway function, was significantly reduced in the Asthma + FAO group (33% [22–40]) compared to the Asthma (66% [52–74]) and Asthma + Smoking (80% [64–102]) groups (*P* < 0.01). The ACO group showed intermediate impairment (49% [37–65]), with no statistically significant difference compared to the other three groups.

Plasma concentrations of inflammatory cytokines among the Asthma, Asthma + Smoking, Asthma + FAO and ACO groups are presented in Table 3. Significant intergroup differences were observed for IL-1β, IL-17 and tumour necrosis factor alpha (TNF-α).

IL-1β levels differed significantly across groups (*P* < 0.01). The ACO group exhibited markedly elevated concentrations (3.8 ng/L [2.7–4.8]), significantly higher than those in the Asthma (1.4 ng/L [0.8–2.7]) and Asthma + FAO (1.0 ng/L [0.81–2.7]) groups (Figure 2).

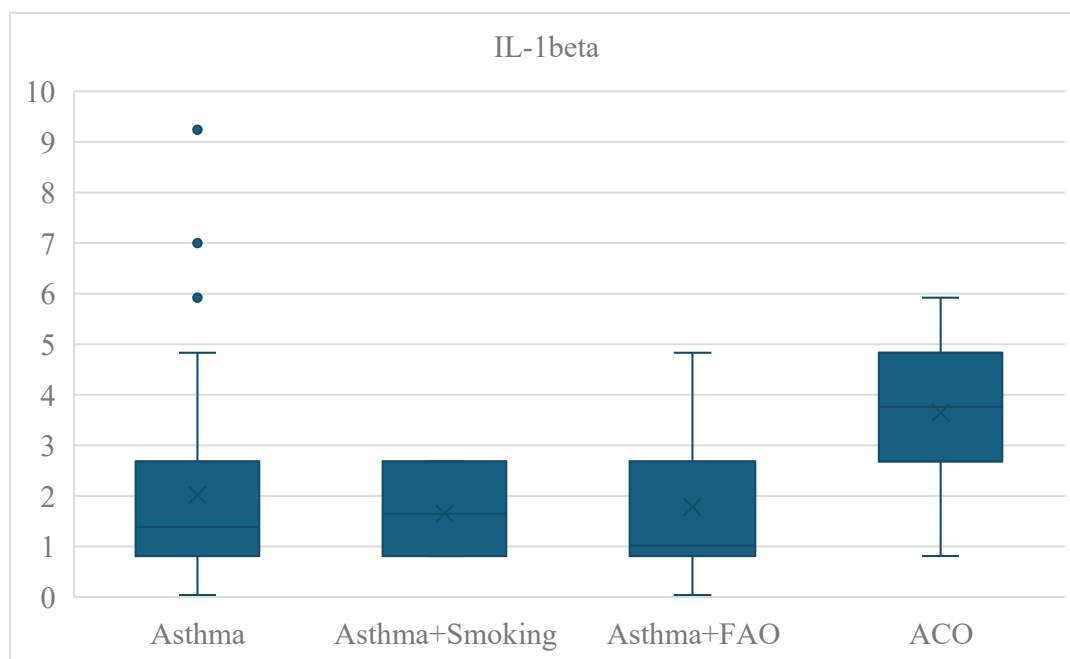


Figure 2. Plasma IL-1 β concentrations across patient groups: asthma, Asthma+Smoking, Asthma+FAO, and ACO.

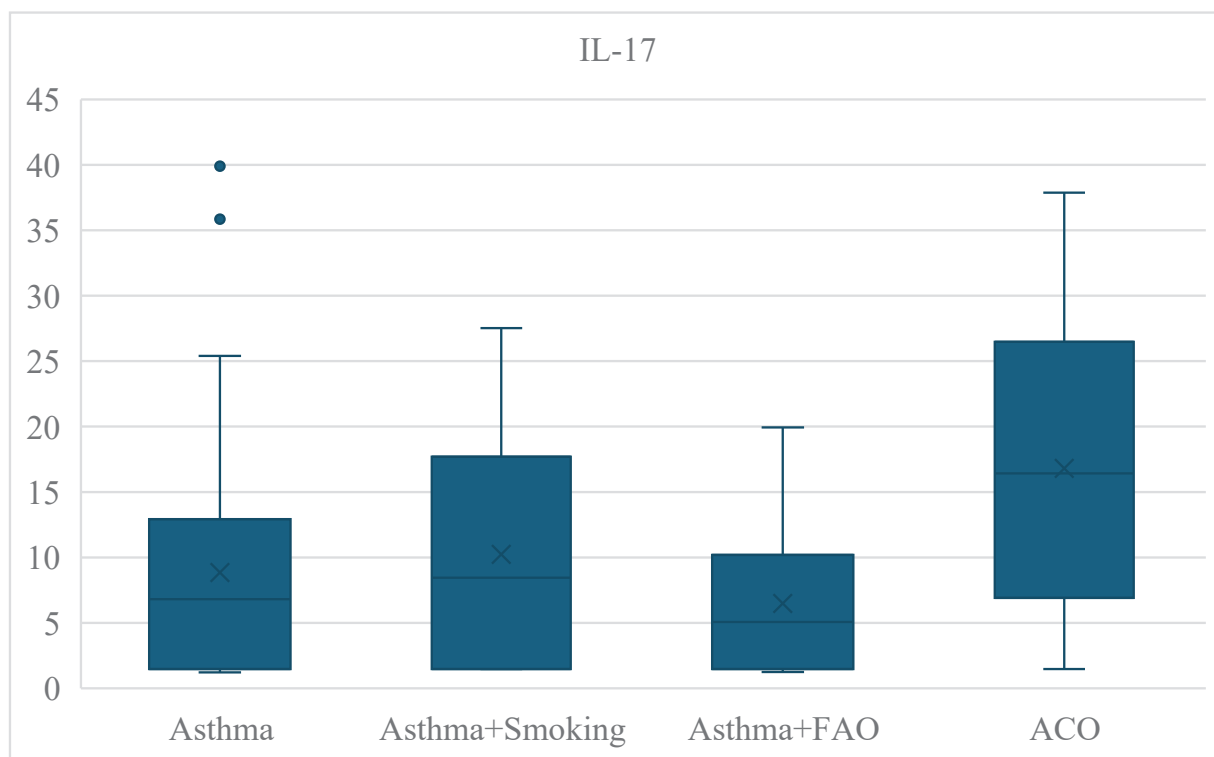


Figure 3. Plasma IL-17 concentrations across patient groups: asthma, Asthma+Smoking, Asthma+FAO, and ACO.

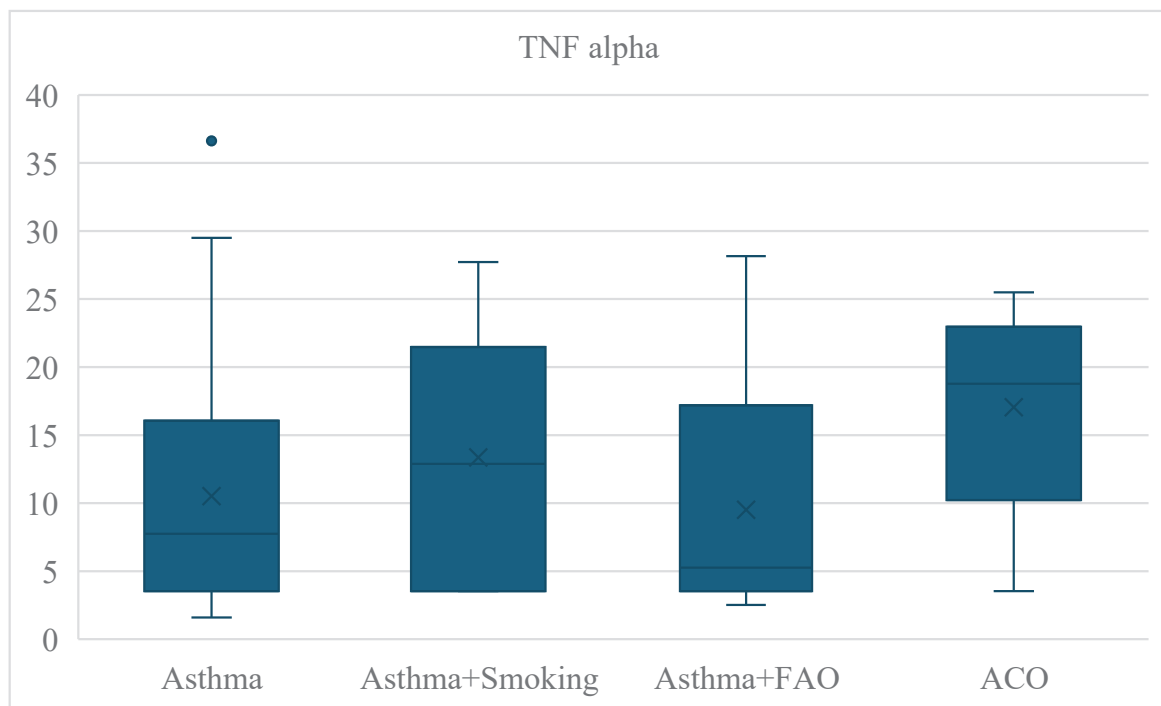


Figure 4. Plasma TNF- α concentrations across patient groups: asthma, Asthma+Smoking, Asthma+FAO, and ACO.

IL-17 levels were also significantly increased in the ACO group (16.4 ng/L [6.9–26.5]) compared to the Asthma (6.8 ng/L [1.5–12.8]) and Asthma + FAO (5.1 ng/L [1.5–10.0]) groups ($P < 0.01$) (Figure 3).

Similarly, TNF- α concentrations were significantly higher in the ACO group (18.8 ng/L [10.2–23.0]) than in the Asthma (7.6 ng/L [3.5–16.0]) and Asthma + FAO (5.3 ng/L [3.5–17.2]) groups ($P = 0.01$) (Figure 4).

No statistically significant differences were observed in IFN- α , IFN- γ , IL-4, IL-12, or IL-13 levels among the four groups ($P > 0.05$).

Discussion

Patients in the ACO and asthma + FAO groups were significantly older than those with typical asthma. This observation is consistent with previous evidence that ACO predominantly manifests in older individuals as a result of cumulative airway remodelling and prolonged inflammatory exposure (7). Similarly, in asthma, FAO represents long-term structural airway alterations driven by chronic inflammation, which are more likely to arise in older patients (8). The predominance of male patients in the ACO and asthma + smoking groups aligns with epidemiological data suggesting higher smoking rates and COPD prevalence among males (7, 9).

No significant differences were found in BMI, allergic history or comorbidities such as diabetes, hypertension, OSA and GERD. This suggests that these factors may not be primary discriminators among asthma phenotypes, although GERD and OSA have been implicated in exacerbation risk and poor asthma control (10). Laboratory parameters – including serum total IgE, FeNO and peripheral blood eosinophil counts – did not differ significantly across groups.

This study revealed significant differences in pulmonary function indices among patients with typical asthma, asthma with smoking history, asthma with FAO and ACO, underscoring the functional heterogeneity that characterises distinct asthma phenotypes.

Patients in the asthma + FAO group exhibited the most pronounced reduction in FEV₁% predicted (median 58%), significantly lower than those in the asthma and asthma + smoking groups, highlighting the severity of fixed airflow limitation in this subgroup. This finding is consistent with previous studies showing that persistent airflow obstruction is a hallmark of long-standing asthma and is often indistinguishable from COPD in clinical practice (2, 8, 11).

Bronchodilator reversibility, expressed as the percentage improvement in FEV₁, was significantly higher in the ACO and Asthma + FAO groups compared with patients with asthma or asthma + smoking. Although the absolute changes in FEV₁ volume did not reach statistical significance, a consistent

trend toward greater reversibility was observed in ACO patients. It is noteworthy that pulmonary function testing was performed during a clinically stable phase, reflecting a potentially more active inflammatory state in these groups compared with patients with typical asthma. Similarly, Erbay et al. (12) reported that the bronchodilator response was significantly higher in ACO patients than in those with asthma or COPD. The greater percentage improvement in FEV₁ observed in the Asthma + FAO group, compared with asthma or asthma + smoking cohorts, may indicate that underlying airway inflammation in this population remains insufficiently controlled.

In our analysis, the pronounced reduction in FEF_{25–75%} observed in the Asthma + FAO group indicates significant involvement of small airway dysfunction. This impairment most likely reflects ongoing airway inflammation and structural remodelling, both of which are key contributors to the pathogenesis of FAO (13). Supporting this interpretation, Chen et al. (11) demonstrated that reduced FEF_{25–75%} values were strongly associated with the presence of FAO in patients with asthma, underscoring its clinical relevance as a marker of small airway disease and progression to irreversible obstruction.

The observed disparities in ventilator function across patient subgroups highlight the critical need for precise characterisation of airway inflammatory phenotypes within each cohort. Such differentiation is not merely academic – it can guide clinicians in selecting targeted therapies that address the underlying pathophysiology more effectively.

In this study, patients with ACO demonstrated significantly higher plasma concentrations of IL-1 β , IL-17 and TNF- α compared with asthma and asthma + FAO groups, while no significant differences were observed for IFN- α , IFN- γ , IL-4, IL-12 or IL-13.

Specifically, IL-1 β levels in ACO (3.8 ng/L [2.7–4.8]) were almost threefold higher than in asthma (1.4 ng/L [0.8–2.7]) and asthma + FAO (1.0 ng/L [0.81–2.7]) groups ($P < 0.01$). This finding is consistent with emerging evidence that IL-1 β is a central mediator in the inflammatory milieu of ACO, reflecting activation of innate immune pathways beyond the classical type 2 inflammation typically observed in asthma. IL-1 β has been implicated in chronic airway inflammation, promoting neutrophilic infiltration and airway remodelling (14). Cigarette-smoke and ambient particulate exposures activate the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome–IL-1 β axis in the airway epithelium and macrophages, amplifying chronic airway inflammation characteristic of smoking-related disease (15). In asthma, IL-1 β elevation has been variably associated with severe and neutrophilic phenotypes, but its levels are lower than in smoking-related COPD or

ACO, suggesting that cigarette smoke and environmental exposures act as potent triggers of the NLRP3–IL-1 β axis (16). By contrast, FAO in asthma has been increasingly attributed to chronic structural and parenchymal alterations that processes less dependent on inflammasome-driven cytokine release, which may explain the relatively lower IL-1 β levels observed in the asthma + FAO group compared with ACO (8).

In this study, plasma IL-17 concentrations were significantly elevated in the ACO group compared to both the asthma and asthma with FAO groups ($P < 0.01$). This finding underscores the distinct inflammatory profile of ACO, characterised by heightened Th17-mediated immune responses. Elevated IL-17 levels have been associated with increased airway hyperresponsiveness, mucus production and recruitment of neutrophils, contributing to persistent airflow limitation in chronic airway diseases (17). Recent experimental models have demonstrated that IL-17 blockade can attenuate airway inflammation and remodelling in ACO. Camargo et al. (18) showed that anti-IL-17 monoclonal antibody treatment significantly reduced inflammatory cell infiltration, oxidative stress markers and extracellular matrix remodelling in an ACO mouse model.

The present study demonstrated significantly elevated TNF- α concentrations in the ACO cohort compared to both the Asthma and Asthma + FAO groups ($P = 0.01$). In ACO, the coexistence of eosinophilic and neutrophilic inflammation may amplify TNF- α production, contributing to persistent airflow limitation and poor therapeutic response. Kubysheva et al. (19) reported that TNF- α concentrations were significantly higher in patients with ACO compared to those with asthma or COPD alone, and these elevations correlated negatively with FEV₁ and FEV₁/FVC ratios, indicating a direct link between TNF- α -mediated inflammation and ventilatory impairment. Furthermore, TNF- α has been shown to synergise with other proinflammatory cytokines such as IL-1 β and IL-17 (17), amplifying the inflammatory cascade and promoting steroid insensitivity. This cytokine network may explain the more severe clinical presentation and reduced treatment responsiveness observed in ACO patients.

The distinct inflammatory profiles observed in ACO and Asthma + FAO phenotypes suggest that, despite similarities in clinical presentation and ventilatory function, these entities represent fundamentally different pathobiological processes. Notably, tobacco smoke exposure appears to exert a decisive influence on the nature of airway inflammation. This underscores the importance of delineating ACO and Asthma + FAO as separate clinical phenotypes, each requiring tailored therapeutic approaches aligned with their respective inflammatory mechanisms.

Conclusion

This study demonstrates that asthma, asthma with smoking exposure, asthma with FAO and ACO represent clinically and immunologically distinct entities. Among these, ACO was characterised by significantly elevated concentrations of IL-1 β , IL-17 and TNF- α .

The marked elevations of IL-1 β and IL-17 in ACO highlight the contribution of innate and Th17-mediated immune pathways, which may be further amplified by environmental exposures such as tobacco smoke. In contrast, the distinct inflammatory and functional signatures observed in the asthma + FAO group reinforce the concept of heterogeneity across obstructive airway diseases and underscore the necessity of precise phenotypic classification for accurate diagnosis and targeted therapy.

Collectively, these findings underscore the clinical value of integrating spirometry indices with biomarker profiling for phenotypic stratification in obstructive airway diseases.

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Author contributions

- Ta Ba Thang: idea of writing an article, writing a text.
- Dao Ngoc B ng, Nguyen Hoang Cuong: Patient recruitment and data collection, writing a text.
- Pham Dac The, Pham Thi Kim Nhung, Nguyen Tien Dung: Laboratory analysis and interpretation.
- Bach Quoc Tuan, Nguyen Hai Cong, Le Thi Dieu Hien: Statistical analysis and literature review.
- Vu Minh Duong: Study design, manuscript drafting and correspondence.

Conflicts of interest

The authors declare not to have any conflicts of interest that may be considered to influence directly or indirectly the content of the manuscript.

Artificial intelligence involvement

No part of this manuscript was produced with the assistance of artificial intelligence software or tools.

Informed Consent Statement

The research protocol was approved by the Ethics Committee of Hai Phong International General Hospital (Approval No. 09/2020/HH-IRB, dated January 6, 2020) and was conducted in full compliance with the principles of the Declaration of Helsinki. All participants provided written informed consent after receiving comprehensive information about the study's objectives, procedures, potential risks, and benefits. The consent process was conducted in accordance with institutional and ethical guidelines. Informed consent was obtained from all subjects involved in the study. Written informed consent was also obtained from the patients for the publication of this paper, including any potentially identifiable images or data contained within.

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