**Online supplementary material**

**Increased serum periostin levels and eosinophils in nasal polyps** **are associated with the preventive effect of endoscopic sinus surgery for asthma exacerbations in chronic rhinosinusitis patients**

Yoshihiro Kanemitsu, MD, PhDa\*, Ryota Kurokawa, MDa\*, Junya Ono, MS, PhDb, Kensuke Fukumitsu, MD, PhDa, Norihisa Takeda, MD, PhDa, Satoshi Fukuda, MD, PhDa, Takehiro Uemura MD, PhDa, Tomoko Tajiri MD, PhDa, Hirotsugu Ohkubo, MD, PhDa, Ken Maeno, MD, PhDa, Yutaka Ito, MD, PhDa, Tetsuya Oguri, MD, PhDa, Masaya Takemura, MD, PhDa, Jennifer Yap, MSa, Hirono Nishiyama MDa, Ayako Masaki, MD, PhDc, Yoshiyuki Ozawa, MD, PhDd, Kenji Izuhara, MD, PhDe, Motohiko Suzuki, MD, PhDf, and Akio Niimi, MD, PhDa.

**Affiliations:**

aDepartment of Respiratory Medicine, Allergy and Clinical Immunology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

bShino-Test Corporation, Sagamihara, Japan

cDepartment of Pathology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

dDepartment of Radiology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

eThe Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan

fDepartment of Otorhinolaryngology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

\*Y.K., and R.K. contributed equally to this work.

**Short title:** Serum periostin and eosinophilic NPs in CRS with asthma

**Corresponding:** Yoshihiro Kanemitsu, MD, PhD, Department of Respiratory Medicine, Allergy and Clinical Immunology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

**Email:** kaney32@med.nagoya-cu.ac.jp

**Tel:** +81-52-853-8216, **FAX:** +81-52-852-0849

**Methods**

This study is a post hoc analysis of our previous study that evaluated the pathophysiological link between upper and lower airways in CRS patients[E1]. We prospectively recruited 56 CRS patients who were subjected to undergo ESS between October 2015 and December 2017 and followed them for 1 year after ESS. Patients were diagnosed as having asthma when they complained of asthma-related symptoms such as cough, dyspnea, chest tightness and wheezing with the presence of clinical reversible airway obstruction or airway hyperresponsiveness to inhaled methacholine[E2]. The diagnosis of CRS was made according to radiological and endoscopic findings with two or more physical symptoms (mucopurulent drainage, nasal obstruction, facial pain, impaired sense of smell) lasting for 12 weeks or longer[E3].

All patients underwent measurements of blood eosinophils, serum IgE, periostin, osteopontin, and YKL-40, and FeNO at enrollment, along with sinus CT scan, olfactory function testing assessed by Open Essence method (ranging from 0 to 12, lower scores indicate worse olfaction), CRS-related QoL questionnaire, the Sino-nasal Outcome Test-22[E4], and pulmonary function testing. Patients were excluded if they were current smokers or had ceased smoking within the previous six months; had other pulmonary diseases including chronic obstructive pulmonary disease; took oral corticosteroids; or experienced an acute respiratory infection within four weeks prior to enrollment. We previously described this methodology in detail[E1], except for the serum biomarkers measures.

This study was approved by the Ethics Committee of Nagoya City University (Number 1165) and was registered on the UMIN Clinical Trials Registry (Registry ID UMIN000018672). Written informed consent was obtained from all participants.

**Measurements of biomarkers**

Serum periostin levels were measured with the use of an enzyme-linked immunosorbent assay developed by Shino-Test (Kanagawa, Japan) as described previously[E5, E6]. Serum osteopontin (R&D systems, Tokyo, Japan) and YKL-40 levels (Quidel, San Diego, Calif., USA) were measured according to the manufacturer instructions. These biomarkers were measured in a blinded manner following the completion of clinical data collection. Detailed methods for the measurements of FeNO have been described previously[E1]. Briefly, we measured FeNO levels with an oral expiratory flow rate of 50 ml/s using a Sievers NOA 280i chemiluminescence analyser (GE Analytical Instruments, Boulder, USA) according to the manufacturers guidelines[E7]. One patient with asthma failed to to undergo the NO measurement at enrollment due to an apparatus failure.

**Histological evaluation of sinus tissues and/or nasal polyps**

One otorhinolaryngology specialist performed all ESS and obtained inflamed sinus tissue and/ or nasal polyps (NPs) samples under general anesthesia. After staining with Hematoxylin-Eosin, one pathologist, blinded to the clinical data, counted the number of eosinophils in NPs and sinus tissue per HPF. Patients were considered to have ECRS if eosinophils were seen ≥70 /HPF in either sinus tissues or NPs[E8]. Otherwise, a diagnosis of non-ECRS was made.

**Definition of asthma exacerbations**

Asthma exacerbations were defined as patients requiring oral corticosteroids for 3 or more days and/or intravenous corticosteroids for one or more days, or experiencing hospitalization due to asthma worsening according to the ERS/ATS severe asthma guidelines[E9]. The change in exacerbations number (Δexacerbations) with ESS intervention was calculated as follows: Δexacerbations = numbers of exacerbations for 1 years before ESS – those for 1 year after ESS.

**Evaluation of asthma-related QoL**

We assessed asthma-related QoL using the Asthma Quality of Life Questionnaire (AQLQ), which consists of 32 items covering four domains; symptoms, environmental stimuli, emotional function, and activity limitation[E10]. Higher scores represent improved asthma-related QoL. We obtained the permission to use the questionnaire for this study from Professor Elizabeth Juniper, McMaster University, Canada.

**Statistics**

Data were analysed using JMP 14.2 Start Statics (SAS Institute Inc., Cary, NC, USA), and are presented as medians (5th percentile, 95th percentile) or n (%). Comparisons of upper/lower airways indices and biomarkers between the two groups [asthma vs without asthma, and non-exacerbators after ESS (Ex- group) vs exacerbators after ESS (Ex+ group)] were made using Wilcoxon rank sum test. Wilcoxon single rank test was adapted when biomarkers were compared between before and after ESS. For categorical variables, Fisher’s exact tests were applied. We investigated the accuracy of biomarkers for the detection of comorbid asthma among CRS patients and the prediction of exacerbation following ESS using receiver operating characteristic (ROC) analysis. A p value ≤0.05 was considered significant when α error was set at 5 %.

Spearman rank correlations were used to assess associations of preoperative upper/lower airways indices and biomarkers with exacerbations numbers for one year post ESS and Δexacerbations. When a ρ value showed ≥ |0.40|, we considered this a meaningful correlation.

**Figure legends:**

**Figure E1:** **Receiver operating characteristic curves for discriminating comorbid asthma among ECRS (A-C) and non-ECRS patients (D-F).**

The sensitivity and specificity of blood eosinophils (A, D), serum periostin (B, E) and FeNO (C, F) are shown. Patients were considered as ECRS if eosinophils were seen ≥70 /HPF in sinus tissue and/or NPs. Otherwise, non-ECRS. One non-ECRS patient with asthma failed to conduct the FeNO measurement because of apparatus failure. Receiver Operating Characteristic analysis was applied to determine the accuracy of biomarkers for the diagnosis of comorbid asthma.

**Figure E2: Receiver operating characteristic curves for predicting the absence of exacerbations for a year after ESS (A, B) or reduction of exacerbations (C, D) by endoscopic sinus surgery (ESS)**

The sensitivity and specificity of serum periostin (A, C) and eosinophils in NPs (B, D) are shown.

**References:**

E1. Kanemitsu Y, Suzuki M, Fukumitsu K, Asano T, Takeda N, Nakamura Y, et al. A novel pathophysiologic link between upper and lower airways in patients with chronic rhinosinusitis: Association of sputum periostin levels with upper airway inflammation and olfactory function. World Allergy Organ J. 2020 Jan;13(1):100094.

E2. 2015 GINA Report, Global Strategy for Asthma Management and Prevention <https://ginasthma.org>.

E3. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. Rhinol Suppl. 2012 03;23:3 p preceding table of contents, 1-298.

E4. Kennedy JL, Hubbard MA, Huyett P, Patrie JT, Borish L, Payne SC. Sino-nasal outcome test (SNOT-22): a predictor of postsurgical improvement in patients with chronic sinusitis. Ann Allergy Asthma Immunol. 2013 Oct;111(4):246-51.e2.

E5. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. J Allergy Clin Immunol. 2013 Aug;132(2):305-12.e3.

E6. Asano T, Kanemitsu Y, Takemura M, Yokota M, Fukumitsu K, Takeda N, et al. Serum Periostin as a Biomarker for Comorbid Chronic Rhinosinusitis in Patients with Asthma. Ann Am Thorac Soc. 2017 May;14(5):667-75.

E7. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011 Sep;184(5):602-15.

E8. Tokunaga T, Sakashita M, Haruna T, Asaka D, Takeno S, Ikeda H, et al. Novel scoring system and algorithm for classifying chronic rhinosinusitis: the JESREC Study. Allergy. 2015 Aug;70(8):995-1003.

E9. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014 Feb;43(2):343-73.

E10. Juniper EF, Guyatt GH, Epstein RS, Ferrie PJ, Jaeschke R, Hiller TK. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. Thorax. 1992 Feb;47(2):76-83.