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References

1. Bhatt SP, Kim Y-i, Harrington KF, Hokanson JE, Lutz SM, Cho MH, et al. Smoking duration alone provides stronger risk estimates of chronic obstructive pulmonary disease than pack-years. Thorax 2018;73:414–421.
2. Inoue-Choi M, Liao LM, Reyes-Guzman C, Hartge P, Caporaso N, Freedman ND. Association of long-term, low-intensity smoking with all-cause and cause-specific mortality in the National Institutes of Health–AARP Diet and Health Study. JAMA Intern Med 2017;177:87–95.
3. Jamal A, Phillips E, Gentzke AS, Homa DM, Babb SD, King BA, et al. Current cigarette smoking among adults—United States, 2016. MMWR Morb Mortal Wkly Rep 2018;67:53.
4. Hackshaw A, Morris JK, Boniface S, Tang JL, Milenkovic D. Low cigarette consumption and risk of coronary heart disease and stroke: meta-analysis of 141 cohort studies in 55 study reports. BMJ 2018;360:j5855.
5. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SB, Jacobs DR Jr, et al. CARDIA: study design, recruitment, and some characteristics of the examined subjects. J Clin Epidemiol 1988;41:1105–1116.
6. Nagin DS, Odgers CL. Group-based trajectory modeling in clinical research. Annu Rev Clin Psychol 2010;6:109–138.
7. Kalhan R, Dransfield MT, Colangelo LA, Cuttica MJ, Jacobs DR Jr, Thyagarajan B, et al. Respiratory symptoms in young adults and future lung disease: the CARDIA Lung Study. Am J Respir Crit Care Med 2018;197:1616–1624.
8. Etter J-F, Perneger T. Measurement of self reported active exposure to cigarette smoke. J Epidemiol Community Health 2001;55:674–680.
9. Lange P, Celli B, Agusti A, Boje Jensen G, Divo M, Faner R, et al. Lung-function trajectories leading to chronic obstructive pulmonary disease. N Engl J Med 2015;373:111–122.
10. Agustí A, Noell G, Brugada J, Faner R. Lung function in early adulthood and health in later life: a transgenerational cohort analysis. Lancet Respir Med 2017;5:935–945.
11. Lee PN. The effect of reducing the number of cigarettes smoked on risk of lung cancer, COPD, cardiovascular disease and FEV1 – A review. Regul Toxicol Pharmacol 2013;67:372–381.

IL-5 Levels in Nasosorption and Sputosorption Correlate with Sputum Eosinophilia in Allergic Asthma

To the Editor:

Monoclonal antibodies that block type 2 cytokines (e.g., IL-4, IL-5, and IL-13) are promising new therapies for asthma (1). However, these agents need to be targeted to selected patients with asthma, where biomarker assessment of airway samples may support

stratification of patients into molecular endotypes (2). Thus, we measured cytokine and chemokine mediator levels in nasosorption samples and by using a novel method of sputosorption from induced sputum. Nasal and sputum mediators were then compared with sputum eosinophil levels. A group of 29 allergic asthmatics (AAs) not receiving oral, inhaled, or intranasal corticosteroids or leukotriene modifiers in the last 3 months (with positive skin prick tests, mean baseline FEV_1 of 79.3 ± 18.0, and FEV_1 reversibility), one of whom did not report a history of allergic rhinitis, were recruited. Dust mite extracts were the most commonly sensitized antigens: *Dermatophagoides pteronyssinus* (93.1%), *Dermatophagoides farinae* (79.3%), and *Blomia tropicalis* (75.8%). These were compared with a control group of 17 nonatopic healthy control patients (HC) with no history of allergic rhinitis and a mean baseline FEV_1 of 94.1 ± 11.4. Participants were nonsmokers and were free of upper or lower respiratory infections for the last 4 weeks. Subjects were excluded if they could not safely provide specimens; by the presence of other medical conditions; because of use of concomitant medication, tobacco, or illicit drugs; and if women were pregnant or breastfeeding. The study was approved by the Ethical Committee of Universidade Federal de Santa Catarina (Brazil) on March 10, 2008.

Methods

Nasosorption was performed by placing two strips of synthetic absorptive matrix (Leukosorb, Pall Life Sciences), measuring 7×30 mm each, against the inferior turbinate for 2 minutes (Conformité Européene–marked nasosorption sampling devices are now available sterile and allergen-free from Mucosal Diagnostics Ltd.) (3, 4). Sputum was induced by inhalation of hypertonic saline, after which the sample was poured onto a Petri dish, and the viscid portions apparently free of salivary contamination were macroscopically selected. One portion of the selected material was used for cytospin preparation and differential cell count. The remaining portion was used for sputosorption, whereby two synthetic absorptive matrix strips, 7×30 mm each, overlaid the sputum for 2 minutes.

Once removed, the synthetic absorptive matrixes were immersed in 200 μl assay buffer (Millipore) + 4 μl DPP Protease Inhibitor-IV (Millipore), placed in the filter cup within a microcentrifuge tube, and centrifuged (10 min, $16,000 \times g$, 4°C); the supernatant aliquots were stored at −80°C. Mediators were quantified using a Luminex 200 IS Analyzer (Luminex Corp.), and results were expressed in picograms per milliliter. Continuous data were evaluated by the Shapiro-Wilk normality test and presented as median and interquartile ranges and tested for significance by Mann-Whitney *U* tests. Correlations were performed by Spearman rank correlation tests with Benjamini-Hochberg *post hoc* correction.

Sputum eosinophil percentage levels from AAs were significantly higher than those of HCs (Figure 1A, left; $P < 0.001$). A panel of cytokines and chemokines were measured in nasosorption and sputosorption samples, and the corresponding levels are shown for individual patients (Figure 1B), noting that sputum and nasal IL-5 levels were significantly higher in AAs relative to HCs (Figure 1A, right; $P < 0.001$), whereas AAs exhibited significantly lower
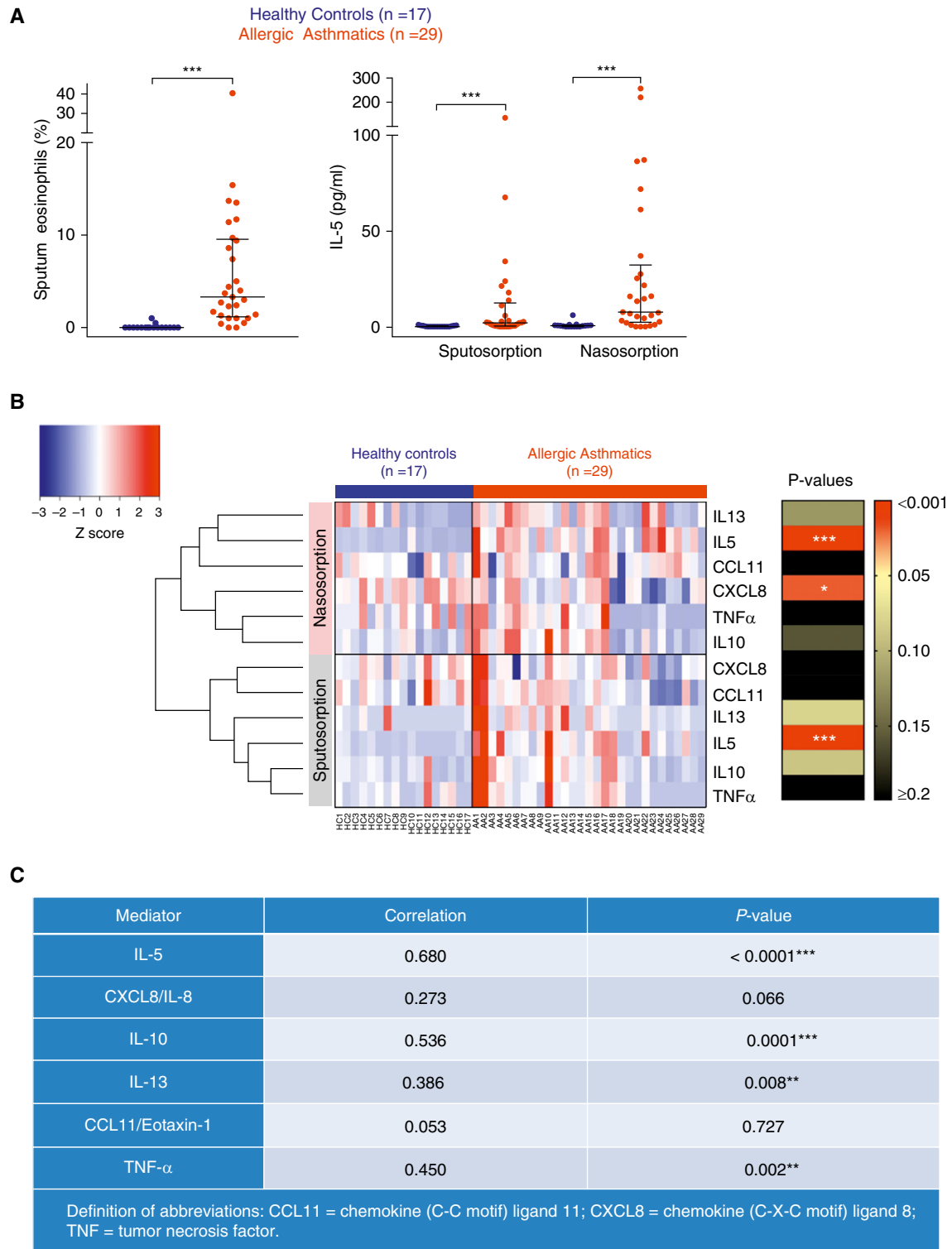
A

B

C

Mediator	Correlation	P-value
IL-5	0.680	< 0.0001***
CXCL8/IL-8	0.273	0.066
IL-10	0.536	0.0001***
IL-13	0.386	0.008**
CCL11/Eotaxin-1	0.053	0.727
TNF-α	0.450	0.002**
Definition of abbreviations: CCL11 = chemokine (C-C motif) ligand 11; CXCL8 = chemokine (C-X-C motif) ligand 8; TNF = tumor necrosis factor.		

Figure 1. Sputosorption and nasosorption reveal IL-5 to be selectively increased in the asthmatic airway. (*A*) Sputum eosinophil levels (left) and nasosorption and sputosorption IL-5 levels (right) in healthy control patients (HCs; *n* = 17) and patients with asthma (*n* = 29). Bars represent median and interquartile range. Between-group comparisons by Mann-Whitney *U* test; ***$P < 0.001$. (*B*) Heat map of nasosorption and sputosorption levels of a panel of cytokines and chemokines (IL-5, IL-10, IL-13, tumor necrosis factor-α, CXCL8/IL-8, and CCL11) in HCs and allergic asthmatics (AAs). Mediator levels were normalized (Z scores) and were hierarchically clustered. Levels of mediators between HCs and AAs were compared using Mann-Whitney test and corresponding *P* values summarized: *$P < 0.05$; ***$P < 0.001$. (*C*) Spearman rank correlations between matched sputosorption and nasosorption samples in HCs and AAs: **$P < 0.01$; ***$P < 0.001$.
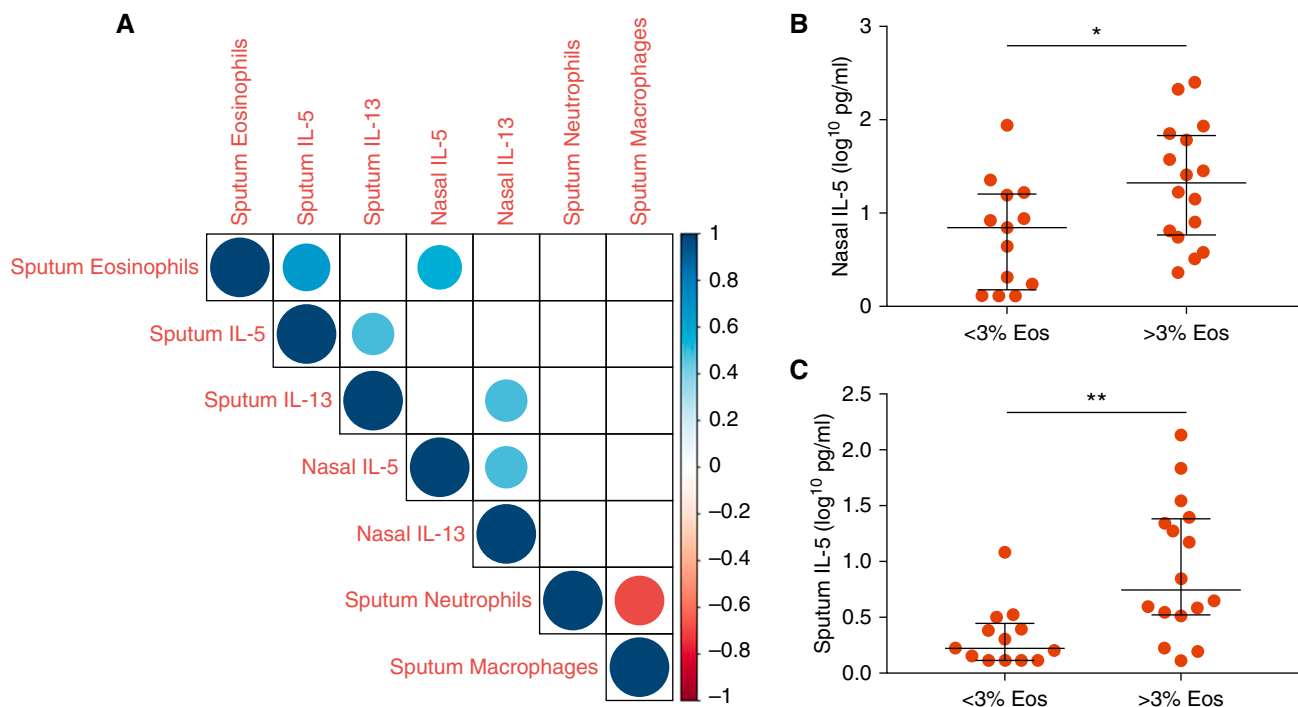
Figure 2. Airway IL-5 levels are closely associated with eosinophilic asthma. (*A*) Correlation matrix of nasosorption and sputosorption ILs and sputum eosinophils, neutrophils, and macrophages in patients with asthma ($n = 29$); blank squares denote insignificant correlations; color denotes Spearman *R* value. (*B*) Nasosorption IL-5 levels in allergic asthmatics stratified on a 3% cut-off for defining sputum eosinophilia (Eos). (*C*) Sputosorption IL-5 levels in allergic asthmatics with the 3% threshold for defining sputum eosinophilia. (<3% Eos: $n = 13$, >3%Eos: $n = 16$). *$P = 0.037$; **$P = 0.001$.

levels of nasosorption CXCL8/IL-8 (Figure 1B; $P < 0.05$). Levels of IL-10, IL-13, tumor necrosis factor-α, and particularly IL-5 were significantly positively correlated between matched nasosorption and sputosorption samples (Figure 1C).

In the AA group, levels of type 2 cytokines (IL-5 and IL-13) were positively correlated in nasosorption ($r_s = 0.500$; $P = 0.021$) and sputosorption ($r_s = 0.502$; $P = 0.021$) samples. In the AAs, levels of IL-13 were also positively correlated between nasosorption and sputosorption samples ($r_s = 0.509$; $P = 0.021$). Similarly, sputum eosinophils were positively correlated with nasal IL-5 ($r_s = 0.560$; $P = 0.01$) and sputum IL-5 ($r_s = 0.657$; $P = 0.001$) levels in AAs. In addition, sputum macrophages were negatively correlated with sputum neutrophils ($r_s = -0.673$; $P = 0.001$; Figure 2A) in AAs. Eosinophilic individuals with asthma (those with ⩾3% sputum eosinophils) had higher nasal IL-5 (Figure 2B; $P = 0.037$) and sputum IL-5 (Figure 2C; $P = 0.001$) levels. These findings support the United Airways Concept (5) and demonstrate the potential of minimally invasive nasal IL-5 measurement as a promising biomarker, representative of lower airway eosinophilia in patients with asthma. Despite the known role of CCL11 in eosinophil recruitment, no significant correlation was observed between sputum eosinophils and levels of nasal or sputum CCL11 ($r_s = 0.341$ [$P = 0.07$] and $r_s = 0.108$ [$P = 0.576$], respectively). This may be because β_2-agonists can inhibit tumor necrosis factor-α–mediated CCL11 induction (6).

Discussion

The utility of nasosorption and sputosorption IL-5 levels as a biomarker for eosinophilic asthma will require direct comparison with existing biomarkers, including fractional exhaled nitric oxide, peripheral blood eosinophil counts, IgE levels, and findings on mucosal bronchial biopsy. In smaller clinical studies, eosinophil counts in induced sputum have been used for the selection of patients with asthma who could benefit from anti-IL-5 therapy (7), whereas peripheral blood eosinophilia has been extensively used in phase III studies as a surrogate for sputum eosinophilia (8). However, blood eosinophil counts not only undergo diurnal variation and increase after exercise (9) but also fail to accurately predict sputum eosinophilia in patients with asthma (10).

As patients in this study were not treated with corticosteroids, the effect of these common therapeutics for asthma on nasosorption and sputosorption IL-5 levels, and the potential consequences for patient stratification, will require further study.

In conclusion, nasosorption and sputosorption sampling methods were minimally invasive and rapidly performed to permit measurement of cytokine levels that reflect lower airway sputum eosinophilia. Although extensive further validation will be required, nasosorption and sputosorption samples have promise for stratification and selection of patients with asthma to receive highly specific therapy with biologics. ∎

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References

1. Israel E, Reddel HK. Severe and difficult-to-treat asthma in adults. *N Engl J Med* 2017;377:965–976.
2. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, et al. After asthma: redefining airways diseases. *Lancet* 2018;391: 350–400.
3. Hansel TT, Tunstall T, Trujillo-Torralbo MB, Shamji B, Del-Rosario A, Dhariwal J, et al. A comprehensive evaluation of nasal and bronchial cytokines and chemokines following experimental rhinovirus infection in allergic asthma: increased interferons (IFN-γ and IFN-λ) and type 2 inflammation (IL-5 and IL-13). *EBioMedicine* 2017;19: 128–138.
4. Thwaites RS, Jarvis HC, Singh N, Jha A, Pritchard A, Fan H, et al. Absorption of nasal and bronchial fluids: precision sampling of the human respiratory mucosa and laboratory processing of samples. *J Vis Exp* 2018;e56413.
5. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al.; World Health Organization; GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;63:8–160.
6. Nie M, Knox AJ, Pang L. beta2-Adrenoceptor agonists, like glucocorticoids, repress eotaxin gene transcription by selective inhibition of histone H4 acetylation. *J Immunol* 2005;175:478–486.
7. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985–993.
8. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380: 651–659.
9. Spector SL, Tan RA. Is a single blood eosinophil count a reliable marker for "eosinophilic asthma?". *J Asthma* 2012;49:807–810.
10. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al.; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. *J Allergy Clin Immunol* 2013;132:72–80.

The Ratio of Peripheral Regulatory T Cells to Lox-1+ Polymorphonuclear Myeloid-derived Suppressor Cells Predicts the Early Response to Anti–PD-1 Therapy in Patients with Non–Small Cell Lung Cancer

To the Editor:

Immune checkpoint inhibitors (ICIs) have emerged as a promising treatment modality for patients with non–small cell lung cancer (NSCLC). However, ICI monotherapy has a relatively low response rate (approximately 20–50% in patients with NSCLC) (1), and consequently it is important to develop predictive biomarkers of response to inform clinical decisions regarding ICI use. Although PD-L1 expression on tumor cells is currently used as a predictive determinant for anti–PD-1 therapy responses, the accuracy of this test is relatively poor. Therefore, better predictive biomarkers for anti–PD-1 therapy in patients with NSCLC are needed.

Myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) play crucial immune-suppressive roles in patients with cancer (2). In addition to promoting tumor growth, the suppressive actions of MDSCs and Tregs hinder the efficacy of cancer immunotherapy. Therefore, we hypothesized that the frequency of immune-suppressive cell subsets might be correlated with the response to anti–PD-1 therapy. The fact that MDSCs and Tregs can be easily detected and affordably quantified in the peripheral blood of patients with NSCLC makes them excellent candidates for predictive biomarkers of ICIs.

To identify correlations between suppressive-cell subsets and the response to anti–PD-1 therapy, we longitudinally analyzed the frequency of immune-suppressive cells, including Tregs and MDSCs, in the blood of patients with NSCLC in an exploratory cohort ($n = 34$) before and after nivolumab treatment. The data were validated in an independent cohort of patients with NSCLC ($n = 29$). We prospectively enrolled patients with NSCLC who had failed platinum-based chemotherapy.

Patients were categorized as responders (complete response, partial response, or stable disease for more than 6 mo) or nonresponders, and blood samples were collected both before and after the first nivolumab treatment (3 mg/kg every 2 wk). The frequency of Tregs and polymorphonuclear (PMN)-MDSCs, which are the dominant population of MDSCs in patients with NSCLC, was quantified using flow cytometry. Expression of the lectin-type oxidized LDL receptor-1 (Lox-1) in PMN-MDSCs was additionally analyzed to distinguish PMN-MDSCs from neutrophils (3). At baseline, the percentage of Tregs was higher in