Letter to the Editor

Combined assessment of serum eosinophil-derived neurotoxin and YKL-40 may identify Asthma-COPD overlap

Dear Editor,

In our previous report,[1] we showed that the combined assessment of serum periostin, a biomarker of type 2 inflammation in asthma, and serum YKL-40, a useful biomarker of COPD, may be beneficial for identifying ACO. We found that the proportion of patients with both high serum periostin and YKL-40 levels was significantly higher in asthma-COPD overlap (ACO) than in asthma or COPD: odds ratio, 2.59 (95% CI, 1.58–4.25); p < 0.001; sensitivity, 38.3%; and specificity, 80.7%. To explore another useful type 2 biomarker, we focused on eosinophil-derived neurotoxin (EDN).[2,3] In this study, we hypothesized that using serum EDN instead of periostin would be useful to identify ACO.

The study subjects included 353 adult patients who visited outpatient clinics at Shizuoka General Hospital or Nihon University Itabashi Hospital for routine check-ups between February 2013 and August 2016. The participants were classified into three groups: asthma (n = 177), COPD (n = 61), and ACO (n = 115). The asthma and COPD patients fulfilled the definition of the Global Initiative for Asthma (GINA)[4] and the Global Initiative for Chronic Obstructive Lung Disease (GOLD),[5] respectively. According to the previous reports,[6–8] ACO was diagnosed if the asthma patients were older than 40 years, had post-bronchodilator forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) < 0.7, and fulfilled at least one of the following criteria: more than 10 pack-years of smoking history, less than 80% of diffusing capacity of the lung for carbon monoxide/alveolar volume, or the presence of a low attenuation area on high-resolution computed tomography. ACO was also diagnosed if the COPD patients fulfilled at least two of the following criteria: a past history of asthma, a blood eosinophil count ≥250 cells/μL, fractional exhaled nitric oxide (FeNO) > 35 ppb, or serum total IgE > 100 IU/mL. The cohorts of this study were identical to our previous report, and the patient flow and clinical characteristics are shown elsewhere.[1]

On the examination day, the study subjects underwent blood draws, FeNO, and spirometry in that order. The study protocols were approved by the ethics committee of each institution (approved number: SGH 15-01-55 and RK-130111, respectively) and written informed consent was obtained from the patients. Serum EDN levels were measured using the EDN ELISA Kit (MBL International, Nagoya, Japan). Serum YKL-40 was determined by the

Human Chitinase 3-like 1 Quantikine ELISA Kit (catalog number DC3L10, R&D systems, Minneapolis, MN, USA). Each ELISA kit was used according to the manufacturer's instructions. All of the analyses were performed with R software (version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria). A two-tailed P value of <0.05 was considered statistically significant.

Serum EDN was higher in ACO than in asthma or COPD (Fig. 1A), whereas serum YKL-40 was higher in both COPD and ACO than in asthma.[1] One hundred and fifteen patients with ACO consisted of 86 patients derived from asthma, denoted as ACO (asthma), and 29 derived from COPD, denoted as ACO (COPD). The results of serum EDN and YKL-40 in these patients are shown in Supplementary Figure 1A, B. Serum EDN levels were significantly higher in patients with ACO (COPD) than in those with ACO (asthma). Serum EDN levels correlated with blood eosinophil counts in patients with asthma, ACO, and COPD (Fig. 2A). There was a weak correlation between serum EDN and total IgE in patients with asthma and between serum EDN and FeNO or YKL-40 levels in patients with COPD, but no correlation was observed in patients with ACO (Fig. 2B–D). There was a weak negative and positive correlation between serum EDN and FEV1/FVC in patients with ACO and patients with COPD, respectively. There also was a modest correlation between serum EDN and FEV1 in patients with COPD (Fig. 2E, F). There was no correlation between serum EDN and FEV1 in patients with asthma and ACO. Multivariate linear regression analysis revealed that higher eosinophil counts and higher YKL-40 levels were significantly associated with higher EDN levels (Supplementary Table 1). ICS or oral corticosteroid use did not affect the concentration of EDN. Based on cutoff values derived by receiver operating characteristics (ROC) analysis (EDN: 23.0 ng/mL; YKL-40: 61.3 ng/mL) (Supplementary Fig. 2), patients were classified into high or low groups. The proportion of patients with both high serum EDN and YKL-40 levels was significantly higher in ACO than in asthma or COPD: odds ratio, 3.85 (95% CI, 2.35–6.36); p < 0.001; sensitivity, 45.2%; specificity, 82.4% (Fig. 1B, C). The area under the curve of the ROC analysis for detecting ACO was significantly higher in serum EDN plus YKL-40 than in serum periostin plus YKL-40 (Supplementary Fig. 3).

This study found that serum EDN levels were higher in ACO than in asthma or COPD. Based on the multivariate linear
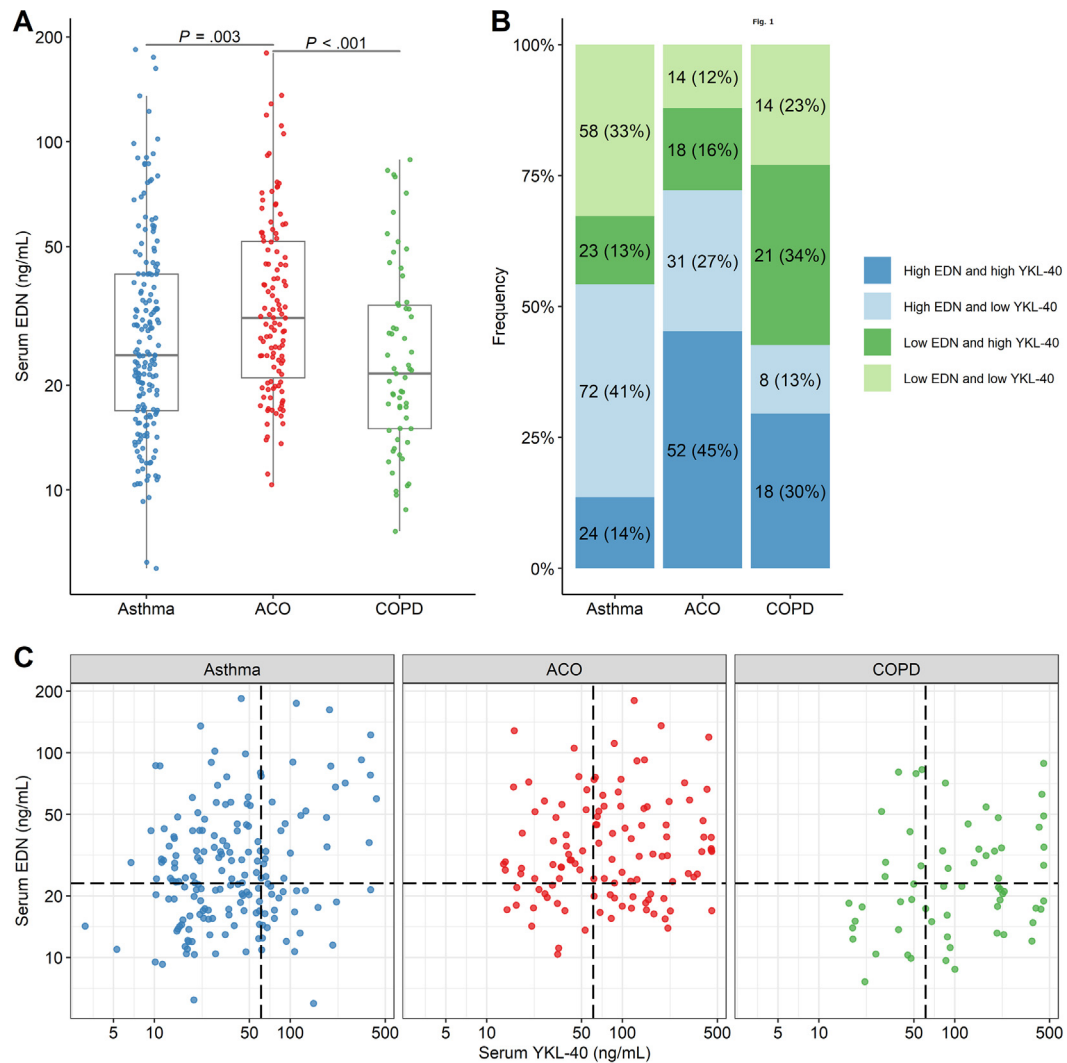
Fig. 1. Comparison of serum EDN among patients with asthma, ACO, and COPD **(A)**. Serum EDN was higher in ACO than in asthma or COPD. Proportions of patients classified as high or low levels of serum EDN (>23.0 ng/mL or ≤23.0 ng/mL) and YKL-40 (>61.3 ng/mL or ≤61.3 ng/mL) **(B)**. The proportion of patients with both high serum EDN and YKL-40 levels was significantly higher in ACO than in asthma or COPD. Scatter plots of serum EDN versus YKL-40 levels in patients with asthma, ACO, and COPD **(C)**. ACO, asthma-COPD overlap; COPD, chronic obstructive pulmonary disease; EDN, eosinophil-derived neurotoxin; YKL-40, chitinase-3-like protein 1.

regression analysis, this finding can be explained by the higher blood eosinophil counts in ACO than in asthma or COPD and higher serum YKL-40 levels in ACO than in asthma. Since EDN is a type 2 biomarker in asthma, the difference between ACO and COPD may be due to the shift toward type 2 inflammation in ACO. A previous study found a negative correlation between serum EDN and FEV1 in asthma, and a significant correlation between a decrease in serum EDN level from baseline and improvement in FEV1 after 8 weeks of omalizumab therapy.[2] This suggests serum EDN as a marker of persistent airflow limitation. However, a constant relationship between serum EDN and lung function was not observed in asthma, ACO, or COPD in this study.

ACO can be diagnosed without serum biomarkers. However, if we diagnose ACO from asthma patients, we have to perform computed tomography or measure diffusing capacity to assess comorbid emphysema. Those modalities are generally unavailable for general physicians in daily clinical practice. If we diagnose ACO from COPD, typical type 2 biomarkers, excluding sputum eosinophils, may be enough because Japanese ACO criteria, for example, consist of these markers. However, if more diagnostic serum biomarkers are available, it would be much helpful. We speculate that ACO has both conditions of type 2 inflammation of asthma as reflected by serum EDN and neutrophilic inflammation of COPD as reflected by serum YKL-40. However, the condition of ACO is heterogeneous with several phenotypes, including non-type 2 or non-eosinophilic asthma and non-emphysematous COPD. At present there is no serum biomarker to reflect effectiveness of drug such as ICS or prognosis of ACO. Thus, the findings of this study should be confirmed in other populations to facilitate more accurate identification of ACO and more targeted interventions for this disorder.
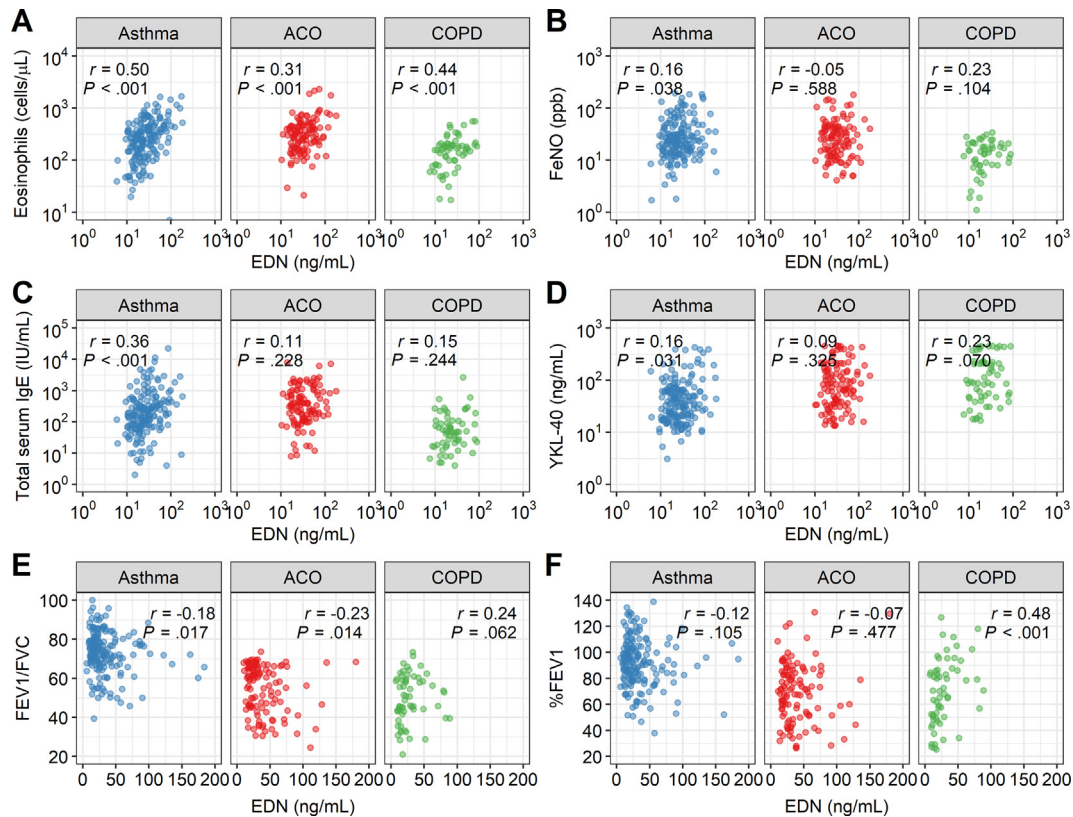
Fig. 2. Correlation between serum EDN and blood eosinophil counts **(A)**, FeNO **(B)**, total serum IgE **(C)**, serum YKL-40 **(D)**, FEV1/FVC **(E)**, and %FEV1 **(F)** in asthma, ACO, and COPD. ACO, asthma-COPD overlap; COPD, chronic obstructive pulmonary disease; EDN, eosinophil-derived neurotoxin; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; YKL-40, chitinase-3-like protein 1.

Overall, the combined assessment of serum EDN and YKL-40 led to the identification of ACO with high specificity despite low sensitivity. The diagnostic accuracy of this study using EDN was better than that of the combined assessment of serum periostin and YKL-40. Recent studies have raised concerns about the use of periostin as a type 2 biomarker.[9,10] Further studies are warranted to find a better type 2 biomarker in identifying ACO in clinical practice.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2020.05.007.

Conflict of interest
TS reports personal fees from AstraZeneca Japan outside of the submitted work. YG reports personal fees from AstraZeneca Japan and personal fees from Boehringer Ingelheim outside of the submitted work. The rest of the authors have no conflict of interest.

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