

Letter to the Editor

Reliable mite-specific IgE testing in nasal secretions by means of allergen microarray

To the Editor:

Because there is evidence for local production of IgE in the nasal mucosa,¹⁻³ methods to measure allergen-specific IgE (sIgE) at nasal level in patients with allergic rhinitis (AR) are of great interest. Nasal secretions (NasSec) are easily collected with various noninvasive methods⁴ and are suitable for IgE measurements.⁵⁻⁷ Nevertheless, IgE “classic” assays, based on immunoenzymatic methods and allergen extracts, require large volumes and are inefficient in detecting the low amounts of IgE in NasSec. Microarray biochips allow simultaneous measurement of IgE against several allergen molecules with excellent sensitivity and specificity.^{8,9} To our knowledge, this technology has never been tested in NasSec of patients with AR. To fill this gap, we tested the performance of a customized microarray containing 15 house dust mite (HDM) allergen molecules based on the Immunoassay capture test (ImmunoCAP) ImmunoCAP solid-phase allergen chip technology (Thermo Fisher Scientific, Uppsala, Sweden) for sIgE detection in NasSec of 30 adult patients with HDM-AR and 29 nonallergic controls (see Table E1 in this article’s Online Repository at www.jacionline.org). All subjects were recruited via the Department of Otorhinolaryngology in Ghent University Hospital (Belgium). HDM-AR was defined by a consistent history and a positive skin prick test (SPT) result to *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae* (ALK Abellø, The Netherlands). Controls were volunteers without history of any allergy, and negative SPT results. Eligible subjects underwent collection of serum and NasSec.

After collection with filter disks (FDs) and sinus packs (SPs) (Fig 1, A and B), NasSec were processed and diluted with saline (“fixed dilution processing,” ie, FD, 1:20; SP, 1:10).

Thirteen patients with AR underwent additional collections of serum and NasSec on a following visit. In this *second test*, NasSec were collected with 3 different absorbent devices: FDs, ear packs (EPs), and SPs. This time, NasSec were diluted with a fixed volume of saline (“fixed volume processing,” ie, FD, 1 mL; SP, 2 mL; EP, 3 mL). Further details are provided in this article’s Online Repository at www.jacionline.org.

Total IgE and sIgE to HDM extract were measured in sera and NasSec with ImmunoCAP-fluorescent enzyme immunoassay (TFS, Uppsala, Sweden). HDM sIgE were detected in serum of all 30 patients with AR, but only in 21 FD and 22 SP samples (see Table E2 in this article’s Online Repository at www.jacionline.org). All controls had undetectable HDM sIgE in both serum and NasSec. Hence, HDM sIgE in NasSec predicted with 100% specificity, but poor sensitivity (FDs, 70%; SPs, 73%) the results in serum.

Specific IgE to nDer p 1, rDer p 2, rDer p 4, rDer p 5, rDer p 7, rDer p 10, rDer p 11, rDer p 14, rDer p 15, rDer p 18, rDer p 21, rDer p 23, clone 16, and nDer f 1 and rDer f 2 were measured with the microarray in serum (cutoff ≥ 0.10 ImmunoCAP solid-phase allergen chip Standardized Units [ISU]) and NasSec (cutoff ≥ 0.03 ISU in diluted NasSec). The cutoff in NasSec was selected on the basis of sensitivity and specificity analyses of sIgE tests in NasSec for different cutoffs (see details in Table E3 in this article’s Online Repository at www.jacionline.org).

Among the patients with AR, the prevalence of sIgE to 13 of the 15 examined HDM molecules was similar in serum and NasSec: 83% to 97% for group 2 molecules (rDer p 2, rDer f 2);

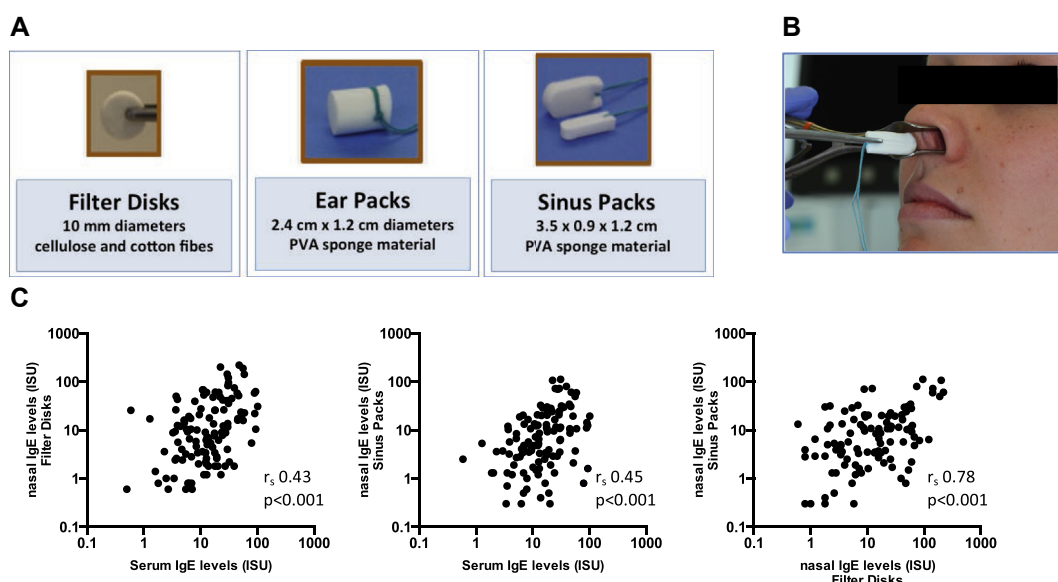


FIG 1. Methodology and results of IgE determination in NasSec. **A**, The 3 devices used to collect NasSec. **B**, Positioning sinus pack in the nostril. **C**, Relationship between levels (ISU) of detectable IgE to the 15 HDM molecules detected in NasSec (y-axis), collected with FDs and SPs, and serum (x-axis) in 30 patients with AR (first test). The Spearman rank correlation coefficients (r_s) and the respective P values are shown.

TABLE I. Prediction of HDM allergy and serum IgE responses by testing IgE in NasSec in 30 HDM-allergic patients and 29 healthy controls

	FD										Accuracy, %	LR+	LR–
	Sensitivity		Specificity		PPV		NPV						
	%	95% CI*	%	95% CI*	%	95% CI*	%	95% CI*					
Any major molecules†	90	73-98	100	83-100	100	82-100	91	75-98	95	∞	0.1		
nDer p 1	46	26-66	100	85-100	100	62-100	73	58-85	78	∞	0.5		
nDer f 1	68	46-85	100	85-100	100	73-100	81	66-91	86	∞	0.3		
rDer p 2	90	73-98	100	83-100	100	81-100	91	76-98	95	∞	0.1		
rDer f 2	90	73-98	100	83-100	100	81-100	91	76-98	95	∞	0.1		
rDer p 23	81	61-93	100	85-100	100	77-100	87	72-96	92	∞	0.2		
Other molecules													
rDer p 4	0	0-53	98	90-100	0	0-99	88	77-95	86	0.0	1.0		
rDer p 5	80	44-97	100	89-100	100	52-100	96	87-100	97	∞	0.2		
rDer p 7	78	40-97	100	90-100	100	47-100	96	87-100	97	∞	0.2		
rDer p 21	57	18-90	100	90-100	100	28-100	95	85-99	95	∞	0.4		
All molecules‡	71	63-77	100	99-100	98	93-100	93	91-95	94	167.4	0.3		

	SP										Accuracy, %	LR+	LR–
	Sensitivity		Specificity		PPV		NPV						
	%	95% CI*	%	95% CI*	%	95% CI*	%	95% CI*					
Any major molecules†	87	69-96	100	83-100	100	81-100	88	72-97	93	∞	0.1		
nDer p 1	58	37-78	100	85-100	100	68-100	78	63-89	83	∞	0.4		
nDer f 1	48	28-69	100	85-100	100	64-100	72	57-84	78	∞	0.5		
rDer p 2	86	68-96	100	83-100	100	80-100	88	73-97	93	∞	0.1		
rDer f 2	86	68-96	100	83-100	100	80-100	88	73-97	93	∞	0.1		
rDer p 23	81	61-93	100	85-100	100	77-100	87	72-96	92	∞	0.2		
Other molecules													
rDer p 4	14	0-58	98	90-100	50	1-99	89	78-96	88	7.4	0.9		
rDer p 5	70	35-93	90	78-97	58	28-85	94	82-99	86	6.9	0.3		
rDer p 7	67	30-93	100	90-100	100	42-100	94	84-99	95	∞	0.3		
rDer p 21	57	18-90	100	90-100	100	28-100	95	85-99	95	∞	0.4		
All molecules‡	68	60-75	99	98-100	95	90-98	93	91-94	94	80.3	0.3		

Values in boldface are the outcomes referred to at least 1 of the major allergen molecules (nDer p 1, Der f 1, nDer p 2, rDer f 2, rDer p 23).

LR+, Positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

*Exact binomial confidence limits (95% CI) for test sensitivity, specificity, PPV and NPV.

†Outcomes referred to at least 1 of the major allergen molecules (nDer p 1, nDer f 1, rDer p 2, rDer f 2, rDer p 23).

‡Including outcomes of rDer p 10, rDer p 11, rDer p 14, rDer p 15, Clone 16, and rDer p 18, all characterized by a low positive sample size ($n < 5$).

70% to 87% for rDer p 23; 20% to 33% for rDer p 5 and rDer p 7; and 0% to 23% for all others. In contrast, sIgE to group 1 molecules (nDer p 1, nDer f 1) was detected frequently in serum (80% to 83%), but less so in NasSec (37% to 60%). In both serum and NasSec of the 29 controls, sIgE was undetectable, except for IgE to rDer p 5 detected in 4 SP samples (see Table E4 in this article's Online Repository at www.jacionline.org).

Detection of IgE to at least 1 of the major allergen molecules (nDer p 1, nDer f 1, rDer p 2, rDer f 2, rDer p 23) in NasSec predicted with high sensitivity and excellent specificity the allergic status of the patient and the outcome in serum (specificity 100% for both FDs and SPs; sensitivity 90% for FDs and 87% for SPs; Table I). With the FD method, the sensitivity of the microarray to identify patients with AR was significantly higher than that of the ImmunoCAP (27 of 30, 90% vs 21 of 30, 70%; $P = .05$).

Levels of IgE to the HDM molecules in NasSec collected with FDs and SPs were highly related to one another (r_s , 0.78; $P < .001$) and to the levels in serum (FDs, r_s , 0.43, $P < .001$; SP, r_s , 0.45, $P < .001$; Fig 1, C).

The results of the *first test* were generally reproduced in the *second test*. Moreover, this time, the prevalence of sIgE to group 1 molecules in serum and NasSec was more similar (serum, 77%; NasSec, 62%-77%; see Tables E5 and E6 in this article's Online Repository at www.jacionline.org). Possible reasons for this difference are discussed in this article's Online Repository at www.jacionline.org. HDM sIgE levels measured in the *first test* and the *second test* were highly correlated in each sample type (serum, r_s , 0.86, $P < .001$; FD, r_s , 0.76, $P < .001$; SP, r_s , 0.71, $P < .001$; see Fig E1 in this article's Online Repository at www.jacionline.org).

We pioneered the use of an allergen microarray chip for sIgE detection in NasSec of patients with AR. Our customized microarray detected sIgE to HDM molecules in NasSec with a good sensitivity, superior to ImmunoCAP-fluorescent enzyme immunoassay in detecting IgE to HDM extracts in the same samples. Moreover, the microarray results in NasSec were very predictive for the serum results. Hence, testing nasal IgE to allergen molecules by a microarray approach may soon become a noninvasive alternative in the diagnostic workup of AR. In addition, our findings may offer new

perspectives for research on “Local Allergic Rhinitis,” a subset of rhinitis characterized by sIgE at the nasal level without systemic allergy.⁵

The high accuracy of the microarray approach was very robust with all the methods used for NasSec collection (FDs, EPs, SPs) and processing (“fixed volume” and “fixed dilution”). The best performance was obtained with FDs, which are smaller in size and more acceptable for the patient. NasSec were quite highly diluted during processing, but high diagnostic accuracy was also obtained in some less diluted samples of the *second test*, raising the question whether a lower dilution (eg, 1:4) might be used to further improve the test sensitivity.

A limitation of our study is the possibility that IgG₄ antibodies—not measured in our samples—may have exerted competitive effects to IgE binding in our microarray. Also, the selected cutoff (ie, 0.03 ISU for diluted NasSec) cannot be automatically applied in other settings or to other microarrays. Large-scale studies are needed to confirm our promising findings. The technique should be investigated in more heterogeneous patient populations and with other airborne allergens.

We thank Gabriele Holtappels (Upper Airway Research Laboratory, Ghent) for her advise on how to process NasSec and how to perform measurements in NasSec. We thank Alexander Rohrbach for his laboratory support (Charité-Universitätsmedizin, Berlin). We thank Sander De Bruyne and Eveline van Mulders (2 medical students of Ghent University) for their help with patient recruitment and sample collection. Finally, we thank all patients for their participation in this study.

Margot Berings, MD^{a,b,*}
Stefania Arasi, MD^{d,e,*}
Natalie De Ruyck, MSc^a
Serena Perna, MSc^d
Yvonne Resch, PhD^f
Christian Lupinek, MD^f
Kuan-Wei Chen, PhD^f
Susanne Vrtala, PhD^{f,g}
Giovanni Battista Pajno, MD^e
Claus Bachert, MD, PhD^{b,c}
Bart N. Lambrecht, MD, PhD^{b,c}
Melissa Dullaers, PhD^{b,c,h}
Rudolf Valenta, MD^f
Paolo Maria Matricardi, MD^d
Philippe Gevaert, MD, PhD^a

From ^athe Upper Airways Research Laboratory, Ghent University, ^bthe Laboratory of Immunoregulation, VIB Inflammation Research Center, and ^cthe Department of Internal Medicine, Ghent University, Ghent, Belgium; ^dthe Department of Pediatric Pneumology & Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany; ^ethe Department of Pediatrics-Allergy Unit, University of Messina, Messina, Italy; ^fthe Division of Immunopathology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, and ^gChristian Doppler Laboratory for the development of allergen chips, Medical University of Vienna, Vienna, Austria; and ^hClinical Immunology Research Lab, Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium. E-mail: margot.berings@ugent.be.

*These authors contributed equally to this work.

Disclosure of potential conflict of interest: M. Berings receives grant support from Flemish Scientific Research Foundation. C. Lupinek receives payment for lectures from ThermoFisher. S. Vrtala receives grant support from Austrian Science Fund and Christian Doppler Research Association. R. Valenta receives grant support from the Austrian Science Fund (Der Wissenschaftsfonds [FWF]) and Christian Doppler Research Association and serves as a consultant for Biomay AG, Thermo Fisher Scientific, and Fresenius Medical Care. P. M. Matricardi receives materials and agents from ThermoFisher Scientific; serves as a consultant for ThermoFisher Scientific and Eutoimmun AG; and receives grant support from ThermoFisher. P. Gevaert receives grant support from the Flemish Scientific Research Foundation. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic rhinitis patients with negative skin tests. *Lancet* 1975;2:148-50.
2. Platts-Mills TA. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979;122:2218-25.
3. Dullaers M, De Bruyne R, Ramadani F, Gould HJ, Gevaert P, Lambrecht BN. The who, where, and when of IgE in allergic airway disease. *J Allergy Clin Immunol* 2012;129:635-45.
4. Howarth PH, Persson CG, Meltzer EO, Jacobson MR, Durham SR, Silkoff PE. Objective monitoring of nasal airway inflammation in rhinitis. *J Allergy Clin Immunol* 2005;115:S414-41.
5. Rondón C, Campo P, Togias A, Fokkens WJ, Durham SR, Powe DG, et al. Local allergic rhinitis: concept, pathophysiology, and management. *J Allergy Clin Immunol* 2012;129:1460-7.
6. Scadding G, Hellings P, Alobid I, Bachert C, Fokkens W, van Wijk RG, et al. Diagnostic tools in Rhinology EAACI position paper. *Clin Transl Allergy* 2011;1:2.
7. Sakaida H, Masuda S, Takeuchi K. Measurement of Japanese cedar pollen-specific IgE in nasal secretions. *Allergol Int* 2014;63:467-73.
8. Reisacher WR. Detecting local immunoglobulin E from mucosal brush biopsy of the inferior turbinates using microarray analysis. *Int Forum Allergy Rhinol* 2013;3:399-403.
9. Lupinek C, Wollmann E, Baar A, Banerjee S, Breiteneder H, Broecker BM, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the McDALL allergen-chip. *Methods* 2014;66:106-19.

<http://dx.doi.org/10.1016/j.jaci.2016.11.047>

METHODS

Subjects

The local ethical committee of Ghent University Hospital approved the study. All subjects gave written informed consent before inclusion.

All subjects underwent an SPT to 8 locally relevant airborne allergen extracts (grass mix, tree mix, *Artemisia vulgaris*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, and *Alternaria*; ALK Abellø). Subjects with acute bacterial/viral rhinosinusitis at the moment of testing were excluded from the study. Intake of oral antihistamines, nasal decongestants, and nasal or systemic corticosteroids was discontinued in the week before the study visits. None of the subjects had ever been treated with allergen-specific immunotherapy or anti-IgE antibodies (omalizumab). All subjects completed questionnaires on general health and allergy.

Sample collection for the *first test* was performed in 30 patients with AR and 29 nonallergic controls between November 2013 and August 2016. Sample collection for the *second test* was performed in 13 patients with AR between January and March 2015, in the 15 patients whose first sample had been taken between November 2013 and March 2014. Patients with AR and control subjects were comparable in terms of age and sex (clinical characteristics are presented in Table E1).

Statistics

Sensitivity and specificity, positive and negative predictive values, accuracy, and positive and negative likelihood ratios of IgE tests in NasSec were calculated with 2-by-2 tables, using the results of IgE tests in serum as a reference. Inpatient comparisons of IgE levels were performed with Spearman rank correlation and a *P* value of less than .05 was considered statistically significant. Statistical analyses were performed with R Core Team (2014), version 3.2.3. Graphs were created with Graphpad Prism version 6.00 (La Jolla, Calif).

Collection and processing of NasSec

Three different absorptive devices were used in this study for collection of NasSec: FDs, EPs, and SPs. FDs are punched out of Shandon filter paper cards (No. 190005; Shandon, Pittsburgh, Pa) using a 10-mm punch. SPs and EPs are commercially available sponges, for this study provided by Fabco (Ivalon; New London, Conn): "Post-Op Sinus Packing" (K9, 4000plus, 3.5 cm long × 0.9 cm wide × 1.2 cm high) and "Small Post-Op Ear Packing" (2.4 cm long × 1.2 cm diameter). The FDs, SPs, and EPs are sealed in pairs in 15-mL tubes (Falcon, Becton-Dickinson Labware, Lincoln Park, NJ) and are preweighed. Under direct visualization, using a surgical headlight, a speculum, and bayonet forceps, the FDs, SPs, or EPs are placed in the nasal cavity (1 per nostril). FDs are placed on the anterior third of the nasal septum,^{E1} whereas SPs and EPs are inserted on the floor of the cavity between the septum and inferior turbinate.^{E2} The devices are left in the nose for 5 minutes, are then removed, placed back in their respective 15-mL tube, and weighed again. The weight of collected NasSec (WNS) is determined by comparing the weights of the devices in their respective Falcon tubes before and after collection, using the same balance.^{E2}

After collection, an amount of physiologic saline (0.9% NaCl) is added to mobilize the NasSec from the device, resulting in diluted samples. For the first test, NasSec were processed with a *fixed dilution*: FD samples were diluted 1:20, SP samples 1:10. To obtain this fixed dilution, the amount of saline to be added, needs to be calculated for each sample: X

(mL) = $WNS \times (DF - 1)$ (DF, dilution factor; WNS, weight of collected NS; X , volume of saline to be added).

For the second test, NasSec were processed with a *fixed volume*: 1 mL was added to FD samples, 2 mL to EP samples, and 3 mL to SP samples. Hence, the dilution differs for each sample and needs to be calculated for each sample: $DF = WNS + X(mL) / WNS$. After addition of saline, samples are stored at 4°C for 2 hours. Subsequently, the devices are placed in the shaft of a 5-mL syringe (Becton-Dickinson S.A.), placed back in their respective tube, and squeezed out with the piston of the syringe. The samples are then centrifuged (1500g, 15 minutes, 4°C) to extract all fluid. Finally, the supernatants are aliquoted and stored at -20°C until analysis.

Because the NasSec samples were diluted during processing, measured levels of total IgE and sIgE needed to be corrected afterward: levels were multiplied with the corresponding dilution factor to estimate the levels in NasSec samples before they were diluted.

For *fixed dilution processing*, the cutoff of 0.2 kU/L (ImmunoCAP) in the diluted samples reflects a cutoff of 4.0 kU/L for FDs and 2.0 kU/L for SPs in the undiluted samples; the cutoff of 0.03 ISU (allergen microarray) in the diluted samples reflects a cutoff of 0.6 ISU for FDs and 0.3 ISU for SPs in the undiluted samples. Because *fixed volume processing* results in a different dilution for each sample, the cutoff points of 0.2 kU/L and 0.03 ISU in the diluted samples correspond to different cutoff points in the undiluted samples.

Selecting the cutoff in NasSec

Sensitivity and specificity of IgE tests in NasSec were calculated for different cutoffs, with IgE tests in serum as reference. The best balance of sensitivity and specificity was achieved with a cutoff of 0.03 ISU or more in diluted NasSec for all the 15 molecules and for at least 1 of the major allergen molecules of groups 1 (nDer p 1, nDer f 1), 2 (rDer p 2, rDer f 2), and 23 (rDer p 23) (Table E3). Therefore, this cutoff was selected for further analyses.

Prevalence of sIgE to group 1 molecules: Difference between *first test* and *second test*

In the *first test*, sIgE to group 1 molecules (nDer p 1, nDer f 1) was detected frequently in serum, but less so in NasSec. In the *second test*, the prevalence of sIgE to group 1 molecules in serum and NasSec was more similar. The second sample was collected 1 year after the first one, and we did not measure exposure to Der p 1 in the home environment of our patients. Therefore, we cannot ascertain whether this variation is due to (1) differences in allergen exposure; (2) an increased local (nasal) production of IgE against group 1 molecules; or (3) a very specific, although unknown, methodological issue in the first determination.

REFERENCES

- E1. Calus L, Devuyst L, Van Zele T, De Ruyck N, Derycke L, Bachert C, et al. The response to nasal allergen provocation with grass pollen is reduced in patients with chronic rhinosinusitis with nasal polyposis and grass sensitization. *Clin Exp Allergy* 2016;46:555-63.
- E2. Watelet JB, Gevaert P, Holtappels G, Van Cauwenberge P, Bachert C. Collection of nasal secretions for immunological analysis. *Eur Arch Otorhinolaryngol* 2004; 261:242-6.

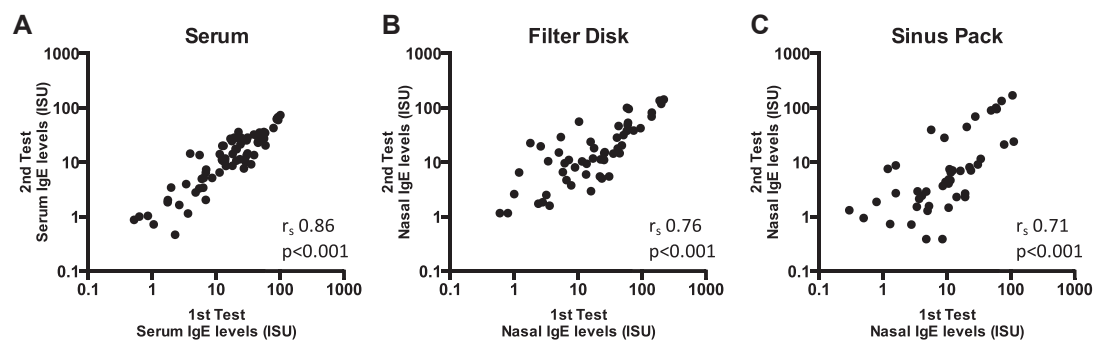


FIG E1. IgE levels (ISU) to all 15 HDM allergen molecules detected in each patient with AR ($n = 13$) at 2 different time points (test 1 and test 2) in the 3 samples, respectively: serum (**A**), FDs (**B**), and SPs (**C**). The Spearman rank correlation coefficients (r_s) and the respective P values are shown.

TABLE E1. Characteristics of study population

Characteristic	Nonallergic subjects (n = 29)		HDM-allergic patients (n = 30)		<i>P</i> value*
	Median/n	IQR/%	Median/n	IQR/%	
Age (y)	26	(24.2-29.8)	26	(23.7-31.0)	.458
Sex: female	22	75.9%	18	60.0%	.267
BMI (kg/m ²)	22	(19.8-24.3)	23	(21.2-24.3)	.110
Nicotin	1	3.4%	3	10.0%	.612
VAS control AR (mm)	0	(0-1.5)	34	(14.0-50.3)	<.001
VAS nasal symptoms (mm)	2	(0-7)	25	(11.8-51.3)	<.001
ARIA, persistent	NA	NA	20	66.7%	NA
ARIA, moderate/ severe	NA	NA	15	50.0%	NA
SS Nose (0-3)	0	(0-0.5)	1	(0.8-1.6)	<.001
SS Nose&Eye (0-3)	0	(0-0.3)	0.8	(0.7-1.5)	<.001
Asthma	0	0.0%	9	30.0%	.002
ACT score (0-25)	25	(25-25)	25	(24.3-25)	.022
Monosensitized to HDM	NA	NA	5	16.7%	NA

ACT, Asthma Control Test; ARIA, Allergic Rhinitis and its Impact on Asthma; BMI, body mass index; IQR, interquartile range; NA, not applicable/available; SS, symptom score; VAS, Visual Analog Scale.

**P* value for difference between nonallergic subjects and HDM-allergic patients (Mann-Whitney *U* test or Fisher exact test).

TABLE E2. IgE responses detected with ImmunoCAP in 30 patients with AR at the first test

Patient ID	Serum (IgE $\geq$ 0.1 kU/L)		FD (IgE $\geq$ 4 kU/L)		SP (IgE $\geq$ 2 kU/L)	
	Total IgE (kU/L)	HDM slgE (kU/L)	Total IgE (kU/L)	HDM slgE (kU/L)	Total IgE (kU/L)	HDM slgE (kU/L)
1	472	46	91	16	65	13
2	595	83	29	10	22	6
3	66	8	4	BDL	BDL	BDL
4	307	34	105	25	30	10
5	134	36	32	15	59	24
6	27	11	5	4	4	2
7	167	33	4	BDL	BDL	BDL
8	194	20	8	4	14	5
9	62	8	8	BDL	73	14
10	56	3	5	BDL	BDL	BDL
11	164	29	21	8	5	2
12	197	36	23	9	11	5
13	28	3	BDL	BDL	2	BDL
14	28	10	BDL	BDL	3	2
15	246	27	90	27	64	18
16	49	17	5	4	3	2
17	402	78	8	4	35	12
18	362	26	39	7	123	14
19	87	2	59	4	10	BDL
20	208	20		13	4	2
21	61	6	BDL	BDL	5	2
22	52	4	25	8	3	BDL
23	273	43		63	19	6
24	473	20	BDL	BDL	4	BDL
25	112	10	26	6	26	9
26	55	8	BDL	BDL	BDL	BDL
27	56	2	118	10	30	3
28	72	17	11	6	5	3
29	82	7	43	9	12	4
30	143	6	79	6	20	2
Median (IQR)	123 (56-253)	17 (7-33)	16 (4-42)	6 (BDL-10)	10 (3-30)	3 (BDL-9)
	0 BDL	0 BDL	5 BDL	9 BDL	4 BDL	8 BDL

Detection limit of 0.2 kU/L in diluted NasSec reflecting a detection limit of 4 kU/L for FDs (1:20 dilution) and 2 kU/L for SPs (1:10 dilution) in the undiluted NasSec. For patient 20 and patient 23, total IgE was not measured in NasSec collected with FDs because not enough volume was available.

BDL, Below detection limit; *IQR*, interquartile range.

TABLE E3. Sensitivity and specificity of the microarray for different cutoffs of sIgE positivity in NasSec, with the results in serum[‡] as reference, considering all the 15 and at least 1 of the major allergen molecules (nDer p 1, rDer p 2, rDer p 23, nDer f 1, rDer f 2)

Collection method	Allergen molecules	sIgE CO 0.01 ISU	sIgE CO 0.02 ISU	sIgE CO 0.03 ISU	sIgE CO 0.1 ISU	sIgE CO 0.3 ISU
Filter disk						
	Any*	90/83	90/100	90/100	87/100	73/100
	All [†]	77/91	72/98	71/100	59/100	44/100
Sinus pack						
	Any*	90/66	90/100	87/100	83/100	73/100
	All [†]	75/89	69/97	68/99	62/100	49/100

Sensitivity/specificity shown for the respective cutoff levels in NasSec. Sensitivity/specificity in boldface for the selected cutoff level of 0.03 ISU in NasSec.

CO, Cutoff.

*Outcomes referred to at least 1 of the major allergenic molecules (nDer p 1, rDer p 2, rDer p 23, nDer f 1, rDer f 2).

[†]Outcomes referred to all 15 molecules, including rDer p 4, rDer p 21, rDer p 11, rDer p 18, Clone 16, rDer p 10, rDer p 15, and rDer p 14, all characterized by a low positive sample size ($n < 5$).

[‡]Cutoff of sIgE positivity in serum: 0.1 ISU.

TABLE E4. Prevalence of detectable IgE antibodies to each of the 15 HDM allergen molecules among the different samples (serum and NasSec) collected from 29 nonallergic controls and 30 HDM-allergic patients with AR at the first test

	Nonallergic subjects (n = 29)						HDM-allergic patients with AR (n = 30)					
	Serum		FD		SP		Serum		FD		SP	
	IgE ≥ 0.1 ISU		IgE ≥ 0.6 ISU		IgE ≥ 0.3 ISU		IgE ≥ 0.1 ISU		IgE ≥ 0.6 ISU		IgE ≥ 0.3 ISU	
	n	%	n	%	n	%	n	%	n	%	n	%
<i>D pteronyssinus</i>												
nDer p 1	0	0	0	0	0	0	24	80	11	37	14	47
rDer p 2	0	0	0	0	0	0	29	97	26	87	25	83
rDer p 4	0	0	0	0	0	0	7	23	1	3	2	7
rDer p 5	0	0	0	0	4	14	10	33	8	27	8	27
rDer p 7	0	0	0	0	0	0	9	30	7	23	6	20
rDer p 10	0	0	0	0	0	0	1	3	0	0	1	3
rDer p 11	0	0	0	0	0	0	1	3	0	0	0	0
rDer p 14	0	0	0	0	0	0	0	0	0	0	0	0
rDer p 15	0	0	0	0	0	0	1	3	1	3	0	0
Clone 16	0	0	0	0	0	0	3	10	1	3	1	3
rDer p 18	0	0	0	0	0	0	1	3	2	7	0	0
rDer p 21	0	0	0	0	0	0	7	23	4	13	4	13
rDer p 23	0	0	0	0	0	0	26	87	21	70	21	70
<i>D farinae</i>												
nDer f 1	0	0	0	0	0	0	25	83	17	57	18	60
rDer f 2	0	0	0	0	0	0	29	97	26	87	25	83

Overall, 4 of 1305 (0.31%) microarray tests performed were positive among the 29 controls, whereas 423 of 1350 (31.33%) microarray tests performed were positive among the 30 patients with AR. Cutoff in diluted NasSec of 0.03 ISU reflecting a cutoff in the undiluted NasSec of 0.6 ISU for FDs (1:20 dilution) and 0.3 ISU for SPs (1:10 dilution).

TABLE E5. Prevalence of detectable IgE antibodies to each of the 15 HDM allergen molecules among the different samples (serum and NasSec) collected from 13 patients with AR at the second test

	Serum		FD		SP		EP	
	n	%	n	%	n	%	n	%
<i>D pteronyssinus</i>								
nDer p 1	10	77	9	69	10	77	10	77
rDer p 2	13	100	11	85	12	92	12	92
rDer p 4	1	8	4	31	3	23	4	31
rDer p 5	4	31	4	31	3	23	5	38
rDer p 7	5	38	3	23	3	23	3	23
rDer p 10	1	8	0	0	0	0	0	0
rDer p 11	0	0	0	0	0	0	4	31
rDer p 14	0	0	0	0	0	0	0	0
rDer p 15	1	8	0	0	0	0	0	0
Clone 16	1	8	1	8	1	8	0	0
rDer p 18	4	31	3	23	2	15	3	23
rDer p 21	2	15	0	0	0	0	0	0
rDer p 23	10	77	8	62	9	69	8	62
<i>D farinae</i>								
nDer f 1	10	77	8	62	9	69	8	62
rDer f 2	13	100	11	85	12	92	12	92

TABLE E6. Prediction of IgE responses in serum by testing IgE responses in NasSec collected with FDs, SPs, and EPs in 13 patients with AR at the second test

	Sensitivity		Specificity		PPV		NPV		Accuracy, %	LR+	LR–	<i>r_s</i>
	%	95% CI*	%	95% CI*	%	95% CI*	%	95% CI*				
FD vs serum												
nDer p 1	90	23-83	100	19-100	100	55-100	75	19-99	92	NA	0.1	0.57
rDer p 2	85	53-97	NA	NA	100	67-100	0	0-80	85	NA	NA	0.28
rDer p 5	100	28-100	100	55-100	100	28-100	100	55-100	100	NA	0.0	0.2
rDer p 7	60	14-94	100	51-100	100	19-100	80	44-97	85	NA	0.4	0.5
rDer p 23	82	48-97	100	9-100	100	55-100	50	6-93	85	NA	0.2	0.72†
nDer f 1	80	44-97	100	19-100	100	51-100	60	14-94	85	NA	0.2	0.48
rDer f 2	85	53-97	NA	NA	100	67-100	0	0-80	85	NA	NA	0.27
All‡	82	72-89	99	97-99	96	88-99	95	92-97	95	86.5	0.2	0.60§
SP vs serum												
nDer p 1	90	55-100	67	9-99	90	55-99	67	9-99	85	2.7	0.2	0.50
rDer p 2	92	62-99	NA	NA	100	69-100	0	0-30	92	NA	NA	0.29
rDer p 5	75	19-99	100	55-100	100	19-100	90	55-99	92	NA	0.3	0.50
rDer p 7	60	14-94	100	51-100	100	19-100	80	44-97	85	NA	0.4	0.50
rDer p 23	73	39-94	100	9-100	100	51-100	40	5-85	77	NA	0.3	0.50
nDer f 1	80	44-97	67	9-99	89	51-99	50	6-93	77	2.4	0.3	0.40
rDer f 2	92	62-99	NA	NA	100	69-100	0	0-30	92	NA	NA	0.17
All‡	76	65-84	98	95-99	92	83-96	94	90-96	93	40.2	0.2	0.46§
EP vs serum												
nDer p 1	100	58-100	100	19-100	100	58-100	100	19-100	100	NA	0.0	0.55
rDer p 2	92	62-99	NA	NA	100	69-100	0	0-30	92	NA	NA	0.30
rDer p 5	100	28-100	89	51-99	80	28-99	100	51-100	92	9.0	0.0	0.40
rDer p 7	60	14-94	100	51-100	100	19-100	80	44-97	85	NA	0.4	0.50
rDer p 23	91	58-99	100	9-100	100	58-100	67	9-99	92	NA	0.1	0.56
nDer f 1	80	44-97	100	19-100	100	51-100	60	14-94	85	NA	0.2	0.40
rDer f 2	92	62-99	NA	NA	100	69-100	0	0-30	92	NA	NA	0.22
All‡	82	71-89	92	86-96	87	77-94	89	81-93	88	10.8	0.2	0.52§

LR+, Positive likelihood ratio; LR–, negative likelihood ratio; NA, not applicable/available; NPV, negative predictive value; PPV, positive predictive value; r_s , Spearman rank correlation coefficient.

*Exact binomial confidence limits (95% CI) for test sensitivity, specificity, PPV, and NPV.

† r_s ($P < .05$).

‡Including outcomes of rDer p 4, rDer p 21, rDer p 11, rDer p 18, Clone 16, rDer p 10, rDer p 15, and rDer p 14, all characterized by a low positive sample size ($n < 5$).

§ r_s ($P < .001$).