Inflammatory Pattern of Eosinophilic COPD Involving 12/15-LOX Lipid Signals Expressed by Eosinophils

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Abstract

Background and objective:

Eosinophilic chronic obstructive pulmonary disease (COPD) has been recognized as an inflammatory pattern which is importance for precise treatment interventions among COPD. However, the studies about eosinophilic COPD show conflicting results and the role of eosinophils in COPD remains unclear. In this study, LC-MS/MS-based mediator lipidomics was to determine the expression status of lipid signals in non-eosinophilic and eosinophilic COPD.

Method:

A totally 80 patients with COPD including 40 eosinophilic COPD and 40 non-eosinophilic COPD were enrolled over 12 months. Clinical characteristics information record, pulmonary function tests, complete blood count, serum metabolites analysis and other clinical tests were performed at baseline and follow-up.

Results:

There were no significant differences in pulmonary function or pulmonary function decline between eosinophilic COPD and non-eosinophilic COPD after follow-up. However, eosinophilic COPD have higher numbers of acute exacerbation patient in the last 1 year. Complete blood count (CBC) data demonstrated that Δblood eosinophil count (BEC) was significantly decreased and correlated with ΔFEV1 (% Predicted) (r = 0.314, P = 0.036) in eosinophilic COPD. Furthermore, compared to non-eosinophilic COPD, a series of 12/15-LOX-derived mediators were found increased in eosinophilic COPD. Among them, 17-HDoHE was found significantly decreased after follow-up and significantly correlated with ΔBEC (r= 0.336, P= 0.023).

Conclusion:

This study demonstrates that metabolic levels of non-eosinophilic COPD and eosinophilic COPD were different due to the huge difference in eosinophil level, which leads to different inflammatory patterns, and the 12/15-LOX metabolic pathway was one of them. The results might help to understand the inflammatory response and lipid metabolism of eosinophilic COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is an irreversible progressive chronic inflammatory disease characterized by incomplete and persistent limitation of airflow (1). Currently, about 3 million people have COPD worldwide, and it is predicted that it will be the third leading cause of death by 2030 (2). Because of its high prevalence, related disability and mortality, COPD became a public health challenge and cause high disease burden (3). Identify the heterogeneity of inflammatory patterns is closely related to the concept that the characteristics of the disease and may be the target for tailored therapeutic intervention. Precise treatment intervention has been recognized as a basis for the management because of the different phenotypes of COPD. (4).
Identification of the inflammatory patterns of COPD is the most important strategy in precise treatment. Recently, eosinophilic COPD have attracted considerable interest as constitutes 32–40% of COPD population and eosinophils may be one of the significant factors for COPD pathogenesis which seems that the role is different from that in asthma (5, 6). However, it is still largely unknown how eosinophils affect the course of the disease and why the studies about eosinophilic COPD show conflicting results (7, 8). Eosinophils are a distinct granulocyte lineage that is involved in inflammatory responses via lipid signals (9). Lipid mediators play essential roles in host defense and restoring homeostasis during inflammatory responses (10). 12/15-Lipoxygenase (12/15-LOX) which major expressing from eosinophils is the enzyme that locally produces polyunsaturated fatty acid-derived lipid mediators such as protectin D1 (PD1), which are classified as specialized pro-resolving mediators (SPMs) and exhibit potent anti-inflammatory and pro-resolving roles in many disease models (11–13).

In this study, LC-MS/MS-based mediator lipidomics was to determine the expression status of lipid signals in non-eosinophilic and eosinophilic COPD. A new perspective was provided to understand the role of these cells in COPD. More importantly, the results may provide a new method for the inflammation pattern recognition in patients with eosinophilic COPD.

**Materials And Methods**

1. Study design and patients

According to the GOLD 2020 diagnostic and classification criteria, 40 of eosinophilic COPD patients (blood eosinophil percentages ≥ 2%) participated in Shenzhen People's Hospital from October 2018 to June 2019. All patients met the criteria: (1) no helminth infections and various allergic diseases; (2) no other primary pulmonary diseases; (3) no other extrapulmonary diseases involving the lungs. In addition, 40 COPD patients with lower blood eosinophil percentages (< 2%) were recruited as non-eosinophilic COPD. Each patient underwent a 12 months follow-up and written signed informed consent was obtained from all patients before enrollment. At baseline and follow-up in all participants, clinical characteristics, mMRC, CAT scores, vital signs, current medications and exacerbation history were recorded, serum samples and stool samples were collected, CBC, six-minute walk test (6MWT) and spirometry according to ATS/ERS guideline were performed. If the follow-up is within 2 weeks after or during the acute exacerbation period, the follow-up visit should be postponed after 2 weeks. During the follow-up period, patients were selectively treated with long-acting muscarinic receptor antagonist (LAMA), long-acting beta 2 agonist (LABA) and inhaled corticosteroid (ICS). In acute exacerbation, theophylline, antibiotics, expectorant and other drugs were used according to the actual situation. According to the Helsinki declaration, all participants signed informed consent authorization before enrollment. This study was approved by the Ethics Committee of Shenzhen People's Hospital (Ethics approval No. KY-LL-2020242).

2. Pulmonary function test

According to the requirements of the American Thoracic Society and the European Respiratory Society (ATS/ERS), participants were tested for pulmonary function using the Jaeger lung function instrument.
Parameters included: FEV1, FVC and FEV1/FVC. These indicators were used to diagnose and monitor COPD progression according to ATS/ERS criteria.

3. Collection and storage of blood samples

A venous blood sample (5mL) was collected from each participant enrolled. After centrifugation at 3000 RPM for 10 min, the serum supernatant was collected. 50uL was taken from each sample for testing. Keep the remaining serum samples at -80°C and not repeatedly freeze or thawed.

4. Measurement of metabolites by choline derived-UHPLC-Q-TOF/MS

The serum samples of the participants were pre-processed and subjected to complete derivatization; hydroxy-eicosatetraenoic acid-d8 and prostaglandin D2-d4 (Cayman) were added to each sample, which was then isolated using an Agilent 1290 Infinity LC system (UHPLC, Santa Clara CA) and quantitated using an accurate mass Agilent 6550 UHD Q-TOF/MS system. The metabolites were identified by comparing KEGG (Kyoto Encyclopedia of Genes and Genomes) with HMDB (The Human Metabolome Database, Canada) database.

Agilent MassHunter Qualitative Analysis software (Agilent Technologies, USA) was used to extract and align the raw LC-MS data. MassHunter Profiler Professional (MPP) software (Agilent Technologies) was used to data measurement. The metabolites were identified based on the comparison of retention time, MS and MS/MS spectra with corresponding standards, and metabolites database including Lipid Maps and METLIN. The Molecular Feature Extractor was used to extract molecular characteristics by accurate masses, retention time and ionic strength and the internal standard was used to normalize each retained peak.

5. Statistical analysis

SPSS (Version 20.0, IBM Corp, USA), RStudio (version 1.2.5019, R Studio Inc, Austria) and GraphPad Prism (version 8.0, GraphPad Software, USA) were employed for statistical analysis and plotting. Non-parametric quantitative data were described as a median (interquartile range) and parametric quantitative data were depicted as the mean ± standard deviation. Between-group differences were tested with independent-samples t-test for normally distributed variables and the Kruskal–Wallis h-test was used for non-Gaussian data. P-value below 0.05 was regarded as statistically significant.

Results

1. Characteristics of the population studied

At the end of the study, there were 75 patients who still participated including 37 eosinophilic COPD patients which blood eosinophil percentages > 2% and 38 COPD patients of non-eosinophilic COPD which blood eosinophil percentages < 2%. Table 1 briefly summarizes the basic information of the participants in the baseline of this study. Smoking status and medication history were similar in both groups at baseline. Pulmonary function showed that there was no significant difference between the two groups in spirometric...
data and GOLD Classification. In the last 1 year, the numbers of acute exacerbation patients with hospitalization showed no significant differences between eosinophilic COPD and non-eosinophilic COPD while eosinophilic COPD have higher numbers of acute exacerbation patient. CBC data demonstrated that blood eosinophil count (BEC) in eosinophilic COPD was predictably higher than in non-eosinophilic COPD. On the contrary, blood leucocyte counts and BNC were found significant higher in non-eosinophilic COPD. The result also demonstrated that there were no significant differences in biochemistry, pulmonary function and functional performance which measured by mMRC, CAT and 6MWD between two groups.
Table 1
Baseline characteristics of non-eosinophilic COPD and eosinophilic COPD.

|                          | Non-eosinophilic COPD | Eosinophilic COPD | P-Value |
|--------------------------|-----------------------|-------------------|---------|
| N                        | 38                    | 37                |         |
| Age (Year)               | 69.38 ± 10.43         | 67.97 ± 9.39      | 0.709   |
| Male (Female)            | 28 (10)               | 30 (7)            | 0.444   |
| BMI (kg/m²)              | 23.74 ± 6.15          | 24.44 ± 6.23      | 0.748   |
| Smoking Status, N (%)    |                       |                   |         |
| No-Smokers               | 4 (10.5%)             | 3 (8.1%)          | 0.675   |
| Past-Smokers             | 26 (68.4%)            | 23 (62.2%)        |         |
| Smokers                  | 8 (21.1%)             | 11 (29.7%)        |         |
| GOLD Classification, N (%)|                       |                   |         |
| 1                        | 10 (26.3%)            | 7 (18.9%)         | 0.894   |
| 2                        | 14 (36.8%)            | 15 (40.5%)        |         |
| 3                        | 8 (21.1%)             | 9 (24.3%)         |         |
| 4                        | 6 (15.8%)             | 6 (16.2%)         |         |
| Clinical History, N (%)  |                       |                   |         |
| AECOPD in Last 1 Year    | 7 (18.4%)             | 15 (40.5%)        | 0.035*  |
| AECOPD with Hospitalization in Last 1 Year | 6 (15.8%) | 11 (29.7%) | 0.149   |
| CBC                      |                       |                   |         |
| Leucocyte (10^9/L)       | 9.12 (6.28–13.6)      | 8.09 (5.31–10.02) | 0.002** |
| Neutrophil (10^9/L)      | 7.72 (5.43–9.95)      | 5.81 (4.02–6.87)  | 0.001***|
| Lymphocyte (10^9/L)      | 0.95 (0.06–2.69)      | 1.07 (0.47–2.13)  | 0.194   |
| Monocyte (10^9/L)        | 0.67 (0.06–1.29)      | 0.59 (0.17–1.25)  | 0.399   |
| Eosinophil (10^9/L)      | 0.05 (0-0.21)         | 0.34 (0.17–0.94)  | 0.001***|
| Basophil (10^9/L)        | 0.03 (0.02–0.05)      | 0.04 (0.01–0.11)  | 0.171   |
| Erythrocyte (10^12/L)    | 3.64 (2.37–5.37)      | 2.92 (2.26–5.21)  | 0.115   |
| Hemoglobin (g/L)         | 142 (125–151)         | 136 (119–144)     | 0.113   |
| Platelet (10^9/L)        | 237.5 (26–560)        | 267.5 (140–482)   | 0.168   |
|                               | Non-eosinophilic COPD | Eosinophilic COPD | P-Value |
|-------------------------------|-----------------------|-------------------|---------|
| Hematocrit (%)                | 33.2 (23.1–49.4)      | 26.5 (18.9–41.2)  | 0.125   |
| **Biochemistry**               |                       |                   |         |
| Blood Glucose (mg/dL)         | 124 (106–176)         | 118 (95–172)      | 0.666   |
| BUN (mg/dL)                   | 41.7 (33.2–62.9)      | 39.32 (30.5–61.0) | 0.704   |
| Serum Creatinine (mg/dL)      | 0.77 (0.60–1.03)      | 0.80 (0.61–1.01)  | 0.680   |
| Albumin (g/dL)                | 3.6 (3.3–3.9)         | 3.7 (3.2–4.0)     | 0.823   |
| AST (U/L)                     | 23 (17–31)            | 22 (16–29)        | 0.775   |
| ALT (U/L)                     | 18 (11–29)            | 16 (12–26)        | 0.723   |
| Sodium (mmol/L)               | 138.9 (135.4–140.6)   | 138.5 (136.0–140.4) | 0.841 |
| Potassium (mmol/L)            | 4.43 (4.11–4.83)      | 4.44 (4.12–4.82)  | 0.921   |
| Total Colesterol (mmol/L)     | 5.2 ± 1.3             | 5.3 ± 1.2         | 0.737   |
| LDL Colesterol (mmol/L)       | 3.0 ± 1.1             | 3.2 ± 1.2         | 0.679   |
| HDL Colesterol (mmol/L)       | 1.3 ± 0.4             | 1.2 ± 0.3         | 0.692   |
| Triglycerides (mmol/L)        | 1.4 ± 0.5             | 1.4 ± 0.4         | 0.804   |
| **Spirometric Data**          |                       |                   |         |
| FEV1 (L)                      | 1.64 ± 0.68           | 1.63 ± 0.71       | 0.956   |
| FEV1 (% Predicted)            | 64.38 ± 23.01         | 61.3 ± 24.25      | 0.579   |
| FVC (L)                       | 2.71 ± 0.93           | 2.54 ± 0.81       | 0.403   |
| FVC (% Predicted)             | 78.73 ± 16.41         | 74.23 ± 15.61     | 0.297   |
| FEV1/FVC%                     | 54.32 ± 8.71          | 53.96 ± 4.25      | 0.659   |
| **Functional Performance**    |                       |                   |         |
| mMRC                          | 1.55 ± 0.75           | 1.58 ± 0.82       | 0.479   |
| CAT                           | 9.18 ± 4.79           | 9.23 ± 4.93       | 0.529   |
| 6MWD                          | 315.72 ± 130.57       | 317.28 ± 133.24   | 0.741   |

2. Lung function, clinical and laboratory characteristics in both groups of subjects at baseline and at follow-up
Lung function, clinical and laboratory parameters of drop-out subjects were similar to those of the subjects participating to the follow-up. All patients had no symptoms of intestinal parasites and were found no parasite by stool exam. As shown in Table 2, the number of current smokers reduced in both groups, after the period of follow-up, it reduced from 8 to 6 in non-eosinophilic COPD and from 11 to 8 in eosinophilic COPD. Therefore, there was an increment of the number of past smokers in both groups. Not unexpectedly, GOLD stage changed in both groups after the follow-up period. The number of patients classified as GOLD 1 and GOLD 2 at baseline was reduced after the follow-up period, therefore, some subjects were classified as GOLD 3 and GOLD 4 after the period of follow-up. As shown in Fig. 1, FEV1 (% Predicted) at follow-up were significantly lower than at baseline in both groups whereas lung function decline expressed as FEV1 (L) was similar between non-eosinophilic COPD patients and eosinophilic COPD subjects. CBC data including BNC and BEC changed significantly in both groups after the follow-up period. BNC was significantly increased in non-eosinophilic COPD while BEC was significantly decreased in eosinophilic COPD. Compared with eosinophilic COPD, non-eosinophilic COPD seem to have better stability of blood eosinophil levels (Fig. 2). Moreover, ΔBEC was significantly correlated with ΔFEV1 (% Predicted) (r = 0.314, P = 0.036) in eosinophilic COPD (Supplemental Fig. 1).
Table 2
Comparison in clinical parameters between baseline and follow-up.

|                           | Non-eosinophilic COPD | Eosinophilic COPD |
|---------------------------|-----------------------|-------------------|
|                           | Baseline  | Follow-Up  | Baseline  | Follow-Up  |
| BMI (kg/m$^2$)            | 23.74 ± 6.15 | 22.87 ± 6.92 | 24.44 ± 6.23 | 23.14 ± 7.03 |
| Smoking Status, N (%)     |           |           |           |           |
| No-Smokers                | 4 (10.5%) | 4 (10.5%) | 3 (8.1%)  | 3 (8.1%)  |
| Past-Smokers              | 26 (68.4%) | 28 (73.7%) | 23 (62.2%) | 26 (70.3%) |
| Smokers                   | 8 (21.1%) | 6 (15.8%) | 11 (29.7%) | 8 (21.6%)  |
| GOLD Classification, N (%)|           |           |           |           |
| 1                         | 10 (26.3%) | 8 (21.1%) | 7 (18.9%) | 6 (16.2%) |
| 2                         | 14 (36.8%) | 12 (31.6%) | 15 (40.5%) | 13 (35.1%) |
| 3                         | 8 (21.1%) | 10 (26.3%) | 9 (24.3%) | 11 (29.7%) |
| 4                         | 6 (15.8%) | 8 (21.1%) | 6 (16.2%) | 7 (18.9%) |
| Clinical History, N (%)   |           |           |           |           |
| AECOPD in Last 1 Year     | 7 (18.4%) | 9 (23.7%) | 15 (40.5%) | 14 (37.8%) |
| AECOPD with Hospitalization in Last 1 Year | 6 (15.8%) | 6 (15.8%) | 11 (29.7%) | 10 (27.0%) |
| Medication, N (%)         |           |           |           |           |
| LABA & ICS                | 29 (76.3%) | 31 (83.8%) |           |           |
| LAMA                      | 22 (57.9%) | 24 (64.9%) |           |           |
| SAMA                      | 3 (7.9%)  | 1 (2.7%)  |           |           |
| SABA                      | 2 (5.3%)  | 3 (8.1%)  |           |           |
| Theophylline              | 17 (44.7%) | 19 (51.4%) |           |           |
| Expectorant               | 12 (31.6%) | 10 (27.0%) |           |           |
| CBC                       |           |           |           |           |
| Neutrophil (10^9/L)       | 7.72 (5.43–9.95) | 8.62 (5.79–10.43) * | 5.81 (4.02–6.87) | 5.34 (4.17–6.82) |
| Eosinophil (10^9/L)       | 0.05 (0.0–0.21) | 0.02 (0.0–0.16) | 0.34 (0.17–0.94) | 0.25 (0.13–0.64) ** |
| Spirometric Data          |           |           |           |           |
### Non-eosinophilic COPD vs. Eosinophilic COPD

|                      | Non-eosinophilic COPD       | Eosinophilic COPD         |
|----------------------|-----------------------------|---------------------------|
| **FEV1 (L)**         | 1.64 ± 0.68                 | 1.38 ± 0.66*              |
|                      | 1.63 ± 0.71                 | 1.41 ± 0.76*              |
| **FEV1 (% Predicted)** | 64.38 ± 23.01               | 56.67 ± 23.8**            |
|                      | 61.3 ± 24.25                | 56.31 ± 23.74*            |
| **FVC (L)**          | 2.71 ± 0.93                 | 2.47 ± 0.85               |
|                      | 2.54 ± 0.81                 | 2.32 ± 0.75               |
| **FVC (% Predicted)**| 78.73 ± 16.41               | 74.81 ± 15.83             |
|                      | 74.23 ± 15.61               | 73.24 ± 15.24             |
| **FEV1/FVC%**        | 54.32 ± 8.71                | 51.58 ± 6.30              |
|                      | 53.96 ± 4.25                | 50.07 ± 5.62              |

### Functional Performance

|        | Non-eosinophilic COPD | Eosinophilic COPD |
|--------|-----------------------|-------------------|
| mMRC   | 1.55 ± 0.75           | 1.64 ± 0.81       |
| CAT    | 9.18 ± 4.79           | 9.24 ± 3.81       |
| 6MWD   | 315.72 ± 130.57       | 310.63 ± 128.40   |

### 3. 12/15-LOX-derived mediators increased in eosinophilic COPD compared to non-eosinophilic COPD

To determine the expression status of lipid signals in non-eosinophilic and eosinophilic COPD, we analyzed lipid metabolites both in baseline and follow-up (Table 3). LC-MS/MS-based mediator lipidomics revealed that a series of 12/15-LOX-derived mediators were increased significantly in eosinophilic COPD compared to non-eosinophilic COPD (Fig. 3). Those mediators including 15-HETE, 15-HEPE and 17-HDoHE which were generated from arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) respectively. Furthermore, the latter two mediators were found significantly decreased after follow-up whereas Δ17-HDoHE was significantly correlated with ΔBEC (r = 0.336, P = 0.023) in eosinophilic COPD (see supplemental Fig. 1, Supplemental Digital Content, which illustrates correlations between ΔBEC with ΔFEV1 (% Predicted) and Δ17-HDoHE in eosinophilic COPD). These results indicated that eosinophils might involve 12/15-LOX-expressing and mediator environment regulating to support resolution of inflammation in eosinophilic COPD (see supplemental Fig. 2, Supplemental Digital Content, which illustrates inflammatory response in eosinophils).
Table 3
LC-MS/MS-based mediator lipidomics between baseline and follow-up in non-eosinophilic COPD and eosinophilic COPD groups.

|               | Non-eosinophilic COPD | Eosinophilic COPD |               |               | P2  | P3  | P4  |
|---------------|-----------------------|-------------------|---------------|---------------|-----|-----|-----|
|               | Baseline | Follow-Up | Baseline | Follow-Up | 0.545 | 0.387 | 0.199 | 0.262 |
| 12-HETE       | 86.81 (63.34-111.27) | 91.14 (77.82-118.14) | 111.74 (90.95-143.11) | 116.71 (97.29-131.48) |     |     |     |     |
| 15-HETE       | 13.81 (8.49-19.48) | 13.06 (9.50-18.77) | 22.47 (17.87-26.72) | 21.94 (17.23-26.83) | 0.272 | 0.306 | 0.027* | 0.039* |
| 14,15-EET     | 0.28 (0.24-0.35) | 0.27 (0.17-0.35) | 0.38 (0.28-0.45) | 0.34 (0.25-0.47) | 0.643 | 0.877 | 0.278 | 0.450 |
| LTB4          | 0.83 (0.58-1.05) | 0.91 (0.75-1.08) | 0.74 (0.58-0.88) | 0.72 (0.55-0.90) | 0.438 | 0.768 | 0.944 | 0.860 |
| PGD2          | 63.54 (51.48-75.91) | 71.67 (64.13-80.85) | 74.63 (67.65-84.05) | 73.33 (65.50-84.65) | 0.527 | 0.877 | 0.568 | 0.697 |
| AA            | 10656.69 (9532.00-12563.51) | 11578.77 (10035.86-13126.51) | 12620.73 (11010.75-14392.13) | 12476.44 (11244.52-13834.62) | 0.275 | 0.612 | 0.224 | 0.195 |
| EPA           | 1094.45 (996.78-1254.41) | 1037.24 (884.11-1184.13) | 1268.79 (1083.82-1479.21) | 1251.34 (1134.56-1365.00) | 0.229 | 0.207 | 0.909 | 0.345 |
| 12-HEPE       | 11.58 (10.81-13.97) | 11.46 (10.76-13.34) | 15.27 (13.57-18.62) | 15.47 (13.11-17.33) | 0.546 | 0.311 | 0.371 | 0.204 |
| 15-HEPE       | 7.61 (4.05-10.70) | 7.21 (5.18-9.80) | 12.13 (10.54-15.63) | 11.99 (9.65-13.65) | 0.153 | 0.037* | 0.014* | 0.020* |
| 5-HEPE        | 0.83 (0.59-1.17) | 0.95 (0.83-1.08) | 0.84 (0.61-1.24) | 0.89 (0.75-1.03) | 0.627 | 0.782 | 0.689 | 0.768 |
| PGD3          | 1.42 (1.31-1.51) | 1.48 (1.38-1.56) | 1.62 (1.53-1.70) | 1.69 (1.61-1.78) | 0.434 | 0.743 | 0.759 | 0.572 |
| 17,18-diHETE  | 15.57 (13.88-18.41) | 16.03 (14.41-18.19) | 17.38 (15.23-20.24) | 18.57 (16.42-21.41) | 0.99 | 0.703 | 0.19 | 0.319 |
|                   | Non-eosinophilic COPD |       | Eosinophilic COPD |       |       |       |
|-------------------|-----------------------|-------|-------------------|-------|-------|-------|
|                   |                       | $P1$  |                   |       | $P2$  | $P3$  | $P4$  |
| DHA               | 1037.40 (978.67-1245.54) | 0.797 | 1248.91 (1136.53-1365.58) | 0.688 | 0.223 | 0.461 |
| 17-HDoHE          | 12.77 (8.57–16.63)     | 0.604 | 25.91 (16.64–32.42)   | 22.42 (19.69–26.41) | 0.072* | 0.006** | 0.044* |
| 14-HDoHE          | 35.54 (23.18–44.86)    | 0.673 | 43.53 (32.10–53.93)   | 44.67 (40.09–51.04) | 0.806 | 0.371 | 0.254 |
| 10-HDoHE          | 0.93 (0.76–1.17)       | 0.632 | 0.73 (0.58–0.96)      | 0.78 (0.68–0.89)    | 0.463 | 0.402 | 0.586 |
| 13-HDoHe          | 0.82 (0.55–1.04)       | 0.504 | 0.77 (0.68–0.83)      | 0.74 (0.66–0.94)    | 0.425 | 0.336 | 0.451 |
| 19,20-HDoHE       | 0.78 (0.68–0.89)       | 0.423 | 0.9 (0.75–1.03)       | 0.89 (0.75–1.04)    | 0.798 | 0.553 | 0.357 |

**Discussion**

In this study, we found that there was no significant difference in lung function between patients with eosinophilic COPD ($\geq$ 2%) at baseline and patients with low blood eosinophil percentages COPD (< 2%). Similarly, Chou K-T et al. found that the airway obstruction was similar between patients with eosinophilic and non-eosinophilic COPD (14). Chronic inflammation was one of the factors in destroying the structure of the lungs and the process in the pathogenesis of COPD (15). There was no difference in lung function between the two groups, probably because of the two types of inflammation models, eosinophilic and neutrophil, although the mechanism of action was different, the degree of damage to lung function was equivalent, and there was no significant difference. In addition, the change of symptom scores, FEV1 (% Predicted) and FEV1 (L) after follow-up between the two groups were found no significant difference, which further confirmed our opinion. There were some controversial findings on the lung function of eosinophilic COPD and non-eosinophilic COPD (16, 17). This may be due to the heterogeneity of COPD. In general, we prefer the view that the two inflammatory models have comparable damage capacity. Our study also demonstrated that eosinophilic COPD were more likely to develop exacerbation than non-eosinophilic COPD, but interestingly, there was no significant difference in spirometric data between two groups. The studies of Schumann DM et al. and Yun, J. H. et al. also showed the same characteristics in eosinophilic COPD (18, 19). We speculate that the reason may be that patients with high blood eosinophilia will prefer to use ICS for the treatment of acute exacerbation, and can benefit from this treatment, resulting in the control of the degree of acute exacerbation.
Peripheral blood eosinophil increased was characteristic of eosinophilic COPD. Our result demonstrated that non-eosinophilic COPD seem to have better stability of blood eosinophil levels compared with eosinophilic COPD. Casanova C et al. studies also show that the stability of blood eosinophil levels appears to be worse in patients with high eosinophil counts than patients with low eosinophil counts (20). Moreover, our study showed positive correlation between $\Delta$BEC and $\Delta$FEV1 (% Predicted) in eosinophilic COPD. Review of Tashkin et al (21) showed diverse results between lung function parameter and BEC from various prior studies. Some studies (22, 23) showed that higher BEC was related to lower pulmonary function parameters but Saiphoklang N study (24) had opposite results. In the Saiphoklang N study, showed positive correlation between BEC and post-broncho-dilator FEV1. The outcomes corresponded to our study. Airway inflammation was a significant host defense mechanism and requires appropriate decomposition to stay homeostasis. The decomposition of inflammation including active molecular and cellular processes that can restore inflammatory tissue to a steady state. Eosinophils have multiple functions in regulating immunological responses and inflammatory processes via lipid signals (25). In this study, LC-MS/MS-based mediator lipidomics revealed that a series of 12/15-LOX-derived mediators were significantly increased in eosinophilic COPD compared to non-eosinophilic COPD.

Lipid metabolism was considered to be a significant factor in disease control and immune regulation (26). Compared to non-eosinophilic COPD, DHA-derived 17-HDoHE was found to be one of the most abundant mediators produced by 12/15-LOX in eosinophilic COPD. Eosinophils were the major cell type expressing 12/15-LOX (9). These results indicate that due to the advantage of eosinophil counts, the levels of 12/15-LOX-derived mediators increased in eosinophilic COPD. Furthermore, $\Delta$BEC was significantly correlated with $\Delta$17-HDoHE and both of them showed decreased after the follow-up in eosinophilic COPD. Eosinophils might actively convert DHA into pro-resolving mediator 17-HDoHE during the development of eosinophilic COPD. As a mediator, 17-HDOHE was a precursor of PD1 and has been reported that can promote antibody-mediated immune response, macrophage phagocytosis and reduce inflammation related obesity (11, 21, 24, 27). 17-HDoHE could reduce inflammatory cytokines and transformed immune cell functions, such as macrophages into M2 polarization and B cells differentiating into antibody secreting phenotypes. The in vivo depletion of eosinophils significantly reduced 12/15-LOX products in resolving exudates and caused a resolution defect characterized by impaired lymphatic drainage to draining lymph nodes (DLNs), along with increased polymorphonuclear leukocyte (PMN) numbers in inflamed sites. Furthermore, eosinophils were recruited to the inflamed site, produced pro-resolving mediators via a 12/15-LOX-initiated biosynthetic route and play roles in promoting resolution of acute inflammation (9). These results indicated that BEC might be involved in the regulation of airway inflammation in eosinophilic COPD.

Taken together, our results suggest that the metabolic levels of neutrophil COPD and eosinophilic COPD were different due to the huge difference in eosinophil level, which leads to different inflammatory patterns, and the 12/15-LOX metabolic pathway was one of them. According to previous reports, 12/15-LOX-derived mediators promoted the regression of inflammation. However, we did not find any difference in lung function between non-eosinophilic COPD and eosinophilic COPD during follow-up. In contrast, we did find that 12/15-LOX-derived mediators were associated with airway inflammation in eosinophilic COPD. This suggests that 12/15-LOX metabolic pathway might be the key to the role of eosinophils in COPD. There were some deficiencies in this study. Firstly, only considering the level of eosinophils, ignoring the
heterogeneity of eosinophil phenotype. Secondly, the sample size was insufficient, and further research needs to increase the sample size. Thirdly, ignoring the sputum eosinophilic counts and might lead to some discrepancies in defining eosinophilic COPD. In recent years, eosinophils as one of the important factors and potential biomarkers in the pathogenesis of COPD had attracted extensive attention. The results of this study might help to understand the inflammatory response and lipid metabolism of eosinophilic COPD.

**Abbreviations**

COPD
chronic obstructive pulmonary disease; CBC:Complete blood count; BEC:blood eosinophil count; BNC:blood neutrophil count; 12/15-LOX:12/15-Lipoxygenase; PD1:protectin D; SPMs:specialized pro-resolving mediators; 6MWT:six-minute walk test; LAMA:long-acting muscarinic receptor antagonist; LABA:long-acting beta 2 agonist; ICS:inhaled corticosteroid; FEV1:Forced expiratory volume in 1st second; COX:cyclooxygenase; LOX:lipoxygenase; HETE:Hydroxyeicosatetraenoic Acid; LT:Leukotriene; PG:Prostaglandin; AA, Arachidonic Acid; EPA, Eicosapentaenoic Acid; HEPE, Hydroxyeicosapentaenoic Acid; DHA, Docosahexaenoic Acid; HDoHE, Hydroxydocosahexaenoic Acid.

**Declarations**

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**Authors’ contributions**

CC; Conceptualization, Writing-original draft, Funding acquisition. SW; Data curation, Writing-review & editing. HY; Formal analysis, Validation. DZ; Project administration. YZ; Methodology. BZ; Investigation. YY; Supervision, Resources. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Shenzhen People's Hospital (Ethics approval No. KY-LL-2020242).

**Consent for publication**
Not applicable.

**Competing interests**

The authors have no potential conflict of interest related to this article.

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Figure 1

Lung function decline in non-eosinophilic COPD and eosinophilic COPD groups after the period of follow-up. Data are expressed as FEV1 (L) and FEV1 (% Predicted) between baseline and follow-up values. Results are mean ± standard deviation. *P < 0.05; ** P < 0.01; *** P < 0.001. Abbreviations: COPD, chronic obstructive pulmonary disease; FEV1, Forced expiratory volume in 1st second
Figure 2

Comparison in BNC and BEC results between baseline and follow-up in non-eosinophilic COPD and eosinophilic COPD groups. Data shown as median (interquartile range). Abbreviations: COPD, chronic obstructive pulmonary disease; BNC, blood neutrophil count; BEC, blood eosinophil count.
Figure 3

LC-MS/MS-based mediator lipidomics between baseline and follow-up in non-eosinophilic COPD and eosinophilic COPD groups. Data shown as median (interquartile range). *P < 0.05; ** P < 0.01; *** P < 0.001. Abbreviations: COPD, chronic obstructive pulmonary disease; COX, cyclooxygenase; LOX, lipoxygenase; HETE, Hydroxyeicosatetraenoic Acid; LT, Leukotriene; PG, Prostaglandin; AA, Arachidonic Acid; EPA, Eicosapentaenoic Acid; HEPE, Hydroxyeicosapentaenoic Acid; DHA, Docosahexaenoic Acid; HDoHE, Hydroxydocosahexaenoic Acid.
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