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Serum periostin, IgE and SE-IgE can be used as biomarkers to identify moderate to severe chronic rhinosinusitis with nasal polyps

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Serum periostin, IgE and SE-IgE can be used as biomarkers to identify moderate to severe chronic rhinosinusitis with nasal polyps

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Capsule Summary

Serum Periostin is elevated in CRSwNP and can, together with serum IgE and serum SE-IgE, be used as biomarker to predict presence of IL-5 and SE-IgE in nasal polyp tissue and hence to identify endotypes.

To the editor:

Chronic rhinosinusitis (CRS) with nasal polyps (CRSwNP) in the Western world is typically characterized by type 2 or eosinophilic inflammation, whereas chronic rhinosinusitis without nasal polyps (CRSsNP) mostly resembles non-type 2 inflammation¹. With the idea of precision medicine gaining ground, recent efforts led to the differentiation of endotypes according to pathomechanisms also in CRS. We recently published the existence of three CRS endotypes representing different inflammatory profiles based on the presence or absence of IL-5 in tissue: IL-5 negative; moderately IL-5 positive; highly IL-5 positive, high serum IgE and often SE IgE positive in tissues². The third endotype represents the most severe type of CRSwNP with a high frequency of asthma co-morbidity and recurrent disease, making repeated surgery necessary.

In the last couple of years, periostin has been identified as marker of eosinophil/type 2 inflammation, specifically in asthma³ and in relation to therapies blocking the IL-13 and/or IL-4 pathway^{4,5}. Recent literature suggests that periostin levels are elevated in tissues from patients with CRSwNP compared to CRSsNP and controls, and periostin mRNA is upregulated and more frequently expressed in nasal mucosa of CRSwNP patients, especially in those with aspirin sensitivity^{6,7}. Whether periostin is elevated in serum of these patients is not known. Although periostin is induced by IL-4 and IL-13, but not IL-5, these cytokines are usually co-expressed and there might be an interplay between those cytokines, eosinophilic inflammation and periostin. Therefore, the aim of this study was to investigate if serum (s) periostin is elevated in CRSwNP compared to CRSsNP and controls, and to evaluate if s-periostin and other inflammatory markers could be used as serum biomarkers to identify CRSwNP patients with IL-5 and/or SE-IgE tissue (t) expression, representing moderate and severe endotypes of CRSwNP according to the formerly mentioned cluster analysis².

In total 377 patients participating in the Global Allergy and Asthma European Network (GA²LEN) study were included, divided in CRSwNP (n=144), CRSsNP (n=123) and controls (n=110) based on history and nasal endoscopy. Baseline characteristics are provided in supplementary Table E1 (online repository). Serum and tissue from these patients were analysed for selected inflammatory markers (for information on materials and methods please refer to the online repository for this article). Results show, as expected and previously reported, significantly elevated concentrations of tissue and serum IgE, SE-IgE, IL-5/sIL-5R

alpha and ECP in CRSwNP compared to CRSsNP and controls. S-periostin was significantly elevated in CRSwNP compared to CRSsNP and controls ($p < 0.0001$) (Table 1). There were no or weak correlations between serum and tissue concentrations for IgE, SE-IgE, Eosinophil cationic protein (ECP) or IL-5, hence none of these serum markers were stand-alone candidates to use for prediction of tissue expressions of these biomarkers.

Next, we performed ROC analyses to evaluate whether serum markers could predict presence of t-IL-5 or t-SE-IgE in patients with CRSwNP only. The first ROC analysis was performed on 108 CRSwNP patients where we had a complete data set for s-periostin, t-IL-5 and t-SE-IgE. Out of the 108 patients, 92 were positive for IL-5 in tissue. ROC-analysis determined the best cut-off for s-periostin regarding sensitivity and specificity to predict presence of IL-5 in tissue (Figure 1A); the cut-off was set at 48.5 ng/ml which gave a corresponding sensitivity and specificity of 93.5% and 62.5% ($p < 0.0001$) (Figure 1 B). 22 of 108 patients were SE-IgE positive in tissue, all of these patients were also IL-5 positive and had s-periostin concentrations > 48.5 ng/ml (Figure 1D). In order to identify CRSwNP patients with SE-IgE positive tissue, we performed ROC analysis of all patients with elevated s-periostin ($n=92$) (Figure 1C) and found that elevated levels of s-IgE (above 96 kU/L) and s-SE-IgE (above 0.28 kUA/L) predict presence of SE-IgE in tissue with a sensitivity of 77.3% and a specificity of 87.1% ($p < 0.0001$) (Figure 1B). Elevated levels of s-IgE or s-SE-IgE alone in CRSwNP patients predicted presence of t-SE-IgE with a sensitivity and specificity of 86.4% and 67.7% and 77.3% and 75.7 %, respectively (Figure 1C). Clinical markers, such as the polyp score or asthma comorbidity did not predict presence of IL-5 or SE-IgE in nasal polyp tissue with reasonable sensitivity or specificity in these patients (please see table E2 in this article online repository).

To conclude, our results show that s-periostin is elevated in patients with CRSwNP. S-periostin, s-IgE and s-SE-IgE may be used as biomarkers to predict expression of IL-5 or SE-IgE in nasal polyp tissue, i.e. identifying CRSwNP patients with moderate and severe type-2 inflammation. S-periostin concentrations below 48.5 ng/ml appeared to rule out SE-IgE presence in nasal polyps and make tissue IL-5 positivity unlikely; in contrast, s-periostin above 48.5 ng/ml predicts IL-5 tissue positivity with high sensitivity, but moderate specificity. Among CRSwNP patients with elevated s-periostin above 48.5 ng/ml, s-IgE and s-SE-IgE combined predicted the presence of t-SE-IgE with a moderate sensitivity and high specificity. The serum biomarkers predicted t-IL5 or t-SE-IgE in a differentiated manner, with periostin being predictive for t-IL-5 positivity, and s-IgE and s-SE-IgE being predictive for t-

SE-IgE positivity. As a possible shortcoming of the current paper, we cannot exclude that other biomarkers such as blood eosinophils might be equally useful.

Our findings suggest that serum biomarkers may be useful to predict the type of inflammation in nasal polyp tissue better than clinical markers and without the necessity of taking biopsies and analysing tissue samples. This approach can possibly be used in clinical work to identify different endotypes of CRSwNP and to determine severity of inflammation according to a recent cluster analysis², parameters which are linked to the prognosis after surgery and the risk of co-morbid asthma, and eventually predict response to corticosteroids and biologics in the future.

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Legends for tables and figures:

Table 1: Median concentrations (M) and inter quartile range (IQR) of inflammatory markers in serum and tissue among patients with chronic rhinosinusitis with nasal polyps (CRSwNP), chronic rhinosinusitis without nasal polyps (CRSsNP) and controls, proportion of patients expressing these inflammatory markers above cut off in serum and tissue.

Figure 1: Serum levels of periostin, IgE and SE-IgE in relation to tissue IL-5 positivity (A) and tissue SE-IgE (B) presented as ROC-curves. The table (C) presents cut-off concentrations, proportions of positive patients, area under the curve and sensitivity, specificity and p values for predicting IL-5 and SE-IgE in tissue using serum periostin, IgE and SE-IgE. P-value calculated with Fisher's exact test. D. Proportional Venn diagram showing the overlap between serum periostin > 48.5 ng/ml, tissue IL-5 positivity and tissue SE-IgE positivity among patients with CRSwNP (N=108).

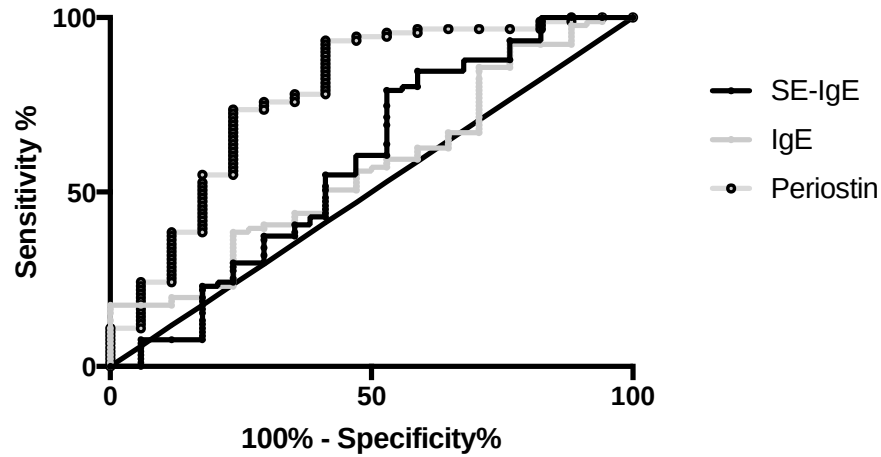
		CRSwNP	CRSSNP	Controls	p-value		CRSwNP vs CRSSNP	CRSwNP vs Controls	CRSSNP vs Controls
Serum concentration		M (IQR)	M (IQR)	M (IQR)					
	Periostin ng/ml	68.9 (57.1,86.6)	53.5 (46.4,64.3)	56.0 (48.3,67.8)	<0.0001 ¹		<0.0001 ²	<0.0001 ²	>0.05 ²
	IgE kU/l	84.5 (36.2,231.4)	44.4 (16.1,113.5)	50.1 (13.4,131.5)	<0.001 ¹		<0.001 ²	<0.01 ²	>0.05 ²
	SE-IgE kUA/l	0.13 (0,0.43)	0.07 (0,0.23)	0.08 (0,0.23)	<0.0001 ¹		<0.001 ²	<0.0001 ²	>0.05 ²
	ECP µg/l	15.3 (7.7,28.9)	13.1 (6.8,21.6)	11.2 (5.5,18.9)	<0.01 ¹		<0.01 ²	<0.01 ²	>0.05 ²
	sIL-5Rα pg/ml	318.5 (205.9,527.6)	239.4 (171.3,363)	219 (142.8,300.4)	<0.0001 ¹		<0.001 ²	<0.0001 ²	>0.05 ²
Proportion of patients above cut-off (numbers) in serum		% (n/N)	% (n/N)	% (n/N)					
	Periostin > 48.5 ng/ml %	93 (129/138)	72.7 (80/110)	75 (78/104)	< 0.0001 ³		<0.0001 ⁴	<0.0001 ⁴	>0.05 ⁴
	IgE >96 kU/l %	44.4 (64/144)	28.4 (35/123)	30 (33/110)	<0.05 ³		<0.01 ⁴	<0.05 ⁴	>0.05 ⁴
	SE-IgE > 0.28 kUA/l %	33.3 (48/144)	26 (32/123)	22.7 (25/110)	>0.05 ³		>0.05 ⁴	>0.05 ⁴	>0.05 ⁴
Tissue concentration		M (IQR)	M(IQR)	M (IQR)					
	IgE U/g tissue	203.2 (72.1,529.7)	25.2 (7.3-76.6)	8.9 (1.9,22.3)	<0.0001 ¹		<0.0001 ²	<0.0001 ²	<0.01 ²
	IL-5 pg/g tissue	132.3 (23.9,519.5)	0 (0,17.5)	0 (0,0)	<0.0001 ¹		<0.0001 ²	<0.0001 ²	<0.0001 ²
	ECP µg/g tissue	4.626 (2.2,9.4)	0.83 (0.20,2.3)	0.11 (0.07,0.35)	<0.001 ¹		<0.0001 ²	<0.0001 ²	<0.0001 ²
Proportion of patients above cut-off (numbers) in tissue		% (n/N)	% (n/N)	% (n/N)					
	IgE >0.35 U/g tissue %	97.3 (110/113)	87.2 (82/94)	71.9 (64/89)	<0.0001 ³		<0.01 ⁴	<0.0001 ⁴	<0.05 ⁴
	SE-IgE > 0.35 UA/g tissue %	20.3 (23/113)	3.2 (3/94)	0 (0/92)	<0.0001 ³		<0.001 ⁴	<0.0001 ⁴	>0.05 ⁴
	IL-5 >6.6 pg/g tissue %	83.9 (94/112)	32.3 (30/93))	2.4 (2/84)	<0.0001 ³		<0.0001 ⁴	<0.0001 ⁴	<0.0001 ⁴

M=Median concentration

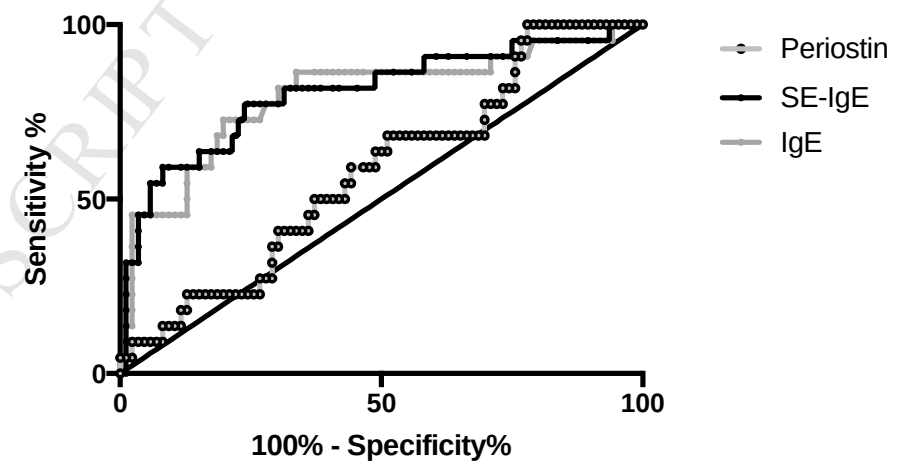
IQR=inter quartile range

- 1) Kruskal-Wallis test
- 2) Mann Whitney U-test
- 3) Chi-square test
- 4) Fisher's exact test

A.



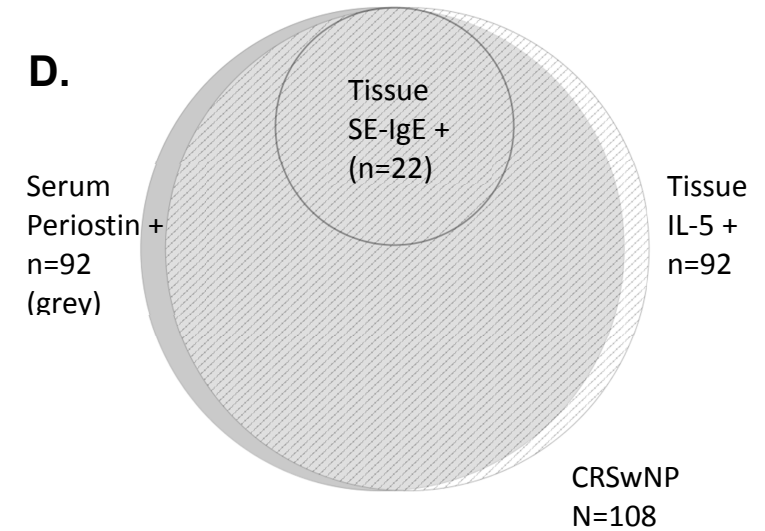
C.



B.

N=108	Cut-off level (proportion positive)	AUC	Sensitivity	Specificity	p-value
Tissue IL-5 positive (n=92)					
serum Periostin	48.5 ng/ml (86/92)	0.78	93.5%	62.5%	<0.0001
N=92					
Tissue SE-IgE positive (n=22)					
serum IgE	96 kUA/l (19/22)	0.8	86.4%	67.7%	<0.0001
serum SE-IgE	0.28 kUA/l (17/22)	0.1	77.3%	75.7%	<0.0001
Serum IgE, SE-IgE	(17/22)		77.3%	87.1%	<0.0001

D.



	CRSwNP	CRSsNP	Control	Total
N	144	123	110	377
Gender male/female/missing	95/47/2	64/57/2	60/47/3	
Age years; mean (range)	45 (25-72)	40(17-70)	32(17-69)	
BMI mean (range)	25.8 (18.1-34.8)	25.5 (17.1-39.6)	24.3 (17.7-33.4)	
Smoking packyears; mean (range)	18.2 (1-40)	17.5 (2-41)	13.5 (1-50)	
Smoking ever, n (%)	83 (58)	80 (65)	52 (47)	215 (57)
Smoking current, n (%)	23 (16)	46 (37)	26 (24)	95 (25)
Asthma, n (%)	60 (42)	17 (14)	15 (10)	92 (24)
Allergy, n (%)	63 (44)	61 (50)	39 (35)	163 (43)
Allergic rhinitis, n (%)	42 (29)	39 (32)	26 (24)	107 (28)
Aspirin intolerance, n (%)	14 (10)	2 (2)	1 (1)	15 (6)

Table E1

Tissue IL-5 positive	Sensitivity	Specificity	p-value
Polyp score > 3/6	60.7	84.6	<0.005
Asthma	38	62.5	>0.05
Tissue SE-IgE positive			
Polyp score > 3/6	61.9	39.4	>0.05
Asthma	54.6	64.3	>0.05

Table E2

Materials and methods:**Study design:**

This is a substudy of the GA²LEN Sinusitis Cohort group (principal investigator C. Bachert), performed in tertiary referral ear, nose and throat departments in Leuven, Ghent, Stockholm, Malmö, Helsinki, Amsterdam and Lodz. Ethical approval was obtained at all participating study centers and written informed consent was obtained from all subjects before sample collection.

Patient population:

Patients were included as previously described². In total 377 patients were included, 144 with CRSwNP, 123 with CRSsNP and 110 controls (Table E1). All patients from whom there was an adequate amount of tissue and serum were included in the first analyses of levels of inflammatory markers, please see table 1. In the second step, for serum biomarkers corresponding to tissue markers in CRSwNP, only patients with valid data on serum periostin, tissue IL-5 or tissue SE-IgE were included, in total 108. In the third step, identifying patients with SE-IgE in tissue, only patients with serum periostin above 48.5ng/ml were included.

Measurement of inflammatory markers:

Nasal tissue of 0.1 g was diluted in 1 mL of 0.9% NaCl solution containing a protease inhibitor cocktail (Complete; Roche Diagnostics, Mannheim, Germany), homogenized at 1000 rpm for 5 minutes, and centrifuged at 1500xg for 10 minutes at 4°C as described before^{E1}. Serum and tissue homogenate concentrations of total IgE, specific IgE to a mixture of *S. aureus* enterotoxins (staphylococcal enterotoxins A and C and toxic shock syndrome toxin 1) and ECP were assayed by using the UniCAP system (Phadia, Uppsala, Sweden). Concentrations of tissue IL-5 were assayed with commercially available Luminex Performance Assay Kits and measured on a Bio-Plex 200 Platform (Bio-Rad Laboratories S.A.-N.V, Nazareth Eke, Belgium). Serum periostin was measured using the clinical trial version of the Elecsys® Periostin assay on the e 601 module of the cobas 6000 system^{E2}. The Elecsys® Periostin assay is a fully automated assay employing two monoclonal antibodies

that target different epitopes of periostin in a sandwich immunoassay configuration. Detection is based on electrochemiluminescence technology.

Statistics:

Graph pad Prism 7 for Mac OS X was used for all analyses. Data were analysed with non-parametric tests: Kruskal-Wallis test to determine if there are statistically significant differences between the three groups (CRSwNP, CRSsNP and controls), and Mann Whitney U-test was used to compare the pairwise groups. A test for multiple comparison was performed. Chi-square test and Fisher's exact test was used to determine if there are statistically significant differences in absolute numbers between the three groups and for each pairwise comparison between groups, respectively. Spearman's rank correlation coefficient test was used to calculate correlations. ROC-curves were used to determine cut off values for serum and nasal secretion markers and p-value for sensitivity and specificity were calculated using Fisher's exact test. Statistical significance was set at $p < 0.05$. For drawing of the proportional Venn diagram euler APE was used (<http://www.eulerdiagrams.org/eulerAPE>).

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Legends for tables:

Table E1: Baseline characteristics of the cohort.

Table E2: Sensitivity and specificity of clinical signs for predicting the presence of IL-5 or SE-IgE in CRSwNP. P-values calculated by Fischer's exact test.