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Nasal specific IgE correlates to serum specific IgE: First steps towards nasal molecular allergy diagnostic

To the Editor,

Up to 40% of the European population suffer from respiratory type I hypersensitivity reactions induced by airborne allergens, such as plant pollen, fungal spores, or dust mite feces.[1] Guidelines for the treatment of AR in children recommend causative treatment, that is, allergen-specific immunotherapy (ASIT), as early as possible.[2] Allergy diagnostics is routinely performed by skin prick test (SPT) or blood test for the detection of allergen-specific immunoglobulin E (sIgE). If specific serum IgE is absent despite a positive history of allergic rhinitis, a nasal allergen provocation test is performed to assess local allergic rhinitis (LAR). Recent developments in microchip technology enabled the simultaneous detection of specific IgE levels against 112 individual allergens using only 30 µL of serum. However, SPTs are still often the method of choice when diagnosing young children, as children are typically afraid of needles. This can lead to improper diagnosis, since SPTs are prone to false-positive results due to the unspecified extracts used.[3] The aim of our study was therefore to adopt the Immuno Solid-phase Allergen Chip (ISAC) for nasal fluid as a noninvasive sampling method and to validate the technology as potential novel allergy test. Our analysis (see details in online supporting information) focused on the most relevant aeroallergens, that is, house dust mite (HDM), Betulaceae trees, including birch, hazel and alder, and grass pollen.[4]

Blood and nasal fluid samples as previously described [5] were obtained from 2 nonsensitized (NS) control subjects and 47 subjects sensitized (Figure S1and Table S2. online supporting information) to aeroallergens such as birch, hazel, alder, grass pollen, or house dust mite (HDM). Specific IgE levels were measured in sera and nasal fluid by the ImmunoCAP ISAC 112 (Table S1. online supporting information) according to the manufacturer's instruction (Thermo Fischer Scientific).

When correlating IgE against single allergen components, we observed a significant positive correlation (n = 49; $P < .001$) between serum and nasal tests (Figure 1, A), with a median of all Spearman correlation coefficients (r_s) across the whole panel of 0.77 (IQR 0.75, 0.85). The highest correlation coefficient was observed for Der p 2 and Aln g 1 ($r_s = .88$), followed by Cor a 1 ($r_s = .87$) and Bet v 1 ($r_s = .85$) (Figure 1, B).

We next determined the global sensitization profile of each subject's serum and nasal fluid and compared the profiles for all

FIGURE 1 Correlation between nasal and serum sIgE levels. A, Spearman correlation coefficients for all tested allergen components. B, Nasal sIgE levels (*y*-axis) plotted against serum sIgE levels (*x*-axis). Dots indicate study subjects. Fitted lines indicate positive linear correlations (Spearman). C, Spearman correlation coefficients per subject over the entire aeroallergen sensitization profile, as shown in panel A. D, Serum sIgE profile (*x*-axis) versus nasal sIgE profile (*y*-axis) shown for selected subjects. Blue dots indicate the 17 allergen-specific IgE tests included in the overall analysis. The red line represents the linear regression curve fit (positive Spearman correlation)

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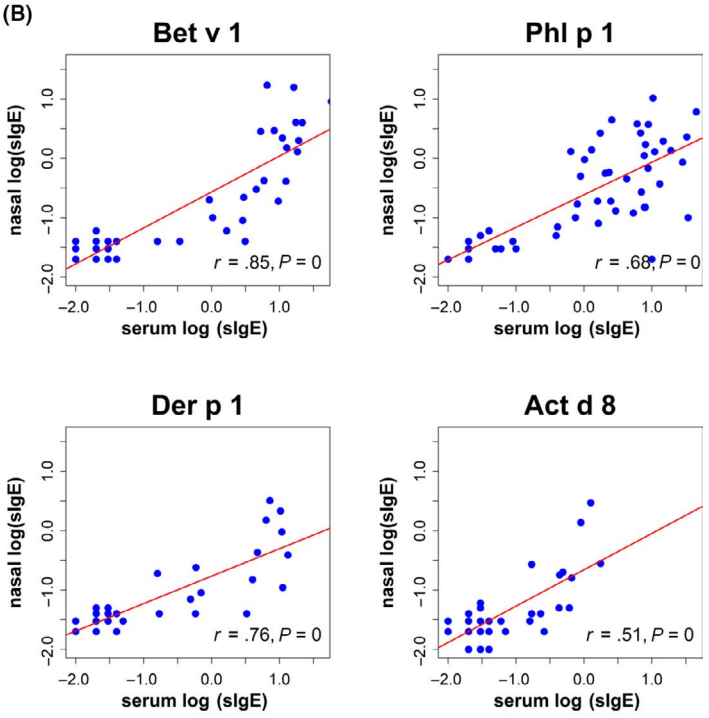
(A)

Allergen	*R*
Aln g 1	0.88
Der p 2	0.88
Cor a 1.0101	0.87
Bet v 1	0.85
Phl p 5	0.85
Mal d 1	0.82
Der f 1	0.79
Phl p 6	0.78
Pru p 1	0.77
Der f 2	0.77
Phl p 2	0.76
Der p 1	0.76
Cor a 1.0401	0.75
Ara h 8	0.74
Cyn d 1	0.72
Phl p 1	0.68
Act d 8	0.51

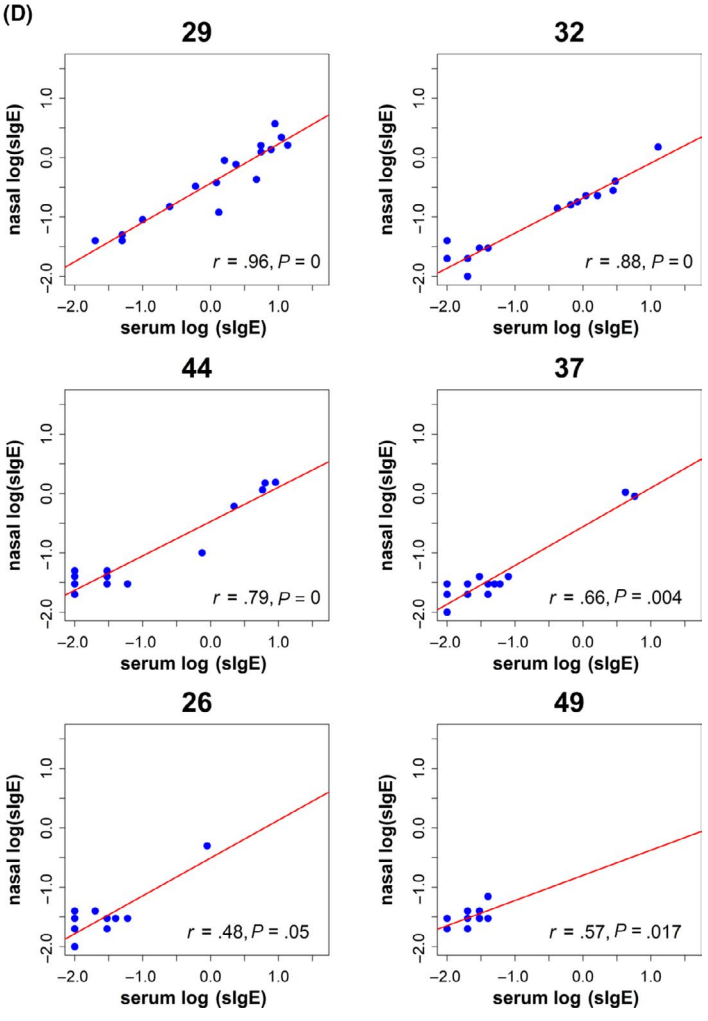
(B)



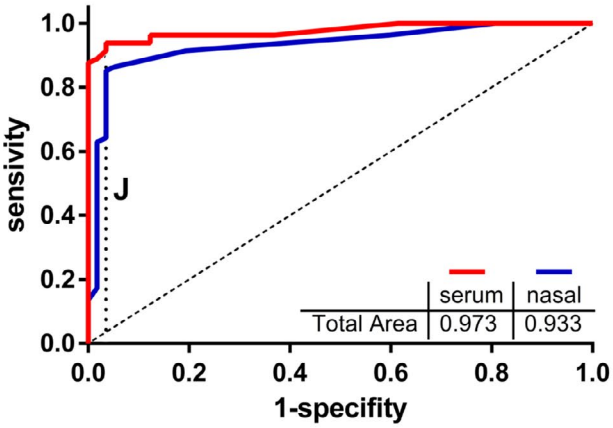
(C)

ID	*R*	ID	*R*
45	0.96	41	0.81
29	0.96	09	0.80
28	0.95	42	0.80
24	0.94	38	0.79
13	0.93	44	0.79
34	0.93	11	0.77
39	0.92	30	0.76
10	0.92	23	0.75
06	0.91	21	0.71
25	0.91	04	0.71
27	0.9	22	0.69
43	0.89	12	0.68
32	0.88	47	0.67
33	0.87	37	0.66
18	0.87	20	0.61
14	0.87	49	0.57
15	0.86	05	0.56
07	0.86	19	0.52
17	0.86	48	0.51
40	0.85	26	0.48
36	0.85	16	0.40
03	0.85	08	0.32
31	0.83	01	0.28
02	0.83	46	0.03
35	0.82		

(D)



(A)



(B)

		Sensitization	
		Yes	No
Serum	Positive test	76	3
	Negative test	5	54
Nasal fluid	Positive test	69	2
	Negative test	12	55

(C)

Serum	Statistical analysis	Nasal
0.94	Sensitivity (TPR)	0.85
0.95	Specificity (TNR)	0.96
0.96	Positive prediction value (PPV)	0.97
0.92	Negative prediction value (NPV)	0.82
0.04	Type I error	0.03
0.06	Type II error	0.15
0.05	False positive rate (FPR)	0.04
0.08	False omission rate (FOR)	0.18
17.83	Likelihood ratio pos. (LR+)	24.28
0.07	Likelihood ratio neg. (LR-)	0.15
273.60	Diagnostic odds ratio (DOR)	158.13
0.95	F1 score	0.91
0.94	Accuracy	0.90
0.88	Matthews correlation coefficient	0.80
0.89	Youden´s Index	0.82
0.88	Markedness	0.79

FIGURE 2 Receiver operating characteristic (ROC) curve for nasal and serum IgE test, and their comparison of ImmunoCAP and ISAC diagnostic. A, The ROC curves are generated by plotting nasal (blue) and serum (red) data. "J" indicates the threshold for positive nasal test. B, Contingency table for tests in serum and nasal fluid. C, Statistical analysis to verify performance of serum and nasal ISAC. Diagnostics via ImmunoCAP served as control

subjects (Figure 1, C). We observed a strong positive correlation between serum and nasal sIgE profiles, and the median Spearman correlation coefficient was r_s = .75, the IQR 0.68, 0.88. The only poor correlations observed were for subjects *No* 01, 08, 16, 26, and 46. The highest correlations were observed for subjects with several sensitizations, whereas nonsensitized subjects showed poorer correlations (see Figure 1, D for overview over selected subjects).

Finally, we evaluated the performance of the ISAC tests in serum and nasal fluid in comparison with the ImmunoCAP method by assessing cutoff, sensitivity, specificity, likelihood ratios, and operating characteristics. First, a nasal fluid cutoff threshold was estimated by a receiver operating characteristics (ROC) curve using Youden's index. The area under the curve (AUC) for nasal fluid (0.93) and serum tests (0.97) was found to be comparable (Figure 2, A). The nasal threshold was calculated to be 0.08 ISU-E. The diagnostic capability of sIgE determination for birch pollen (Bet v 1, Bet v 2, and Bet v 4), grass pollen (Cyn d 1, Phl p 1, Phl p 2, Phl p 5, and Phl p 6), and HDM (Der f 1, Der f 2, Der p 1, and Der p 2) was assessed by a two-by-two table (Figure 2, B). Specificity (serum: 0.95 and, nasal: 0.96) and positive prediction value (serum: 0.96 and nasal: 0.97) were similar for both methods. The diagnostic sensitivity and negative prediction value of serum diagnostic were higher than nasal diagnostic (TPR serum: 0.94 vs TPR nasal: 0.85; NPV serum: 0.92 vs NPV nasal: 0.82). In addition, diagnostic accuracy was determined by calculating likelihood ratios for serum (LR+ 17.83, LR– 0.07) and nasal biosampling (LR+ 24.28, LR– 0.15). Further statistical analyses were done to complement the results (Figure 2, C).

We demonstrate similar specificities of ISAC for serum and nasal fluid tests, whereas the sensitivity in the serum test is higher than in the nasal test. A likely explanation for the lower sensitivity in our nasal tests is that the manufacturer's instructions are optimized for the serum matrix. Consequently, the experimental setup (eg, incubation times, fluorescent marker, sampling methods) should be improved further to raise sensitivity of nasal fluid diagnostic. Moreover, the threshold for the nasal fluid test, as assessed in the current study, could be set to an even lower value (0.08) than recommended for serum by the manufacturer (0.3) without losing specificity. Overall, sIgE levels against all tested allergen components were significantly and positively correlated between nasal fluid and serum, and the inter-sample correlation was best for sIgE against birch and grass pollen and HDM (Figure S2. online supporting information). Recent studies comparing ISAC tests in serum and nasal fluid have shown similar results[6-9]; however, these studies compared only tests for single allergens, that is, house dust mite, Japanese cedar, mugwort pollen, and fungal spores, instead of a whole aeroallergen panel.

To conclude, we present the first study to assess whole patterns of IgE specific to aeroallergens in serum and nasal fluid and to systematically evaluate the novel, ISAC-based method in comparison with clinical standard diagnostics. Our results could be of high

relevance for the future improvement of clinical diagnostics, especially in children with allergic airway disease.

KEYWORDS

allergy diagnosis, IgE, pollen, rhinitis

CONFLICTS OF INTEREST

The authors declare no conflicting interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Cadherin-related family member 3 upregulates the effector functions of eosinophils

To the Editor,

Acute respiratory infections including rhinovirus (RV) infections are a major cause of asthma exacerbations.[1] Recent studies suggest that eosinophils play important roles in the development of asthma exacerbation.[2] Not only neutrophils but also eosinophils increase in asthmatic airways during viral infection,[3] suggesting that eosinophils are indeed recruited to and activated in the airways during virus-related asthma exacerbations.

Cadherin-related family member 3 (CDHR3), a member of the cadherin superfamily, is a transmembrane protein with six extracellular cadherin domains. However, the biological function of CDHR3 is still unknown. Recently, Bønnelykke et al[4] reported that