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ORIGINAL ARTICLE

Comparison of serum biomarkers between patients with asthma and with chronic obstructive pulmonary disease

Seon-Sook Han, MD, PhD^{1,2}, Won Ho Lee, MD¹, Yoonki Hong, MD^{1,2}, Woo Jin Kim, MD, PhD^{1,2}, JeongHee Yang, MD, PhD³, Myoung Nam Lim, MD⁴, Seung-Joon Lee, MD, PhD^{1,2}, and Jae-Woo Kwon, MD^{1,2,5}

¹Department of Internal Medicine, Kangwon National University College of Medicine, Chuncheon, Korea, ²Environmental Health Center, Kangwon National University Hospital, Chuncheon, Korea, ³Department of Family Medicine, Kangwon National University School of Medicine, Chuncheon, Korea, ⁴Regional Center for Respiratory Diseases, Kangwon National University Hospital, Chuncheon, Korea, and ⁵Department of Allergy and Clinical Immunology, Kangwon National University Hospital, Chuncheon, Korea

ABSTRACT

Objective: Asthma and chronic obstructive pulmonary disease (COPD) have distinct pathophysiological mechanisms but sometimes share similar clinical manifestations. Distinguishing between these diseases is important. This study compared the profiles of serum biomarkers between patients with asthma and those with COPD. **Methods:** Serum levels of the chitinase like protein YKL-40, periostin, interleukin (IL)-18, and chemokine (C–C motif) ligand 18 (CCL18) were measured in asthma patients ($n = 20$), COPD patients ($n = 16$), and normal controls ($n = 20$). **Results:** Serum levels of YKL-40 were higher in COPD patients [median (range), 55 (17–565) versus 208 (74–922) ng/mL, $p < 0.0001$], but no differences were observed between asthma and COPD patients after adjusting for age and forced expiratory volume in 1 s (FEV₁). No differences in serum levels of periostin, IL-18, or CCL18 were observed between the patient groups. Total IgE and airway hypersensitivity were negatively correlated ($r = -0.485$, $p = 0.007$). CCL18 levels were related to patients' age in asthmatic patients ($r = -0.562$, $p = 0.010$). Serum levels of CCL18 and IL-18 were positively correlated in patients with COPD ($r = 0.696$, $p = 0.003$). **Conclusions:** No differences in the serum profiles of periostin, IL-18, or CCL18 were observed between patients with asthma and those with COPD. Serum levels of YKL-40 were not different between asthma and COPD patients after adjusting for age and FEV₁. There were negative correlation between CCL18 and age in patients with asthma and positive correlation between IL-18 and CCL18 in patients with COPD.

Keywords

Asthma, COPD, CCL18, IL-18, periostin, YKL-40

History

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Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are chronic inflammatory airway diseases that are suspected to have different pathogenic mechanisms and usually present different clinical features of each disease [1]. Asthma is caused by sensitizing agents and Th2 inflammation characterized by interleukin (IL)-4, IL-13, mast cells, and eosinophils dominant in the airway. COPD is caused by noxious agents such as smoking and Th1 inflammation characterized by neutrophils and macrophages dominant in the airway. Clinically, asthma usually develops in childhood or young adulthood and more often in female [2]. Typical asthmatic symptoms are characterized by episodic and fluctuating dyspnea, wheezing, and cough [3]. On the contrary, COPD is prevalent in the elderly and closely related with smoking. Symptoms of COPD are characterized by relatively continuous and exertional dyspnea. However, despite the distinct pathophysiological

mechanisms of these airway diseases, they often share similar clinical manifestations in real practice [3]. It is difficult to distinguish between COPD and asthma with fixed airway obstruction, and many patients show clinical characteristics of both diseases, so-called asthma–COPD overlap syndrome. Therefore, a differential diagnosis between asthma and COPD based on pathophysiological mechanisms is needed for a correct diagnosis and efficient treatment. There have been many studies about biomarkers related to each disease but there have been no studies that compared with the profiles of serum biomarkers between different diseases.

This study was conducted to compare the profiles of serum biomarkers between patients with clinically typical asthma and those with typical COPD. Asthmatic patients were young or mid-life non-smoking female with typical asthmatic history and patients with COPD were middle- or older-aged heavy smokers with typical clinical history of COPD. Although asthma with Th2 inflammation consists major portion among asthmatics [4,5], there is an increasing appreciation of heterogeneity within asthma primarily on cluster analyses of clinical phenotypes and different responses to conventional treatments or targeted therapeutics [4,6]. There are several

Correspondence: Jae-Woo Kwon, MD, Department of Internal Medicine, Kangwon National University Hospital, Baengnyeong-ro 156, Chuncheon-Si, Gangwon-Do, 200-722, Republic of Korea. Tel: +82 33 258 9370. Fax: +82 33 258 2404. E-mail: legent@hanmail.net

successful therapeutic trials of targeted therapies using biomarkers for Th2 inflammation such as periostin which is secreted from bronchial epithelial cells induced by IL-13. On the contrary, asthma without Th2 inflammation is poorly defined and clinically heterogeneous and there are no specific biomarkers. COPD is the chronic inflammatory airway disease with Th1 inflammation and several serum biomarkers have been studied in COPD patients. However, there have been no studies to discriminate COPD from asthma by serum biomarkers. The serum level of YKL-40, a member of the human chitinase-like protein family, is elevated in both patients with asthma and those with COPD. YKL-40 is a key regulator of Th2 inflammation with close relation to IL-13 and its role is well described in asthma [7–9]. On the other hands, the expression levels of YKL-40 are elevated in COPD [10]. YKL-40 is a critical regulator of oxidant injury [11] and plays a protective role in the pathogenesis of cigarette smoke-induced inflammation via an IL-18-dependent pathway [12]. Thus we hypothesized that YKL-40 might increase in both asthma and COPD but via different mechanisms such as Th2 inflammation, reflected by periostin [13] in asthma and oxidative stress related with IL-18 signaling in COPD. In addition, serum chemokine (C–C motif) ligand 18 (CCL18) which is another biomarker elevated in both asthma and COPD was evaluated in this study [14–16]. Finally, we compared serum levels of YKL-40, periostin, IL-18, and CCL18 between patients with asthma and those with COPD.

Methods

Study subjects

Patients with asthma ($n = 20$) or COPD ($n = 16$) and healthy controls ($n = 20$) were enrolled after informed consent was obtained from each subject, and clinical data and serum samples were collected. All patients with asthma or COPD were enrolled by a pulmonologist in out-patient clinic of Kangwon National University Hospital. Asthma was defined as a diagnosis by a pulmonologist based on clinical symptoms and a positive methacholine bronchial provocation test (MBPT), i.e., the concentration of methacholine that causes a 20% decrease in forced expiratory volume in 1 s (PC_{20}) ≤ 8 mg/mL. MBPT was performed using the 2-min tidal breathing method according to American Thoracic Society (ATS) Guidelines [17]. Asthmatic patients with a history of smoking were excluded. COPD was defined as a diagnosis by a pulmonologist based on typical symptoms, a smoking history of at least 10 pack-years, and a ratio of pre-bronchodilator forced expiratory volume in 1 s (FEV_1) to forced vital capacity $< 70\%$, and a pre-bronchodilator FEV_1 of $< 80\%$ of the predicted value. The patients with COPD over age 40 were enrolled [18]. Spirometry was performed with Vmax 22 spirometer (SensorMedics, Yorba Linda, CA) as recommended by the ATS [19]. The enrolled patients were in stable condition when the serum samples were collected. Patients who suffered exacerbations of asthma or COPD were excluded. Healthy control subjects who had no history of smoking and no symptoms of asthma or COPD were enrolled, and demographic characteristics were collected. Demographic characteristics,

smoking status, medication history for asthma, or COPD during the previous 4 weeks and the results of pre-bronchodilator lung function tests were collected from patients with COPD or asthma. Results of the MBPT as PC_{20} , skin prick tests for 21 common inhalant allergens and serum levels of total IgE were collected from patients with asthma. Commercial extracts of 21 common aeroallergens (Allergopharma, Reinbeck, Germany) were used for skin prick tests: tree pollen mixture, grass pollen mixture, mold mixture, cereal mixture, mixture of feathers, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium*, *Cladosporium*, *Hemithosporium*, *Botrytis*, *Curvularia*, mugwort, ragweed, wheat, cat epithelia, dog epithelia, sheep wool, and cockroach. Atopy was defined as a positive skin prick test result (a mean wheal diameter ≥ 3 mm) for one or more of the inhalant allergens. Blood samples for measurement of total IgE levels were kept standing for 30 min at room temperature for coagulation and then centrifuged within 2 h. Serum total IgE was measured by chemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). This study protocol was approved by the Institutional Review Board of Kangwon National University Hospital (no. KWNH 2012-03-004).

Sample collection and biochemical analysis

Serum samples for biomarker analysis were stored at -70°C until analysis. Serum levels of YKL-40, IL-18, CCL18, and periostin were measured by enzyme-linked immunosorbent assay using a YKL-40 ELISA kit (Quidel Corp., San Diego, CA), an IL-18 Human ELISA kit (Invitrogen, Carlsbad, CA), a CCL18/PARC DuoSet ELISA kit (R&D Systems, Minneapolis, MN), and a Human Periostin/OSF-2 DuoSet ELISA kit (R&D Systems, Minneapolis, MN) according to the instructions of the manufacturer. All assays were performed in duplicate for each sample, and mean values are reported.

Statistical analysis

Patient characteristics and serum biomarkers are presented as medians (range) for continuous variables and as relative frequencies for categorical variables; they were compared using the Kruskal–Wallis H test or the Mann–Whitney U -test. Spearman's correlations were calculated for the variables of interest that did not follow a normal distribution. Comparing the serum levels of YKL-40 between patients with asthma and those with COPD after adjusting for age and FEV_1 was done by using General Linear Model. The power analysis of study population was preformed because the study population was small. Statistical significance was accepted for p values < 0.05 . Statistical power analysis was conducted according to method suggested by Cohen using mean and standard deviation of data from current study with significance level of 5%. [20,21] All statistical analyses were performed using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the characteristics of the study populations. Patients with COPD were older and had poorer lung function. Patients with COPD were all male, and the patients with asthma

Table 1. Baseline characteristics.

Characteristics	COPD (n = 16)	Asthma (n = 20)	Control (n = 20)	p value
Age, year	73 (49–89)	50 (22–67)	50 (40–57)	<0.0001
Sex, % female	0%	95%	100%	<0.0001
Smoking, pack year	40 (10–96)	0	0	<0.0001
Current	6	0	0	
Former	10	0	0	
Never	0	20	20	
Lung function tests				
FEV ₁ , L	1.17 (1–2)	2.21 (2–3)		<0.0001
FEV ₁ , %	46 (32–91)	95 (55–133)		<0.0001
FVC, L	2.78 (1–4)	2.57 (2–4)		0.582
FVC, %	76 (48–121)	86 (57–136)		0.132
FEV ₁ /FVC, %	44.1 (32.7–56.5)	83.7 (60.1–98.0)		<0.0001
Eosinophil count (/μL) ^a	157 (0–688)	169 (0–539)		0.972
PC ₂₀		2.04 (0–4)		
Atopy		71% (12/17) ^c		
Total IgE		22 (4–643)		

Median (range) FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s.

^aPeripheral blood eosinophil count.

^bConcentration of methacholine that causes a 20% decrease in FEV₁.

^cNo skin prick test data for three patients.

or controls were all female except one. All patients with asthma had not been treated for asthma at least more than 3 months. Seven patients with COPD had been treated with long acting inhaled anticholinergics, tiotropium bromide, while 9 patients had not been treated for COPD at least more than 3 months.

Serum levels of YKL-40 were higher in patients with COPD (median [range], controls versus asthma versus COPD, 60 [18–624] versus 55 [17–565] versus 208 [74–922] ng/mL, $p < 0.0001$; Figure 1), but no differences were observed between patients with asthma and those with COPD after adjusting for age and FEV₁ (L). Analysis after transforming the crude values into log values provided the same results. In YKL-40 values were higher in patients with COPD than in patients with asthma and controls ($p < 0.0001$). Figure 2 shows serum levels of YKL-40, age, and FEV₁ (%) in patients with asthma and those with COPD.

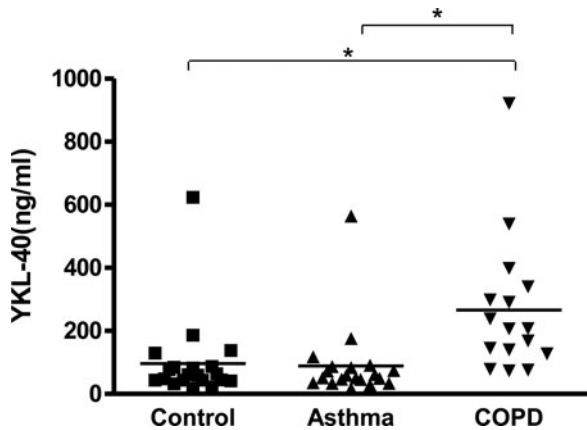


Figure 1. Serum levels of YKL-40 in patients with asthma, those with chronic obstructive pulmonary disease (COPD), and controls. Levels were higher in patients with COPD (60 [18–624] versus 55 [17–56] versus 208 [74–922] ng/mL, controls versus COPD versus asthma, respectively), but no differences were observed between patients with asthma and those with COPD after adjusting for age and forced expiratory volume in 1 s (FEV₁[L]). * $p < 0.0001$.

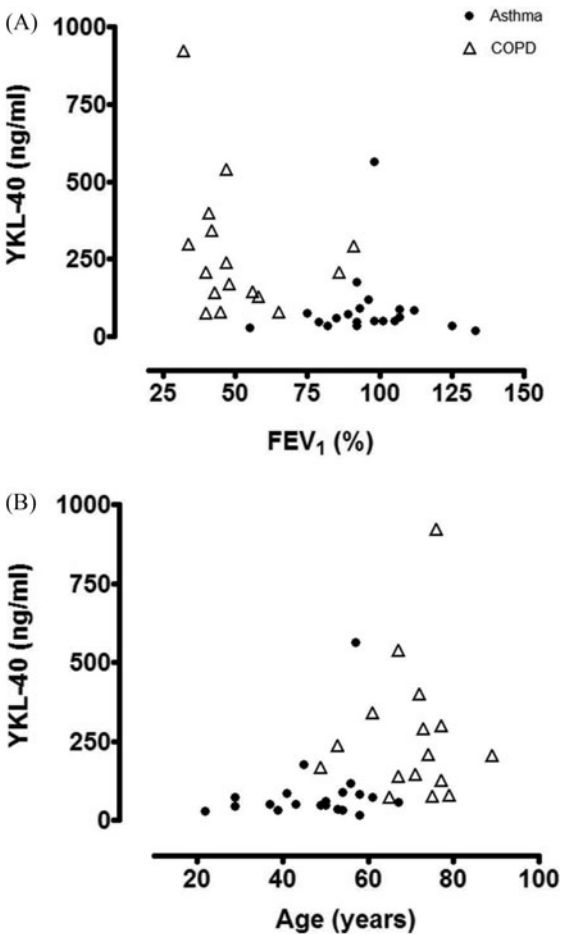


Figure 2. Serum YKL-40 and age or forced expiratory volume in 1 s (FEV₁) (%) from patients with asthma and those with COPD.

No differences in serum levels of periostin, IL-18, or CCL18 were observed among asthma patients, COPD patients, and controls (Table 2). No differences in the ratio of the levels of serum biomarkers (YKL-40/IL-18, YKL-40/periostin, CCL18/IL-18, and CCL18/periostin) were observed among

Table 2. Serum levels of biomarkers.

Serum biomarkers	COPD (n = 16)	Asthma (n = 20)	Control (n = 20)	p Value ^a
YKL-40, ng/mL	208 (74–922)	55 (17–565)	60 (18–624)	<0.0001
Periostin, pg/mL	106 (40–189)	97 (26–198)	84 (36–200)	0.525
IL-18, pg/mL	199 (87–238)	210 (86–319)	168 (101–265)	0.208
CCL18, pg/mL	53 (20–86)	47 (29–73)	47 (30–85)	0.947

Median (range).

^aKruskal–Wallis H test.

the groups. In patients with asthma, total IgE and PC₂₀ were negatively correlated ($r = -0.485$, $p = 0.007$). Atopy was not related with the serum levels of biomarkers, total IgE, and peripheral blood eosinophil count. CCL18 levels were related to patients' age in asthmatic patients ($r = -0.562$, $p = 0.010$; Figure 3). There were no relations among biomarkers in patients with asthma. In patients with COPD, serum levels of CCL18 and IL-18 were positively correlated ($r = 0.696$, $p = 0.003$; Figure 4). Amount of smoking (pack-years), current smoking, patients' age and COPD treatments were not associated with any serum biomarkers in patients with COPD.

Discussion

Typical clinical manifestation of asthma or COPD is a key factor in the diagnosis of each disease. However, clinically typical phenotype of each disease did not mean the differences in pathophysiology reflected by four serum biomarkers in this study. There were different relationships among biomarkers and dermatographics in the patients with each disease. Serum levels of YKL-40 were higher in COPD patients, but no differences were observed between asthma and COPD patients after adjusting for age and FEV₁. The serum levels of CCL18 were negatively related to patients' age in asthmatics. Serum levels of CCL18 and IL-18 were positively correlated in COPD patients.

YKL-40 is a highly phylogenetically conserved chitin-binding glycoprotein that belongs to the family of chitinase-like

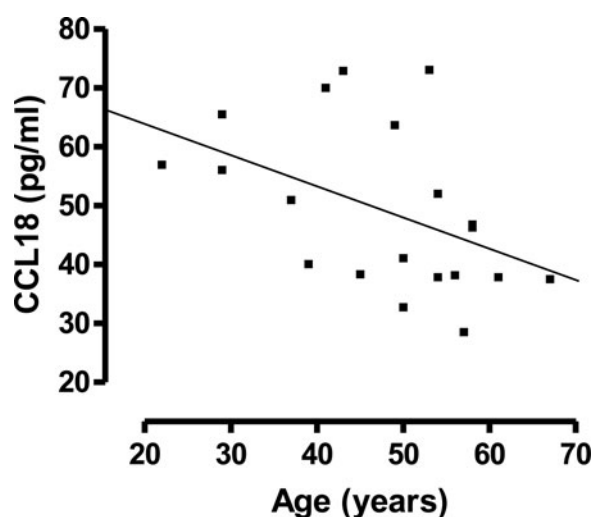


Figure 3. Serum levels of CCL18 and age in patients with asthma. Serum levels of CCL18 were related to patients' age in asthmatics ($r = -0.562$, $p = 0.010$).

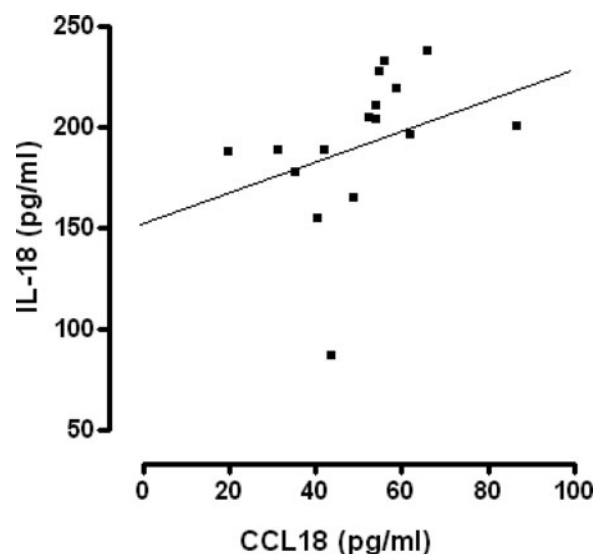


Figure 4. Serum biomarkers in patients with chronic obstructive pulmonary disease (COPD). Serum levels of chemokine (C–C motif) ligand 18 (CCL18) and interleukin (IL)-18 were positively correlated with each other in patients with COPD ($r = 0.696$, $p = 0.003$).

proteins. Prominent expression of YKL-40 has been associated with pathologic conditions characterized by tissue inflammation and remodeling and by aberrant cell growth, such as rheumatic arthritis, atherosclerosis, asthma, liver fibrosis, and several malignancies [7,10]. The serum levels of YKL-40 showed inverse correlation with FEV₁ in both COPD and asthma [8,10,20]. Furthermore YKL-40 is positively correlated with asthma severity and airway remodeling [8,21]. YKL-40 is essential for IL-13 mediated Th2 inflammation and related with antigen sensitization and IgE induction in animal models [9], but the relations between YKL-40 and markers of Th2 inflammation such as total serum IgE and peripheral blood eosinophils are controversial in patients with asthma [8,22]. Current study suggests that serum levels of YKL-40 were different according to FEV₁ but not specific diseases. Furthermore, there were no differences in serum levels of YKL-40 between patients with asthma and healthy controls. It may be because asthmatic patients in this study had near normal FEV₁ just as Chupp et al. [8] previously showed that there were no differences in serum YKL-40 levels between patients with mild asthma based on FEV₁ and healthy controls. These findings could suggest that YKL-40 might be more related with remodeling than Th2 inflammation in asthma.

Periostin, a matricellular protein secreted from bronchial epithelial cells, is induced by IL-13 [23]. Periostin may contribute to airway remodeling in asthma and has been successfully used in asthma treatment as a screening marker for selecting good responders to lebrikizumab, an anti-IL-13 therapy [13,24]. No studies about periostin in patients with COPD have been conducted. It was expected that serum periostin would be lower in patients with COPD than those with asthma, as periostin is a surrogate marker for IL-13. However, our results showed no differences in serum periostin levels between the patient groups. This could be because the asthma patients in this study had low levels of Th2 inflammation according to the percentage or absolute counts of peripheral blood eosinophils

which were similar between the two groups in this study. Meanwhile, since there were no direct evaluations for airway inflammation, there was possibility that asthmatics with neutrophilic Th1 inflammation which is pathophysiologically more similar to COPD were enrolled in this study. Asthma with neutrophilic Th1 inflammation is a less common phenotype and usually known to be associated with low FEV₁ and characteristic of older patients with a late onset of the disease [22]. In contrast, enrolled patients in this study were young and had relatively normal FEV₁, and, furthermore, they showed relations between total IgE and airway hyperresponsiveness which is closely related with Th2 inflammation. So this study suggests that periostin may not be more sensitive biomarker for Th2 inflammation than peripheral blood eosinophils and serum total IgE.

Current study reported the relationship between CCL18 and IL-18 in COPD. Although CCL18 is elevated in COPD and also elevated in asthma [14–16], the role of CCL18 in the pathogenesis is still not clear. Since IL-18 is known as mediator related with oxidative stress [12], this finding would be the clue for further studies and help to evaluate the role of CCL18 in COPD. IL-18, originally described as an interferon (IFN)- γ -releasing factor, enhances Th1 responses and has synergistic effects with IL-12 in inducing IFN- γ release and inhibiting Th2 inflammation [25]. Corren et al. [26] showed that serum levels of IL-18 in COPD patients and smokers are significantly higher than those in non-smokers. They also found a significant negative correlation between serum IL-18 level and FEV₁ (%) in patients with COPD. However, IL-18 has autoimmune regulatory effects on both Th1 and Th2 cytokines [27], and several studies have demonstrated that IL-18 activity increases in Th2 type diseases such as asthma exacerbations and allergic rhinitis [28–30]. CCL18 is known to be constitutively expressed in the lung and induced in inflammatory conditions [14]. CCL18 is mainly produced by antigen-presenting cells, and may exhibit dual functions, with both pro- and anti-inflammatory properties including the generation of adaptive regulatory T cells in healthy subjects. There have been several studies that suggested the function of CCL18 in allergic asthma was to favor and amplify a Th2 immune response [14]. Meanwhile, serum levels of CCL18 are associated with fibrosis and elevated in COPD and significantly associated with clinical severity of COPD, based on reduced FEV₁ levels, higher symptom scores, and acute exacerbations [15,16]. However, since there is the lack of data on the nature of its putative receptor and the absence of murine orthologue, it is difficult to know the role of CCL18 in COPD and asthma pathogenesis. Current study also reported that there was negative relation between serum levels of CCL18 and age in patients with asthma. In contrast, several studies reported that serum CCL18 concentrations were significantly increased with age in COPD [15]. Different relationship between age and CCL18 in asthma and COPD might be related with different pathogenesis of each disease. Further studies on the relationship between CCL18 and age in asthma and COPD are needed.

Several limitations of this study should be discussed. First, study population was small. The result of power analysis using study population was 72.3%. As 70% of statistical power with significance level of 5% means 12–20 people per group as the

minimum sample size [31,32] statistical power of this study seemed to be acceptable in spite of small study population. Second, there were differences in severity based on FEV₁ and age between patients with asthma and those with COPD. Defining equal severity between two different diseases is difficult. There could be overlap of asthma and COPD in patients with similar FEV₁ and age [24]. We approached to severity based on symptoms which was severe enough to visit the tertiary hospital because all patients with asthma or COPD were enrolled in out-patient clinic of tertiary hospital based on typical symptoms and history, which is the way doctors usually discriminate each disease and could minimize the overlap of asthma and COPD with typical lung function tests such as persistent airflow limitation in COPD and reversible airflow limitation with normal FEV₁ between symptoms in asthma. Third, there could be concerns about confounding effects by gender although the results were analyzed by adjusting for age and sex. However, serum levels of YKL-40 were evaluated in two large cohorts of asthma and several groups of COPD patients, but there were no gender differences [8,10]. There have been no reports on gender difference of periostin in asthmatics [33], while there have been no studies about periostin in COPD. On the contrary, one study for school children with asthma or allergic rhinitis reported that male showed higher serum levels of IL-18 than female, but since serum total IgE levels were much higher in male, it is not clear the differences of serum IL-18 came from gender difference or not [34], meanwhile another study reported that there were no gender differences in IL-18 production of lung tissues in patients with COPD [28]. For serum levels of CCL18, several studies reported that serum CCL18 concentrations were significantly related to increasing age, male sex, BMI, and smoking status in COPD [15], but for asthma, there had been a few studies and there had been no report on gender difference. However, there were no differences in CCL18 levels in current study although COPD patients were male and older than asthmatics.

Conclusion

There were no significant differences in serum levels or profiles of four biomarkers among the patients with typical phenotypes of asthma and COPD. However, there were negative relations between CCL18 and age in asthmatics and positive relations between CCL18 and IL-18 in patients with COPD. A larger, well-designed study is needed to evaluate the role of biomarkers in the differential diagnosis of these two conditions.

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Declaration of interest

The authors report that they have no conflicts of interest.

References

- Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 2008;118:3546–3556.
- Andersen H, Lampela P, Nevanlinna A, Saynajakangas O, Keistinen T. High hospital burden in overlap syndrome of asthma and copd. *Clin Respir J* 2013;7:342–346.
- Kim SR, Rhee YK. Overlap between asthma and copd: where the two diseases converge? *Allergy Asthma Immunol Res* 2010;2:209–214.
- Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008;178:218–224.
- Cianchetti S, Bacci E, Ruocco L, Pavia T, Bartoli ML, Cardini C, Costa F, et al. Are sputum eosinophil cationic protein and eosinophils differently associated with clinical and functional findings of asthma? *Clin Exp Allergy* 2014;44:673–680.
- Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: The next steps toward personalized care. *J Allergy Clin Immunol* 2015;135:299–310.
- Lee CG, Elias JA. Role of breast regression protein-39/ykl-40 in asthma and allergic responses. *Allergy Asthma Immunol Res* 2010;2:20–27.
- Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007;357:2016–2027.
- Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, et al. Role of breast regression protein 39 (brp-39)/chitinase 3-like-1 in th2 and il-13-induced tissue responses and apoptosis. *J Exp Med* 2009;206:1149–1166.
- Letuve S, Kozhich A, Arouche N, Grandsaigne M, Reed J, Dombret MC, Kiener PA, et al. Ykl-40 is elevated in patients with chronic obstructive pulmonary disease and activates alveolar macrophages. *J Immunol* 2008;181:5167–5173.
- Sohn MH, Kang MJ, Matsuura H, Bhandari V, Chen NY, Lee CG, Elias JA. The chitinase-like proteins breast regression protein-39 and ykl-40 regulate hyperoxia-induced acute lung injury. *Am J Respir Crit Care Med* 2010;182:918–928.
- Matsuura H, Hartl D, Kang MJ, Dela Cruz CS, Koller B, Chupp GL, Homer RJ, et al. Role of breast regression protein-39 in the pathogenesis of cigarette smoke-induced inflammation and emphysema. *Am J Respir Cell Mol Biol* 2011;44:777–786.
- Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, Harris JM, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088–1098.
- Tsicopoulos A, Chang Y, Ait Yahia S, de Nadai P, Chenivesse C. Role of ccl18 in asthma and lung immunity. *Clin Exp Allergy* 2013;43:716–722.
- Sin DD, Miller BE, Duvoix A, Man SF, Zhang X, Silverman EK, Connett JE, et al. Serum parc/ccl-18 concentrations and health outcomes in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011;183:1187–1192.
- Prasse A, Probst C, Bargagli E, Zissel G, Toews GB, Flaherty KR, Olschewski M, et al. Serum cc-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:717–723.
- Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the american thoracic society was adopted by the ats board of directors, july 1999. *Am J Respir Crit Care Med* 2000;161:309–329.
- Vestbo J, Edwards LD, Scanlon PD, Yates JC, Agusti A, Bakke P, Calverley PM, et al. Changes in forced expiratory volume in 1 second over time in copd. *N Engl J Med* 2011;365:1184–1192.
- Standardization of spirometry, 1994 update. American thoracic society. *Am J Respir Crit Care Med* 1995;152:1107–1136.
- Lachin JM. Introduction to sample size determination and power analysis for clinical trials. *Control Clin Trials* 1981;2:93–113.
- Konradsen JR, James A, Nordlund B, Reinius LE, Soderhall C, Melen E, Wheelock AM, et al. The chitinase-like protein ykl-40: A possible biomarker of inflammation and airway remodeling in severe pediatric asthma. *J Allergy Clin Immunol* 2013;132:328–335, e325.
- Rufo J, Taborda-Barata L, Lourenco O. Serum biomarkers in elderly asthma. *J Asthma* 2013;50:1011–1019.
- Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, Muller SJ, Fahy JV. Roles of epithelial cell-derived periostin in tgf-beta activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci USA* 2010;107:14170–14175.
- Kim HB, Kim CK, Iijima K, Kobayashi T, Kita H. Protein microarray analysis in patients with asthma: elevation of the chemokine parc/ccl18 in sputum. *Chest* 2009;135:295–302.
- Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. *Semin Nephrol* 2007;27:98–114.
- Imaoka H, Hoshino T, Takei S, Kinoshita T, Okamoto M, Kawayama T, Kato S, et al. Interleukin-18 production and pulmonary function in copd. *Eur Respir J* 2008;31:287–297.
- Kohno K, Kataoka J, Ohtsuki T, Suemoto Y, Okamoto I, Usui M, Ikeda M, Kurimoto M. Ifn-gamma-inducing factor (igif) is a costimulatory factor on the activation of th1 but not th2 cells and exerts its effect independently of il-12. *J Immunol* 1997;158:1541–1550.
- Kurt E, Aktas A, Aksu K, Keren M, Dokumacioglu A, Goss CH, Alatas O. Autologous serum skin test response in chronic spontaneous urticaria and respiratory diseases and its relationship with serum interleukin-18 level. *Arch Dermatol Res* 2011;303:643–649.
- Tanaka H, Miyazaki N, Oashi K, Teramoto S, Shiratori M, Hashimoto M, Ohmichi M, Abe S. Il-18 might reflect disease activity in mild and moderate asthma exacerbation. *J Allergy Clin Immunol* 2001;107:331–336.
- Verhaeghe B, Gevaert P, Holtappels G, Lukat KF, Lange B, Van Cauwenberge P, Bachert C. Up-regulation of il-18 in allergic rhinitis. *Allergy* 2002;57:825–830.
- Cohen J. Statistical power analysis for the behavioral sciences, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
- Tang H, Fang Z, Sun Y, Li B, Shi Z, Chen J, Zhang T, Xiu Q. Ykl-40 in asthmatic patients, and its correlations with exacerbation, eosinophils and immunoglobulin e. *Eur Respir J* 2010;35:757–760.
- Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, Shikotra A, et al. Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma Study G. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012;130:647–654, e610.
- Ando M, Shima M. Serum interleukins 12 and 18 and immunoglobulin e concentrations and allergic symptoms in japanese schoolchildren. *J Investig Allergol Clin Immunol* 2007;17:14–19.