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Serum Galectin-10: A biomarker for persistent airflow limitation in adult asthmatics

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ABSTRACT

Background: Inhaled corticosteroids (ICS) are primary anti-inflammatory medications to control eosinophilic airway inflammation, and prevent asthma exacerbation. However, persistent airflow limitation (PAL) presents in some asthmatics even on ICS treatment, leading to lung function decline. Thus, we evaluated clinical associations of serum galectin-10 (Gal10) and galectin-3 (Gal3) levels in adult asthmatics who had maintained anti-asthma medication.

Methods: Sixty-seven asthmatics and 78 healthy controls (HCs) were recruited. Serum Gal10 and Gal3 levels were measured by enzyme-linked immunosorbent assay, and their clinical relevance with inflammatory and lung function parameters was evaluated. Spirometry was performed to assess PAL and small airway dysfunction (SAD). Airway epithelial cells were cocultured with eosinophils/neutrophils, and were exposed to house dust mites to assess the production of Gal10 and Gal3.

Results: Serum Gal10 (not Gal3) levels were significantly higher in asthmatics than in HCs ($P < 0.001$), in asthmatics with PAL than in those without PAL ($P = 0.005$), and in those with SAD than in those without SAD ($P = 0.004$). The Gal10-high group had significantly higher levels of peripheral $CD66^+$ neutrophil counts, serum periostin and Gal3, and lower values of FEV_1% and MMEF% than the Gal10-low group ($P < 0.050$ for all). The production of Gal10 and Gal3 was increased in eosinophilic airway model, while Gal10 (not Gal3) levels were increased in neutrophilic airway model as well as house dust mite stimulation.

Conclusion: Our findings suggest that serum Gal10 level may be a potential biomarker for PAL in adult asthmatics.

Keywords: Asthma, Galectin-10, Inflammation, Airway remodeling, Eosinophils, Neutrophils, Epithelial cells

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INTRODUCTION

Asthma is a heterogeneous airway inflammation with reversible airway obstruction and hyperresponsiveness to distinct pathogens (eg, allergens, air pollutants, and microbes).[1,2] Asthma phenotypes are clinically classified into type 2-high and type 2-low on the basis of blood/sputum eosinophil counts, fractional exhaled nitric oxide (FeNO), and serum total IgE levels.[3] Despite maintenance of anti-asthma medication, such as inhaled corticosteroids (ICS), long-acting beta-2 agonists (LABA), and/or leukotriene receptor antagonists (LTRA), asthmatics who have persistent airway inflammation with small airway dysfunction (SAD), lung function decline, or infection-related neutrophilic inflammation suffer from uncontrolled symptoms.[4,5] Persistent eosinophilic inflammation with the formation of eosinophil extracellular traps (EETs) affects airway epithelial cells (AECs), resulting in AEC dysfunction, persistent airflow limitation (PAL), and lung function decline.[6–8] In addition, neutrophil activation with the formation of neutrophil extracellular traps (NETs) further contribute to steroid insensitivity/uncontrolled asthma status.[9–11] Therefore, the identification of persistent airway inflammation and airflow limitation by using serum biomarkers would be beneficial in the management of chronic asthma.

Galectin-10 (Gal10) is a prototype of the galectin superfamily and is expressed in immune cells (eg, eosinophils, mast cells, basophils, and T lymphocytes).[12] Gal10 is a predominant protein expressed in human eosinophils, involving eosinophil-related diseases such as eosinophilic granulomatosis with polyangiitis (EGPA), asthma, and chronic rhinosinusitis (CRS).[13–15] The high levels of serum Gal10 and ratios of serum Gal10 to blood eosinophils were reported to be associated with EGPA activity, suggesting that serum Gal10 may play a potential role in predicting eosinophil activation.[13] Moreover, elevated levels of Gal10 were detected in nasal lavage fluid from patients with aspirin-exacerbated respiratory disease/aspirin-tolerant asthma before and after aspirin challenges, suggesting that Gal10 may be released from immune and/or structural cells (AECs and lung fibroblasts).[14] Additionally, Gal3 is a chimera type of the galectin superfamily and is primarily produced from structural cells.[16] According to an allergen-challenged mouse model, Gal3 knockout mice showed lower degrees of eosinophilic airway inflammation and remodeling than wild-type mice, suggesting a potential role of Gal3 in the development of allergic asthma.[17] Extracellular Gal3 has been reported to induce IgE cross-linking through binding to glycans expressed on IgE molecules.[18] Thus, the present study sought to evaluate: 1) clinical relevance of serum Gal10 and Gal3 levels with inflammatory parameters according to PAL and SAD in adult asthmatics who had maintained anti-asthmatic medication; and 2) the production of Gal10 and Gal3 from AECs co-cultured with eosinophils/neutrophils, or exposed to house dust mites (HDM).

MATERIALS AND METHODS

Study subjects

To assess clinical relevance of Gal10 in adult asthmatics, 78 healthy controls (HCs) and 67 asthmatic patients were recruited to measure serum Gal10 levels. Asthma was diagnosed according to the Global Initiative for Asthma (GINA) guideline; airway hyperresponsiveness was confirmed by methacholine bronchial challenge test, and reversible airway obstruction was observed after the inhalation of a short-acting β2-agonist.[3] All the asthmatic subjects had maintained anti-asthma medication, including the medium-to-higher doses of ICS plus LABA with/without LTRA, for more than 3 months prior to the study. Uncontrolled asthma status was assessed according to the GINA guideline.[3] CRS was diagnosed in terms of typical symptoms and sinus CT scan/sinus radiograph. All the subjects provided written informed consent. Serum and sputum samples were collected at the enrollment, and were kept at frozen status (−70 °C) until immunoassays as previously described.[4]

Atopy status was defined when subjects showed positive response to at least 1 inhalant allergen on the skin prick test using 55 common inhalant allergens. The ImmunoCAP system (ThermoFisher Scientific; Waltham, CA, USA) was used to assess serum total IgE levels. Total eosinophil count (TEC)

and Siglec8$^+$ eosinophil/CD66$^+$ neutrophil counts (flow cytometry analysis) were assessed from peripheral blood.[4] Sputum eosinophils, neutrophils, and inflammatory cytokines were assessed in the study.[19]

Lung function of asthmatics was assessed by spirometry with forced expiratory volume in first second (FEV_1), forced vital capacity (FVC), and maximal mid-expiratory flow (MMEF). PC20 methacholine value, which is the concentration of methacholine needed to produce a 20% decrease in FEV_1%, was calculated. Asthmatics with FEV_1 <80% and/or FEV_1/FVC <80% even on maintenance medication were identified with PAL (n = 20).[20,21] Asthmatics with MMEF <65% were identified with SAD (n = 29).[22]

Immune cell isolation and stimulation

Immune cells (neutrophils [Neu] and eosinophils [Eos]) were isolated from whole bloods to coculture with AECs or induce EETs and NETs. Cells were cultured in media containing phenol red-free RPMI-1640 supplemented with 2% fetal bovine serum (FBS), 100 U/mL penicillin G sodium, and 100 μg/mL streptomycin sulfate from Gibco (Grand Island, NY, USA). For the formation of EETs/NETs, Eos/Neu were stimulated with 100 nM phorbol 12-myristate 13-acetate (PMA) from Sigma-Aldrich (St. Louis, MO, USA) for 3 h as previously described.[23] Briefly, cells-free supernatants were removed, and cells were gently washed 3 times with phosphate buffered saline. Next, 1 mL phenol red-free RPMI-1640 supplemented with 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, and 1 U/mL micrococcal nuclease (ThermoFisher Scientific) was added and incubated for 20 min at 37 °C. The quantification of dsDNA in EETs and NETs was measured by a Quant-IT pico-green dsDNA kit (ThermoFisher Scientific) according to the manufacturer's instructions.

Treatment protocols of human AECs and immune cells

To assess the productions of Gal10 and Gal3, A549 cells (human alveolar type II epithelial cell line) (2 x 10^5 cells) were treated with EETs or NETs in a dose-dependent manner (0, 0.1, and 1 μg/mL), or co-cultured with Eos (5 × 10^5 cells) or Neu (1 × 10^6 cells) obtained from asthmatics for 24 h.[23] To assess the effects of *Dermatophagoides pteronyssinus* (Der p) on Gal10 and Gal3 production, A549 cells were stimulated with 20 μg/mL Der p from Prolagen (Yonsei University College of Medicine, Seoul, Korea) in a time-dependent manner (0, 12, 24, 48, and 72 h). A549 cells were cultured at 37 °C with 5% CO_2 in humidified air in complete media RPMI-1640 supplemented with 10% FBS, 100 U/mL penicillin G sodium, and 100 μg/mL streptomycin sulfate.

Enzyme-linked immunosorbent assay (ELISA)

Serum and sputum samples of the study subjects and supernatants *in vitro* studies were collected to measured markers by using human ELISA kits: human Gal10/Charcot Leyden Crystal protein from Novus Biologicals (Littleton, CO, USA); human Gal3, periostin, tissue inhibitor of metalloproteinase-1 (TIMP1), matrix metalloproteinase-9 (MMP9), and myeloperoxidase (MPO) ELISA kits from R&D systems (Minneapolis, MN, USA).

Western blot analysis

To detect protein expression of markers as follow: Gal10 (Abcam; Cambridge, MA, USA); E-cadherin and vimentin (Cell Signaling; Danvers, MA, USA); Gal3 and β-actin (Santa Cruz Biotechnology; Dallas, TX, USA) in cell lysis, cells were lysed with RIPA buffer containing protease/phosphatase inhibitor cocktail (Cell Signalling). The equal quantities of protein samples were separated by 10% SDS-PAGE, followed by transferred onto PVDF membranes (Sigma-Aldrich). Signals were developed and visualized by the ECL Western blotting substrate.

Immunofluorescence assay

To detect Gal10 expression in AECs in response to Der p, A549 cells were seeded on a 24-well plate and were stimulated with 20 μg/mL Der p in a time-dependent manner (0, 24, 48, and 72 h). After the incubation with primary antibody (rabbit anti-human Gal10 antibody). The cells were stained with secondary antibody (Alexa Fluor 488 donkey anti-rabbit) from Thermo Life Technology (Waltham, MA, USA) for 1 h at room temperature, followed by stained with 1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) from Abcam for 5 min.

For the detection of intracellular Gal10 by confocal microscope, Neu obtained from HCs were stimulated with 10 nM PMA in a time-dependent manner (0, 10, and 30 min). Cells were intracellularly stained with rabbit anti-human Gal10 and mouse anti-human neutrophil elastase (NE) antibodies (Santa Cruz Biotechnology), followed by stained with Alexa Fluor 488 donkey anti-rabbit and 594 donkey anti-mouse antibodies from Thermo Life Technology. Fluorescent images were acquired using confocal laser scanning microscopy at the Three-Dimensional Immune System Imaging Core Facility (LSM710, Cal Zeiss Microscopy GmbH; Jena, Germany).

Flow cytometry

For the detection of intracellular Gal10 by flow cytometry, Neu obtained from HCs were stimulated with 10 nM PMA in a time-dependent manner (0, 10, and 30 min). Cells were extracellularly stained with CD45, CD11b, and CD16 (BD Biosciences; San Diego, CA, USA) and intracellularly stained with rabbit anti-human Gal10 antibody for 30 min, followed by stained with APC-conjugated anti-rabbit antibody for 30 min eBioscience™ Intracellular Fixation & Permeabilization Buffer Set (ThermoFisher Scientific) was used in the process of the intracellular stain. Stained cells were analyzed with BD FACSCantoTM II (BD Bioscience), and graphs were produced using FlowJo Software version 10.8.1 (Ashland, Oregon, USA).

Statistical analysis

SPSS software version 22.0 (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. The graphs were created using the GraphPad Prism 8.0 software (GraphPad Inc.; San Diego, CA, USA). Fisher's exact tests were used for categorical variables. For continuous variables, the D'Agostino-Pearson omnibus tests were used to check normal distribution. For nonnormally distributed variables, the Mann–Whitney *U* tests and the Kruskal-Wallis tests were used to compare between groups. Spearman's rank correlation coefficients were calculated to examine correlations between continuous clinical variables. Receiver operating characteristic (ROC) curve analysis of serum Gal10 was performed to discriminate the PAL and SAD groups from the non-PAL and non-SAD groups, respectively. Significant differences were set at $P < 0.050$.

RESULTS

Clinical characteristics of the study subjects

A total of 67 adult asthmatics and 78 HCs were recruited. Demographics of the study subjects are shown in Supplementary Table 1. There were no differences in age and sex between the asthma and HC groups. Asthmatics had significantly higher prevalence rates of atopy and levels of serum total IgE than HCs ($P < 0.050$ for both). Median [interquartile range] of TEC in asthmatics was 301.6 [142.6 − 478.1] cells/μL.

Elevated levels of serum Gal10 in asthmatics with PAL

Serum Gal10 levels were higher in asthmatics (4.7 [3.7 − 7.0] ng/mL) than in HCs (2.9 [2.2 − 3.8] ng/mL, $P < 0.001$; **Fig. 1**A), but there were no differences in serum Gal3 levels (data not shown). We identified 67 adult asthmatics, of whom 20 (29.9%) fulfilled the criteria for PAL. Serum Gal10 levels were significantly higher in the PAL group than in the non-PAL group ($P = 0.005$; Fig. 1B), but no difference in serum Gal3 levels was observed ($P = 0.635$; Fig. 1C). The ROC curve analysis showed that serum Gal10 levels could discriminate the PAL group from the non-PAL group (area under the curve [AUC]: 0.719, sensitivity: 70%, specificity: 66%, $P = 0.005$; Fig. 1D). A negative correlation was found between serum Gal10 levels and FEV_1% ($r = -0.290$, $P = 0.017$; Fig. 1E).

Asthmatics with PAL were reported to have a distinct asthma phenotype.[21] Clinical characteristics of the asthmatic subjects according to PAL are summarized in Supplementary Table 2. The PAL group was older and had lower lung functions (FEV_1%, FVC%, FEV_1/FVC, and MMEF%) than the non-PAL group ($P < 0.010$ for all). Moreover, higher prevalence of uncontrolled asthma, higher serum periostin levels and peripheral $CD66^+$ neutrophil counts (%) ($P < 0.050$ for all) were observed in the

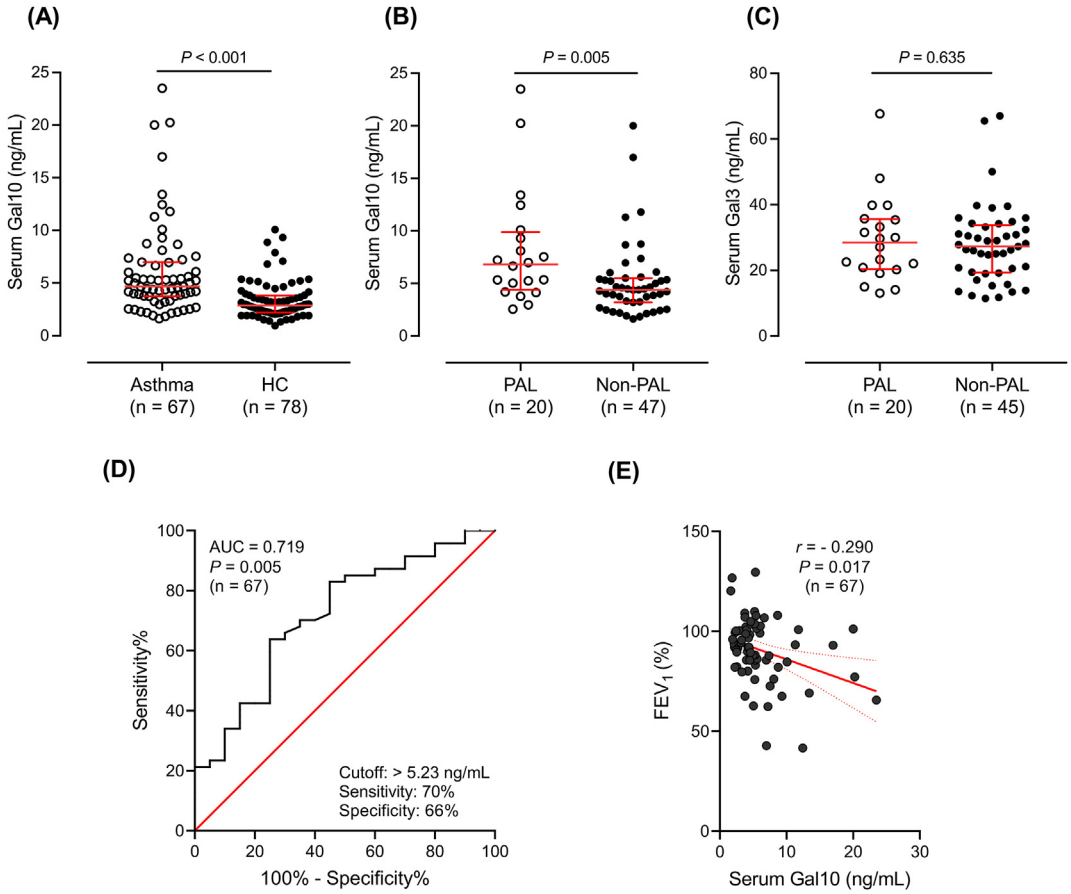

Fig. 1 Elevated levels of serum galectin-10 (Gal10) in adult asthmatics with persistent airflow limitation (PAL). Comparisons of serum Gal10 levels between the asthma and healthy control (HC) groups (A). Serum Gal10 (B) and galectin-3 (Gal3) (C) levels in asthmatic subjects according to PAL. Data are presented as median (interquartile range). *P* values were obtained by the Mann-Whitney *U* tests. Receiver operating characteristic curve (ROC) analysis of serum Gal10 levels for discriminating the PAL group from the non-PAL group (D). Spearman correlations between serum Gal10 levels and FEV_1% in asthmatics (E), depicted with coefficient *r* and *P* value. AUC, area under the ROC curve; FEV_1, forced expiratory volume in the first second

PAL group except for FeNO. PC20 methacholine levels tended to be lower in the PAL group (1.6 [0.8 – 4.2] mg/mL) than in the non-PAL group (3.1 [1.2 – 9.9] mg/mL, $P = 0.069$).

Elevated levels of serum Gal10 in asthmatics with SAD

Small airways exhibit an important role in asthma pathogenesis, whereas SAD has been reported to be from 50% to 90% in asthma.[22,24] In the present study, among asthmatics, 29 (43.3%) had SAD even on anti-asthma medication. Clinical characteristics of the asthmatic subjects according to SAD are shown in Supplementary Table 3. Patients with SAD were older ($P = 0.007$), and had lower PC20 methacholine values (1.6 [0.9 – 4.1] vs 3.4 [1.3 – 10.0] mg/mL, $P = 0.036$), and had higher prevalence rates of PAL (62.1% vs 5.3%, $P = 0.001$). Serum Gal10 levels were significantly higher in the SAD group than in the non-SAD group ($P = 0.004$; **Fig. 2**A), but no difference in serum Gal3 levels was noted ($P = 0.786$; Fig. 2B). The ROC curve analysis showed that serum Gal10 levels could discriminate the SAD group from the non-SAD group (AUC: 0.688, sensitivity: 72%, specificity: 66%, $P = 0.009$; Fig. 2C). A negative correlation was noted between serum Gal10 levels and MMEF% ($r = -0.428$, $P < 0.001$; Fig. 2D).

Clinical characteristics of asthmatics with elevated Gal10 levels

Asthmatics were classified into the Gal10-high and Gal10-low groups based on the cutoff point of serum Gal10 (6.95 ng/mL: mean plus 2 standard deviations) in the HC group. The comparison of

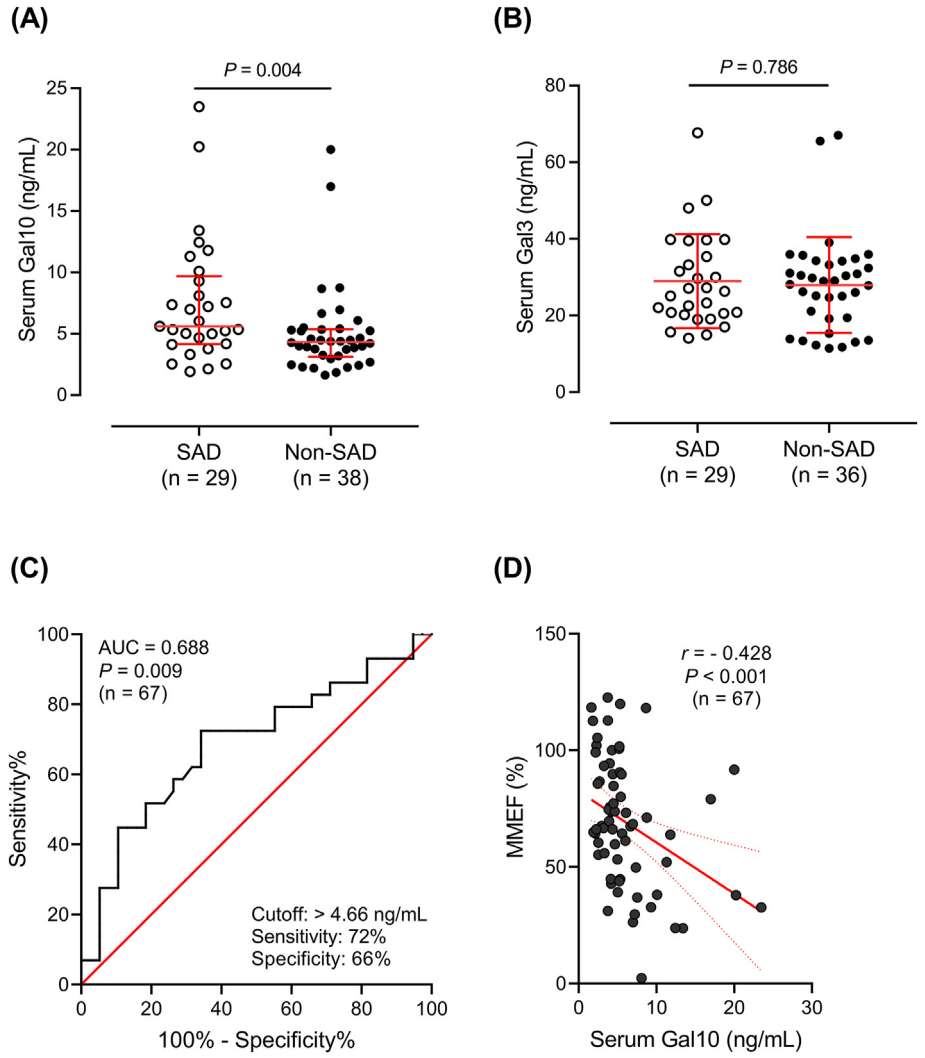

Fig. 2 Elevated levels of serum galectin-10 (Gal10) in adult asthmatics with small airway dysfunction (SAD). Serum Gal10 (A) and galectin-3 (Gal3) (B) levels in asthmatic subjects according to SAD. Data are presented as median (interquartile range). *P* values were obtained by the Mann-Whitney *U* tests. Receiver operating characteristic curve (ROC) analysis of serum Gal10 for discriminating the SAD group from the non-SAD group (C). Spearman correlations between serum Gal10 levels and MMEF% in asthmatics (D), depicted with coefficient *r* and *P* value. AUC, area under the ROC curve; MMEF, maximal mid-expiratory flow

clinical characteristics between the 2 groups are shown in **Table 1**. The Gal10-high group was older and had lower prevalence rates of atopy than the Gal10-low group ($P < 0.050$ for both), while no differences in smoking status, FeNO, total IgE, and TEC were noted. Moreover, the Gal10-high group had lower lung functions (FEV_1%, FVC%, FEV_1/FVC, MMEF%, and PC20 methacholine), and had higher levels of peripheral $CD66^+$ neutrophil counts (%), serum periostin levels, and serum Gal3 levels than the Gal10-low group ($P < 0.050$ for all). Serum Gal10 levels had positive correlations with peripheral $CD66^+$ neutrophil counts ($r = 0.386$, $P = 0.014$), serum periostin levels ($r = 0.267$, $P = 0.029$), and serum Gal3 levels ($r = 0.376$, $P = 0.002$) as shown in **Fig. 3**A–C.

Sputum samples were collected from 51 asthmatics to measure Gal10 and Gal3 levels (Supplementary Fig. 1). Sputum Gal10 levels were higher in the PAL group than in the non-PAL ($P = 0.007$; Supplementary Fig. 1A) and in the SAD group than in the non-SAD group ($P = 0.013$; Supplementary Fig. 1D), but no differences in the sputum Gal3 levels were noted (Supplementary Fig. 1B and E). Sputum Gal10 levels had negative correlations with FEV_1% ($r = -0.377$, $P = 0.006$) and MMEF%

Variables	Gal10-high[a] (n = 17)	Gal10-low[a] (n = 50)	*P* value
Age (years)	63.0 (57.8–73.8)	43.5 (30.3–54.0)	0.001
Female, sex (n, %)	10/17 (58.8%)	34/50 (68.0%)	0.491
Atopy (n, %)	7/17 (41.2%)	36/50 (72.0%)	0.021
Ex-smokers (n, %)	11/17 (64.7%)	44/50 (88.0%)	0.061
Chronic rhinosinusitis (n, %)	14/17 (82.4%)	21/50 (42.0%)	0.004
Uncontrolled asthma (n, %)	4/17 (23.5%)	4/50 (8.0%)	0.088
Baseline FEV_1 (%)	79.1 (66.1–93.2)	93.8 (85.9–101.9)	0.001
Baseline FVC (%)	83.4 (78.6–92.9)	93.7 (85.4–102.6)	0.003
FEV_1/FVC (%)	75.1 (69.7–84.4)	85.2 (79.6–92.6)	0.003
MMEF (%)	37.5 (27.1–69.3)	73.8 (60.8–93.9)	0.001
PC20 methacholine (mg/mL)	1.1 (0.9–4.8)	3.1 (1.8–9.8)	0.039
FeNO (ppb)	14.5 (5.8–44.0)	19.0 (10.5–42.0)	0.400
Total IgE (kU/L)	301.5 (111.8–610.8)	180.5 (43.8–558.8)	0.258
TEC (cells/μL)	240.1 (148.2–471.0)	304.0 (132.6–494.7)	0.802
Sputum eosinophils (%)	1.0 (0.0–33.0)	15.0 (0.0–46.5)	0.889
Sputum neutrophils (%)	85.0 (38.5–93.5)	73.0 (45.5–90.5)	0.535
$Siglec8^+$ eosinophils (%)	6.5 (4.6–12.1)	6.9 (2.4–12.6)	0.577
$CD66^+$ neutrophils (%)	37.9 (28.1–95.5)	29.6 (25.1–39.1)	0.043
Serum periostin (ng/mL)	79.0 (48.7–97.4)	67.5 (53.0–86.4)	0.041
Serum Gal3 (ng/mL)	34.8 (23.4–39.7)	25.7 (19.0–31.0)	0.020
Serum MPO (ng/mL)	399.2 (332.6–607.4)	317.9 (215.2–630.1)	0.183

Table 1. Clinical characteristics of asthmatic subjects according to serum Gal10 levels. *Note: Data are expressed as median (interquartile range) for continuous variables and as number (%) for categorical variables. The comparisons between 2 groups were corrected by Mann-Whitney U tests and Fisher's exact tests for continuous and categorical variables, respectively. Abbreviations: FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; MMEF, maximal mid-expiratory flow; PC20 methacholine, the concentration of methacholine needed to produce a 20% decrease in FEV_1%; TEC, total eosinophil count. [a]Gal10-high and Gal10-low groups were identified at cutoff point at 6.95 ng/mL (mean plus 2 standard deviation of serum Gal10 in the healthy control group)*

($r = -0.300$, $P = 0.033$) (Supplementary Fig. 1C and F) and positive correlations with sputum levels of TIMP1 ($r = 0.515$, $P < 0.001$), MMP9 ($r = 0.485$, $P < 0.001$), and $CD66^+$ neutrophil counts (%) ($r = 0.465$, $P = 0.001$; Supplementary Fig. 1G–I).

Gal10 production *in vitro* settings

When A549 cells were cocultured with Eos or stimulated with EETs, Gal10 levels was increased (Fig. 4A). Protein expression of Gal10 was increased in A549 cells in response to EETs (Fig. 4E), but no change in NETs-stimulated A549 was noted (Fig. 4F). Low Gal10 levels were detected in the component of NETs (Fig. 4B), while Gal10 levels did not change from NETs-stimulated A549 cells. Significantly higher values of $Gal10^{high}$-expressing Neu (%) and Gal10 (but not Gal3) levels released from Neu in response to PMA were noted (Supplementary Fig. 2A–E).

Meanwhile, Gal3 is primarily released from structural cells (eg, fibroblast and epithelium).[16]

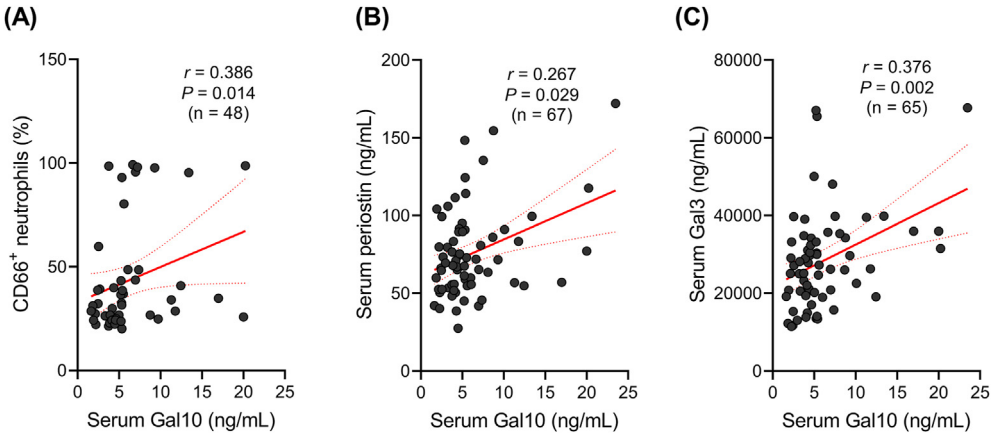

Fig. 3 Correlations between serum galectin-10 (Gal10) levels and inflammatory biomarkers in asthmatic subjects. Spearman correlations between serum Gal10 levels and peripheral CD66$^+$ neutrophil counts (A), serum periostin levels (B) and serum galectin-3 (Gal3) levels (C) in asthmatics, depicted with coefficient *r* and *P* values

The release of Gal3 from A549 cells cocultured with Eos and stimulated with EETs were increased (**Fig. 4**C). However, Gal3 levels released from A549 in coculture with Neu or in stimulation with NETs were decreased (Fig. 4D), which was consistent with Gal3 protein expression in NETs-stimulated A549 (Fig. 4F).

NETs and EETs function as an anti-microbial agent through releasing peptides, proteases, and reactive oxygen species.[25] NETs contain high levels of serine proteases, which are absent or not functionally expressed in EETs.[25] In addition, Der p contains allergens functioning as serine proteases (Der p3 and Der p9) and activates AECs through protease-activated receptor 2.[26] The present study demonstrated that Gal10 production from A549 cells in response to Der p was increased in a time-dependent manner (0, 24, 48, and 72 h) (Fig. 5A-C), but Gal3 levels were decreased (**Fig. 5**D).

DISCUSSION

The present study demonstrates that one-third of asthmatic subjects displayed PAL even on maintenance medications including medium-to-high doses of ICS. Patients with PAL exhibited distinct features, including a higher prevalence of uncontrolled asthma, reduced lung function, elevated peripheral CD66$^+$ neutrophil counts, and elevated serum periostin levels. Moreover, asthmatic subjects with PAL had higher Gal10 levels in serum and sputum samples. The *in vitro* experiments unveiled that Gal10 is released from AECs in response to HDM exposure as well as through interactions with Eos/Neu. These findings propose that elevated serum Gal10 could potentially serve as a valuable biomarker for identifying PAL in adult asthmatics.

Emerging evidence suggests that the presence of persistently activated eosinophils and their mediators within the airways are hallmarks of PAL.[27] Eosinophil-derived neurotoxin acts as a selective chemotactic factor for dendritic cells, leading to their activation and subsequent release of proinflammatory cytokines.[28] Increased EETs released from activated eosinophils induced the disruption of tight junction-related proteins and alarmin (such as IL33 and thymic stromal lymphopoietin [TSLP]) production from AECs, enhancing type 2 airway inflammation in patients with severe asthma.[8] Gal10 was shown to act as an adjuvant in its crystallized form, amplifying type 2 airway inflammation in mouse models of ovalbumin-induced asthma.[29,30] Periostin that is released from AECs in response to interleukin-13 (IL13) and transforming growth factor beta-1 (TGFβ1) acts as a biomarker for type 2 inflammation with a close correlation with airway remodeling in asthma.[27] The present study showed the production of Gal10 from AECs in response to eosinophils/EETs. Serum Gal10 levels were positively correlated with periostin levels, but were negatively correlated with FEV_1%. These findings suggest that serum Gal10 may play a potential role in airway remodeling in asthmatic airways.

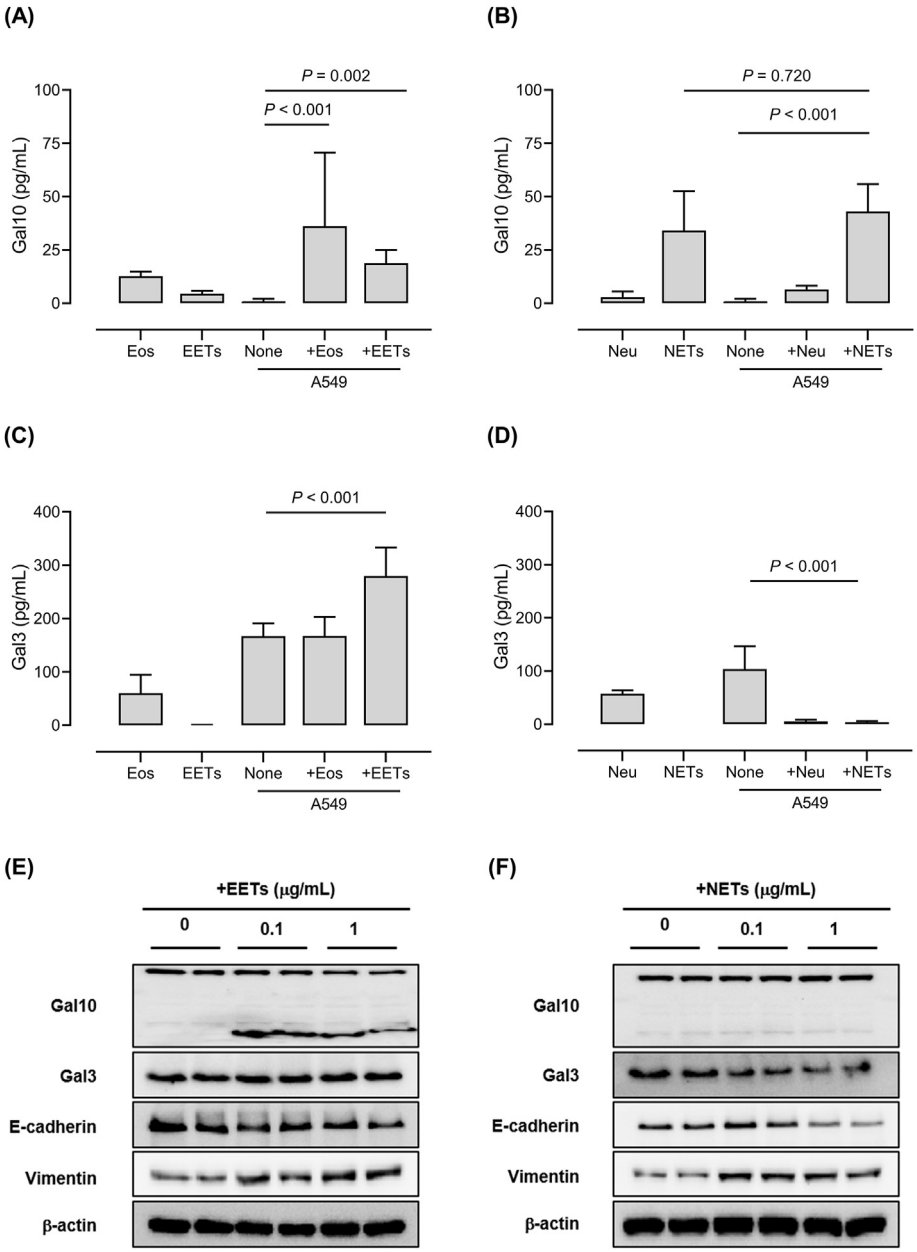

Fig. 4 The production of galectin-10 (Gal10) and galectin-3 (Gal3) upon eosinophilic and neutrophilic airway inflammation. The levels of Gal10 released from A549 cells in coculture with Eos/Neu or in response to EETs/NETs (n = 6 for each group) (A–B). The levels of Gal3 released from A549 in coculture with Eos/Neu or in response to EETs/NETs (n = 6 for each group) (C–D). Data are presented as geometric mean with 95% CI. *P* values were obtained by the Kruskal-Wallis tests. The protein expression of Gal10, Gal3, E-cadherin and Vimentin in A549 cells in response to EETs and NETs, respectively (E–F). Eosinophils, Eos; Eosinophil extracellular traps, EETs; Neutrophils, Neu; Neutrophil extracellular traps, NETs; PMA, phorbol 12-myristate 13-acetate

In addition to eosinophils, neutrophil migration and activation may play a role in the pathophysiological mechanisms of PAL. The chronic activation of neutrophils could induce airway obstruction and increase airway hyperresponsiveness in patients with severe asthma.[31] Our findings align with previous studies, showing that asthmatic subjects with PAL had elevated blood $CD66^{+}$ neutrophils even on anti-inflammatory medications including ICS.[4,20,21] Neutrophils strengthen their impacts by releasing NETs (composed of DNA-histone complexes and granule proteins) and contribute to steroid resistance, AEC damage, proinflammatory cytokines release from AECs, and lead to eosinophil activation, thereby promoting both eosinophilic and neutrophilic inflammation in patients with uncontrolled asthma.[4,32] Noteworthy, Gal10 (in its crystallized form) could influence NET formation via 2 primary

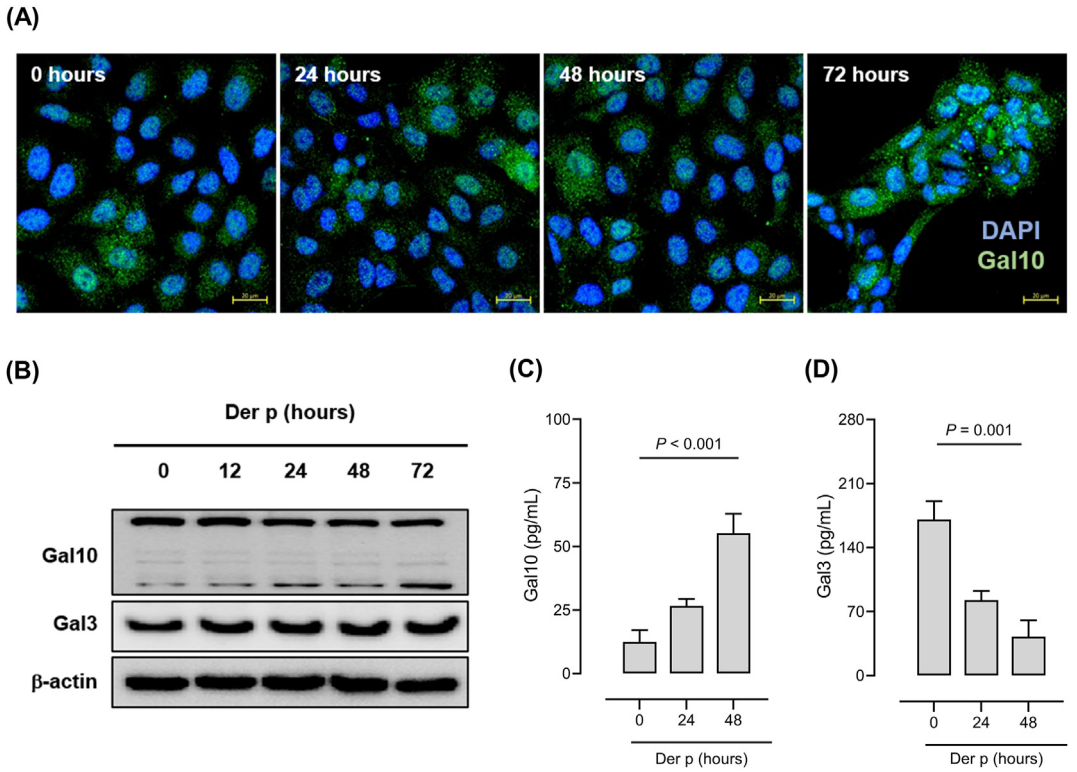

Fig. 5 Galectin-10 (Gal10) expression in human airway epithelial cells (AECs) in response to house dust mite. The intracellular expression of Gal10 in A549 cells in response to Der p, evaluated by confocal microscopy with DAPI (nuclear, blue) and Gal10 immunofluorescence (green) staining (A). The protein expression of Gal10 and galectin-3 (Gal3) in A549 cells in response to Der p in a time-dependent manner (0, 12, 24, 48, and 72 h), evaluated by Western blot analysis (B). The levels of Gal10 (C) and Gal3 (D) released from A549 cells in response to Der p (n = 6 for each group). Data are presented as geometric mean with 95% CI. *P* values were obtained by the Kruskal-Wallis tests. DAPI, 4′,6-diamidino-2-phenylindole; Der p, *Dermatophagoides pteronyssinus*.

pathways: 1) directly activating neutrophils to release NETs, and 2) promoting NLRP3 inflammasome activation, leading to IL1β accumulation in human macrophages.[31,33] Our study further revealed the presence of Gal10 within NET components. Taken together, the interplay between Gal10 and neutrophils/NETs may contribute to development of PAL in adult asthmatics.

Gal3, a member of the galectin family, has the ability to bind β-galactoside sugars on glycoproteins.[34,35] Gal3, primarily secreted by IL4/IL13-stimulated activated macrophages, is assumed to play a multifaceted role in the development of asthma.[36] Specifically, Gal3 serves as a chemoattractant, recruiting monocytes and macrophages, while supporting the survival of adaptive immune cells like $CD4^+$ T cells and activated B cells.[17,34,37,38] Furthermore, Gal3 is associated with epithelial-mesenchymal transition (EMT), a process that transforms epithelial cells into mesenchymal-like cells, contributing to airway remodeling.[17] In addition, Gal3 overproduction triggers M2-like macrophage differentiation, resulting in the production of TGFβ and arginine.[17] Extracellular Gal3 could also induce the EMT process by facilitating the nuclear translocation of β-catenin.[39] Here, we found that EETs are key factors to induce the release of Gal3 from AECs *in vitro*, indicating that the Gal3-EETs axis may amplify eosinophilic inflammation and airway remodeling in asthma. Considering that intracellular Gal3 might exerts anti-apoptotic effects by interacting with mitochondrial apoptosis regulators; NETs induce AEC death and detachment.[34,39] Therefore, reduced expression of Gal3 within AECs may be derived from NET production in the present study. Taken together, Gal3 opens up exciting possibilities for novel therapeutic interventions in patients with uncontrolled asthma.

While asthma has traditionally been associated with airflow obstruction and inflammation in large airways and there has been a close association between PAL and SAD, recent reports suggest a relationship between SAD and poor clinical outcome of asthma.[22,40] SAD could be the consequence of small airway obstruction associated with chronic airway inflammation.[22] However, direct relationships between SAD and clinical features of asthma did not show consistent results.[40] There has been a study showing no association between SAD and asthmatic symptoms,[41] while the others reported an association with worse asthma control and more severe exacerbations.[22,40] That may be derived from different methods applied (impulse oscillometry and spirometry), effects of anti-inflammatory medications used or differences in study subjects. The present study showed significant associations between SAD and lower lung function parameters (lower FEV_1%/PC20 methacholine values). Higher serum/sputum Gal10 levels were noted in patients with SAD. These findings suggest that Gal10 could potentially serve as a predictor of airway obstruction in both small and large airways in asthma.

The present study has a few limitations. First, this study was done in a smaller sample size based on a single tertiary center; additional replication studies are required in larger and multicenter cohorts. Secondly, direct correlations between serum/sputum Gal10 levels and blood/sputum eosinophil counts or FeNO values were not observed. Longitudinal studies with monitoring serum Gal10 levels are required. Thirdly, the relationship between neutrophils and Gal10 release in the form of Charcot-Leyden crystals are still unclear, requiring further investigations. Despite these challenges, our study highlights the significant contribution of Gal10 to the pathophysiology of PAL.

In conclusion, the results suggest that serum Gal10 level may be a potential biomarker for PAL. Additional studies on potential controllers are required to completely modulate Gal10 production.

Abbreviations

AECs: airway epithelial cells; AERD: aspirin-exacerbated respiratory disease; AUC: area under the curve; CRS: chronic rhinosinusitis; DAPI: 4′,6-diamidino-2-phenylindole; Der p: *Dermatophagoides pteronyssinus*; EETs: eosinophil extracellular traps; EGPA: eosinophilic granulomatosis with polyangiitis: Eos: eosinophils; ETs: extracellular traps; FBS: fetal bovine serum; FeNO: fractional exhaled nitric oxide; FEV_1: forced expiratory volume in first second; FVC: forced vital capacity; Gal: galectin; HCs: healthy controls; HDM: house dust mites; ICS: inhaled corticosteroid; IL: interleukin; LABA: long-acting beta-2 agonists; LTRA: leukotriene receptor antagonists; MBP: eosinophil major basic protein; MMEF: maximal mid-expiratory flow; MMP9: matrix metalloproteinase-9; MPO: myeloperoxidase; NE: neutrophil elastase; NETs: neutrophil extracellular traps; Neu: neutrophils; PAL: persistent airflow limitation; PMA: phorbol 12-myristate 13-acetate; ROC: receiver operating characteristic; SAD: small airway dysfunction; TEC: total eosinophil count; TGFb1: transforming growth factor beta-1; TIMP1: tissue inhibitor of metalloproteinase-1.

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Availability of materials and data

The data that support the findings of the study are available in the supplementary material of this article.

Author contributions

Thi Bich Tra Cao analyzed clinical results, conducted *in vitro* experiments and wrote the first draft of manuscript. Quang Luu Quoc analyzed clinical results and revised the manuscript. Jae-Hyuk Jang, Min Sook Ryu, and Youngwoo Choi collected clinical data and samples. Eun-Mi Yang measured serum and sputum levels of galectin-10 and galectin-3. Hae-Sim Park supervised all the process.

Ethics approval

This study was approved by the Institutional Review Board of Ajou University Hospital (AJIRB-BMR-SUR-15-498).

Consent to publish

All authors agree to publish this manuscript in World Allergy Organization Journal.

Declaration of competing interest

The authors confirmed that there are no financial or other issues that might lead to conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.100955.

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REFERENCES

1. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: eosinophilic airway inflammation in nonallergic asthma. *Nat Med*. 2013;19(8):977–979.
2. Agache I, Eguiluz Gracia I, Cojanu C, et al. Advances and highlights in asthma in 2021. *Allergy*. 2021;76(11):3390–3407.
3. 2022 GINA report. Global strategy for asthma management and prevention. https://ginasthma.org/gina-reports/; 2022. Accessed December 22, 2023.
4. Kim SH, Jung HW, Kim M, et al. Ceramide/sphingosine-1-phosphate imbalance is associated with distinct inflammatory phenotypes of uncontrolled asthma. *Allergy*. 2020;75(8):1991–2004.
5. Jung CG, Cao TBT, Quoc QL, Yang EM, Ban GY, Park HS. Role of club cell 16-kDa secretory protein in asthmatic airways. *Clin Exp Allergy*. 2023;53(6):648–658.
6. Hellings PW, Steelant B. Epithelial barriers in allergy and asthma. *J Allergy Clin Immunol*. 2020;145(6):1499–1509.
7. Choi Y, Lee DH, Trinh HKT, et al. Surfactant protein D alleviates eosinophil-mediated airway inflammation and remodeling in patients with aspirin-exacerbated respiratory disease. *Allergy*. 2019;74(1):78–88.
8. Choi Y, Kim YM, Lee HR, et al. Eosinophil extracellular traps activate type 2 innate lymphoid cells through stimulating airway epithelium in severe asthma. *Allergy*. 2020;75(1):95–103.
9. Park SY, Kang SY, Song WJ, Kim JH. Evolving concept of severe asthma: transition from diagnosis to treatable traits. *Allergy Asthma Immunol Res*. 2022;14(5):447–464.
10. Lee JH, Cho YS. Eosinophil extracellular traps pave the way for the identification of novel therapeutics in severe asthma. *Allergy Asthma Immunol Res*. 2022;14(5):441–443.
11. Bich TCT, Quoc QL, Choi Y, et al. Serum Amyloid A1: a biomarker for neutrophilic airway inflammation in adult asthmatic patients. *Allergy Asthma Immunol Res*. 2022;14(1):40–58.
12. Liu FT, Stowell SR. The role of galectins in immunity and infection. *Nat Rev Immunol*. 2023;23(8):479–494.
13. Fukuchi M, Kamide Y, Ueki S, et al. Eosinophil ETosis-mediated release of galectin-10 in eosinophilic granulomatosis with polyangiitis. *Arthritis Rheumatol*. 2021;73(9):1683–1693.
14. Negrete Garcia MC, Jiménez Torres CY, Alvarado Vásquez N, et al. Galectin-10 is released in the nasal lavage fluid of patients with aspirin-sensitive respiratory disease. *Sci World J*. 2012;2012, 474020.
15. Gelardi M, Netti GS, Giancaspro R, et al. Chronic rhinosinusitis with nasal polyposis (CRSwNP): the correlation between expression of Galectin-10 and Clinical-Cytological Grading (CCG). *Am J Rhinol Allergy*. 2022;36(2):229–237.
16. Slack RJ, Mills R, Mackinnon AC. The therapeutic potential of galectin-3 inhibition in fibrotic disease. *Int J Biochem Cell Biol*. 2021;130, 105881.
17. Ge XN, Bahaie NS, Kang BN, et al. Allergen-induced airway remodeling is impaired in galectin-3-deficient mice. *J Immunol*. 2010;185(2):1205–1214.
18. Schroeder JT, Adeosun AA, Do D, Bieneman AP. Galectin-3 is essential for IgE-dependent activation of human basophils by A549 lung epithelial cells. *J Allergy Clin Immunol*. 2019;144(1):312–315.e1.
19. Luu QQ, Cao TBT, Shin YS, Yang EM, Moon JY, Park HS. Sputum antinuclear antibody serves as a biomarker for severe asthma. *Allergy*. 2021;76(12):3832–3835.
20. Bumbacea D, Campbell D, Nguyen L, et al. Parameters associated with persistent airflow obstruction in chronic severe asthma. *Eur Respir J*. 2004;24(1):122–128.
21. Konstantellou E, Papaioannou AI, Loukides S, et al. Persistent airflow obstruction in patients with asthma: characteristics of a distinct clinical phenotype. *Respir Med*. 2015;109(11):1404–1409.
22. Postma DS, Brightling C, Baldi S, et al. Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study. *Lancet Respir Med*. 2019;7(5):402–416.
23. Cao TBT, Quoc QL, Yang EM, et al. Tissue inhibitor of metalloproteinase-1 enhances eosinophilic airway inflammation in severe asthma. *Allergy Asthma Immunol Res*. 2023;15(4):451–472.
24. Pornsuriyasak P, Khiawwan S, Rattanasiri S, Unwanatham N, Petnak T. Prevalence of small airways dysfunction in asthma with- and without-fixed airflow obstruction and chronic obstructive pulmonary disease. *Asian Pac J Allergy Immunol*. 2021;39(4):296–303.
25. Sprenkeler EGG, Goetschalckx I, Fernández Hermira S, et al. Lack of eosinophil extracellular trap formation due to failure of plasma membrane breakdown in the absence of elastase. *Blood Adv*. 2023;7(19):5868–5876.
26. Sun G, Stacey MA, Schmidt M, Mori L, Mattoli S. Interaction of mite allergens Der p3 and Der p9 with protease-activated receptor-2 expressed by lung epithelial cells. *J Immunol*. 2001;167(2):1014–1021.
27. Lee Y, Quoc QL, Park HS. Biomarkers for severe asthma: lessons from longitudinal cohort studies. *Allergy Asthma Immunol Res*. 2021;13(3):375–389.
28. Tota M, Łacwik J, Laska J, Sędek Ł, Gomułka K. The role of eosinophil-derived neurotoxin and vascular endothelial growth factor in the pathogenesis of eosinophilic asthma. *Cells*. 2023;12(9):1326.
29. Persson EK, Verstraete K, Heyndrickx I, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science*. 2019;364(6442).
30. Rodríguez Alcázar JF, Ataide MA, Engels G, et al. Charcot-Leyden crystals activate the NLRP3 inflammasome and cause IL-1β inflammation in human macrophages. *J Immunol*. 2019;202(2):550–558.

31. Shaw DE, Sousa AR, Fowler SJ, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J*. 2015;46(5):1308–1321.
32. Pham DL, Ban GY, Kim SH, et al. Neutrophil autophagy and extracellular DNA traps contribute to airway inflammation in severe asthma. *Clin Exp Allergy*. 2017;47(1):57–70.
33. Gevaert E, Delemarre T, De Volder J, et al. Charcot-Leyden crystals promote neutrophilic inflammation in patients with nasal polyposis. *J Allergy Clin Immunol*. 2020;145(1):427–430.e4.
34. Nangia Makker P, Nakahara S, Hogan V, Raz A. Galectin-3 in apoptosis, a novel therapeutic target. *J Bioenerg Biomembr*. 2007;39(1):79–84.
35. Gupta A. Galectin-3: a cancer marker with therapeutic applications. In: Gupta GS, ed. *Animal Lectins: Form, Function and Clinical Applications*. Vienna: Springer Vienna; 2012:291–310.
36. MacKinnon AC, Farnworth SL, Hodkinson PS, et al. Regulation of alternative macrophage activation by galectin-3. *J Immunol*. 2008;180(4):2650–2658.
37. Sano H, Hsu DK, Yu L, et al. Human galectin-3 is a novel chemoattractant for monocytes and macrophages. *J Immunol*. 2000;165(4):2156–2164.
38. de Oliveira FL, Gatto M, Bassi N, et al. Galectin-3 in autoimmunity and autoimmune diseases. *Exp Biol Med*. 2015;240(8):1019–1028.
39. Perez Moreno E, Oyanadel C, de la Peña A, et al. Galectins in epithelial-mesenchymal transition: roles and mechanisms contributing to tissue repair, fibrosis and cancer metastasis. *Biol Res*. 2024;57(1):14.
40. Cottini M, Lombardi C, Comberiati P, Landi M, Berti A. Small airway dysfunction and impulse oscillometry in adult patients with asthma: recent findings. *Explor asthma allergy*. 2023;1(4):163–173.
41. van der Wiel E, Postma DS, van der Molen T, Schiphof Godart L, ten Hacken NHT, van den Berge M. Effects of small airway dysfunction on the clinical expression of asthma: a focus on asthma symptoms and bronchial hyper-responsiveness. *Allergy*. 2014;69(12):1681–1688.