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Hemopexin: A Novel Anti-inflammatory Marker for Distinguishing COPD From Asthma

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ABSTRACT

Purpose: Systemic inflammatory biomarkers can improve diagnosis and assessment of chronic obstructive pulmonary disease (COPD) and asthma. We aimed to validate an airway disease biomarker panel of 4 systemic inflammatory biomarkers, α2-macroglobulin, ceruloplasmin, haptoglobin and hemopexin, to establish their relationship to airway disease diagnosis and inflammatory phenotypes and to identify an optimized biomarker panel for disease differentiation.

Methods: Participants with COPD or asthma were classified by inflammatory phenotypes. Immunoassay methods were used to measure levels of validation biomarkers in the sera of participants with disease and non-respiratory disease controls. Markers were analyzed individually and in combination for disease differentiation and compared to established biomarkers (C-reactive protein, interleukin-6, and white blood cell/blood eosinophil count).

Results: The study population comprised of 141 COPD, 127 severe asthma, 54 mild-moderate asthma and 71 control participants. Significant differences in ceruloplasmin, haptoglobin and hemopexin levels between disease groups and between systemic inflammatory phenotypes were observed. However, no differences were found between airway inflammatory phenotypes. Hemopexin was the best performing individual biomarker and could diagnose COPD versus control participants (area under the curve [AUC], 98.3%; 95% confidence interval [CI], 96.7%–99.9%) and differentiate COPD from asthmatic participants (AUC, 97.0%; 95% CI, 95.4%–98.6%), outperforming established biomarkers. A biomarker panel, including hemopexin, haptoglobin and other established biomarkers, could diagnose asthma versus control participants (AUC, 87.5%; 95% CI, 82.8%–92.2%).

Conclusions: Hemopexin can be a novel biomarker with superior diagnostic ability in differentiating COPD and asthma. We propose an anti-inflammatory axis between the airways and systemic circulation, in which hemopexin is a protective component in airway disease.

Keywords: Asthma; COPD; biomarker; anti-inflammation; hemopexin; haptoglobin; diagnosis; airway inflammation

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INTRODUCTION

Inflammation is a significant feature of chronic airway disease pathogenesis, playing a key role in asthma and chronic obstructive pulmonary disease (COPD). These common diseases cause a major burden worldwide and require significant advances in assessment and treatment.[1-3] Inflammation in these diseases is complex, with individuals presenting with heterogeneous patterns of airway and systemic inflammation,[4-6] impacting clinical outcomes and treatment responses.[7,8] Improving the assessment of inflammatory phenotypes and diagnosis in asthma and COPD, resulting in precise treatment, is a priority. Advancing chronic airway disease to a precision-medicine and treatable traits approach requires identification and validation of novel biomarkers.

Novel biomarkers are valuable in pathophysiological research, discovering mechanisms underlying phenotypes, known as endotypes.[9] Asthma and COPD pathology involves an underlying complex network of pro- and anti-inflammatory components that drive and resolve inflammation. However, an innate inflammatory-axis that links the airways and systemic circulation is not yet elucidated, with both supporting and disputing evidence.[10]

In a previous proteomic discovery study, we identified a biomarker panel of 4 circulating acute-phase proteins measurable in serum, which differentiate between asthma, COPD and controls.[11] The biomarker proteins, $\alpha 2$-macroglobulin (A2M), ceruloplasmin (Cp), haptoglobin (Hp) and hemopexin (Hpx), were significantly elevated in the platelet-depleted plasma of asthma patients and had trending elevation in COPD patients, compared to controls. However, these biomarkers have not yet been validated in a larger population of individuals with COPD and severe asthma. These biomarkers may also provide a better understanding of an airway-systemic inflammatory-axis in airway disease.

We aimed to validate the panel of 4 protein markers in a population of COPD, mild, moderate and severe asthma as well as non-respiratory control participants. We also established the relationship between the panel of protein biomarkers with airway and systemic inflammatory phenotypes. We hypothesized that the panel of biomarkers relates to airway and systemic inflammatory phenotypes and has the ability to distinguish between asthma, COPD and non-respiratory control populations.

MATERIALS AND METHODS

Study populations

All participants with respiratory disease were recruited between July 2012 and February 2017 via the respiratory ambulatory care clinics and research database of the Department of Respiratory Medicine at John Hunter Hospital (NSW, Australia), or by media advertisement. All control participants were recruited by media advertisement or through the research register of the Hunter Medical Research Institute. The studies were approved by the Hunter New England Health Human Research Ethics Committee (mild-moderate asthma, (12/12/11/3.06), severe asthma (08/08/20/3.10), COPD (12/12/12/3.06) and control (8/08/20/3.10) populations). All participants gave written informed consent.

Participants

This validation study involved 4 groups (n = 393): mild-moderate asthma, severe asthma, COPD and non-respiratory controls. All participants were aged ≥ 18 years. Asthma and COPD participants with a confirmed doctor diagnosis of stable asthma and COPD were recruited (**Supplementary Table S1**). Mild-moderate asthmatic participants had to have variable airflow limitation meeting at least one of the following conditions: 1) airway hyper-responsiveness (AHR): 15% fall in forced expiratory volume in one second (PD15) < 15 mL to hypertonic saline **OR** AHR to another indirect or direct standard challenge agent; 2) bronchodilator response (BDR): Change post-bronchodilator forced expiratory volume in one second (FEV1) > 12% **OR** 200 mL; 3) peak flow variability > 12% over at least 1 week of monitoring; and 4) FEV1 variability > 12% (2 values measured within 2 months of each other).

Severe asthmatic participants had to demonstrate a history of, or current variable airflow limitation—meeting each of the following conditions: 1) Previous evidence of BDR ≥ 12% or AHR or peak flow diary (diurnal variation ≥ 15% or > 50 mL); 2) being on maximal inhaled corticosteroid (ICS)/long-acting β2-agonist (LABA) therapy (Global Initiative for Asthma [GINA] step 4 treatment—defined as 1,000 mcg ICS as well as LABA)[12] **OR** maintenance prednisone; 3) post-bronchodilator FEV1% predicted values < 80% and forced expiratory ratio [FER] < 0.70) **OR** asthma control questionnaire (ACQ) score ≥ 1.5 **OR** experiencing a severe exacerbation in the previous 12 months requiring OCS use.

COPD participants had to have incompletely reversible airflow limitation—meeting each of the following conditions: a) post-BD FEV1% predicted < 80% **AND** b) FER < 0.70 **OR** physician diagnosis and objective confirmation from chest computed tomography or pulmonary function test.

Stable disease was defined as an absence of recent respiratory infection, acute exacerbation or change in maintenance therapy within 4 weeks prior to recruitment.

Participants were excluded if they were pregnant, breast-feeding or had a primary diagnosis of another significant respiratory disease (*i.e.* bronchiectasis, pulmonary fibrosis, active tuberculosis or lung cancer). COPD participants were excluded if current smoking was reported, although current smoking was not an exclusion criterion for severe asthmatic participants. Control participants were without a diagnosed respiratory disease (*e.g.* asthma, COPD or bronchiectasis). Control participants were excluded if currently smoking, had a respiratory disease (*e.g.* asthma, COPD or bronchiectasis) diagnosis or presented with airway hyperresponsiveness. Control participants were not excluded based on the presence of non-respiratory comorbidities.

Clinical procedures

Participants underwent a multidimensional assessment[13] that involved blood collection to assess systemic inflammatory markers and induced sputum to assess airway inflammation: 1) spirometry (FEV1% predicted, forced vital capacity (FVC)% predicted, and FEV1/FVC%); 2) assessments of exacerbation severity (emergency presentations, hospital admissions, unscheduled doctor visits and oral corticosteroids (OCS) courses in the past 12 months); 3) exercise tolerance (6 minute walk distance [6MWD]); 4) health status (St. George Respiratory Questionnaire [SGRQ] and Asthma Quality of Life Questionnaire [AQLQ]); 5) asthma control (ACQ); airway T2 inflammation (fractional exhaled nitric oxide [FeNO]); 6) dyspnoea (modified Medical Research Council [mMRC] dyspnoea score); 7) atopic status (skin prick test to common

aeroallergens); and 8) anxiety and depression symptoms (Hospital Anxiety and Depression Scale [HADS]) and a comorbidity measure (Charleston Comorbidity Index [CCI]).

Laboratory procedures

Induced sputum was processed as described previously[14] (see **Supplementary Data 1**). Serum biomarkers (interleukin [IL]-6, A2M, Cp, Hp, and Hpx) were measured in duplicate by immunoassay via commercially available enzyme-linked immunosorbent assay (ELISA) kits as per manufacturers' instructions (see **Supplementary Data 1**). The high-sensitivity C-reactive protein was measured by NSW Health Pathology. Due to batch effects yielding inconsistent results when using the Human α-2 macroglobulin ELISA kit, this serum biomarker was excluded from further analysis.

Inflammatory phenotyping

Airway inflammatory phenotypes were determined based on the presence or absence of elevated sputum eosinophil and/or neutrophil proportion: 1) eosinophilic airway inflammation: < 61% neutrophils, ≥ 3% eosinophils; 2) neutrophilic airway inflammation: ≥ 61% neutrophils, < 3% eosinophils; 3) mixed granulocytic inflammation: ≥ 61% neutrophils, ≥ 3% eosinophils and 4) pauci-granulocytic inflammation: < 61% neutrophils, < 3% eosinophils.[4] A systemic inflammatory phenotype was defined as having 2 or more of the following markers increased[6]: C-reactive protein (CRP) > 3 mg/L, white blood cell (WBC) count > 9×10^9/L[15] and IL-6 > 1.55 pg/mL.[16]

Statistical analyses

STATA v.15 software was used to conduct statistical analyses. Parametric results are presented as mean (standard deviation) and non-parametric as median (Q_1, Q_3). Student's *t* test and the 2-sample Wilcoxon rank sum test were used for parametric and non-parametric data, respectively. Analysis of variance or the Kruskal–Wallis test was used for > 2 groups with Bonferroni *post hoc* correction. Categorical data were analyzed using Fisher's exact test, with Fisher's *P* value reported when expected counts were < 5. Correlation analysis performed with Spearman's correlation coefficients. Modelling of biomarkers was completed using multiple logistic regression (adjusted for sex, age and body mass index [BMI]), where each individual was assigned a predictive value for individual and combined biomarkers. Biomarker-predicted values were used to generate receiver operator characteristic (ROC) curves and optimal cutoff values determined by Youden Index analysis. A *P* value of <0.05 was considered statistically significant.

RESULTS

Participant characteristics

The clinical characteristics of the participants are presented in **Table 1**. Disease groups had a higher BMI, airflow limitation, increased FeNO levels, dyspnoea scores, HADS anxiety and depression scores, comorbidity status and reduced exercise tolerance compared to controls (**Table 1**). The COPD population was significantly older, with a mean age of 70.5 years (range, 43.9–88.6) and had a higher proportion of ex-smokers (95.0%) compared to mild-moderate and severe asthmatic populations. COPD participants reported worse quality of life, with a higher mean SGRQ total score (55.5 ± 17.0) compared to severe asthma (47.2 ± 19.0) (*P* = 0.0002). AQLQ and ACQ scores indicated poorer quality of life and poorer asthma control (respectively)

in severe asthma versus mild-moderate asthma participants (**Table 1**). COPD participants had the most severe airflow limitation. Both COPD and severe asthma populations reported increased exacerbations over the past 12 months compared to mild-moderate asthma. COPD participants walked significantly less meters (368.0 ± 117.8) than severe asthma (454.5 ± 105.7) ($P \leq 0.0001$), and reported higher levels of depressive symptoms and CCI.

Table 1. Clinical characteristics of the study populations by diagnosis

Characteristic	Controls	Mild-Moderate asthma	Severe asthma	COPD	P value
Sample number	71	54	127	141	
Demographics					
Female sex	37 (52.1)	30 (55.6)	82 (64.6)§	61 (43.3)	**0.006**
Age (yr)	56.3 (19.1–82.5)	61.7 (24.3–80.2)	59.1 (18.6–82.3)	70.5 (43.9–88.6)*†‡	**0.0001**
BMI (kg/m²) (n = 392)	25.3 (23.2, 27.7) (n = 71)	32.1 (25.4, 35.5)* (n = 54)	30.5 (25.9, 36.8)* (n = 126)	29.0 (24.6, 32.1)* (n = 141)	**0.0001**
Current smoker (n = 180)	0	2 (3.7) (n = 54)	9 (7.1) (n = 126)	0	-
Ex-smoker (n = 391)	23 (32.4) (n = 71)	20 (37.0) (n = 54)	59 (46.8) (n = 126)	133 (95.0)*†‡ (n = 140)	**< 0.0001**
Pack years (n = 234)	3.0 (0.6, 15.0) (n = 23)	10.0 (3.9, 20.0) (n = 19)	9.0 (2.0, 18.0) (n = 59)	45.5 (32.5, 68.8)*†‡ (n = 133)	**0.0001**
ICS use (n = 321)	0	51 (94.4) (n = 54)	126 (100.0)§ (n = 126)	129 (91.5) (n = 141)	**0.001**
ICS dose (Beclomethasone equiv. µg/day) (n = 302)	-	1,000 (1,000, 2,000) (n = 51)	2,000 (2,000, 2,000)§† (n = 125)	2,000 (1,000, 2,000) (n = 126)	**0.0001**
OCS use (n = 321)	0	1 (1.9)‖ (n = 54)	35 (27.8)§† (n = 126)	1 (0.7) (n = 141)	**< 0.0001**
OCS dose (mg/day) (n = 302)	-	1 (1, 1) (n = 1)	10 (5, 25) (n = 35)	5 (5, 5) (n = 1)	0.17
Macrolide use (n = 320)	-	6 (11.1)§ (n = 54)	10 (8.0)§ (n = 125)	0 (0) (n = 141)	**< 0.0001**
LABA use (n = 321)	-	49 (90.7) (n = 54)	126 (100.0)†§ (n = 126)	123 (87.2) (n = 141)	**< 0.0001**
Monoclonal anti-IgE use (n = 320)	-	2 (3.7) (n = 54)	0 (0) (n = 125)	0 (0) (n = 141)	**0.03**
Monoclonal anti-IL-5 use (n = 320)	-	3 (5.6) (n = 54)	4 (3.2) (n = 125)	0 (0) (n = 141)	**0.01**
SGRQ total (n = 264)	-	-	47.2 ± 19.0 (n = 124)	55.5 ± 17.0‡ (n = 140)	**0.0002**
AQLQ score (n = 178)	-	6.2 (5.7, 6.5) (n = 53)	4.9 (4.1, 5.8) (n = 125)	-	**< 0.0001**
ACQ score (n = 180)	-	0.8 (0.5, 1.7) (n = 54)	2.0 (1.3, 2.8) (n = 126)	-	**< 0.0001**
Pulmonary					
Post β₂ FEV1 % predicted (n = 389)	101.3 ± 14.4 (n = 69)	83.7 ± 18.3* (n = 54)	75.1 ± 21.3*† (n = 125)	51.6 ± 17.7*†‡ (n = 141)	**< 0.0001**
Post β₂ FVC % predicted (n = 387)	97.1 ± 13.4 (n = 69)	89.9 ± 15.2 (n = 53)	85.7 ± 16.1* (n = 125)	76.7 ± 16.8*†‡ (n = 140)	**< 0.0001**
Post β₂ FEV1/FVC% (n = 387)	81.9 ± 6.1 (n = 69)	72.2 ± 10.3* (n = 53)	67.6 ± 13.5* (n = 125)	50.3 ± 15.4*†‡ (n = 140)	**< 0.0001**
FeNO (ppb) (n = 210)	12.6 (6.7, 18.9) (n = 59)	17.8 (8.4, 30.4) (n = 40)	16.0 (7.0, 37.4)* (n = 111)	-	**0.04**
mMRC dyspnoea score (n = 338)	0 (n = 71)	-	2 (1, 3)* (n = 126)	2 (1, 3)* (n = 141)	**0.0001**
Exacerbation history past 12 months (n = 321)		(n = 54)	(n = 126)	(n = 141)	
Casualty/emergency presentations	-	0	0 (0, 1)	1 (0, 1)†‡	**0.0001**
Hospital admissions	-	0	0	0 (0, 1)†‡	**0.0001**
Unscheduled doctor visits	-	0 (0, 1)	1 (0, 2)†	1 (0, 2)†	**0.002**
Oral corticosteroid courses	-	1 (0, 2)	2 (1, 5)†	2 (1, 3)†	**0.0001**
Extra-pulmonary					
Atopic status (n = 243)	43 (61.4) (n = 70)	40 (74.1) (n = 54)	95 (79.8)* (n = 119)	-	**0.02**
6MWD (metres) (n = 297)	603.9 ± 81.0 (n = 70)	-	454.4 ± 105.3* (n = 125)	368.0 ± 117.8*‡ (n = 102)	**< 0.0001**
HADS anxiety score (n = 337)	4 (2, 6) (n = 70)	-	6 (4, 9)* (n = 126)	6 (4, 9)* (n = 141)	**0.0001**
HADS depression score (n = 337)	1 (0, 2) (n = 70)	-	4 (2, 7)* (n = 126)	6 (3, 9)*‡ (n = 141)	**0.0001**
CCI score (n = 338)	0 (n = 71)	-	0 (0, 3)* (n = 126)	4 (3, 5)*‡ (n = 141)	**0.0001**
Induced sputum inflammatory characteristics					
Viability (%) (n = 319)	70.0 (50.0, 81.3) (n = 42)	66.6 (48.9, 85.7) (n = 46)	67.7 (47.4, 80.4) (n = 102)	78.3 (50.0, 90.5) (n = 129)	0.12
Total cell count (× 10⁶/mL) (n = 319)	2.0 (1.1, 3.3) (n = 42)	3.2 (1.9, 4.8) (n = 46)	4.1 (2.5, 7.9)* (n = 102)	4.6 (2.6, 8.2)* (n = 129)	**0.0001**
Neutrophils (%)	25.7 (13.0, 45.3)	36.2 (15.0, 58.5)	38.3 (18.9, 61.6)	52.5 (29.5, 70.3)*†‡	**0.0001**
Eosinophils (%)	0.5 (0, 1.0)	3.5 (0.8, 9.3)*	3.5 (0.8, 12.7)*	1.5 (0.8, 4.3)*	**0.0001**
Macrophages (%)	54.0 (41.1, 72.3)	40.0 (22.8, 60.0)*	43.1 (28.3, 60.5)*§	36.5 (18.3, 50.3)*	**0.0001**
Lymphocytes (%)	2.3 (0.4, 5.3)	1.3 (0.3, 4.2)‡	0.8 (0, 1.8)*	1.0 (0.3, 2.0)*	**0.0002**
Columnar epithelial cell (%)	2.3 (0.5, 4.8)	3.8 (1.0, 12.0)	2.8 (1.0, 6.6)	3.0 (0.5, 7.0)	0.45

Data are presented as number (%), mean ± standard deviation or median (Q₁, Q₃). Bold-faced *P* values are considered significant.
COPD, chronic obstructive pulmonary disease; BMI, body mass index; ICS, inhaled corticosteroid; OCS, oral corticosteroid; LABA, long-acting β2-agonist; SGRQ, St. George Respiratory Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; ACQ, asthma control questionnaire; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide; mMRC, modified Medical Research Council; 6MWD, 6 minute walk distance; HADS, Hospital Anxiety and Depression Scale; CCI, Charleston Comorbidity Index.
Bonferroni *post hoc* test and Kruskal Wallis *post hoc* test. *P < 0.008 vs. control participants; †P < 0.008 vs. mild-moderate asthma participants; ‡P < 0.008 vs. severe asthma participants; §P < 0.008 vs. COPD participants. ‖Prescribed maintenance OCS indicated for fibromyalgia.

Induced sputum inflammatory characteristics

Of the 393 participants, 346 (88.0%) had quality sputum samples adequate for analysis (see **Supplementary Data 1** for detailed sputum quality assessment). Differential cell counts are described in **Table 1**. COPD participants had a higher sputum neutrophil percentage compared to severe asthmatics, mild-moderate asthmatics and controls. All disease groups had higher sputum eosinophil percentages compared to controls.

Serum inflammatory biomarkers

By airway disease status

All disease groups showed higher WBC and peripheral blood eosinophil (PBE) counts, CRP and IL-6 compared to controls (**Table 2**). Median PBE counts ($\times 10^9$/L) were higher in mild-moderate asthma (0.3 [0.2, 0.4]) and severe asthma (0.2 [0.1, 0.4]) compared to COPD (0.2 [0.1, 0.3]). Higher levels of IL-6 (pg/mL) were observed in COPD (2.9 [1.8, 4.9]) compared to severe asthma (2.3 [0.9, 4.1]). Cp levels (µg/mL) were higher in COPD (576.9 [448.7, 812.0]) and in severe asthma (686.8 [354.5, 987.8]) compared to mild-moderate asthma (302.2 [243.5, 426.5]) ($P = 0.0001$). Cp was higher in controls (373.8 [319.0, 696.5]) compared to mild-moderate asthma. Hpx levels (µg/mL) were higher in COPD (2,179 [1,738, 2,751]) compared to severe asthma (1,161 [965, 1,353]), mild-moderate asthma (790 [664, 1,102]) and controls (834 [759, 1,060]) ($P = 0.0001$). Additional correlation analysis investigating the relationship between post β2 FEV1% predicted values and Hpx measures shows that FEV1% predicted was not correlated in COPD (Spearman's $r = 0.05$, $P = 0.59$) but was moderately negatively correlated in severe asthma (Spearman's $r = -0.24$, $P = 0.007$) and moderately positively correlated in mild-moderate asthma (Spearman's $r = 0.36$, $P = 0.007$). Hp (µg/ml) was significantly higher in disease groups compared to controls ($P = 0.0001$), but did not differ between the disease groups (**Table 2** and **Fig. 1**). Additional analysis, where mild-moderate and severe asthma groups were merged into a single group (**Supplementary Table S2**), revealed similar results, where COPD had higher levels of Cp, Hp and Hpx compared to the merged asthma group and controls. The merged asthma group also showed higher levels of Hp and Hpx compared to controls.

By airway inflammatory phenotype

We pooled the data from the mild-moderate asthma, severe asthma and COPD groups and classified the population by airway inflammatory phenotypes described earlier. We compared airway disease groups to non-respiratory controls. Clinical characteristics and differential cell counts of these phenotypes can be found in the online repository (**Supplementary Data 2** and **Supplementary Tables S3** and **S4**).

Table 2. Serum inflammatory biomarkers of the study populations by diagnosis

Biomarker	Controls	Mild-moderate asthma	Severe asthma	COPD	P value
Sample number	71	54	127	141	
CRP (mg/L) (n = 385)	1.2 (0.6, 2.4) (n = 67)	2.6 (1.0, 7.0)* (n = 54)	3.0 (1.3, 7.1)* (n = 125)	3.9 (1.9, 9.3)* (n = 139)	**0.0001**
WBC count (× 10⁹/L) (n = 389)	6.2 (5.4, 7.1) (n = 67)	7.3 (6.4, 8.7)* (n = 54)	7.6 (6.3, 9.3)* (n = 127)	7.6 (6.4, 9.2)* (n = 141)	**0.0001**
IL-6 HS (pg/mL)	0.5 (0.1, 1.2)	2.0 (1.0, 3.9)*	2.3 (0.9, 4.1)*	2.9 (1.8, 4.9)*‡	**0.0001**
PBE count (× 10⁹/L) (n = 389)	0.1 (0.1, 0.3) (n = 67)	0.3 (0.2, 0.4)*§ (n = 54)	0.2 (0.1, 0.4)*§ (n = 127)	0.2 (0.1, 0.3)* (n = 141)	**0.0001**
Cp (µg/mL)	373.8 (319.0, 696.5)†	302.2 (243.5, 426.5)	686.8 (354.5, 987.8)*†	576.9 (448.7, 812.0)*†	**0.0001**
Hp (µg/mL)	980 (716, 1,305)	1,586 (1,277, 1,899)*	1,579 (1,167, 2,218)*	1,948 (1,288, 2,874)*	**0.0001**
Hpx (µg/mL)	834 (759, 1,060)	790 (664, 1,102)	1,161 (965, 1,353)*†	2,179 (1,738, 2,751)*†‡	**0.0001**

Data are presented as median (Q₁, Q₃). Bold-faced *P* values are considered significant.
COPD, chronic obstructive pulmonary disease; WBC, white blood cell count; IL-6, interleukin-6; HS, high sensitivity; PBE, peripheral blood eosinophil count; Cp, ceruloplasmin; Hp, haptoglobin; Hpx, hemopexin.
Bonferroni *post hoc* test and Kruskal Wallis *post hoc* test; *$P < 0.008$ vs. control participants; †$P < 0.008$ vs. mild-moderate asthma participants; ‡$P < 0.008$ vs. severe asthma participants; §$P < 0.008$ vs. COPD participants.
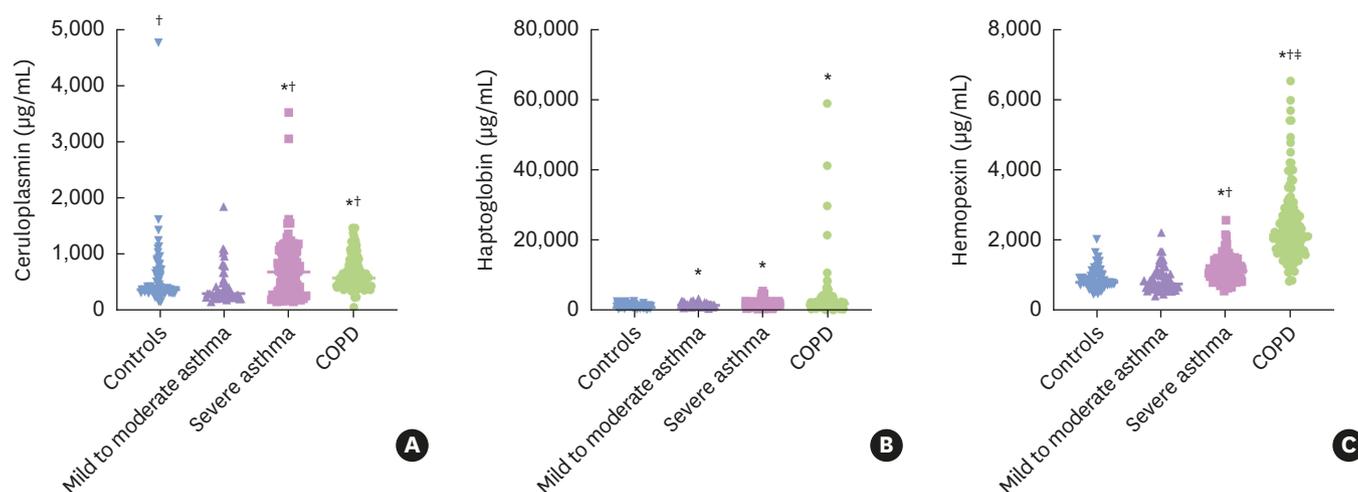
Fig. 1. Serum inflammatory biomarkers between diagnoses. (A) Ceruloplasmin, (B) Haptoglobin and (C) Hemopexin. Scatter graphs show medians (Q_3).
COPD, chronic obstructive pulmonary disease.
*$P < 0.008$ vs. control participants; †$P < 0.008$ vs. mild-moderate asthma participants; ‡$P < 0.008$ vs. severe asthma participants.

Serum CRP, WBC counts and IL-6 were elevated in all airway phenotypes compared to controls. Median PBE counts ($\times 10^9$/L) were higher in eosinophilic (0.3 [0.2, 0.5]) and mixed granulocytic phenotypes (0.3 [0.2, 0.6]) compared to neutrophilic (0.2 [0.1, 0.3]) and pauci-granulocytic phenotypes (0.1 [0.1, 0.2]) (**Table 3**). Higher Hp and Hpx levels were measured in all airway inflammatory phenotypes compared to controls ($P = 0.0001$). Cp levels were higher in neutrophilic and pauci-granulocytic phenotypes compared to controls, but did not differ between the airway inflammatory phenotypes ($P = 0.007$) (**Table 3** and **Fig. 2**).

By systemic inflammatory phenotype

Disease groups were combined and classified as 'systemic inflammation' or 'non-systemic inflammation' phenotypes. Of all participants with airway disease, systemic inflammation (defined as any 2 markers increased: CRP > 3 mg/L, WBC count > 9 × 10^9/L or IL-6 > 1.55 pg/mL) was present in 50.6% (n = 163). Clinical characteristics and differential cell counts according to the presence or absence of systemic inflammation can be found in the online repository (**Supplementary Data 2** and **Supplementary Tables S5** and **S6**). Systemic and non-systemic inflammatory phenotypes had significantly higher total airway inflammatory cell counts compared to controls. However, airway inflammation remained unaltered between the systemic and non-systemic inflammatory phenotypes.

Table 3. Serum inflammatory biomarkers of airway phenotypes

Biomarker	Controls	Neutrophilic	Eosinophilic	Mixed granulocytic	Pauci-granulocytic	P value
Sample number	71	67	113	26	88	
CRP (mg/L) (n = 357)	1.2 (0.6, 2.4) (n = 67)	3.5 (1.8, 10.5) (n = 66)*	3.2 (1.5, 7.9) (n = 110)*	5.1 (3.1, 9.8) (n = 26)*	3.1 (1.6, 6.9) (n = 88)*	**0.0001**
WBC count (× 10^9/L) (n = 361)	6.2 (5.4, 7.1) (n = 67)	7.7 (6.5, 9.2) (n = 67)*	7.7 (6.7, 9.4) (n = 113)*	7.6 (6.4, 9.4) (n = 26)*	7.4 (6.1, 8.4) (n = 88)*	**0.0001**
IL-6 HS (pg/mL)	0.5 (0.1, 1.2)	3.0 (1.3, 5.5)*	2.5 (1.3, 4.4)*	3.1 (1.8, 4.9)*	2.4 (1.3, 4.2)*	**0.0001**
PBE count (× 10^9/L) (n = 361)	0.1 (0.1, 0.3) (n = 67)	0.2 (0.1, 0.3) (n = 67)	0.3 (0.2, 0.5) (n = 113)*†‡	0.3 (0.2, 0.6) (n = 26)*†‡	0.1 (0.1, 0.2) (n = 88)	**0.0001**
Cp (µg/mL)	373.8 (319.0, 696.5)	610.8 (426.5, 960.3)*	512.8 (338.3, 806.5)	584.0 (342.7, 1,130.1)	545.4 (408.8, 857.7)*	**0.007**
Hp (µg/mL)	980 (716, 1,305)	1,792 (1,117, 2,532)*	1,819 (1,257, 2,568)*	1,887 (1,140, 2,504)*	1,644 (1,322, 2,243)*	**0.0001**
Hpx (µg/mL)	834 (759, 1,060)	1,677 (1,143, 2,301)*	1,280 (992, 1,895)*	1,502 (1,011, 2,353)*	1,441 (1,078, 2,140)*	**0.0001**

Data are presented as median (Q_1, Q_3). Bold-faced *P* values are considered significant.
WBC, white blood cell count; IL-6, interleukin-6; HS, high sensitivity; PBE, peripheral blood eosinophil count; Cp, ceruloplasmin; Hp, haptoglobin; Hpx, hemopexin.
Kruskal Wallis *post hoc* test. *$P < 0.005$ vs. control participants; †$P < 0.005$ vs. neutrophilic participants; ‡$P < 0.005$ vs. pauci-granulocytic participants.
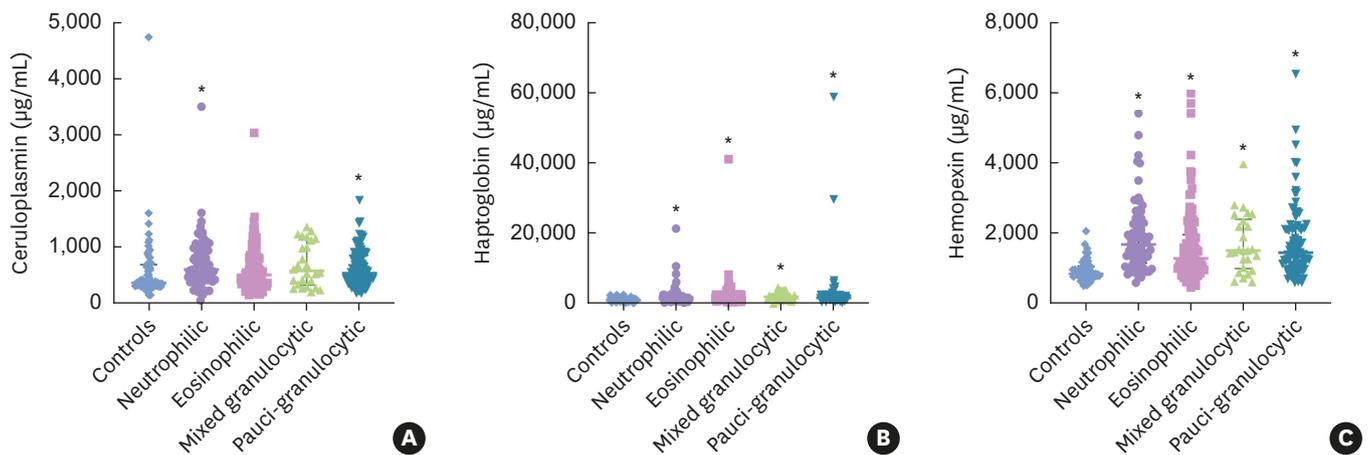
Fig. 2. Serum inflammatory biomarkers between airway inflammatory phenotypes. (A) Ceruloplasmin, (B) Haptoglobin and (C) Hemopexin. Scatter graphs show medians (Q₃).
*P < 0.005 vs. control participants.

Participants with systemic inflammation had higher levels of serum CRP, WBC counts and IL-6 compared to no systemic inflammation and controls. PBE counts were higher in both systemic and no systemic inflammatory groups compared to controls (**Table 4**). Higher Cp (615.5 [420.6, 948.1]), Hp (2,054 [1,398, 2,669]) and Hpx (1,537 [1,143, 2,316]) levels were measured in the systemic inflammatory phenotype compared to those without systemic inflammation and controls ($P = 0.0001$). Hp (1,480 [1,033, 1,844]) and Hpx (1,285 [902, 1,883]) were higher in the group without systemic inflammation compared to controls (980 [716, 1,305]) (834 [759, 1,060]) ($P = 0.0001$) (**Table 4** and **Fig. 3**).

Analysis of biomarker diagnostic ability

ROC curve analysis examined the diagnostic ability of the 3 protein biomarkers, individually and in combination with other systemic inflammatory markers (CRP, IL-6 and WBC and PBE counts) to distinguish between COPD, asthma and control participants (**Table 5** and **Fig. 4**). Between COPD and control participants, Hpx (area under the curve [AUC], 98.3%; 95% confidence interval [CI], 96.7%–9.9%) diagnostic performance was statistically superior to Cp (AUC, 86.6%; 95% CI, 81.0%–92.1%) and Hp (AUC, 89.4%; 95% CI, 85.0%–93.9%; $P \leq 0.01$) and other systemic inflammatory markers ($P \leq 0.05$ vs. CRP and $P \leq 0.01$ vs. WBC, IL-6 and PBE) (**Fig. 4A**). Youden Index analysis determined a Hpx cutpoint of 1,311.37 µg/mL produced a sensitivity = 0.96, specificity = 0.90 and an AUC = 93.0% for differentiating between COPD and control participants (**Table 6**). Combinatorial marker analysis showed that the best performing combination was of Hpx, Cp, Hp, CRP, IL-6, WBC and PBE count (AUC, 98.9%;

Table 4. Serum inflammatory biomarkers for systemic phenotypes

Biomarker	Controls	No systemic Inflammation	Systemic inflammation	P value
Sample number	71	159	163	
CRP (mg/L) (n = 385)	1.2 (0.6, 2.4) (n = 67)	1.6 (0.9, 2.6) (n = 159)	7.1 (4.0, 11.2)*† (n = 159)	**0.0001**
WBC count (× 10⁹/L) (n = 389)	6.2 (5.4, 7.1) (n = 67)	6.8 (6.1, 7.8)* (n = 159)	8.7 (7.2, 10.1)*† (n = 163)	**0.0001**
IL-6 HS (pg/mL)	0.5 (0.1, 1.2)	1.3 (0.5, 2.7)*	3.7 (2.4, 6.4)*†	**0.0001**
PBE count (× 10⁹/L) (n = 389)	0.1 (0.1, 0.3) (n = 67)	0.2 (0.1, 0.3)* (n = 159)	0.2 (0.1, 0.4)* (n = 163)	**0.0002**
Cp (µg/mL)	373.8 (319.0, 696.5)	494.0 (327.2, 757.9)	615.5 (420.6, 948.1)*†	**0.0001**
Hp (µg/mL)	980 (716, 1,305)	1,480 (1,033, 1,844)*	2,054 (1,398, 2,669)*†	**0.0001**
Hpx (µg/mL)	834 (759, 1,060)	1,285 (902, 1,883)*	1,537 (1,143, 2,316)*†	**0.0001**

Data are presented as median (Q₁, Q₃). Bold-faced P values are considered significant.
WBC, white blood cell count; IL-6, interleukin-6; HS, high sensitivity; PBE, peripheral blood eosinophil count; Cp, ceruloplasmin; Hp, haptoglobin; Hpx, hemopexin.
Bonferroni *post hoc* test and Kruskal Wallis *post hoc* test. *P < 0.02 vs. control participants; †P < 0.02 vs. no systemic inflammation.
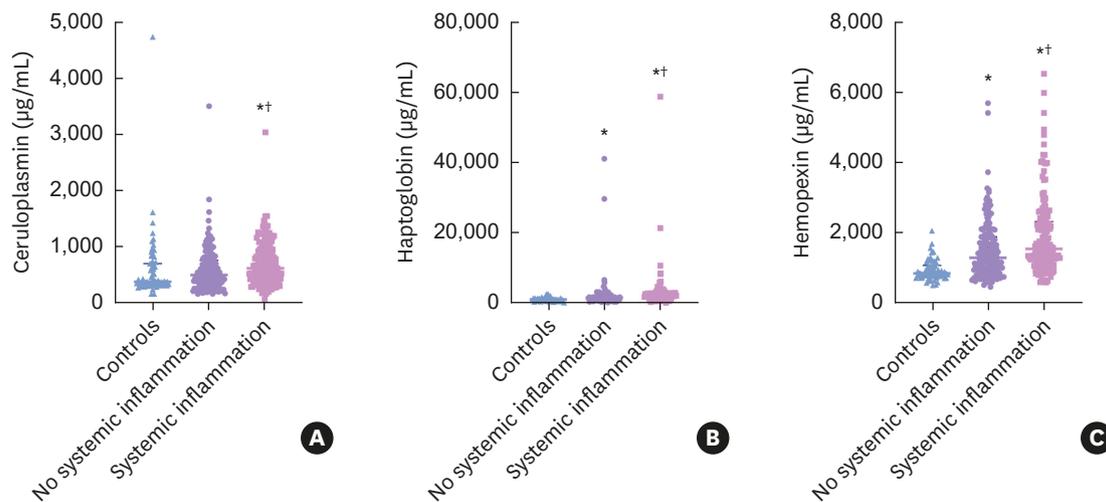
Fig. 3. Serum inflammatory biomarkers between systemic inflammatory phenotypes. (A) Ceruloplasmin, (B) Haptoglobin and (C) Hemopexin. Scatter graphs show medians (Q3).
*$P < 0.02$ vs. control participants; †$P < 0.02$ vs. no systemic inflammation.

95% CI, 97.6%–1.00%), but this did not perform significantly better than Hpx alone ($P = 0.07$). Between asthma and control participants, Hp (AUC, 81.1%; 95% CI, 75.6%–86.6%) diagnostic performance was statistically superior to Cp (AUC, 75.4%; 95% CI, 69.4%–81.5%; $P \le 0.01$), but not Hpx (AUC, 77.6%; 95% CI, 71.6%–83.6%; $P = 0.12$) (**Fig. 4B**). Hp diagnostic performance was not statistically different to other systemic inflammatory markers (WBC [$P = 0.71$], IL-6 [$P = 0.87$] and PBE [$P = 0.76$]), with the exception of CRP ($P = 0.03$). For differentiating between asthma and control participants, a Hp cutpoint of 1,305.01 μg/mL produced a sensitivity = 0.70, specificity = 0.76 and an AUC = 73.0% (**Table 6**). Combinatorial marker analysis showed the combination of Hp, Hpx, CRP, WBC, IL-6 and PBE (87.5% [82.8%, 92.2%]) returned the highest AUC and performed significantly better than Hp alone ($P \le 0.01$).

Table 5. Analysis of diagnostic value of systemic inflammatory biomarkers between study groups

Marker combination	COPD vs. Control			Asthma vs. Control			COPD vs. Asthma		
	No.	AUC	95% CI	No.	AUC	95% CI	No.	AUC	95% CI
Cp	212	0.866	0.810, 0.921	251	0.754	0.694, 0.815	321	0.793	0.745, 0.840
Hp	212	0.894	0.850, 0.939	251	0.811	0.756, 0.866	321	0.815	0.770, 0.861
Hpx	212	0.983	0.967, 0.999	251	0.776	0.716, 0.836	321	0.970	0.954, 0.986
CRP	206	0.878	0.823, 0.933	246	0.764	0.703, 0.826	318	0.805	0.758, 0.851
WBC	208	0.903	0.852, 0.953	247	0.818	0.762, 0.874	321	0.794	0.746, 0.841
IL-6	212	0.897	0.849, 0.944	251	0.807	0.749, 0.865	321	0.797	0.749, 0.844
PBE	208	0.880	0.827, 0.933	247	0.816	0.763, 0.869	321	0.794	0.746, 0.841
Cp + Hp	212	0.894	0.850, 0.939	251	0.812	0.757, 0.868	321	0.816	0.770, 0.861
Cp + Hpx	212	0.984	0.969, 0.999	251	0.776	0.716, 0.835	321	0.970	0.954, 0.986
Hp + Hpx	212	0.986	0.972, 0.999	251	0.817	0.763, 0.871	321	0.972	0.956, 0.987
Cp + Hp + Hpx	212	0.987	0.975, 0.999	251	0.818	0.763, 0.872	321	0.972	0.956, 0.987
Cp + CRP + WBC + IL-6 + PBE	206	0.916	0.869, 0.964	246	0.865	0.814, 0.917	318	0.808	0.762, 0.854
Hp + CRP + WBC + IL-6 + PBE	206	0.925	0.882, 0.967	246	0.875	0.828, 0.923	318	0.825	0.781, 0.869
Hpx + CRP + WBC + IL-6 + PBE	206	0.987	0.972, 1.000	246	0.869	0.819, 0.919	318	0.973	0.957, 0.988
Cp + Hp + CRP + WBC + IL-6 + PBE	206	0.925	0.882, 0.967	246	0.875	0.827, 0.923	318	0.826	0.782, 0.870
Cp + Hpx + CRP + WBC + IL-6 + PBE	206	0.988	0.972, 1.000	246	0.869	0.819, 0.920	318	0.973	0.957, 0.988
Hp + Hpx + CRP + WBC + IL-6 + PBE	206	0.989	0.974, 1.000	246	0.875	0.828, 0.922	318	0.974	0.959, 0.989
Cp + Hp + Hpx + CRP + WBC + IL-6 + PBE	206	0.989	0.976, 1.000	246	0.875	0.828, 0.923	318	0.974	0.959, 0.989

Multivariate logistic regression adjusted for age, sex and body mass index.
COPD, chronic obstructive pulmonary disease; Cp, ceruloplasmin; Hp, haptoglobin; Hpx, hemopexin; CRP, C-reactive protein; WBC, white blood cell count; IL-6, interleukin-6; PBE, peripheral blood eosinophil count; AUC, area under the curve; CI, confidence interval.
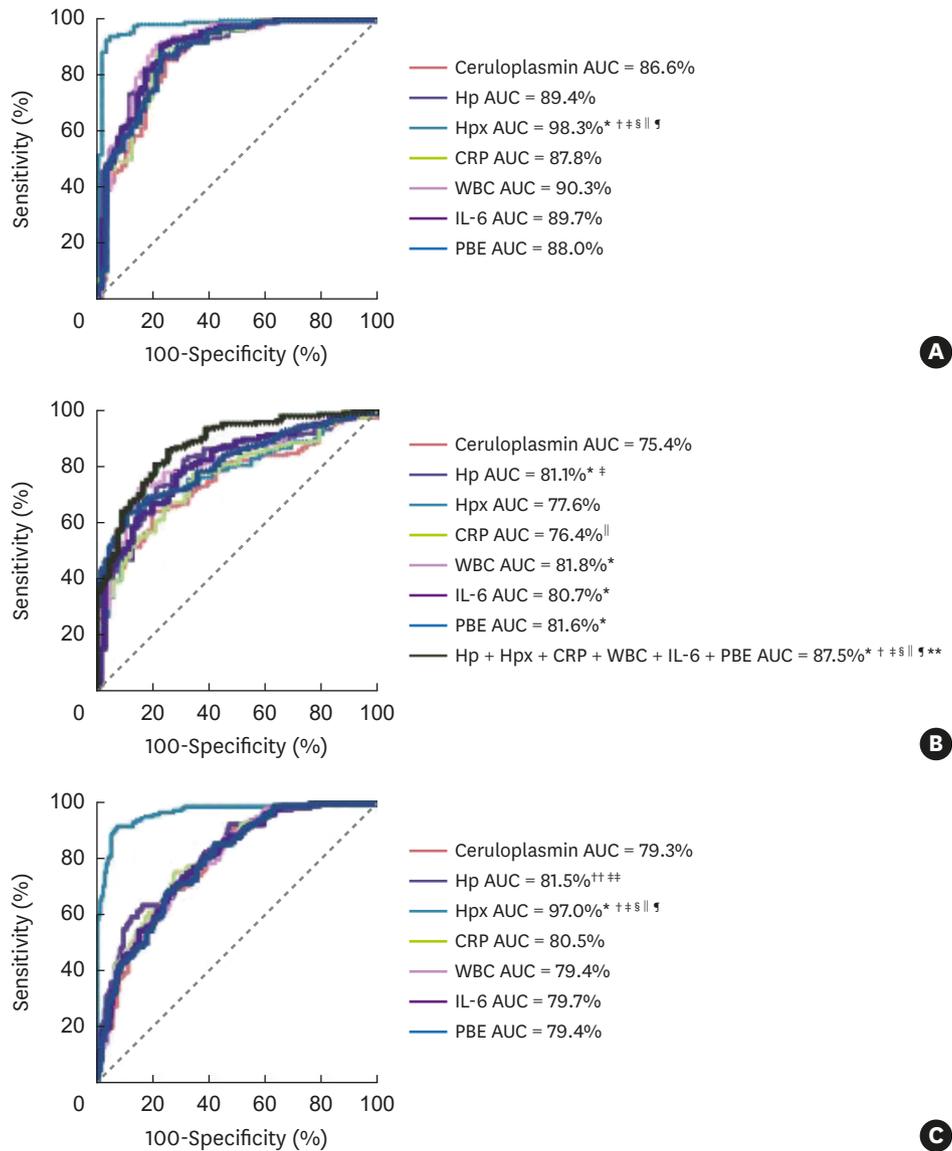
Fig. 4. ROC curve analysis of systemic biomarkers to predict (A) COPD vs. Controls, (B) Asthma vs. Controls, and (C) COPD vs. Asthma.
ROC, receiver operator characteristic; COPD, chronic obstructive pulmonary disease; AUC, area under the curve; CRP, C-reactive protein; WBC, white blood cell; IL-6, interleukin-6; PBE, peripheral blood eosinophil; Hp, haptoglobin; Hpx, hemopexin.
*$P \le 0.01$ vs. Ceruloplasmin; [†]$P \le 0.01$ vs. Haptoglobin; [‡]$P \le 0.05$ vs. CRP; [§]$P \le 0.01$ vs. WBC count; [∥]$P \le 0.01$ vs. IL-6; [¶]$P \le 0.01$ vs. PBE count; **$P < 0.001$ vs. Hemopexin; [††]$P < 0.05$ vs. Ceruloplasmin; [‡‡]$P < 0.05$ vs. WBC.

Table 6. Optimal cutoff values for individual protein markers

Study group	Marker	Empirical optimal cutpoint	Sensitivity	Specificity	Youden Index*	AUC at cutoff
COPD vs. Control	Ceruloplasmin	399.23	0.89	0.59	0.48	0.74
	Haptoglobin	1,414.50	0.70	0.85	0.54	0.77
	Hemopexin	1,311.37	0.96	0.90	0.87	0.93
Asthma vs. Control	Ceruloplasmin	426.37	0.56	0.63	0.20	0.60
	Haptoglobin	1,305.01	0.70	0.76	0.46	0.73
	Hemopexin	962.96	0.64	0.68	0.32	0.66
COPD vs. Asthma	Ceruloplasmin	343.79	0.98	0.36	0.34	0.67
	Haptoglobin	1,724.09	0.60	0.62	0.22	0.61
	Hemopexin	1,572.16	0.86	0.91	0.77	0.88

*Youden Index summarises the performance of a diagnostic test. A value of 1.00 indicates a test with no false positives or false negatives.
COPD, chronic obstructive pulmonary disease; AUC, area under the curve.

Between COPD and asthma participants, Hpx diagnostic performance (AUC, 97.0%; 95% CI, 95.4%–98.6%) was significantly better than Cp (AUC, 79.3%; 95% CI, 74.5%–84.0%) and Hp (AUC, 81.5%; 95% CI, 77.0%–86.1%) ($P \leq 0.01$) and other systemic inflammatory markers ($P \leq 0.01$) (**Fig. 4C**). For differentiating between COPD and asthma participants, a Hpx cutpoint of 1,572.16 μg/mL produced a sensitivity = 0.86, specificity = 0.91 and an AUC = 88.0% (**Table 6**). Combining all systemic inflammatory markers (Cp, Hp, Hpx, CRP, WBC, IL-6 and PBE) produced the highest AUC to differentiate COPD from asthma (AUC, 97.4%; 95% CI, 95.9%–98.9%); however, this was not statistically different compared to Hpx alone ($P = 0.12$). Additional analyses were conducted, where asthma participants were divided based on severity (mild-moderate and severe asthma) and diagnostic performance was measured versus COPD (**Supplementary Table S7** and **Figs. S4** and **S5**). Hpx significantly predicted mild-moderate and severe asthma versus COPD (**Supplementary Figs. S4** and **S5**). Individual ROC curves can be found in the online repository (**Supplementary Figs. S1**-**S5**). Additional analyses were conducted, adjusting for maintenance OCS as well as biological factors (sex, age and BMI) and results were not affected. Additional adjusted and unadjusted analyses can be found in the online repository (**Supplementary Tables S8** and **S9**).

DISCUSSION

In this study, we validated and established the relationship between novel acute-phase protein biomarkers and 1) airway disease diagnosis, 2) airway inflammatory phenotypes and 3) systemic inflammatory phenotypes in participants with COPD, mild-moderate and severe asthma and controls. We found significant differences in Cp, Hp and Hpx between the disease groups and between the systemic and non-systemically inflamed phenotypes. However, we did not demonstrate differences between the airway inflammatory phenotypes. Hpx was the best performing biomarker in differentiating between COPD participants and controls/asthma participants compared to Cp, Hp and other systemic inflammatory markers. Hp was the best performing marker in differentiating asthmatics from control participants compared to Cp. However, it was not significantly better than other systemic inflammatory markers, with the exception of CRP. Combinatorial marker analysis revealed the best combination to differentiate asthma from control participants included Hp, Hpx, CRP, IL-6 and WBC and PBE counts.

Hpx was higher in COPD compared to mild-moderate asthma, severe asthma and controls. Hpx was also higher in severe asthma compared to mild-moderate asthma and controls as well as in airway disease featuring systemic inflammation. We also found that Hpx was a useful marker differentiating between COPD and control participants and COPD and asthma participants. Hpx plays anti-inflammatory roles in heme-iron recovery by binding to haemoglobin, modulating hemo-oxygenase, ferritin and transferrin receptor expression[17] and reducing the pro-inflammatory response to free haemoglobin.[18] Given its anti-inflammatory roles in oxidative stress, Hpx may play a protective role in chronic airway disease as shown in other inflammatory diseases.[19-21] A previous study found that treatment with purified human plasma Hpx in a mouse model of chronic lung injury reduced endoplasmic reticulum stress, airway fibrosis and emphysema.[22] No studies have been conducted in asthma. Our results are similar to those of the original proteomic study, where Hpx was elevated in asthma and COPD participants compared to controls. However, Verrills et al.[11] found no difference in Hpx levels between asthma and COPD groups. They also found that the marker combination of Hpx and Cp or Hpx and A2M was useful in differentiating COPD from control participants and that the

marker combination of Hpx, A2M and Hp was as useful in differentiating between asthma and COPD participants. Differences may be explained by a smaller sample size in the original study. Our results, showing higher Hpx levels in disease compared to controls and between asthma severities, are consistent with its potentially protective role in airway disease.

Hp, released mainly by the liver during inflammation, also plays a role in iron homeostasis, mediating hepatic recycling of heme iron,[23] preventing iron-induced kidney damage[24] and stabilising haemoglobin, protecting globin from oxidative damage.[25] We found that Hp was higher in airway disease and in systemic inflammation. Although we found that Hp was the best performing marker in differentiating asthma from control participants, this was not statistically different to other systemic inflammatory markers. Furthermore, the Youden index revealed that Hp was not a useful diagnostic marker for asthma. Combinatorial marker analysis revealed the best combination to differentiate asthma from control participants included Hp, Hpx, CRP and IL-6 as well as WBC and PBE counts, which performed significantly better than Hp alone. Despite elevated Hp levels among disease groups, we showed no difference in Hp between diseases, suggesting Hp may be a marker for chronic inflammatory disease. Elevated Hp has been reported in cancer,[26-28] psoriasis,[29] obesity and metabolic syndrome.[30,31] The increase in Hp in systemic inflammation may be explained by its induction by IL-6.[32] Previous studies and our prior study[11] have shown higher serum concentrations in COPD [32] and asthma [33] and downregulation in non-atopic asthma.[34] Verrills et al.[11] also reported marker the combination of Hp and Cp or Hp and Hpx could differentiate asthma participants from controls. Our data confirm the chronic inflammatory nature of airway disease, reflected by elevated levels of Hp in airway disease.

Cp levels were higher in COPD patients, severe asthmatics and participants with systemic inflammation compared to control participants. Unexpectedly, we observed reduced Cp in mild-moderate asthma participants compared to controls. ROC curve analysis showed that Cp was not a useful marker in differentiating disease and controls. Cp is a copper-binding glycoprotein with multiple anti-inflammatory functions. Consistent with our previous results,[11] elevated serum Cp has been reported in COPD[35] and asthma.[36] Higher levels of Cp could result from the presence of systemic inflammation, as Cp and Cp-oxidase activity have been shown to correlate with CRP in COPD[35] and non-treated severe asthma-allergic rhinitis,[37] respectively. Our data are consistent with previous studies, suggest that elevated levels represent a host response to greater inflammatory burden on the airways such as in severe asthma and COPD. This requirement only becomes apparent when severe disease develops as Cp was not elevated in mild-moderate asthma.

In asthma and COPD, inflammatory triggers, including allergens, infections, cigarette smoke and pollution, initiate immune responses, releasing pro-inflammatory cytokines and chemokines from the airway epithelium and from inflammatory cells to recruit eosinophils, neutrophils and further immune effector cells to the airways.[38,39] A systemic inflammatory response can also occur with increased circulating levels of pro-inflammatory factors such as CRP and IL-6.[6] We hypothesized that an innate inflammatory-axis between the airways and systemic circulation comprised of anti- and pro-inflammatory components. Our study provides evidence against a pro-inflammatory axis, suggesting that airway and systemic inflammation can occur independently. Specifically, we found elevated systemic inflammation in the absence of airway inflammation, i.e. pauci-granulocytic participants, and unaltered airway inflammatory patterns in the presence of systemic inflammation. The release of anti-inflammatory factors is also important for resolving and regulating

inflammation. However, few anti-inflammatory factors are known in asthma and COPD with airway origin, except annexin A1.[40], [41] Anti-inflammatory acute-phase proteins, such as Hpx, are primarily released from the liver in response to injury, trauma or infection to resolve inflammation. The fact that Hpx is elevated in airway disease highlights a potential link between systemic anti-inflammatory mediators and airway inflammation. Thus, we propose an innate anti-inflammatory axis between the airways and systemic circulation in asthma and COPD involving Hpx as a protective mechanism in responses to oxidative stress and airway damage. Hpx may be a novel anti-inflammatory diagnostic marker and potential functional marker with therapeutic value. Further research will elucidate the innate anti-inflammatory axis in chronic airway disease.

A strength of our study is the panel of 3 serum protein markers tested. Serum is an advantageous source of biomarkers for disease management, offering accessibility, non-invasiveness and standardised processing, easily applicable to most laboratories.[42,43] Few studies have explored whether increased serum levels of acute-phase proteins could differentiate between asthma and COPD,[11] with our study being the first to compare these markers in COPD, mild-moderate and severe asthma. Our study is also the first to combine these markers with established clinical markers of systemic inflammation in composite panels. We believe that our findings, where systemic inflammatory biomarkers could differentiate between COPD and asthma and between airway disease and controls, are useful from a clinical and biological perspective. Clinically, the fact that a single systemic biomarker (Hpx) could distinguish between the 2 airway diseases (COPD and asthma) is a novel finding. Additionally, the finding that Hpx (as well as others) was not associated with airway inflammation and is in fact an anti-inflammatory mediator, improves our biological understanding of the origins and characteristics of inflammation in airway disease. The link between airway and systemic inflammation in airway disease is yet to be elucidated [10] and our study provides evidence for the importance of systemic inflammation in asthma and COPD pathogenesis.

Our study was limited by its cross-sectional design which does not allow the temporal stability of the markers to be established. Longitudinal studies to investigate stability over time are necessary. The longitudinal relationship of systemic inflammation and relevant clinical outcomes could not be established. Also, due to this study's retrospective nature and differing exacerbation definitions, a 12-month history of past exacerbation frequency could not be quantified and the ability to predict exacerbations could not be performed. Due to the non-specific nature of the markers, it is not possible to differentiate other inflammatory diseases with the markers only. Therefore, the markers would need to be used in conjunction with current clinical criteria, as a component of multidimensional assessment.[13] This will provide insight into the systemic inflammatory status of the individual. Finally, we acknowledge the limitation of this being a single-center study. Future multi-center studies to externally validate these markers are suggested to support clinical applicability.

In conclusion, we found significant differences in Cp, Hp and Hpx between airway disease groups and between systemic and non-systemically inflamed phenotypes. However, no differences were found between airway inflammatory phenotypes. We found that Hpx had superior diagnostic ability to distinguish between COPD and asthma, outperforming established systemic inflammatory markers. This supports its use as a single clinical marker in diagnosing COPD, but not asthma. In asthma, a combination panel of diagnostic inflammatory markers may be more useful. We also propose an innate airway and systemic anti-inflammatory axis involving Hpx as a protective pathway.

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ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIALS

Supplementary Data 1

Methods

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Supplementary Data 2

Results

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Supplementary Table S1

Diagnostic criteria for different diagnoses

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Supplementary Table S2

Serum inflammatory biomarkers of study sample populations

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Supplementary Table S3

Clinical characteristics of airway inflammatory phenotypes

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Supplementary Table S4

Induced sputum inflammatory characteristics of airway inflammatory phenotypes

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Supplementary Table S5

Clinical characteristics of systemic inflammatory phenotypes

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Supplementary Table S6
Induced sputum inflammatory characteristics of systemic phenotypes

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Supplementary Table S7
Additional analysis of diagnostic value of systemic inflammatory biomarkers between disease groups

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Supplementary Table S8
Additional unadjusted analysis of diagnostic value of systemic inflammatory biomarkers between disease groups

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Supplementary Table S9
Additional adjusted analysis of diagnostic value of systemic inflammatory biomarkers between disease groups

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Supplementary Fig. S1
ROC curve analysis of systemic biomarkers to predict COPD versus controls. (A) Ceruloplasmin, (B) Haptoglobin, (C) Hemopexin, (D) CRP, (E) WBC count, (F) IL-6, (G) PBE count. (H) Represents graphs (A-G) compared on a single graph.

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Supplementary Fig. S2
ROC curve analysis of systemic biomarkers to predict Asthma vs. Controls. (A) Ceruloplasmin, (B) Haptoglobin, (C) Hemopexin, (D) CRP, (E) WBC count, (F) IL-6, (G) PBE count. (H) Represents graphs (A-G) compared on a single graph.

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Supplementary Fig. S3
ROC curve analysis of systemic biomarkers to predict Asthma vs. COPD. (A) Ceruloplasmin, (B) Haptoglobin, (C) Hemopexin, (D) CRP, (E) WBC count, (F) IL-6, (G) PBE count. (H) Represents graphs (A-G) compared on a single graph.

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Supplementary Fig. S4
ROC curve analysis of systemic biomarkers to predict Mild-moderate asthma vs. COPD. (A) Ceruloplasmin, (B) Haptoglobin, (C) Hemopexin, (D) CRP, (E) WBC count, (F) IL-6, (G) PBE count. (H) Represents graphs (A-G) compared on a single graph.

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Supplementary Fig. S5

ROC curve analysis of systemic biomarkers to predict Severe asthma vs. COPD. (A) Ceruloplasmin, (B) Haptoglobin, (C) Hemopexin, (D) CRP, (E) WBC count, (F) IL-6, (G) PBE count. (H) Represents graphs (A-G) compared on a single graph.

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REFERENCES

1. Masoli M, Fabian D, Holt S, Beasley RGlobal Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004;59:469-78.
   PUBMED | CROSSREF

2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095-128.
   PUBMED | CROSSREF

3. Song WJ, Lee JH, Kang Y, Joung WJ, Chung KF. Future risks in patients with severe asthma. Allergy Asthma Immunol Res 2019;11:763-78.
   PUBMED | CROSSREF

4. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology 2006;11:54-61.
   PUBMED | CROSSREF

5. McDonald VM, Higgins I, Wood LG, Gibson PG. Multidimensional assessment and tailored interventions for COPD: respiratory utopia or common sense? Thorax 2013;68:691-4.
   PUBMED | CROSSREF

6. Agustí A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. PLoS One 2012;7:e37483.
   PUBMED | CROSSREF

7. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.
   PUBMED | CROSSREF

8. Siva R, Green RH, Brightling CE, Shelley M, Hargadon B, McKenna S, et al. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. Eur Respir J 2007;29:906-13.
   PUBMED | CROSSREF

9. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet 2008;372:1107-19.
   PUBMED | CROSSREF

10. Agustí A. Systemic effects of chronic obstructive pulmonary disease: what we know and what we don't know (but should). Proc Am Thorac Soc 2007;4:522-5.
    PUBMED | CROSSREF

11. Verrills NM, Irwin JA, He XY, Wood LG, Powell H, Simpson JL, et al. Identification of novel diagnostic biomarkers for asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2011;183:1633-43.
    PUBMED | CROSSREF

12. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald JM, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008;31:143-78.
    PUBMED | CROSSREF

13. McDonald VM, Clark VL, Cordova-Rivera L, Wark PA, Baines KJ, Gibson PG. Targeting treatable traits in severe asthma: a randomised controlled trial. Eur Respir J 2020;55:1901509.
    PUBMED | CROSSREF

14. Belda J, Hussack P, Dolovich M, Efthimiadis A, Hargreave FE. Sputum induction: effect of nebulizer output and inhalation time on cell counts and fluid-phase measures. Clin Exp Allergy 2001;31:1740-4.
    PUBMED | CROSSREF

15. Thomsen M, Ingebrigtsen TS, Marott JL, Dahl M, Lange P, Vestbo J, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. JAMA 2013;309:2353-61.
    PUBMED | CROSSREF

16. Fu JJ, McDonald VM, Gibson PG, Simpson JL. Systemic inflammation in older adults with asthma-COPD overlap syndrome. Allergy Asthma Immunol Res 2014;6:316-24.
PUBMED | **CROSSREF**

17. Tolosano E, Fagoonee S, Morello N, Vinchi F, Fiorito V. Heme scavenging and the other facets of hemopexin. Antioxid Redox Signal 2010;12:305-20.
PUBMED | **CROSSREF**

18. Lin T, Kwak YH, Sammy F, He P, Thundivalappil S, Sun G, et al. Synergistic inflammation is induced by blood degradation products with microbial Toll-like receptor agonists and is blocked by hemopexin. J Infect Dis 2010;202:624-32.
PUBMED | **CROSSREF**

19. Janz DR, Bastarache JA, Sills G, Wickersham N, May AK, Bernard GR, et al. Association between haptoglobin, hemopexin and mortality in adults with sepsis. Crit Care 2013;17:R272.
PUBMED | **CROSSREF**

20. Vinchi F, Costa da Silva M, Ingoglia G, Petrillo S, Brinkman N, Zuercher A, et al. Hemopexin therapy reverts heme-induced proinflammatory phenotypic switching of macrophages in a mouse model of sickle cell disease. Blood 2016;127:473-86.
PUBMED | **CROSSREF**

21. Mehta NU, Grijalva V, Hama S, Wagner A, Navab M, Fogelman AM, et al. Apolipoprotein E$^{-/-}$ mice lacking hemopexin develop increased atherosclerosis via mechanisms that include oxidative stress and altered macrophage function. Arterioscler Thromb Vasc Biol 2016;36:1152-63.
PUBMED | **CROSSREF**

22. Aggarwal S, Ahmad I, Lam A, Carlisle MA, Li C, Wells JM, et al. Heme scavenging reduces pulmonary endoplasmic reticulum stress, fibrosis, and emphysema. JCI Insight 2018;3:e120694.
PUBMED | **CROSSREF**

23. Buehler PW, Abraham B, Vallelian F, Linnemayr C, Pereira CP, Cipollo JF, et al. Haptoglobin preserves the CD163 hemoglobin scavenger pathway by shielding hemoglobin from peroxidative modification. Blood 2009;113:2578-86.
PUBMED | **CROSSREF**

24. Delanghe JR, Langlois MR. Haptoglobin polymorphism and body iron stores. Clin Chem Lab Med 2002;40:212-6.
PUBMED | **CROSSREF**

25. Marieb EN, Hoehn K. Blood. In: Human anatomy & physiology. 8th ed. San Francisco (CA): Benjamin Cummings; 2010. 641.

26. Arthur R, Williams R, Garmo H, Holmberg L, Stattin P, Malmström H, et al. Serum inflammatory markers in relation to prostate cancer severity and death in the Swedish AMORIS study. Int J Cancer 2018;142:2254-62.
PUBMED | **CROSSREF**

27. Lemańska-Perek A, Lis-Kuberka J, Lepczyński A, Dratwa-Chałupnik A, Tupikowski K, Kątnik-Prastowska I, et al. Potential plasma biomarkers of bladder cancer identified by proteomic analysis: a pilot study. Adv Clin Exp Med 2019;28:339-46.
PUBMED | **CROSSREF**

28. Sollie S, Michaud DS, Sarker D, Karagiannis SN, Josephs DH, Hammar N, et al. Chronic inflammation markers are associated with risk of pancreatic cancer in the Swedish AMORIS cohort study. BMC Cancer 2019;19:858.
PUBMED | **CROSSREF**

29. Tian FJ, Zhang YY, Liu LQ, Xiong Y, Wang ZS, Wang SZ. Haptoglobin protein and mRNA expression in psoriasis and its clinical significance. Mol Med Rep 2016;14:3735-42.
PUBMED | **CROSSREF**

30. Rodrigues KF, Pietrani NT, Carvalho LM, Bosco AA, Sandrim VC, Ferreira CN, et al. Haptoglobin levels are influenced by Hp1-Hp2 polymorphism, obesity, inflammation, and hypertension in type 2 diabetes mellitus. Endocrinol Diabetes Nutr 2019;66:99-107.
PUBMED | **CROSSREF**

31. Minović I, Eisenga MF, Riphagen IJ, van den Berg E, Kootstra-Ros J, Frenay AS, et al. Circulating haptoglobin and metabolic syndrome in renal transplant recipients. Sci Rep 2017;7:14264.
PUBMED | **CROSSREF**

32. Lee PL, Lee KY, Cheng TM, Chuang HC, Wu SM, Feng PH, et al. Relationships of haptoglobin phenotypes with systemic inflammation and the severity of chronic obstructive pulmonary disease. Sci Rep 2019;9:189.
PUBMED | **CROSSREF**

33. Kauffmann F, Frette C, Annesi I, Oryszczyn MP, Dore MF, Neukirch F. Relationships of haptoglobin level to FEV1, wheezing, bronchial hyper-responsiveness and allergy. Clin Exp Allergy 1991;21:669-74.
    PUBMED | CROSSREF

34. Ejaz S, Nasim FU, Ashraf M, Ahmad S. Serum proteome profiling to identify proteins promoting pathogenesis of non-atopic asthma. Protein Pept Lett 2018;25:933-42.
    PUBMED | CROSSREF

35. Milevoj Kopčinović L, Domijan AM, Posavac K, Čepelak I, Žanić Grubišić T, Rumora L. Systemic redox imbalance in stable chronic obstructive pulmonary disease. Biomarkers 2016;21:692-8.
    PUBMED | CROSSREF

36. Vural H, Uzun K. Serum and red blood cell antioxidant status in patients with bronchial asthma. Can Respir J 2000;7:476-80.
    PUBMED | CROSSREF

37. Yalcin AD, Gumuslu S, Parlak GE, Bisgin A, Yildiz M, Kargi A, et al. Systemic levels of ceruloplasmin oxidase activity in allergic asthma and allergic rhinitis. Immunopharmacol Immunotoxicol 2012;34:1047-53.
    PUBMED | CROSSREF

38. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. Am J Respir Crit Care Med 2009;180:388-95.
    PUBMED | CROSSREF

39. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016;138:16-27.
    PUBMED | CROSSREF

40. Lai T, Li Y, Mai Z, Wen X, Lv Y, Xie Z, et al. Annexin A1 is elevated in patients with COPD and affects lung fibroblast function. Int J Chron Obstruct Pulmon Dis 2018;13:473-86.
    PUBMED | CROSSREF

41. Lee SH, Lee PH, Kim BG, Seo HJ, Baek AR, Park JS, et al. Annexin A1 in plasma from patients with bronchial asthma: its association with lung function. BMC Pulm Med 2018;18:1.
    PUBMED | CROSSREF

42. Indovina P, Marcelli E, Pentimalli F, Tanganelli P, Tarro G, Giordano A. Mass spectrometry-based proteomics: the road to lung cancer biomarker discovery. Mass Spectrom Rev 2013;32:129-42.
    PUBMED | CROSSREF

43. Yang J, McDowell A, Seo H, Kim S, Min TK, Jee YK, et al. Diagnostic Models for atopic dermatitis based on serum microbial extracellular vesicle metagenomic analysis: a pilot study. Allergy Asthma Immunol Res 2020;12:792-805.
    PUBMED | CROSSREF