

ORIGINAL ARTICLE

Serum lactoferrin level as a serologic biomarker for allergic rhinitis

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Summary

Background Allergic rhinitis (AR) is a very common disease and a risk factor for allergic asthma. The discovery of new biomarkers for the early detection of AR would improve the clinical outcomes and reduce socio-economic burden. We sought to identify a novel serologic marker for detection of AR using a proteomic approach.

Methods To identify the proteins involved in AR, comparative proteomics was applied using nasal lavage fluids (NLFs) taken before and after a nasal provocation test (NPT) with *Dermatophagoides pteronyssinus* (*Dpt*) in a subject with AR sensitized to *Dpt*. The clinical relevance of the identified proteins was evaluated by ELISA using NLFs and sera from the three study groups: *Dpt*-sensitive AR; asymptomatic *Dpt*-sensitive controls; and non-atopic healthy controls. The sensitivities and specificities of the candidate proteins for predicting AR were determined using receiver operating characteristic (ROC) curves.

Results In proteomic analysis, lactoferrin expression was up-regulated after NPT. The validation study using ELISA showed a significantly lower serum lactoferrin level in the AR group than those of the other two groups ($P < 0.05$, respectively). To discriminate between subjects with or without AR, the optimal serum cut-off level of lactoferrin was set at < 307 ng/mL using the ROC curve. The sensitivity and specificity for predicting AR were 81.4% and 58%. When combined with serum *Dpt*-specific IgE level, the sensitivity and specificity for predicting AR were 76.7% and 79.2%.

Conclusion These results suggest that the serum lactoferrin level is associated with the phenotype of *Dpt*-sensitive AR, and in combination with the serum *Dpt*-specific IgE level, may be a potential serologic marker for early detection of AR.

Key words allergic rhinitis, biomarker, lactoferrin, proteomics

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Introduction

Allergic rhinitis (AR) is a very common disease with significant morbidity. A conservative estimate suggests that more than 500 million people are afflicted with AR worldwide [1–3], and the prevalence of AR is expected to increase. Therefore, AR has a profound socio-economic impact, incurring substantial direct medical costs and considerable indirect costs [1, 4–6]. Moreover, previous studies have demonstrated that AR is a risk factor for allergic asthma and that rhinitis appears before or in the very early stages of asthma [1, 7], which suggests that early detection of AR might produce a better clinical outcome.

Generally, the diagnosis of AR is based on a clinical history of symptoms [1, 3]. However, sneezing and a

runny nose often occur as related to viral infection, not AR [8]. In this case, it is difficult to diagnose AR and it was sometimes delayed. Although skin prick tests (SPTs) and measurements of allergen-specific IgE may help to diagnose AR, these tests cannot discriminate AR from asymptomatic sensitized controls. Besides, the nasal provocation test (NPT) with an allergen, a method to definitively establish the diagnosis in sensitized subjects [9], is clinically impractical for most community clinicians and it is rarely performed because it requires special equipment and training as well as the procedure is invasive. Therefore, a serologic biomarker that can be assayed rapidly and simply for the detection of AR is required.

Proteomics involves the purification and identification of individual proteins from all the proteins expressed in a cell or a tissue [10]. Changes in the nasal lavage fluids

(NLFs) protein profile following NPT with *Dermatophagoides pteronyssinus* (*Dpt*) in the patient with AR provide new insights into the mechanisms of nasal allergic mucosal inflammation, as the house dust mite is the most important indoor allergen inducing sensitization and the development of AR.

In the present study, we need to identify a new serologic biomarker for the diagnosis of AR using a proteomic approach in NLFs and to validate a candidate biomarker, lactoferrin, in both NLFs and sera of individual subjects.

Methods

Study subjects

In total, 117 subjects who attended our clinic were enrolled in the present study. The subjects were divided into three groups: *Dpt*-sensitive AR (group I; $n = 43$); asymptomatic *Dpt*-sensitive controls (group II; $n = 22$); and non-atopic healthy controls (group III; $n = 52$). All subjects were given an NPT with *Dpt* allergen and monitored for nasal symptoms (e.g. rhinorrhoea, nasal itching, nasal obstruction, and sneezing). The subjects in group I had a history of rhinitis and a positive response to NPT with *Dpt*, while the subjects in group II were sensitized to *Dpt*, but showed a negative response to NPT with *Dpt*. The group III subjects were non-atopic healthy volunteers with a negative response to NPT. Individuals with chronic sinusitis and/or nasal polyposis, respiratory infection during the previous 4 weeks (purulent sputum or rhinorrhoea, fever), or a history of immunotherapy were excluded from the present study. None of the subjects had received any antihistamine, leukotriene receptor antagonist, or topical steroid for 4 weeks before the study. This study was reviewed and approved by the Institutional Review Board of Ajou Medical Center, Suwon, Korea, and informed consent was obtained from each study subject.

Skin prick test and assays for total immunoglobulin E and *Dpt*-specific immunoglobulin E

The SPT, which included nine common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat fur, dog fur, tree mixture, grass mixture, mugwort, ragweed, and *Alternaria*; Bencard, Bradford, UK), was performed on all the subjects. The result of each test was reported as the A/H ratio (the mean wheal diameter for the allergen compared with that for histamine). The serum levels of total IgE and *Dpt*-specific IgE were measured by fluoroimmunoassay using the Pharmacia CAP system (Pharmacia-Upjohn, Uppsala, Sweden). Atopy was defined as an A/H ratio > 1 to the common inhalant allergens on the SPT or positive *Dpt*-specific IgE.

Nasal provocation test and nasal lavage

After anterior rhinoscopy, NPT with nasal lavage was performed according to the previously described methods [11]. In brief, the subjects were placed at room temperature for 30 min in the morning, to minimize the effects of daily-life stimuli, and then given a saline challenge, to exclude nasal hyper-reactivity. In the absence of non-specific hyper-responsiveness, NPT was performed by applying an 8 mm filter paper disk that was impregnated with the allergen solution (5000 BU/ μ L *Dpt*; prepared by Allergopharma, Reinbek, Germany) to the anterior tip of the inferior turbinate on the wider side of the nose for 10 min or until the appearance of allergic symptoms. Nasal symptom scores for rhinorrhoea, nasal itching, nasal obstruction, and sneezing were determined at baseline, 10, 30, 180, and 360 min after NPT using a graded scale of 0 (none), 1, 2, and 3 (severe), as described previously [11]. A positive response to NPT with *Dpt* was defined as an increase of more than three points in the total nasal symptom score, which corresponded to the sum of sneezing, itching, rhinorrhoea, and nasal obstruction symptom scores during the provocation test.

Nasal lavage was also performed bilaterally at baseline and at 10, 30, 180, and 360 min after NPT by scoring nasal symptoms. Saline (5 mL) was instilled into one nostril using a syringe, with the head tilted backwards during closure of the soft palate, and the solution was recovered after 10 s by anterior flexion of the head together with pushing out of the solution into a polypropylene tube. This process was repeated in the contralateral nostril. To maximize cell viability, the samples were immediately placed on ice and centrifuged at $300 \times g$ for 10 min. The supernatants were stored at -80°C until the final analysis, while the cell pellets were used to prepare slides for analyses of cellular morphology.

Measurement of inflammatory markers in the nasal lavage fluids

Inflammatory cells including eosinophils, neutrophils, lymphocytes, and monocytes were counted at two different sites by a microscope ($\times 1000$ magnification) and calculated as percentages. The eosinophil cationic protein (ECP) levels in the NLFs were measured using the Pharmacia CAP system.

Two-dimensional electrophoresis and image analysis

To identify proteins involved in AR, NLFs obtained before and 30 min after NPT with *Dpt* in a patient with AR were used. Each sample was prepared by trichloroacetic acid/acetone precipitation for desalting and concentration, and Two-dimensional electrophoresis (2-DE) was performed as described previously [12, 13]. In the first dimension

(isoelectric focusing, IEF), 1 mg protein per sample was applied to the anodic side of the immobilized pH gradient (IPG) strips that contained Immobiline, yielding a non-linear pH gradient from 3 to 10 (Amersham Pharmacia Biotech, Uppsala, Sweden), and IEF was carried out for a total of 80 kVh. Separation in the second dimension, SDS-PAGE, was carried using 9%–17% gradient polyacrylamide gels until the dye front reached the lower end of the gel. At the end of each run, to quantify the relative abundance of the proteins, the gel was stained with Coomassie brilliant blue G-250 (Bio-Rad, Hercules, CA, USA). The stained gels were then scanned using the GS-710 imaging densitometer (Bio-Rad) and analysed with Image Master Platinum 5 (GE Healthcare, Piscataway, NJ, USA).

Intra-gel digestion and mass spectrometric analysis

For mass spectrometry, protein spots that increased or decreased more than twofold in spot intensity, comparing the 2-DE images before and after NPT with *Dpt*, were selected. The spots were excised from the gels, cut into smaller pieces, and digested with trypsin (Promega, Madison, WI, USA), as described previously [14]. Analyses of peptide masses were performed using the model 4800 MALDI TOF/TOF analyzer (Applied Biosystems, Foster City, CA, USA) equipped with a 355-nm laser and using delayed extraction and operation in the reflector mode. Data processing of the spectra was performed using the Data Explorer version 4.4 software (PerSeptive Biosystems, Framingham, MA, USA). Peptide matching and protein searches were performed by database searching of the Swiss-Prot and National Center for Biotechnology Information (NCBI) databases with the Matrix Science (<http://www.matrixscience.com>) search engine.

Measurement of lactoferrin

Lactoferrin was assayed by ELISA using the Bioxytech kit (Oxis International, Portland, OR, USA) for NLFs collected serially during NPT and sera before NPT in each group. The manufacturer's directions were followed, although the diluted standards were not re-used, as they lost activity overnight due to adsorption to the vial surface. The sera and NLF samples were diluted 1:50 and 1:100, respectively, just before the assay, to ensure that the measured values were within the optimal range of the standard curve. The minimum detectable level of lactoferrin is 1.0 ng/mL.

Immunohistochemical detection of lactoferrin

The immunohistochemical study was used to identify the location and expression difference of the lactoferrin in nasal mucosa of the subjects with AR and asymptomatic sensitized controls. The primary antibody against lacto-

ferrin (1 : 500; Oxis International) was incubated for 1 h at room temperature. The Ultravision detection system (Thermo Scientific, Fremont, CA, USA) was used and the NovaRed substrate (Vector Laboratories, Burlingame, CA, USA) was applied as a chromogen, and then the sections were counterstained with hamatoxylin and mounted.

Statistical analyses

Values are given as the mean $\pm$ SD. The significance of the differences between groups was evaluated by ANOVA with Bonferroni correction. Wilcoxon's signed-rank test was used to compare the lactoferrin levels obtained serially during the NPT within a group. A receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of serum lactoferrin and *Dpt*-specific IgE for the diagnosis of AR, and the area under the curve (AUC) with a 95% confidence interval (CI) was computed. Sensitivity and specificity were calculated according to the identified optimal cut-offs. All analyses were carried out using the SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). A *P*-value < 0.05 was considered statistically significant.

Results

Characteristics of the study subjects

Based on sensitization status, history of rhinitis, and the results of the NPT, the subjects were classified into three groups: *Dpt*-sensitive AR (group I, *n* = 43); asymptomatic *Dpt*-sensitive controls (group II, *n* = 22); and non-atopic healthy controls (group III, *n* = 52). The clinical characteristics of the subjects are summarized in Table 1. No significant differences were noted for age or sex across the three groups (*P* > 0.05 , respectively). The parameters of skin reactivity for *Dpt*, *Dpt*-specific IgE, and total IgE showed significant differences between groups I or II and III, although there were no significant differences in these parameters between groups I and II (*P* > 0.05 , respectively).

Proteomic analysis of nasal lavage fluids

To investigate whether the proteins might be markers of AR, proteomic analyses were conducted using NLFs obtained before and after NPT with *Dpt* in a patient diagnosed with AR. The 2-DE results are shown in Fig. 1. There are 311 and 308 spots in the respective gels. Image analysis was performed for 209 grouped protein spots. The protein spots increased or decreased more than twofold on comparing the spot intensity of 2-DE, identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. Spot 1 showed a significant increase in intensity after NPT with *Dpt* compared with the baseline intensity (ratio, 5.5;

Table 1. Clinical characteristics of the subjects in the three groups

	Group I (n = 43)	Group II (n = 22)	Group III (n = 52)	P-value
Age (years)	30.37±8.37	25.72±4.14	29.75±11.5	0.148
Sex (male/female)	31/12	14/8	32/20	0.380
Skin reactivity to <i>Dpt</i>	4.09±1.31	3.81±1.44	0*	<0.001
<i>Dpt</i> -specific IgE (KU/mL)	17.70±23.64	13.98±24.65	0.06±0.11*	<0.001
Total IgE (IU/mL)	391.3±411.1	329.3±373.4	64.8±111.2*	<0.001
Baseline total nasal symptom score	0.76±1.09	0.50±1.0	0	0.506

Skin reactivity to *Dpt* is presented as the weal size ratio of the allergen to histamine (1 mg/mL). The results were expressed as mean±SD.

* $P < 0.05$ (group I or II vs. group III).

Significant differences were determined by ANOVA with Bonferroni's correction. There were no significant differences in all parameters between groups I and II.

Group I, *Dpt*-sensitive AR; group II, asymptomatic *Dpt*-sensitive controls; group III, non-atopic healthy controls; *Dpt*, *Dermatophagoides pteronyssinus*; AR, allergic rhinitis.

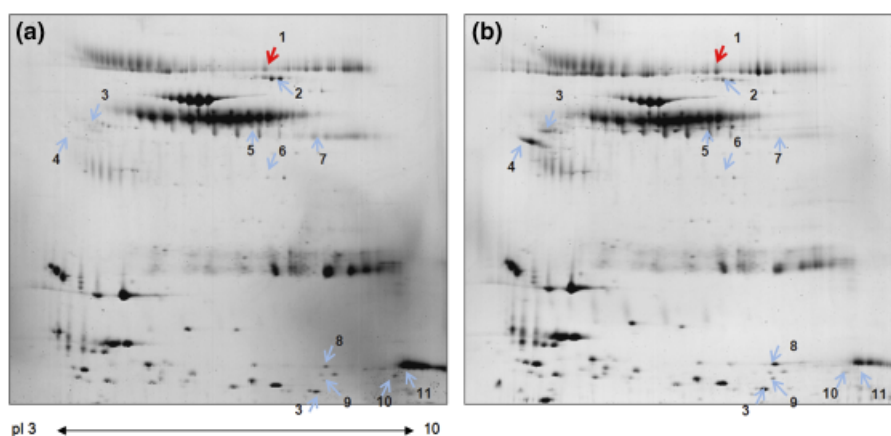


Fig. 1. Two-dimensional electrophoresis (2-DE) separation of nasal lavage fluids (NLFs) collected before (a) and after (b) the nasal provocation test (NPT) with *Dermatophagoides pteronyssinus* (*Dpt*) allergen in a patient with allergic rhinitis. Among the 209 protein spots grouped in the two gels, twofold increased or decreased protein spots in comparing spot intensity of 2-DE were analysed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Spot 1, remarkably up-regulated after NPT with *Dpt* (ratio, 5 : 5), was identified as lactoferrin. The following proteins were also identified: spot 2, transferrin; spot 3, keratin 10; spot 4, keratin 13; spot 5, zinc finger protein; spot 6, squamous cell carcinoma antigen protein; spot 7, immunoglobulin heavy chain; spot 8, plectin-1; spot 9, cystatin B; spot 10, haemoglobin chain; spot 11, lysozyme.

Fig. 1) and was identified as lactoferrin. In addition, transferrin (spot 2; ratio, -2.0), keratin 10 (spot 3; ratio, 12 & 2.1), keratin 13 (spot 4; ratio, 46), zinc finger protein (spot 5; ratio, 2.2), squamous cell carcinoma antigen protein (spot 6; ratio, -2.2), immunoglobulin heavy chain (spot 7; ratio, -2.2), plectin-1 (spot 8; ratio, 4.6), and cystatin B (spot 9; ratio, 3.0), and haemoglobin chain (spot 10; ratio, -2.0), and lysozyme (spot 11; ratio, -2.3) were identified.

Changes in lactoferrin levels in the nasal lavage fluids during the nasal provocation test

To evaluate the relevance of lactoferrin, the serial changes in lactoferrin levels in the NLFs after NPT with *Dpt* were evaluated. There were no significant differences across the three groups in the baseline levels of lactoferrin collected before NPT. In group I, the levels of lactoferrin increased significantly 10 min after NPT with *Dpt* ($P = 0.035$) and tended to increase again from 3 h, whereas no significant

changes were noted for groups II and III. There were no significant correlations between the lactoferrin levels and the ECP levels, inflammatory cells counts including eosinophil cell in the NLFs or total nasal symptom scores.

Serum lactoferrin levels

To validate the lactoferrin results obtained by MALDI-TOF MS, we measured the lactoferrin levels in the sera of the subjects in the three groups using ELISA (Fig. 2). We found that the lactoferrin levels were significantly lower in group I (235.3 ± 91.9 ng/mL) than in groups II and III (374.7 ± 170.1 ng/mL and 355.9 ± 159.3 ng/mL; $P < 0.05$, respectively).

Diagnostic value of serum lactoferrin

Using the ROC curve method, we evaluated the value of serum lactoferrin as a diagnostic tool for AR (Fig. 3). To

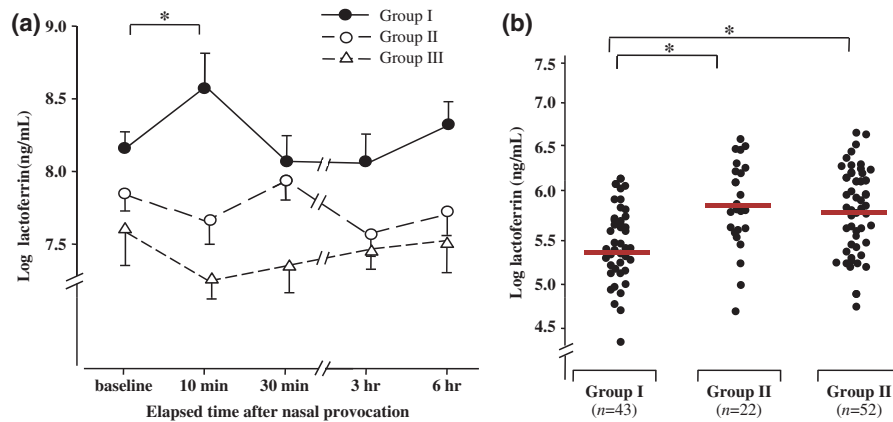


Fig. 2. (a) Changes in the lactoferrin levels in nasal lavage fluids (NLFs) during the nasal provocation test (NPT) with *Dermatophagoides pteronyssinus* (*Dpt*) allergen. Solid circles, group I (*Dpt*-sensitive AR); open circles, group II (asymptomatic *Dpt*-sensitive controls); open triangle, group III (non-atopic healthy controls). Significant changes in the lactoferrin levels after NPT with *Dpt* are noted for group I, but not for groups II and III. Statistical significance was evaluated by Wilcoxon's signed-rank test. (b) Comparison of the serum lactoferrin levels, as measured by ELISA, in the three study groups. Statistically significant differences between the groups were assessed by ANOVA. The line indicates the mean value for each group. * $P < 0.05$. AR, allergic rhinitis.

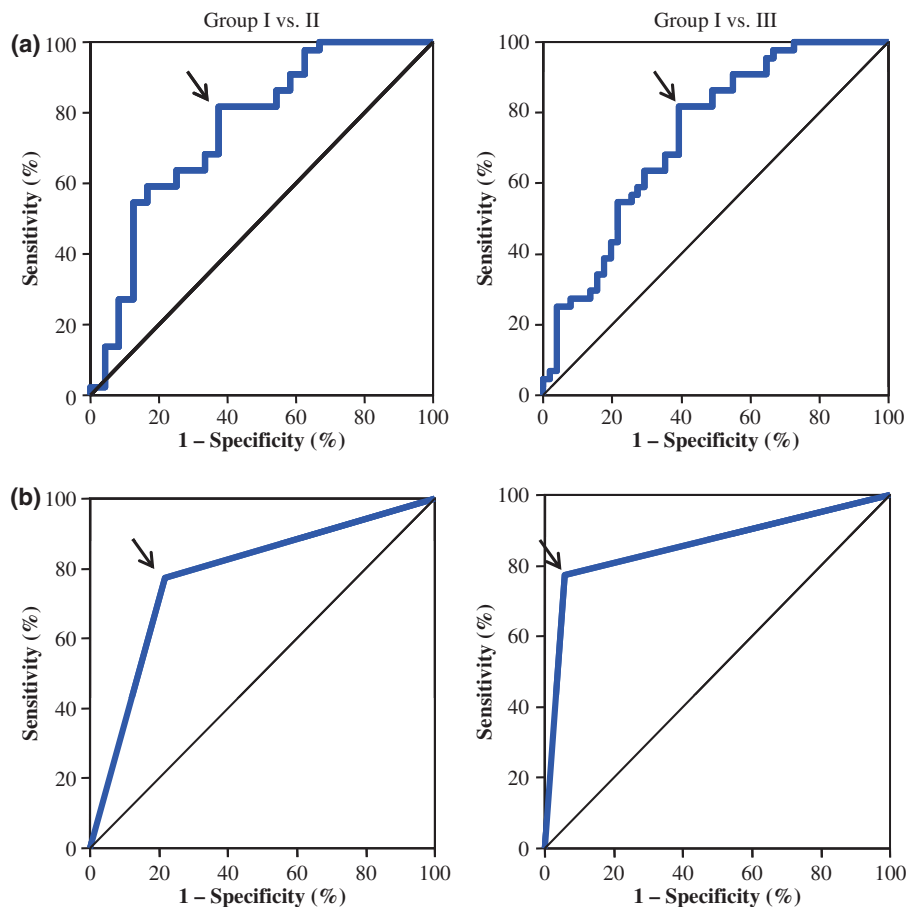


Fig. 3. Receiver operating characteristic (ROC) curves for the serum levels of lactoferrin (a) and the combined values for lactoferrin and serum *Dermatophagoides pteronyssinus* (*Dpt*)-specific IgE (b), which were used to determine the optimal cut-offs for discriminating group I from the other groups. Arrows indicate the optimal value for lactoferrin and the combined value (lactoferrin < 307 ng/mL and *Dpt*-specific IgE > 0.35 kU/L).

generate the highest levels of sensitivity and specificity, the optimal cut-off value from the ROC curve, lactoferrin <307 ng/mL, was chosen. When this value was set, the odds ratio for lactoferrin in AR was 7.29 (95% CI: 2.36–22.53), the sensitivity was 81.4%, and the specificities ranged 62.5% between and 58% for discriminating group I from groups II and III (Table 2). When the cut-off serum *Dpt*-specific IgE level was set at >0.35 kU/L (considering the normal range of 0–0.35 kU/L), high levels of sensitivity and specificity were obtained for discriminating group I from group III, whereas a specificity of only 17% was obtained for discriminating group I from group II (Table 2).

To improve this diagnostic power, the lactoferrin level was combined with the serum *Dpt*-specific IgE level and re-evaluated using the ROC curve (Fig. 3). Thus, the sensitivity was 76.7% and the specificities were 79.2% and 94% for the discrimination of group I from groups II and III, respectively (Table 2).

Discussion

Although many studies have been conducted on the pathogenesis of AR, to the best of our knowledge, no simple serologic test for the diagnosis of AR has been reported to date. Considering the high prevalence and socio-economic burden of AR, the development of novel biomarkers for the early identification of AR would improve clinical outcomes and reduce the economic impact of this disease. We adopted a proteomics approach to identify candidate biomarkers. The protein profiles of human NLFs, as analysed by 2-DE, have been described over the past 10 years and NLF proteins identified by proteomic analyses have been implicated in airway inflammatory and immune responses [15–18]. However, there have been no attempts to develop biomarkers for the diagnosis of AR using the proteomic approach. We examined the protein changes in the NLFs after NPT with *Dpt* in the representing subject who was diagnosed with AR, using proteomic analysis to develop a new biomarker. In the present study,

lactoferrin (spot 1), keratin 10 & 13 (spot 3, 4), zinc finger protein (spot 5), plectin-1 (spot 8), and cystatin B (spot 9), and haemoglobin chain (spot 10) showed increased production after NPT, while transferrin (spot 2), squamous cell carcinoma antigen protein (spot 6), immunoglobulin heavy chain (spot 7), and lysozyme (spot 11) showed decreased production after NPT. These proteins are reported to be involved in inflammatory reactions in previous proteomic analysis using NLFs [16–18]. Additional studies are needed to evaluate the clinical relevance of each protein for AR.

Especially, lactoferrin showed remarkably up-regulated expression after NPT with *Dpt* in 2-DE. Lactoferrin, 80 kDa glycosylated protein, is commonly found in secretions from the submucosal glands of both lower and upper human airways [19–22] and is present in neutrophil granules [23, 24]. This protein is known to possess a variety of biologic functions, such as promotion of iron absorption, and antibacterial, antifungal, and antiviral activities [22, 25], as well as to be related to immunoregulatory and anti-inflammatory activities [16–18, 24, 26]. With our proteomic data, it was suggested that lactoferrin is involved in allergic inflammation of the nasal mucosa following exposure to an allergen. Moreover, it is supported by our finding of significantly increased lactoferrin levels in the NLFs of *Dpt*-sensitive AR subjects during NPT with *Dpt*, whereas the levels of lactoferrin during NPT in asymptomatic *Dpt*-sensitive controls and non-atopic healthy controls were unchanged. Therefore, lactoferrin has potential as a novel biomarker. But, in the present study, the levels of lactoferrin could not be correlated directly with disease severity, as no significant relationships were noted between the lactoferrin levels and the nasal symptom scores, ECP levels, or inflammatory cell counts in the NLFs.

To investigate the possibility of using lactoferrin as a serologic biomarker, the lactoferrin levels in the sera of the study subjects were measured by ELISA. A significant decrease in the lactoferrin level was observed in the *Dpt*-sensitive AR group, compared with the corresponding levels in asymptomatic *Dpt*-sensitive controls and

Table 2. Sensitivities, specificities and the values of the area under the ROC with 95% CI

Cutoff	Group	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P-value
Lactoferrin <307 ng/mL	Group I vs. II	79.5	65.21	81.4	62.5	0.757 (0.627–0.888)	0.001
	Group I vs. III	62.5	78.4	81.4	58	0.729 (0.628–0.830)	<0.001
<i>Dpt</i> -specific IgE >0.35 kU/mL	Group I vs. II	67.2	50	90.7	17.4	0.616 (0.470–0.762)	0.337
	Group I vs. III	92.9	92.2	90.7	94	0.948 (0.895–1.001)	<0.001
Lactoferrin <307 ng/mL plus <i>Dpt</i> -specific IgE >0.35 kU/mL	Group I vs. II	86.8	65.5	76.7	79.2	0.780 (0.660–0.899)	<0.001
	Group I vs. III	91.7	82.5	76.7	94	0.854 (0.769–0.939)	<0.001

Group I, *Dpt*-sensitive AR; group II, asymptomatic *Dpt*-sensitive controls; group III, non-atopic healthy controls. Using the serum level of lactoferrin alone, the specificity is low and the sensitivity is high. When the lactoferrin level is combined with the level of serum *Dpt*-specific IgE (lactoferrin <307 ng/mL and *Dpt*-specific IgE >0.35 kU/L), the specificity is significantly improved.

Dpt, *Dermatophagoides pteronyssinus*; ROC, receiver operating characteristic; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; AR, allergic rhinitis.

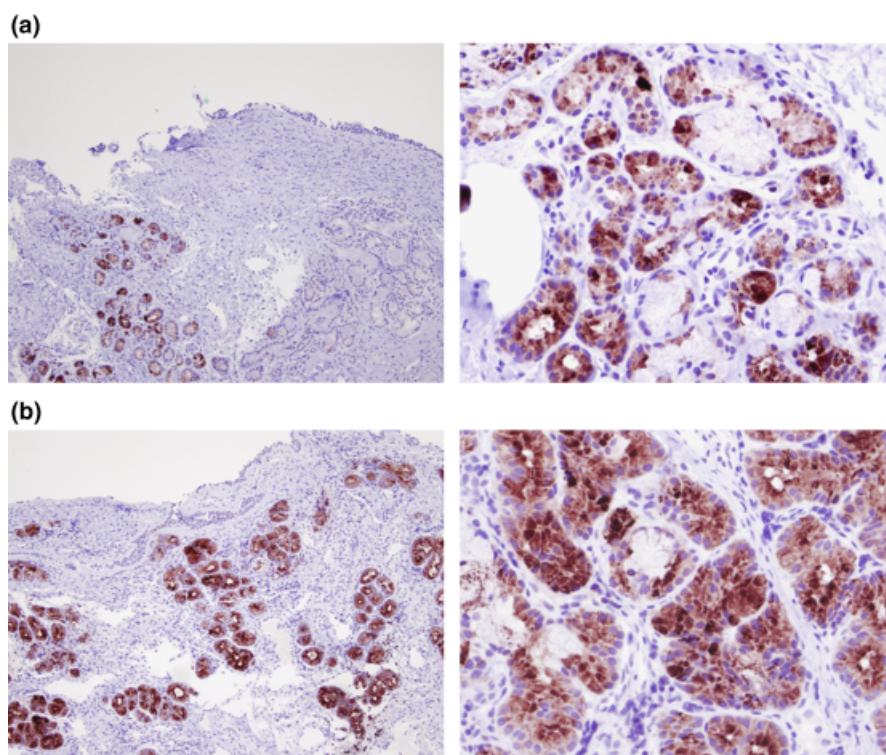


Fig. 4. Immunohistochemical analyses of lactoferrin expression in the nasal mucosa of the subjects in groups I (a) and II (b). Lactoferrin expression is lower in group I [*Dermatophagoides pteronyssinus* (*Dpt*)-sensitive AR] than in groups II (asymptomatic *Dpt*-sensitive controls). Magnification: right panels, $\times 100$; left panels, $\times 400$. AR, allergic rhinitis.

non-atopic healthy controls, despite increased production of lactoferrin in nasal secretions after the NPT. A possible explanation as to why discrepancies of lactoferrin levels between sera and NLFs were found in this study might be that persistent exposure to *Dpt* allergen results in chronic allergic inflammation of the nasal mucosa, with increased nasal secretion of lactoferrin, leading to a decreased serum level of lactoferrin by depletion or negative feedback. A recent study reported that lactoferrin expression of nasal mucosa was down-regulated at both the mRNA and the protein level in patients with chronic rhinosinusitis [27, 28]. Our immunohistochemical analysis also showed that lactoferrin expression was decreased in the nasal mucosa of the *Dpt*-sensitive AR group, compared with those of the asymptomatic *Dpt*-sensitive control groups (Fig. 4), although confirmation of this finding is required in an expanded study with a larger cohort.

In the present study, we evaluated the serum lactoferrin level in terms of its diagnostic value for the identification of AR patients among susceptible subjects. We used ROC curves to calculate the optimal values and selected those areas with $>60\%$ sensitivity and $>50\%$ specificity. At these optimal AUC values, the specificity (58%–62.5%) of lactoferrin was not satisfactory, although sensitivity (81.4%) was high. To improve specificity, we considered a combination of the lactoferrin level and the serum

Dpt-specific IgE level. Because HDM is known to be an important indoor allergen inducing sensitization and development of AR, and the *Dpt*-specific IgE level was easily measured. With this combination (lactoferrin <307 ng/mL and *Dpt*-specific IgE >0.35 kU/L), the specificity was significantly improved to 79.2%. On the basis of these findings, we propose that the combination of serum lactoferrin level and serum *Dpt*-specific IgE level can be used as a serologic marker for early detection of *Dpt*-sensitive AR. But this study was limited only to *Dpt*-sensitive subjects; additional validation in seasonal AR patients should be considered.

Despite its potential as a diagnostic marker for AR, the pathologic role of lactoferrin in AR remains largely unknown. AR involves IgE-mediated allergic inflammation of the nasal mucosa, and the activation of mast cells is an important triggering mechanism in the pathophysiology of AR [29–31]. Also, it has been previously reported that lactoferrin reduces mast cell degranulation and inhibits tryptase activity [32, 33]. Therefore, we speculate that lactoferrin secreted into the nasal mucosa exerts a protective effect to inhibit mast cell-driven inflammation in AR.

In conclusion, this is the first study to identify a candidate biomarker for detection of AR using a proteomic approach and we found that the serum level of lactoferrin,

a candidate marker identified by proteomics, combined with the levels of serum *Dpt*-specific IgE could be used as a serologic marker for detection of AR from asymptomatic sensitized or healthy subjects. Additional studies are needed to elucidate the role of lactoferrin in the pathogenesis of AR.

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