Clara Cell 10-kDa Protein is a Sensitive Biomarker to Identify Th2 Subtype Inflammatory Phenotypes of Asthma

Meijia Wang

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
<https://orcid.org/0000-0002-3596-1229>

Kun Tang

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Pengfei Gao

Department of Respiratory and Critical Care Medicine, the First Affiliated Hospital of Henan University of Science and Technology, Luoyang

Yanjiao Lu

Department of Respiratory and Critical Care Medicine, National Clinical Research Center of Respiratory Disease, Key Laboratory of Pulmonary Disease of Health Ministry, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology

Shanshan Wang

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Jianping Zhao

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Jungang Xie (✉ xiejgg@hotmail.com)

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
<https://orcid.org/0000-0001-9037-3027>

Research

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Abstract

Background: Clara cell 10-kDa protein (CC10) is one of the most abundant proteins in bronchoalveolar lavage fluids and has been described as a biomarker for airway obstructive diseases. CC10 possesses the properties of suppression Th2 cell differentiation and Th2 cytokine production. In this study, we aimed to determine whether CC10 can be a sensitive biomarker to identify Th2 phenotypes of asthma.

Methods: Adults with asthma (n = 50) were categorized as Th2-high asthma or Th2-low asthma according to serum IgE, blood eosinophils and fractional exhaled nitric oxide (FeNO). Patients were classified as Th2-high asthma when two or more biomarkers (high IgE ≥ 100 IU/mL, high Eos $\geq 300/\mu\text{L}$, or high FeNO ≥ 30 ppb) were elevated, and classified as Th2-low asthma when one or no biomarker was elevated. Enzyme-linked immunosorbent assay (ELISA) was used to assess the CC10 and periostin levels in plasma. All participants underwent sputum induction, and different types of inflammatory cells were counted.

Results: The plasma CC10 levels from patients with Th2-low asthma were higher than patients with Th2-high asthma ($P < 0.001$). The receiver-operating characteristic (ROC) analysis showed a sensitivity of 0.73 and specificity of 0.74 for plasma CC10 of 19.76 ng/ml to distinguish asthmatic patients with Th2-high phenotype or Th2-low phenotype. Correlation analysis indicated that the plasma CC10 levels were inversely correlated with plasma periostin, sputum eosinophil and negative logPD20 ($p < 0.05$), however positively correlated with sputum neutrophil percentages and FEV1 % predictions ($p < 0.05$).

Conclusions: The plasma CC10 was potentially useful in predicting Th2-high and Th2-low phenotypes in patients with asthma. Lower plasma CC10 was associated with enhanced airway hyperresponsiveness, Th2-high inflammation and subsequent airflow limitation.

Introduction

Bronchial asthma is commonly occurred in population, however the pathophysiological mechanisms and phenotypes are highly diverse. Despite the significant effort that has been directed at defining the various phenotypes of asthma, the biomarkers of these different phenotypes were not clear^[1]. Phenotype-specific therapies for targeted and personalized approaches according to defined phenotypes had been suggested to improve the disease outcomes in asthmatic patients^[2]. Therefore, the discovering of accurate biomarkers for different phenotypes of asthma was of critical importance.

Asthma historically was characterized as Th2-mediated inflammation disease, however researches on the pathogenesis of asthma had demonstrated that only a subgroup of asthmatic patients exhibited increased Th2 inflammation in the airway^[3]. Th2-high asthma demonstrated enhanced bronchial hyperresponsiveness, higher serum IgE, increased blood and airway eosinophilia, and reduced lung function and frequent exacerbations. Patients with Th2-high asthma were sensitive to the treatments of corticosteroid therapy and Th2-biologic agent^[3,4]. This “Th2 signature” was identified by genome-wide

transcriptional analysis of bronchial epithelial brushings, in which three genes: periostin, chloride channel accessory 1 (CLCA1) and Serpin β 2 (SerpB2), were found specifically induced by interleukin (IL)-4 or IL-13 in vitro^[5]. However, bronchial epithelial brushing from patients with asthma was considered to be an invasive, costly, complex to perform, therefore had not been widely used for clinics and researches^[6]. Many other noninvasive markers such as serum IgE, blood and sputum eosinophil count, FeNO and serum periostin were stable indicators of the inflammatory states of asthmatics and surrogate biomarkers of Th2-driven asthma^[7, 8]. However, their diagnostic accuracies in detecting Th2-high asthma remains controversial. The predicting results were potentially influenced by factors other than asthma, therefore had not been widely recognized^[3]. For this reason, it is necessary to explore alternative and accurate biomarkers for distinguishing Th2-high and Th2-low asthma.

Club cell 10-kDa protein (CC10), belongs to the secretoglobin family, is primarily produced by nonciliated club cells in the distal airway and nasal epithelial cells, and can be detected in the circulation^[9, 10]. It was reported that CC10 can inhibit inflammatory through the suppression of phospholipase A2 activity, downregulation of Th2 cell differentiation, and inhibition of inflammatory cytokine production^[11]. Previous studies suggested that adeno-associated virus (AAV)2/9-CC10 vector virus significantly reduced airway hyperresponsiveness, Th2 cytokines and eosinophilia in the lungs of OVA-sensitized mice^[12]. And serum CC10 levels in asthmatic patients had been shown significantly lower than in control subjects^[13]. However, whether the expression of CC10 differ in Th2-high asthma versus Th2-low asthma were still unknown. Therefore, in this study, we aimed to examine the ability of serum CC10 to predict asthma inflammatory subtypes.

Materials And Methods

Subjects

Fifty asthmatic patients aged 18–65 years were recruited at Tongji Hospital. The diagnosis of asthma was performed based on the Global Initiative for Asthma (GINA) guidelines. All participants were diagnosed with symptomatic asthma and demonstrated symptoms of airways hyperresponsiveness (provacative dose of methacholine causing a 20% drop in FEV1 < 2.5 mg, PD20 FEV1 < 2.5 mg) and/or bronchodilator responsiveness (> 12% improvement in FEV1% predicted following inhalation of 200 μ g salbutamol). No participant were current smokers or had a history over 10 pack-years. Participants were excluded when oral corticosteroids were performed or a respiratory tract infection was diagnosed in the previous 4 weeks. Blood and sputum samples were collected from all participates at their visits for this study. Written informed consents were performed for all participates. This study was approved by the ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Sputum Induction and Analysis

Sputum induction with hypertonic saline (4.5%) was performed as described previously^[14]. Sputum portions were isolated and dispersed using dithiothreitol. Total inflammatory cell counts and viability tests were performed. Aliquots of sputum samples were prepared by Cytospin and stained with May-Grunwald Giemsa, subsequently, differential cell counts for positive stained cells were performed for every 400 nonsquamous cells. Results for different sputum cell type proportions were displayed as percentage over total nonsquamous cell count.

Enzyme-linked immunosorbent assay (ELISA)

Plasma CC10 (R&D Systems, Minneapolis, MN, USA) and periostin (Eton Bioscience, San Diego, CA, USA) were measured with a sandwich ELISA according to the manufacturer's protocols.

Statistical analysis

All data were displayed as means $\pm$ SD. All statistical analyses were carried out by GraphPad Prism 5 software (GraphPad, San Diego, CA, USA). Unpaired t-tests were performed for normal distributed data, while nonparametric tests were applied for nonnormal distributed data. Correlation analysis were performed by Spearman Rank Order Correlation. Receiver-operating characteristic (ROC) curves were generated for the determination of the cut-off value for the ability of plasma CC10 levels to differentiate between Th2-high and Th2-low phenotype asthma according to curve points closest to (0, 1). Sensitivities and specificities were calculated according to the identified optimal cut-points. P value < 0.05 were considered as statistically significant.

Results

Subjects characteristics

Clinical characteristics of all participants were summarized as in Table 1. In this study patients were defined as Th2-high asthma or Th2-low asthma according to the levels of serum IgE, blood eosinophils, and FeNO. Participants were classified as Th2-high asthma as follows elevation in two or more biomarkers: high IgE $\geq$ 100 IU/mL, high blood eosinophils $\geq$ 300/ μ L, high FeNO $\geq$ 30 ppb, and Th2-low asthma as elevation in one or no biomarkers. As the results, no significant difference in demographics, duration of asthma and mean FEV1 at baseline between Th2-high and Th2-low asthma were demonstrated. Meanwhile, significantly higher sputum eosinophil percentages were found in Th2-high asthmatic patients than Th2-low asthmatic patients. However, no significant difference was observed in sputum neutrophil percentages between the two groups.

Table 1
Patient characteristics

	Th2-high asthma (n = 24)	Th2-low asthma (n = 26)
Age(y)	39.04 ± 9.37	44.92 ± 12.18
Sex (male/female)	14/10	10/16
Duration of asthma (y)	6.33 ± 1.59	5.73 ± 1.29
FEV1 (% predicted)	80.18 ± 20.28	85.63 ± 23.03
FEV1/FVC (%)	64.51 ± 12.54	67.36 ± 14.05
Sputum eutrophil,%	47.42 ± 5.71	57.56 ± 5.17
Sputum eosinophil,%	24.31 ± 4.62	9.88 ± 1.81**
Values are presented as mean ± SD. FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity; **P < 0.001 compared with Th2-high asthma		

Distinct CC10 expression in Th2-high and Th2-low phenotype asthma

Plasma CC10 concentrations were measured by ELISA. Plasma CC10 concentrations were higher in Th2-low asthmatic patients compared with Th2-high asthmatic patients (27.07 ± 2.05 ng/ml versus 17.87 ± 1.68 ng/ml, respectively, p < 0.01).

ROC curves for the sensitivity and specificity evaluations of plasma CC10 to detect airway Th2 inflammation

To estimate the diagnostic value of the plasma CC10 for discriminating between Th2-high asthmatic patients and Th2-low asthmatic patients, ROC curves analysis was performed. As shown in the Fig. 4, the area under the curve (AUC) of CC10 was 0.78. When the cutoff for plasma CC10 was selected as 19.76 ng/ml, the sensitivity and specificity for differentiating Th2-high asthmatic patients from asthma patients were 73% and 74%, respectively.

Signal correlation analyses between plasma CC10 with plasma periostin and sputum differential inflammatory cell counts in asthmatic patients

Periostin is a systemic biomarker of airway eosinophilia in asthmatic patients, by showing a significant correlation with sputum eosinophils^[15]. To investigate the association between plasma CC10 levels and eosinophilic airway inflammation, we analysed the correlation between plasma CC10 levels, plasma periostin and sputum differential inflammatory cell counts in asthma. We found that the plasma CC10 levels were negatively correlated with plasma periostin levels (r = -0.4008, p = 0.0085) and sputum eosinophil percentages (r = -0.3578, p = 0.0343), and positively correlated with sputum neutrophil

percentages ($r = 0.3136$, $p = 0.0431$). However, no association between plasma CC10 levels and sputum macrophage percentages was found.

Signal correlation analyses between plasma CC10 with pulmonary lung function in asthmatic patients

We further analyzed the association between plasma CC10 levels and pulmonary function and found that plasma CC10 levels were positively correlated with FEV1% pred ($r = 0.343$, $p = 0.02$), and negatively correlated with negative log PD20 ($r = 0.349$, $p = 0.04$), however not correlated with FVC% pred or FEV1/FVC ratio in asthmatic patients.

Discussion

The present study demonstrates that plasma CC10 levels in Th2-high asthma were significantly lower than that in Th2-low asthma. To evaluate the potential diagnostic usage of plasma CC10 for prediction asthma phenotypes, a ROC curve analysis was performed. When the cut off value for plasma CC10 to distinguish asthmatic patients to Th2-high or Th2-low asthma was 19.76 ng/mL, the sensitivity and specificity were 0.73 and 0.74, respectively. The single analysis revealed that CC10 was negatively related with airway eosinophilic inflammation and airway hyperresponsiveness, and positively correlated with neutrophilic inflammation and airway obstruction. Collectively, plasma CC10 level was demonstrated as a potential clinical diagnostic biomarker for the distinguish of Th2-high and Th2-low asthma.

The aim of personalized medicine in asthma was that individualized treatment based on noninvasive biomarkers predicted clinical course and therapeutic efficacy ^[16]. It was not a research strategy, but rather an up-to-date clinical practice that clinicians classify patients into more homogeneous groups with similar prognosis and/or treatment needs ^[17]. Although airway sampling from bronchoscopy and induced sputum enabled the direct characterizations of airway inflammation, these modalities were invasive to patients, time consuming, labor intensive, technically variable across facilities and difficult to implement widely in primary care settings. Also, lack of consensus on technical procedures, quality assurance and diagnostic markers in airway cytology limited the use of such technique ^[8]. Thus, it was potentially beneficial to develop accessible methods for the assessments of airway inflammation.

CC10 has been used as a potential surrogate biomarker due to the reasons such as accessible to assess, reproducible, unaffected by steroids and associated with disease pathophysiology ^[18]. Studies demonstrated that the concentration of CC10 in circulation was a sensitive biomarker of epithelium damage ^[10], while decreased CC10 had been consistently observed in chronic airway diseases such as chronic obstructive pulmonary disease (COPD) and asthma ^[19, 20]. In this study, we demonstrated that plasma CC10 was significantly decreased in Th2-high asthma. Also, single analysis revealed that plasma CC10 was negatively correlated with sputum eosinophil and negatively with sputum neutrophils, supporting our findings that the level of CC10 is decreased in eosinophil-dominated inflammations (Th2-high asthma) and increased in neutrophil-associated inflammations (Th2-low asthma). Previous studies suggested that CC10 inhibited Th2 cell differentiation, Th2 cytokine generation and pulmonary

eosinophilia [21,22]. Thus, eosinophilia in patients with Th2-high asthma was ascribed to deficits of circulating CC10. Alternatively, the plasma CC10 levels was negatively correlated with serum periostin, which was considered to be a sensitive biomarker to reflect airway eosinophilia among several biomarkers, including blood eosinophils and exhaled nitric oxide [23]. Taken together, a negative correlation was found between airway eosinophilic inflammation and plasma CC10 levels, providing evidence that CC10 deficits was a reflection of Th2-driven inflammation in the airway. Other studies found that CC10 expression in lung tissue was regulated by inflammatory cytokines. IL-4 and IL-13 inhibited CC10 production [24], whereas interferon (INF) - γ and IL-10 could promote CC10 production [25, 26]. The counter effect of Th1 and Th2 cytokines on CC10 production may ensure the stability of discrepant expression in different asthma phenotypes. Thus, the plasma CC10 is an important index for the classification of inflammation in asthmatic patients.

In addition to airway inflammation, plasma CC10 levels might be a surrogate predictor of airway obstruction and bronchial hyperresponsiveness. Previous study demonstrated that serum CC10 levels were positively correlated with lung function in patients with asthma and COPD [27,28]. In our study, we observed that decreased plasma CC10 levels were associated with airflow limitation and damaged lung functions among asthmatic patients. Concurrently, we found a negatively correlation between plasma CC10 levels and bronchial hyperresponsiveness in asthmatic patients, which was in line with a recently report showing that low serum CC16 enhanced airway hyperresponsiveness to methacholine challenge in adults [29]. This scenario demonstrated that CC10 alleviated airway obstruction and hyperresponsiveness as a result of inhibition of Th-2 inflammation in asthma.

Several clinical studies demonstrated that low circulating CC10 levels were a risk factor for accelerated lung function decline, and associated with the progressions of COPD [30,31]. However, it was still unclear that if the levels of circulation CC10 was correlated with the progression of asthma. Also, the recombinant CC10 protein was verified to be valuable in the treatments of COPD mice through the inhibition of the pro-inflammatory factor productions [32]. Meanwhile, no study was performed to evaluated whether recombinant CC10 was valuable in the treatment of asthma patients, especially in Th2-high asthma. Therefore, further studies were necessary for the investigation of their efficacy in monitoring disease progressions and treatments in asthma.

Conclusion

In summary, plasma CC10 levels differed significantly between Th2-high asthma and Th2-low asthma. In addition, low CC10 levels were potentially used as biomarkers for Th2-high inflammation, subsequent airflow limitation and airway hyperresponsiveness enhancement in asthmatic patients.

Abbreviations

CC10: Clara cell 10-kDa protein; FeNO: fractional exhaled nitric oxide; ELISA: enzyme-linked immunosorbent assay; ROC: receiver-operating characteristic; CLCA1: chloride channel accessory 1; SerpinB2: Serpin β 2; AAV 2/9: adeno-associated virus 2/9; GINA: Global Initiative for Asthma guidelines; FEV1: forced expiratory volume in one second; PD20: provocative dose of methacholine causing a 20% drop in FEV1; SD: standard deviation; AUC: area under the curve; FVC: forced vital capacity; COPD: chronic obstructive pulmonary disease

Declarations

Ethics approval and consent to participate

The ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology approved this study. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee

Consent for publication

Not applicable

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

WM recruited patients, collected, analyzed, and interpreted the data, and wrote the draft. TK, GP, LY, WC recruited patients, collected data. ZJ and XJ conceived and designed the study, recruited patients, and provided overall supervision and critically revised the manuscript. All authors read and approved the final manuscript.

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Figures

Fig.1

Figure 1

Comparison of plasma CC10 in Th2-high asthma and Th2-low asthma. Data were expressed as means $\pm$ SD. **p < 0.01.

Fig.1

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Comparison of plasma CC10 in Th2-high asthma and Th2-low asthma. Data were expressed as means $\pm$ SD. **p < 0.01.

Fig.2

Figure 2

Receiver operating characteristics (ROC) curves of plasma CC10 to assess its potential usage in differentiating Th2-low asthma and Th2-high asthma. The cutoff value on the ROC curves closest to (0, 1) was 19.76 ng/ml.

Fig.2

Figure 2

Receiver operating characteristics (ROC) curves of plasma CC10 to assess its potential usage in differentiating Th2-low asthma and Th2-high asthma. The cutoff value on the ROC curves closest to (0, 1) was 19.76 ng/ml.

Fig.3

Figure 3

Single correlations between plasma CC10 with plasma periostin and sputum cell differentiations in asthmatic patients. (a) Correlation between plasma CC10 and plasma periostin. (b) Correlation between plasma CC10 and sputum eosinophils ratio (c) Correlation between plasma CC10 and sputum neutrophils ratio. (d) Correlation between plasma CC10 and sputum macrophages ratio.

Fig.3

Figure 3

Single correlations between plasma CC10 with plasma periostin and sputum cell differentiations in asthmatic patients. (a) Correlation between plasma CC10 and plasma periostin. (b) Correlation between plasma CC10 and sputum eosinophils ratio (c) Correlation between plasma CC10 and sputum neutrophils ratio. (d) Correlation between plasma CC10 and sputum macrophages ratio.

Fig.4

Figure 4

Single correlations between plasma CC10 and respiratory functions in asthmatic patients. (a) Correlation between plasma CC10 and forced volume in one second (FEV1.0), %pred. (b) Correlation between plasma CC10 and FVC, %pred. (c) Correlation between plasma CC10 and FEV1/FVC. (d) Correlation between plasma CC10 and negative log PD20.

Fig.4

Figure 4

Single correlations between plasma CC10 and respiratory functions in asthmatic patients. (a) Correlation between plasma CC10 and forced volume in one second (FEV1.0), %pred. (b) Correlation between plasma CC10 and FVC, %pred. (c) Correlation between plasma CC10 and FEV1/FVC. (d) Correlation between plasma CC10 and negative log PD20.