ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
December 2024; 23(6):625-640.
DOI: 10.18502/ijaai.v23i6.17373

The Role of Th17/Treg Imbalance, FeNO, Eosinophils, IgE and Their Correlation with Lung Function Parameters with Asthma-chronic Obstructive Pulmonary Disease

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Received: 1 May 2024; Received in revised form: 27 July 2024; Accepted: 27 July 2024

ABSTRACT

This study explored the link between clinical features, immune markers, and asthma-chronic obstructive pulmonary disease overlap (ACO), aiming to enhance diagnostic precision and tailor treatment.

The study included 60 patients per group: COPD patients, ACO patients, and healthy controls. Biological indicators such as fractional exhaled nitric oxide (FeNO), eosinophils, immunoglobulin E (IgE), T helper (Th) 17 cell counts, regulatory T-cell (Treg) counts, and cytokine levels of interleukin-17 (IL-17) and interleukin-10 (IL-10) were measured using standard enzyme-linked immunosorbent assay and flow cytometry techniques.

Elevated Th17 cells, IL-17, and Th17/Treg ratio, alongside reduced IL-10 and Treg levels, were observed in COPD and ACO patients. ACO patients showed worse lung function, with a negative correlation between FeNO, Th17 cells, Th17/Treg ratio, IL-17, and lung function indices, and a positive correlation with residual volume/total lung capacity (RV/TLC) ratio.

The study suggests that Th17/Treg imbalance, FeNO, eosinophils, and IgE could be key in ACO pathogenesis, potentially aiding early diagnosis and targeted treatment. Future research may utilize these findings to develop preventative and therapeutic strategies for ACO.

Keywords: Asthma-COPD overlap syndrome; Cytokines; Lung function; Regulatory T cells; Systemic inflammation; Th17 cells

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INTRODUCTION

Asthma-chronic obstructive pulmonary disease overlap (ACO) is a chronic inflammatory airway disease characterized by incompletely reversible airflow limitation, encompassing overlapping features of both asthma and chronic obstructive pulmonary disease (COPD).[1] Recent studies have estimated that the ACO prevalence at 15% to 30% of chronic airway disease cases,[2,3] representing a major public health burden.[4] Epidemiological data indicate a higher prevalence in middle-aged and elderly populations,[2] with important implications for underdiagnosis and management. COPD involves chronic parenchymal and airway inflammation mediated predominantly by neutrophils, macrophages, and cytokines like tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, and IL-10.[5,6] Inflammation triggers small airway fibrosis and parenchymal destruction, leading to incompletely reversible airflow limitation that is usually progressive with disease advancement.[7] In contrast, patients with asthma demonstrate eosinophilic airway inflammation driven by T helper (Th) type 2 cytokines like IL-4, IL-5, and IL-13.[8] The cardinal symptoms are variable expiratory airflow limitations, intermittent wheezing, breathlessness, and chest tightness.[9]

ACO patients possess both inflammatory profiles, with elevated eosinophils and neutrophils.[10] These patients display persistent airflow limitations and exacerbations akin to COPD, along with symptoms like wheezing and breathlessness similar to asthma.[11] This heterogeneity and symptom variability make clinical recognition and timely intervention difficult.[12] Compared to COPD, ACO leads to accelerated lung function decline, worsened quality of life, increased healthcare utilization, and higher mortality.[4,13] Approaches for patient stratification and targeted management remain unclear given the heterogeneity. Likewise, the pathogenesis of ACO has not been fully elucidated, and there are currently no universally accepted criteria for ACO.[14]

Some studies have begun to elucidate the immunopathogenesis of ACO, suggesting that immune dysregulation is a key feature. In ACO, the coexistence of Th2-driven eosinophilic inflammation and Th1/Th17-driven neutrophilic inflammation creates a complex inflammatory environment[15,16]. Th17 cells, in particular, are implicated in driving the chronic inflammation observed in ACO by secreting proinflammatory cytokines like IL-17, IL-21, and IL-22[17]. These cytokines promote extracellular matrix degradation and inflammatory cell infiltration, perpetuating airway and lung tissue inflammation. Conversely, regulatory T cells (Tregs), which secrete immunoregulatory factors like IL-10 and transforming growth factor (TGF)-beta, are typically reduced in ACO, leading to a loss of immune homeostasis and unchecked inflammation.[18]

Airway inflammation and airway responsiveness are key pathophysiological bases for disease progression in ACO,[1,19,20] with chronic inflammation control dependent on limiting bystander activation of autoreactive and potentially pathogenic T cells in vivo[21]. Two critical players in this process are Th17 cells and Tregs, both derived from $CD4^+$ T cells. Functionally antagonistic, these cells play vital roles in maintaining the body's immune equilibrium. Inflammatory cytokines like IL-17, IL-21 and IL-22 secreted by Th17 cells can stimulate extracellular matrix degradation and inflammatory cell infiltration, perpetuating airway and lung tissue inflammation. In contrast, Tregs predominantly secrete immunoregulatory factors like IL-10 and TGF-beta to suppress excessive activation of self and effector T cells, thereby maintaining immune homeostasis.[18] The balance between Th17 and Tregs is crucial in the context of many chronic inflammatory diseases.[22,23] In COPD patients, heightened Th17 and depleted Treg levels systemically and in airway samples disrupt this immune equilibrium to worsen inflammation and airway remodeling. Notably, the Th17/Treg ratio in peripheral blood correlates with the degree of airflow limitation in COPD patients, suggesting that this imbalance serves as a key immunological driver of disease advancement.[24,25] Expanding on the role of Th17 and Tregs in ACO, Th17 cells contribute to the inflammatory damage observed in ACO by secreting inflammatory cytokines. On the other hand, the number of Tregs, which inhibit autoimmune responses, is decreased in ACO, further exacerbating the inflammatory response. This imbalance, therefore, plays a crucial role in the progression of ACO, although the exact mechanism of how Th17 and Tregs regulate the immune response in ACO is still unclear. Correcting the Th17/Treg imbalance may represent a promising therapeutic approach for treating ACO. Some emerging studies are exploring the application of cytokine therapy

or Treg modulatory treatments to re-establish immune tolerance by downregulating Th17 or upregulating Treg reactivity.[26,27] Such immunomodulatory strategies hold promise for unveiling novel clinical interventions for improved ACO management.

Multiple factors, including genetic, environmental, and infectious factors, influence the immunopathology of ACO.[28] Genetic studies have implicated key genes such as those encoding IL-17A, IL-17F, IL-10, and TGF-β1 in ACO susceptibility and severity.[29] These genes play crucial roles in immune responses, and their variations may lead to an imbalance in the immune system, contributing to the pathogenesis of ACO.[30] Environmental factors also significantly contribute to ACO. Smoking, for instance, is a well-known risk factor for ACO.[28] It can induce airway inflammation and oxidative stress, leading to airway remodeling and lung function decline.[31] Air pollution, another environmental factor, has been associated with an increased risk of lung disease. Exposure to pollutants can trigger inflammatory responses in the airways, exacerbating the symptoms of ACO.[32] In addition to genetic and environmental factors, infectious factors, particularly viral infections, play a pivotal role in triggering or exacerbating ACO symptoms.[33] They can influence immune responses and cytokine production in ACO patients, leading to an imbalance in the immune system and contributing to the progression of the disease.[28]

ACO is complex due to its mixed features and standard treatments often fall short, necessitating reliable biomarkers for better diagnosis, monitoring, and treatment.[2,10,34] The mixed inflammatory profile of ACO complicates diagnosis, and biomarkers can help differentiate ACO from asthma and COPD, aiding in accurate diagnosis and appropriate treatment. ACO patients are prone to rapid lung decline and severe exacerbations;[35] biomarkers can identify at-risk patients, allowing early intervention.[36] The heterogeneity of ACO requires personalized treatment, and biomarkers can guide targeted therapies based on specific inflammatory pathways.[37] Moreover, improved diagnostic accuracy and targeted treatments through biomarkers can decrease emergency interventions and hospitalizations, thus lowering healthcare costs. Therefore, biomarkers are essential for enhancing diagnosis, predicting progression, personalizing treatments, and reducing healthcare costs in ACO management.

Despite progress in understanding the role of these factors in ACO pathogenesis, the integration of these factors remains incompletely understood.[29] Furthermore, the roles of other immune cells and cytokines in ACO pathogenesis remain underexplored. Genetic factors influencing immune responses in ACO and the influence of environmental and infectious triggers on immune dysregulation are also areas that warrant further investigation. This highlights a significant gap in current research and underscores the need for further exploration of the molecular and cellular mechanisms involved.

In the current study, we aimed to advance the understanding of ACO, particularly in terms of the immune response and pulmonary function. We explored the roles of Th17 and Tregs, and the expression levels of inflammatory cytokines in peripheral blood lymphocytes in the development of COPD and ACO. We further analyzed their correlations with pulmonary function indicators in patients with COPD and ACO. Our research objectives were to elucidate ACO pathogenesis and to identify potential therapeutic targets for ACO.

MATERIALS AND METHODS

Rigorous Participant Selection and Criteria

A total of 266 subjects were initially considered for the study, including 166 outpatients and inpatients diagnosed with COPD and ACO in acute exacerbation. These cases were recorded between March 2019 and June 2021 at the Affiliated Dongguan Hospital of Jinan University (Binhaiwan Central Hospital of Dongguan). The participants underwent a rigorous screening process based on specific inclusion and exclusion criteria aligned with the 2021 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. After the screening, we had 60 eligible patients each for the COPD and ACO groups. Additionally, we enrolled 60 healthy individuals to form a control group. The detailed selection process is illustrated in Figure 1. Participants were included if they met all the following conditions: (I) diagnosed with COPD or ACO according to the diagnostic standards set by the 2021 GOLD guidelines; (II) mentally sound and capable of communication; (III) chest imaging revealed no significant shadows; (IV) exhibited symptoms like chronic coughing, sputum production, chest tightness, or shortness of breath; and (V) provided signed informed consent. Participants were excluded under these circumstances: (I) failure to meet all diagnostic criteria mentioned above; (II) history of

glucocorticoid treatment within 1 month prior to enrollment; (III) presence of pulmonary embolism, pulmonary interstitial fibrosis, or active pulmonary tuberculosis; (IV) existence of severe diseases related to the blood system or digestive system, critical conditions involving the heart, liver, or kidney, or presence of malignant tumors; (V) underwent major surgery within the past 6 months; (VI) inability to complete examination items pertinent to this study.

General Information

Demographic data, including age, sex, and body mass index (BMI), were collected using customized questionnaires. These variables were chosen due to their potential influence on the health status and disease progression of patients with COPD or ACO. Each patient provided written informed consent prior to data collection, ensuring ethical compliance. The study received approval from the Ethics Committee of Bihaiwai Central Hospital of Dongguan (2021013). Additionally, a control group was established, consisting of 60 subjects who were admitted within the same period, and matched the patients with COPD or ACO in terms of gender and age. Statistical comparisons of the general information among the groups revealed no significant difference (all $p>0.05$, as shown in Table 1).

Pulmonary function indicators

We utilized the German YAEGER lung function detector to conduct a series of pulmonary function tests. These tests were chosen due to their ability to provide comprehensive insights into the respiratory health of the participants. The tests included the measurement of the forced expiratory rate in the first second (FEV1) after bronchodilator inhalation and the ratio of FEV1 to forced vital capacity (FEV1/FVC), forced expiratory volume in 1 second for the expected percentage (FEV1% pred), forced vital capacity for the expected percentage (FVC% pred), the average expiratory flow rate at 25% to 75% of the vital capacity, also known as forced expiratory flow 25–75% (FEF 25–75%), and the ratio of residual volume for total lung capacity (RV/TLC). These specific indicators were selected to assess airflow limitation severity in alignment with the GOLD guidelines for COPD and ACO diagnosis. Lung function tests were conducted using the same personnel and equipment across all groups.

Flow Cytometry Analysis of Th17 and Tregs

The Th17/Treg ratio in patients with COPD and ACO was detected using a Th17/Treg phenotyping kit (BD Biosciences, Mountainview, CA, USA). The process began with the isolation of peripheral blood mononuclear cells from whole blood samples prior to the beginning of therapy. This isolation was achieved using Isopaque-Ficoll (Lymphoprep, Nycomed Pharma, Oslo, Norway). Following isolation, the cells were subjected to gradient centrifugation and were washed twice with Dulbecco's phosphate-buffered saline (DPBS)/10% fetal bovine serum (FBS, Bovogen, East Keilor, Vic, Australia) at 300*g* for 10 minutes at room temperature. The cells were then resuspended in 1 mL of DPBS/10% FBS. At this point, the cells were either cryopreserved for future use or immediately activated and processed for flow cytometry. A Th17/Treg phenotyping kit was used to identify Th17 and Tregs. The kit provides an easy-to-use three-color cocktail of fluorescent antibodies specific for human CD4 and IL-17A, for Th17 cells, and forkhead box P3 (FoxP3), for Tregs. The cells were either stimulated by culturing in the presence of PMA and ionomycin and the protein transport inhibitor BD GolgiStop for 5 hours or left unstimulated. The cells were then fixed, permeabilized, and stained with the Human Th17/Treg Phenotyping Cocktail as described in the protocol. Two-color dot plots showing the correlation of FoxP3 and IL-17A expression in $CD4^+$ T cells were generated from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD FACSCalibur System.

ELISA for Inflammatory Biomarkers

The serum immunoglobulin E (IgE) levels, peripheral blood eosinophil counts, and fractional exhaled nitric oxide (FeNO) levels were measured via enzyme-linked immunosorbent assay (ELISA). These biomarkers were selected due to their relevance in indicating the inflammatory response in studied populations. Patient serum samples were first diluted 1:100 using sample diluent buffer and 100 μL of calibrators, controls, or diluted samples were added per well in the 96-well microtiter plates precoated with analyte-specific antibodies. After incubation for 2 hours at 37°C with shaking at 200 rpm and washing 4 times, 100 μL of detection antibody was added to each well, incubated for 1 hour, washed, and incubated with 100 μL of avidin-HRP conjugate for 30 minutes. TMB

substrate was then added and incubated for 10 to 15 minutes and the reaction was terminated by adding 50 µL stop solution (Thermo Fisher Scientific, Waltham, MA, USA). The optical density was measured at 450 nm within 30 minutes using a microplate reader set and a standard curve was generated using the kit standards for quantitative determination of sample analyte concentrations.

Statistical Analysis

All statistical analyses were performed using the Statistic Package for Social Science (SPSS) software version 22.0 (IBM, Armonk, NY, USA). The normality of the distribution for each dataset was determined using the Shapiro-Wilk test. For normally distributed data, the mean±standard deviation was presented.

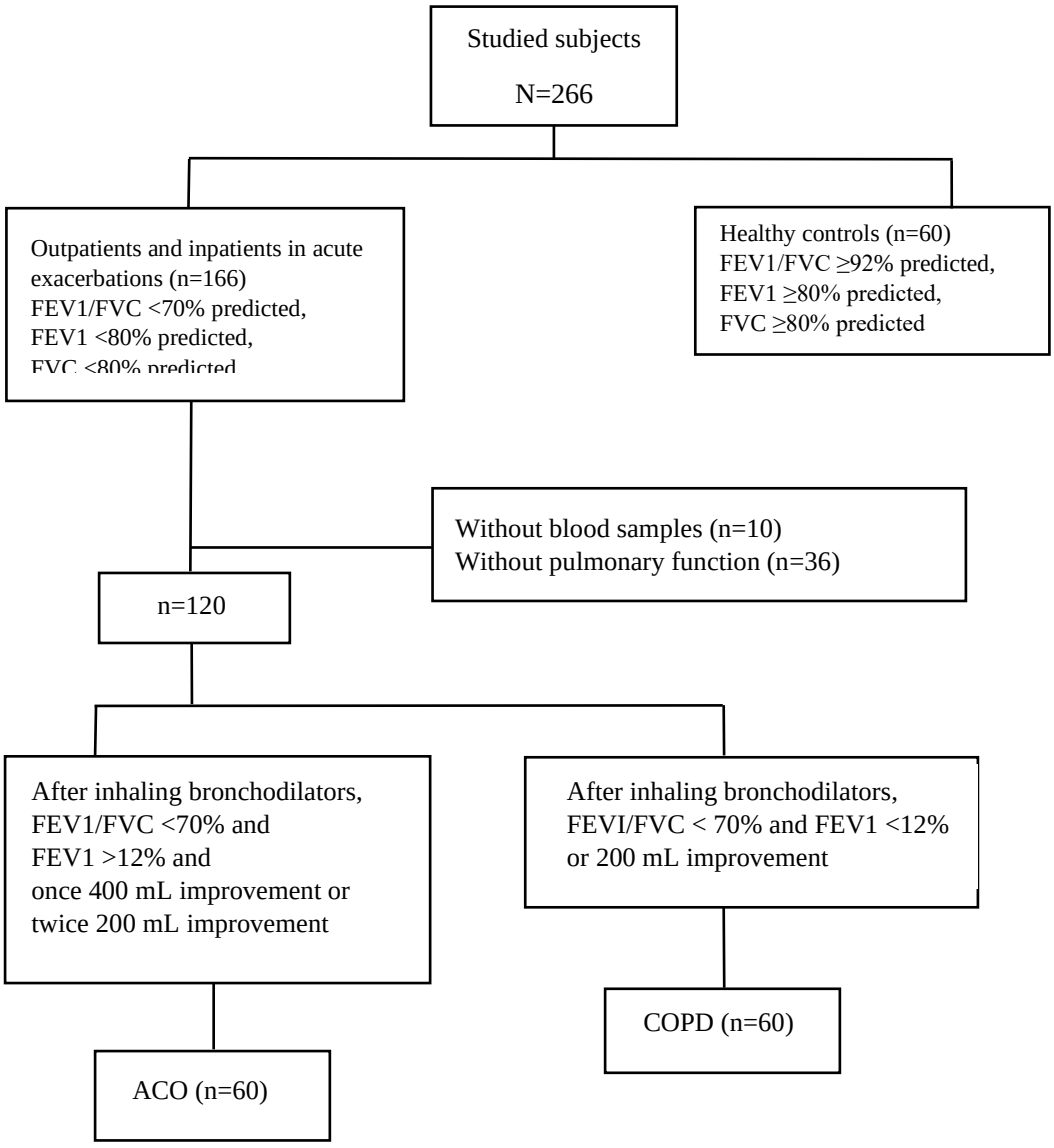

Figure 1. The flowchart of participant selection. After the screening of conditions, a total of 60 individuals were included in each of the asthma-COPD overlap (ACO), chronic obstructive pulmonary disease (COPD), and healthy control groups. FEV1: Forced Expiratory Volume in 1 Second; FVC: Forced Vital Capacity.

Table 1. The comparisons of baseline information and clinical characteristics in the studied participants.

	Control	COPD	ACO	*p* value[a]	*p* value[b]	*p* value[c]	*p* value[d]
Sociodemographic Details							
Age (years)	67.78 ± 3.68	69.03 ± 3.32	67.15 ± 10.18	0.205			
Sex (male/female)	40/20	40/20	40/20	1.000			
Body mass index (kg/m^2)	25.09 ± 3.46	22.84 ± 4.74[a]	22.44 ± 4.02[a]	0.0001	0.003	0.002	0.590
Lung Function Indicators							
FEV1% pred (%)	91.45 (78.47, 97.12)[b]	40.53 (32.19, 54.77)[a]	48.38 (34.69, 60.07)[a]	2.42×10^{-25}	1.11×10^{-22}	4.86×10^{-17}	0.240
FVC% pred (%)	80.50 (71.40, 89.11)[b]	54.40 (44.61, 66.34)[a,b]	70.30 (54.39, 82.60)[a]	9.82×10^{-12}	1.68×10^{-12}	2.25×10^{-4}	0.0013
FEV1% variation rate (%)	4.05 (3.18, 5.43)[b]	2.48 (1.02, 4.39)[b]	12.17 (10.32, 14.16)[a]	6.20×10^{-25}	0.047	5.21×10^{-15}	1.70×10^{-23}
FEV1/FVC (%)	81.86 (77.61, 86.46)[b]	60.14 (51.40, 65.75)[a]	56.65 (48.61, 64.86)[a]	1.12×10^{-20}	1.82×10^{-14}	2.38×10^{-18}	0.428
FEF 25–75% (L)	2.23 (1.76, 2.81)[b]	0.62 (0.44, 0.97)[a]	0.53 (0.38, 0.68)[a]	3.28×10^{-26}	8.87×10^{-18}	2.71×10^{-23}	0.266
RV/TLC (%)	26.51 (23.39, 30.47)[b]	65.72 (55.56, 76.14)[a]	58.33 (51.85, 71.15)[a]	6.94×10^{-27}	2.49×10^{-23}	6.40×10^{-19}	0.433
Inflammatory indices							
FeNO (ppb)	14.50 (6.25, 21.00)[b]	22.00 (17.25, 28.00)[ab]	33.50 (20.75, 46.75)[a]	1.87×10^{-14}	6.01×10^{-6}	3.70×10^{-15}	0.0014
Peripheral eosinophil count (10^9/L)	0.05 (0.02, 0.08)[b]	0.13 (0.06, 0.22)[a]	0.16 (0.05, 0.38)[a]	1.01×10^{-9}	3.28×10^{-7}	5.59×10^{-9}	0.0671
Serum IgE (10^9/L)	105.32 (49.29, 135.87)[b]	329.5 (179.3, 475.8)[a,b]	555.92 (501.96, 596.25)[a]	2.18×10^{-30}	2.68×10^{-9}	2.26×10^{-33}	2.14×10^{-8}
Immune Indices							
Th17 (%)	0.30 (0.21, 0.47)[b]	1.49 (1.13, 1.77)[ab]	2.36 (1.69, 2.68)[a]	2.51×10^{-28}	5.52×10^{-14}	4.60×10^{-28}	8.47×10^{-4}
Treg (%)	8.70 (7.80, 9.30)[b]	6.80 (6.15, 7.50)[ab]	4.68 (3.97, 5.56)[a]	1.45×10^{-28}	9.05×10^{-9}	1.54×10^{-29}	5.52×10^{-8}
Th17/Treg (%)	0.04 (0.02, 0.06)[b]	0.21 (0.17, 0.28)[ab]	0.49 (0.38, 0.61)[a]	1.82×10^{-30}	3.52×10^{-12}	4.90×10^{-31}	6.11×10^{-6}
IL-17 levels (pg/mL)	0.32 (0.20, 0.39)[b]	1.55 (1.02, 1.87)[ab]	4.56 (3.44, 6.75)[a]	1.14×10^{-34}	2.03×10^{-10}	1.11×10^{-35}	1.80×10^{-9}
IL-10 levels (pg/mL)	3.40 (2.64, 3.86)[b]	1.88 (1.68, 2.25)[ab]	1.55 (0.98, 1.78)[a]	2.44×10^{-27}	2.28×10^{-12}	1.51×10^{-27}	1.85×10^{-4}
Peripheral lymphocyte count (10^9/L)	2.45 (1.75, 2.76)[b]	1.34 (1.02, 1.63)[a]	1.14 (0.78, 1.52)[a]	3.72×10^{-16}	1.53×10^{-09}	9.27×10^{-16}	0.072

Table 1. Continued...

	Control	COPD	ACO	*p* value[a]	*p* value[b]	*p* value[c]	*p* value[d]
Peripheral monocyte count (10^9/L)	0.46 (0.38, 0.56)	0.50 (0.37, 0.64)	0.55(0.40, 0.76)	0.063			
Infection Indices							
Peripheral leukocyte count (10^9/L)	6.26 (5.42, 8.24)[b]	7.34 (5.66, 10.10)[ab]	10.04 (6.75, 12.07)[a]	5.14×10^{-7}	0.032	1.22×10^{-7}	0.0033
Peripheral neutrophil count (10^9/L)	3.86 (3.14, 5.35)[b]	5.09 (3.40, 8.18)[a]	7.27 (4.66, 9.79)[a]	2.90×10^{-7}	0.0068	6.18×10^{-8}	0.0012
PCT level (μg/L)	0.03 (0.03, 0.05)[b]	0.05 (0.03, 0.31)[a]	0.07 (0.05, 0.65)[a]	1.76×10^{-5}	0.002	3.76×10^{-6}	0.0049
CRP level (mg/L)	6.54 (4.83, 7.54)[b]	8.96 (6.61, 25.78)[a]	15.45 (9.06, 56.45)[a]	9.72×10^{-7}	0.0067	4.64×10^{-8}	2.75×10^{-6}
Vascular embolism index							
Fbg level (g/L)	3.75 (2.81, 4.83)	3.61 (2.98, 4.44)	3.93 (3.10, 4.98)	0.451			
plasma d-dimer level (mg/L)	0.48 (0.34, 0.65)[b]	0.51 (0.25, 0.96)	0.60 (0.25, 1.68)[a]	0.004	0.225	0.011	0.048
Heart failure index							
TNT level (ng/L)	8.41 (7.04, 10.19)	14.30 (10.83, 24.45)[a]	10.15 (7.79, 19.67)	0.0046	0.0092	0.067	0.054
BNP level (pg/ml)	39.50 (30.46, 56.40)[b]	78.23 (45.36, 225.78)	270.8 (65.1, 1876.5)[a]	1.49×10^{-8}	0.078	5.93×10^{-10}	0.0004

presented as mean ± standard deviation, median (25th percentile, 75th percentile), count, or percentage, as appropriate.

[a] Comparisons among the three groups; [b] comparison for COPD vs. Control group; [c] comparison for ACO vs. Control group; [d] comparison for ACO vs. COPD group.

ACO: asthma-COPD overlap; BNP: B-type natriuretic peptide; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; Fbg: fibrinogen; FeNO: fractional exhaled nitric oxide; FEV1% pred: forced expiratory volume in 1 second, % predicted; FVC% pred: forced vital capacity, % predicted; IL: interleukin; IU/mL: international units per milliliter; PCT: procalcitonin; ppb: parts per billion; RV/TLC: residual volume/total lung capacity; Th17: T helper 17 cells; TnT: cardiac troponin T; Treg: regulatory T cells.

Comparisons between 2 groups were performed using Student's *t* test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by the post-hoc Student-Newman-Keuls (SNK) test. ANOVA and *t* tests were chosen for their robustness in handling normally distributed data, and the SNK test was chosen for its ability to control the type I error rate when performing multiple comparisons. For non-normally distributed data, the median and interquartile range were presented. The Mann-Whitney test was used for comparisons between 2 groups, while the Kruskal-Wallis test was used for comparisons among multiple groups, followed by post-hoc pairwise comparisons using Dunn's test. These tests were chosen for their ability to handle non-normally distributed data. Correlations were determined using Pearson's correlation test for normally distributed data and Spearman's correlation test for non-normally distributed data. These tests were selected for their ability to accurately measure the strength and direction of the linear relationship between two variables. A two-sided test with $p<0.05$ was considered statistically significant.

RESULTS

Participant Characteristics

Table 1 presents the demographic and clinical characteristics of the participants. There were no significant differences in age or sex among the control, COPD, and ACO groups. Interestingly, the BMI in the control group was significantly greater than that in the COPD and ACO groups ($p=0.0001$). However, the COPD and ACO groups did not significantly differ in BMI.

Pulmonary Function Parameters among Different Groups

Figure 2 illustrates the pulmonary function parameters among the control, COPD, and ACO groups. Patients with COPD or ACO had significantly lower FEV1% pred, FVC% pred, FEV1/FVC, and FEF25–75%, and a higher RV/TLC ratio than healthy controls. These findings highlight impaired pulmonary function in COPD and ACO patients. For instance, the mean FEV1% pred in the COPD group was 40.53, which was significantly lower than that in the control group 91.45 ($p=1.11\times10^{-22}$). Similarly, the mean FEV1% pred of the ACO group was 48.38, which was also significantly lower than the control group ($p=4.86\times10^{-17}$). Interestingly, compared to those in the COPD group, patients in the ACO group had a significantly greater percentage of FVC% pred (70.30 vs. 54.40, $p=0.0013$) and FEV1% variation rate (12.17 vs. 2.48, $p=1.70\times10^{-23}$) after bronchodilator inhalation. However, there were no significant differences in FEV1% pred, FEV1/FVC, FEF25–75%, or RV/TCL between the ACO and COPD groups.

Inflammatory and Immunological Biomarkers among Different Patient Groups

Table 1 and Figure 3 present the levels of FeNO, peripheral blood eosinophil count, and serum IgE among the control, COPD, and ACO groups. All 3 biomarkers significantly differed among the groups. Specifically, the levels of FeNO, peripheral eosinophil count, and serum IgE were significantly higher in the COPD and ACO groups than in the control group. For instance, the mean FeNO level in the COPD group was 22.00, which was significantly higher than in the control group's 14.50 ($p=6.01\times10^{-6}$). Similarly, the mean FeNO level in the ACO group was 33.50, which was also significantly higher than that in the control group ($P=3.70\times10^{-15}$). The levels of FeNO (33.50 vs. 22.00, $p=0.0014$) and IgE (555.92 vs. 329.5, $p=2.14\times10^{-8}$) were higher in the ACO group than in the COPD group. However, there was no significant difference in peripheral blood eosinophil counts between the ACO and COPD groups ($p=0.0671$), indicating similar eosinophilic inflammation levels in these patient groups.

Figure 4 presents the flow cytometry results of the peripheral blood Th17 and Treg levels in the COPD, ACO, and healthy control groups. In Table 1 and Figure 4, Th17, Treg, Th17/Treg, IL-17, and IL-10 significantly differed among the control, COPD, and ACO groups. The levels of Th17, Th17/Treg, and IL-17 were all significantly higher in the COPD and ACO groups than in the control group, whereas Treg and IL-10 levels were distinctly lower in the patients with COPD or ACO compared to those in the control group. In addition, patients in the ACO group had higher Th17, Th17/Treg, and IL-17 levels, but lower Treg and IL-10 levels than those in the COPD group. These findings suggest distinct immune profiles in COPD and ACO patients, which could have implications for their diagnosis and treatment.

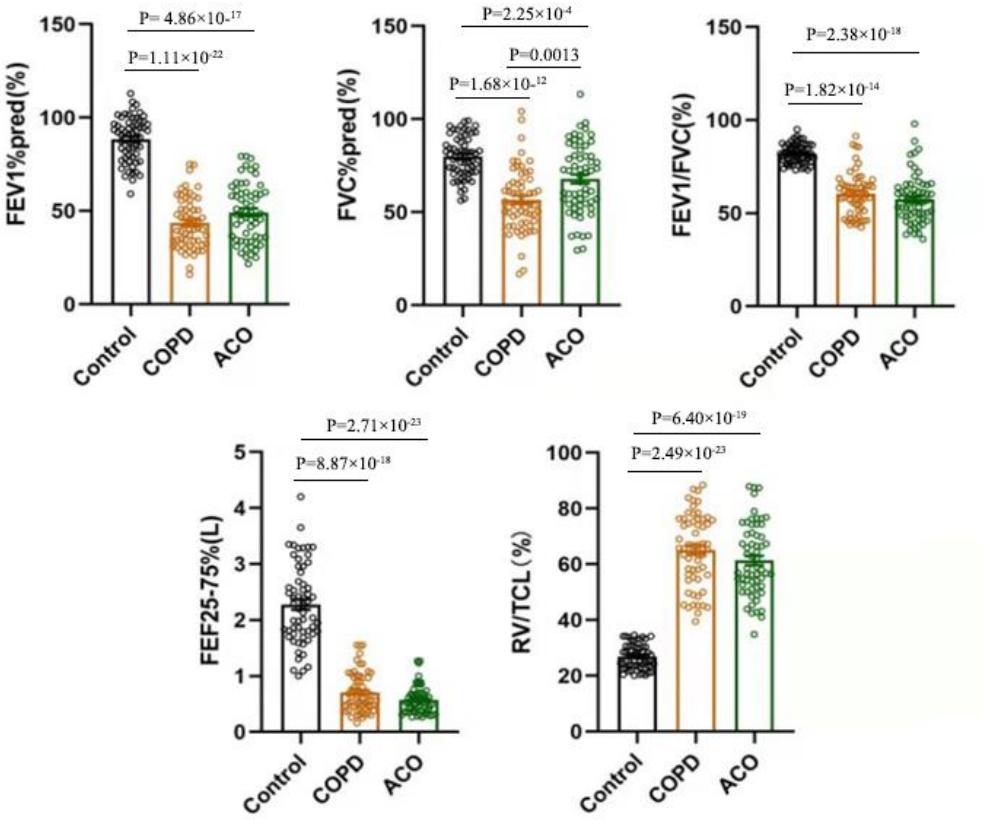

Figure 2. The comparisons of lung function indicators among the COPD, ACO, and control groups. Data were presented as mean±SD; the error bars indicate the standard deviation (SD), and each dot presents a test result for an individual. Each experiment was performed in triplicate.

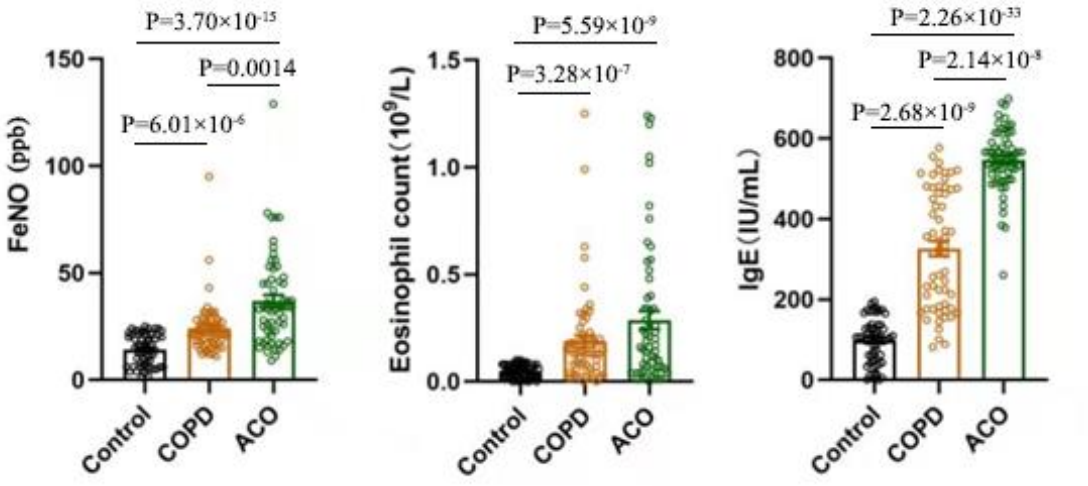

Figure 3. Comparisons of FeNO, peripheral blood EOS count, and serum IgE levels among the COPD, ACO, and control groups. Data were presented as mean±SD; the error bars indicate SD, and each dot presents a test result for an individual. Each experiment was performed in triplicate.

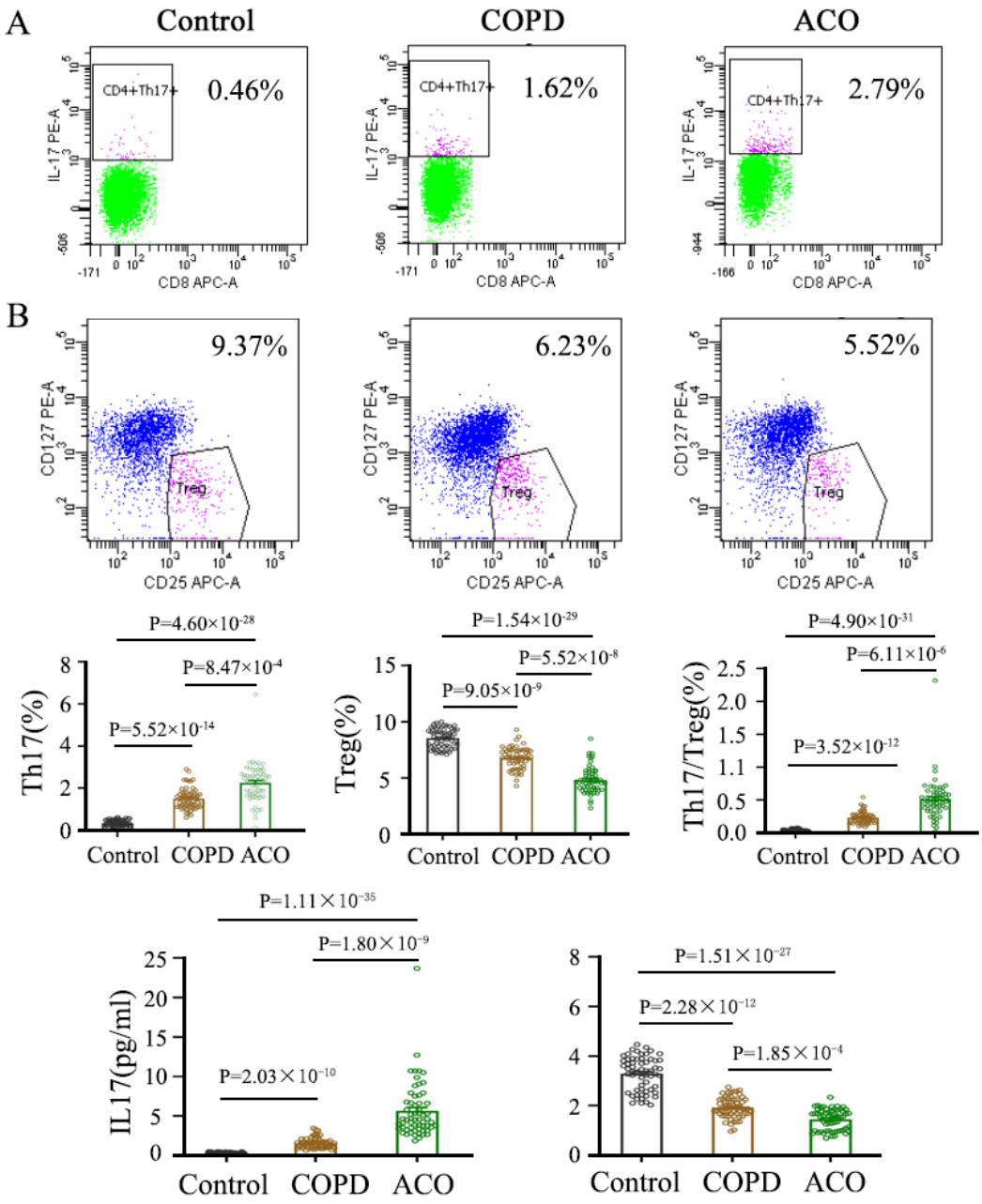

Figure 4. Peripheral blood Thl7, Treg, Thl7Treg, IL-17, and IL-10 levels in each group. (A) Representative flow cytometry result of Thl7 levels, Treg in each group. (B) Representative flow cytometry result of Treg levels in each group. Note: one-way ANOVA followed by SNK post hoc test was used to compare the differences among the groups. a indicates

Correlation of Lung Function with Immunologic and Inflammatory Markers

To further explore the roles of inflammatory factors and immune cells in lung diseases, we analyzed the associations of lung function parameters with inflammatory factors, immune cells, and other peripheral blood indexes in different disease groups. As shown in Figure 5, correlation analysis revealed several significant associations. In the COPD group, peripheral blood Treg was positively associated with FEV1% pred (r=0.463, p=0.0001) and FVC% pred (r=0.376, p=0.003), indicating a potential protective role of Tregs in preserving lung function in COPD. Conversely, the peripheral leukocyte count (r=−0.287, p=0.032) and

neutrophil counts (NEU) (r=−0.277, *p*=0.026) were negatively related to FEV1/FVC (%), suggesting a detrimental role of systemic and neutrophilic inflammation in COPD. In patients with ACO, FeNO (r=−0.461, *p*=0.0001), Th17 (r=−0.287, *p*=0.026), Th17/Treg (r=−0.318, *p*=0.013), and IL-17 (r=−0.309, *p*=0.016) were negatively correlated with FEV1% pred. Similarly, FeNO (r=−0.418, *p*=0.001), Th17/Treg (r=−0.281, *p*=0.030), and IL-17 (r=−0.363, *p*=0.004) had negative relationships with FEV1/FVC. These findings suggest that increased airway inflammation, Th17 response, and Th17/Treg imbalance are associated with worse lung function in ACO. Interestingly, FeNO (r=−0.388, *p*=0.002) and IL-17 (r=−0.362, *p*=0.004) were negatively correlated with FEF 25–75%, a measure of small airway function. This suggests that airway inflammation and Th17 response may particularly affect the small airways in ACO. On the other hand, FeNO (r=0.387, *p*=0.002), Th17 (r=0.313, *p*=0.015), Th17/Treg (r=0.354, *p*=0.005), and IL-17(r=0.489, *p*=0.0001) were positively associated with RV/TCL, a measure of air trapping and hyperinflation. This indicates that airway inflammation, Th17 response, and Th17/Treg imbalance may contribute to air trapping and hyperinflation in ACO. Treg was positively associated with FEV1% pred (r=0.287, *p*=0.026) and FEV1/FVC (r=0.276, *p*=0.033), and IL-10 was positively associated with FEV1/FVC (r=0.658, *P*=0.0001) and FEF 25–75% (r=0.5765, *p*=0.0001), and was negatively correlated with RV/TCL (r=–0.556, *p*=0.00001). These findings suggest that Tregs and IL-10 may have protective roles in ACO. However, there were no significant associations between lung function parameters and other peripheral blood indexes. All these results suggested that there were remarkable associations between lung function parameters and Th17/Treg immunologic dissonance in patients with ACO.

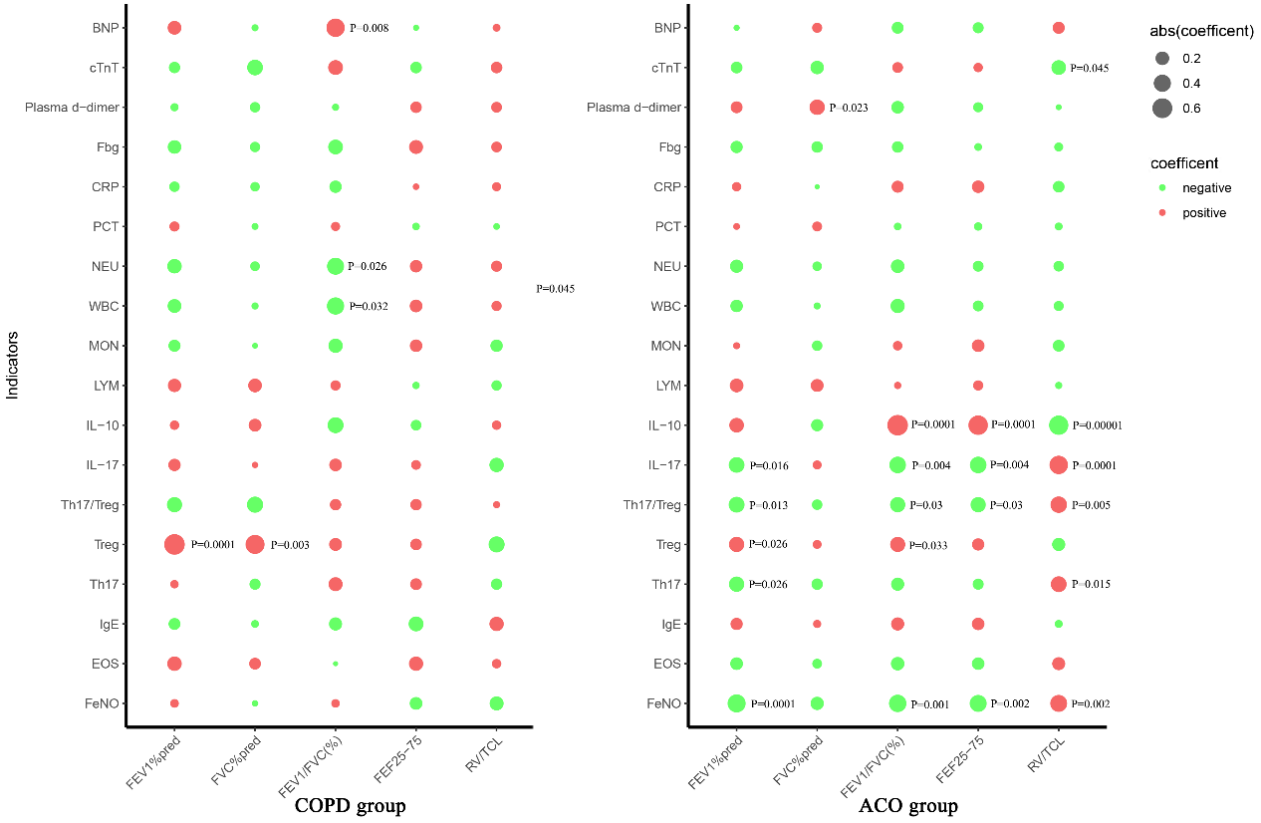

Figure 5. The correlations of lung function parameters with inflammatory factors and immune cells, as well as other peripheral blood indexes in the COPD and ACO group.

The circle size indicates the absolute value of the correlation coefficient. Circles in green represent r<0, and circles in red represent r>0.

DISCUSSION

The present study investigated the intricate relationship between immune-inflammatory markers and lung function parameters in patients with ACO. By examining the roles of Th17/Treg imbalance, FeNO, eosinophils, and IgE levels, this study aimed to enhance our understanding of ACO pathogenesis and identify potential biomarkers for better disease management. Our findings provide valuable insights into the systemic and airway-specific inflammatory processes that contribute to the unique clinical presentation and progression of ACO, highlighting the importance of targeted therapeutic strategies to improve patient outcomes.

ACO, characterized by a chronic airway inflammatory process with features of both COPD and asthma, remains a challenging condition associated with abnormal pulmonary inflammatory responses.[38] The prevalence of ACO in patients with asthma or COPD underscores the need for effective biomarkers to aid in diagnosis and disease progression assessment. Overreliance on clinical manifestations and pulmonary function alone may lead to inappropriate treatment decisions, necessitating a more comprehensive approach for a nuanced understanding of ACO.[39] In this study, we investigated the distinct characteristics of patients with ACO, shedding light on the complex interplay of clinical, immunologic, and inflammatory factors. Notably, ACO patients exhibited worse pulmonary ventilation and more pronounced airway obstruction than COPD patients, as evidenced by the increased rate of FEV1% variation and decreased FEV1/FVC after bronchodilator inhalation. Small airway dysfunction, reflected by a lower FEF 25–75% and RV/TLC, was more severe in ACO. Individuals with ACO exhibited a unique immune profile characterized by elevated Th17 and Th17/Treg ratio and increased IL-17 levels coupled with reduced Treg and IL-10 levels. These immunological imbalances were more pronounced in ACO patients than in COPD patients, suggesting potential implications for diagnostic differentiation and targeted therapeutic interventions.

Pulmonary function analyses unveiled significant differences between ACO patients, COPD patients, and healthy controls. ACO patients demonstrated worse lung function than COPD counterparts, as evidenced by higher variation in FEV1% rate and a lower FEV1/FVC after bronchodilator inhalation. The decrease in FEV1% pred, previously associated with increased airway wall area and thickness alongside decreased airway cavity area,[40] further confirmed the unique characteristics of ACO. The FEV1/FVC ratio, recognized as a pivotal parameter reflecting airflow obstruction, reinforced our observations that ACO is associated with compromised pulmonary ventilation and heightened airway obstruction compared to COPD. Moreover, the lower postbronchodilator FEV1/FVC and higher FEV1% variation rate in the ACO group underscored the increased severity of airflow obstruction and greater reversibility in ACO patients. It is crucial to note that our exclusion of patients with pulmonary fibrosis ensured a focused investigation into obstructive ventilatory dysfunction, particularly in the context of COPD and ACO. Contrasting our COPD and ACO cohorts, we observed a lower FEF 25–75% in ACO patients, indicating a more pronounced small airway dysfunction. Additionally, the lower RV/TLC observed in ACO patients aligns with prior reports highlighting the heightened severity of emphysema in ACO patients compared to COPD patients alone.[41] These findings contribute valuable insights into the nuanced aspects of ACO pathophysiology, distinguishing it from COPD and emphasizing the importance of considering small airway involvement.

IgE plays a crucial role in immune responses, particularly in binding to mast cells and basophils. It is considered the strongest indicative marker of allergic disease.[42] FeNO is recognized as a common biomarker of airway inflammation. Eosinophils are integral to immune and allergic reactions. In our study, we found that the levels of FeNO, peripheral eosinophil counts, and serum IgE were significantly higher in the COPD and ACO groups than in the control group. Notably, the FeNO levels were higher in the ACO patients than in the COPD patients. Concurrently, the plasma IgE levels in the ACO group were significantly higher than those in the COPD group. These findings suggest that the levels of FeNO and IgE in serum correlate with the degree of disease response, and changes in these indices could better support the evaluation of ACO efficacy.[43] However, we observed no prominent differences in the peripheral blood eosinophil counts between COPD patients and ACO patients. We speculate that this could be due to the induced sputum eosinophil counts differing from the peripheral blood eosinophil counts.

In our investigation, we explored the intricate relationship between Th17 and Tregs and their impact on immune response in ACO patients, shedding light on potential immunologic mechanisms underlying this disease. Our study uncovered several key findings that align, and in some instances, contrast with existing literature. Existing literature has established the association of Th17/Treg imbalance with pulmonary function and disease progression in various respiratory conditions, including COPD and asthma.[44] Our study corroborates these associations, revealing elevated Th17, Th17/Treg ratio, and IL-17 levels in both ACO and COPD patients compared to healthy controls. Importantly, we observed a more pronounced Th17/Treg imbalance and heightened inflammatory response in ACO patients than in other groups, contributing novel insights into the immunologic aspects of ACO. Our investigation highlights the significant role of IL-17 in the occurrence and progression of ACO inflammation. Elevated IL-17 levels, coupled with reduced IL-10 levels, suggest an abnormal enhancement of the Th17 immune response and a relative deficiency in Treg-mediated immune modulation.[45] This imbalance, which is evident in ACO patients, disrupts the delicate equilibrium between proinflammatory and inflammatory suppressive immunity, leading to local and systemic inflammatory responses in the airway.[46]

Correlation analysis results underscore the association between immune imbalance and inflammatory response in ACO patients. As pulmonary function declines, peripheral Treg cell numbers decrease, while total serum IgE and peripheral blood eosinophil counts increase in ACO patients. Additionally, peripheral blood Th17 cells, Th17/Treg ratio, and IL-17 increase, while Treg cell numbers and IL-10 levels decrease, further highlighting the intricate immune dysregulation in ACO. The observed Th17/Treg imbalance may contribute to the decline in pulmonary function in ACO patients. Possible mechanisms include the proinflammatory cytokines produced by Th17 cells, such as IL-17, IL-21, and IL-22, inducing airway inflammation, obstruction, and remodeling.[17] Moreover, the suppressive role of Tregs, mediated by anti-inflammatory cytokines like IL-10 and TGF-β, may be compromised, allowing excessive inflammation and tissue damage in the lungs.[47] The aforementioned findings present potential implications for the diagnosis and management of ACO. Understanding Th17/Treg imbalances could aid in differentiating ACO from COPD and guide tailored treatment strategies.[48] Further exploration of these immunologic aspects may open avenues for targeted interventions, offering a more nuanced approach to ACO diagnosis and management.

While our study contributes valuable insights into the immunologic aspects of ACO, several limitations should be acknowledged, which may influence the interpretability of our findings. First, the sample size was relatively small, and the single-center design introduces potential sample and selection biases. Therefore, the generalizability of our results to broader populations may be limited. Additionally, the strict inclusion and exclusion criteria applied to our patient selection may have limited the external validity of our results. Clinicians should exercise caution when extrapolating our findings to a more diverse patient population. Moreover, while our study focused on the association of peripheral blood Th17/Treg immune imbalance with ACO lung functions, the systemic nature of our approach might not fully capture the localized immunopathology within airway/lung compartments, which is crucial for a comprehensive understanding of ACO. To address these limitations, future research endeavors should prioritize multicenter collaborations with larger and more diverse patient cohorts. Additionally, incorporating analyses of airway-specific immune responses in future studies will provide a more comprehensive understanding of ACO immunopathogenesis. Bridging the gap between systemic and local immune dynamics is essential for a more nuanced comprehension of ACO and its impact on lung function. Considering the clinical implications of our findings, the incorporation of Th17/Treg markers in the assessment and management of ACO patients holds promise. These markers could potentially serve as diagnostic and prognostic indicators, aiding clinicians in tailoring treatment strategies. However, it is essential to recognize potential challenges, including the standardization of assays and the need for further validation in diverse patient populations. Our study lays the groundwork for considering the Th17/Treg immune balance as a potential target. Further research is warranted to explore the therapeutic modulation of these markers on ACO. Promising avenues for drug development or therapy optimization may emerge, contributing to more targeted and effective treatments for ACO patients.

In summary, our investigation underscores the significant imbalance in the Th17/Treg ratio in the peripheral blood of both COPD patients and ACO patients, with ACO individuals exhibiting more pronounced immune dysregulation compared to COPD patients. Importantly, we established an inverse association between Th17/Treg imbalance and lung function in ACO patients, offering a valuable metric for assessing disease severity. Moreover, our findings highlight the potential of the Th17/Treg ratio as a discriminating factor between ACO and COPD, guiding more tailored clinical treatments. Future research should delve deeper into elucidating the intricate underlying mechanisms through in vivo and in vitro assays. Clarifying the role of Th17/Treg immune imbalance in ACO pathogenesis and disease progression will not only enhance our understanding of this condition but also pave the way for targeted interventions. These efforts are pivotal in advancing precision medicine approaches for ACO, ultimately improving patient outcomes and refining the clinical management of this complex respiratory disorder.

STATEMENT OF ETHICS

The Ethics Committee of Bihaiwai Central Hospital of Dongguan approved the study (202113). All procedures were performed in accordance with the Declaration of Helsinki. Signed informed consent was obtained from all patients.

FUNDING

Natural Science Foundation of Guangdong Province (2023A1515011627), Dongguan Science and Technology of Social Development Program (20221800905212, 20231800935542, 20221800905222), National Natural Scientific Foundation of China (82073628), Medical Science and Technology Research Fund project of Guangdong Province (A2020357, A2021118).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

Not applicable

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