

warts (P22). In addition, P34 exhibited an ongoing systemic autoimmune disorder, which required long-term IL-1 blockade. Posttransplantation complications were more frequent, although not significantly, for patients with a WAS score of 3 or greater versus those with a score of less than 3 (50% vs 17%, $P = .06$). Graft source, patient age, and conditioning did not influence the development of posttransplantation complications. Although also nonsignificant, patients splenectomized before transplantation had less reported complications than nonsplenectomized patients (23% vs 43%, $P = .32$; see Fig E2 in this article's Online Repository at www.jacionline.org).

We advocate effective and expedient control of pretransplantation morbidities, including autoimmunity, bleeding, and infections. In our cohort 11 boys were splenectomized, and although this has been associated with a risk of severe infections⁶ and mortality,³ safeguards included long-term penicillin prophylaxis and meningococcal/pneumococcal vaccination. In some cases we used specialized avenues, such as preimplantation genetic diagnosis, for selection of an unaffected HLA-matched sibling donor ($n = 1$) and autologous lentiviral gene therapy ($n = 3$, data not included in this cohort), as previously described.⁷ The advantages of the latter include avoiding delays in donor searching and obviation of the risk of GvHD. In other patients without matched donors, mismatched or haploidentical transplantation was undertaken as soon as practicable after stabilization. Although alternative donor sources have previously been associated with higher rates of posttransplantation complications³ and death,² application of reduced-intensity regimens supported sustained engraftment with manageable GvHD, and this is consistent with emerging experiences from other nonmalignant conditions.⁸ In this cohort the 2 patients who received a T-cell receptor $\alpha\beta$ /CD19 depletion haploidentical transplant had reduced toxicity but myeloablative conditioning to support engraftment. Balashov et al⁹ demonstrated in a pilot study that the addition of granulocyte colony-stimulating factor/plerixafor to treosulfan/fludarabine/melphalan conditioning regimen before transplantation ensured stable full donor engraftment in patients with WAS after T-cell receptor $\alpha\beta$ haploidentical transplantations. Our cohort will continue to be monitored for long-term effects, including infertility and secondary malignancy.

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Underestimation of house dust mite-specific IgE with extract-based ImmunoCAPs compared with molecular ImmunoCAPs



To the Editor:

House dust mites (HDMs) are one of the most important allergen sources causing severe forms of respiratory and skin allergy.¹ A major problem associated with the use of natural allergen extracts for diagnostic testing is that they show large variability regarding the contents of the major HDM allergens groups 1 and 2 and might lack clinically important allergens, such as Der p 5, Der p 7, Der p 21, and Der p 23.² In addition to skin testing, intradermal and provocation testing, measurement of allergen-specific IgE levels in serum is a valid method to detect HDM-specific IgE sensitization. However, there are also problems with HDM allergen extract-based serologic tests. HDM allergen extracts can contain bacterial antigens, which can cause false-positive IgE test results in subjects.³ With the introduction of component-resolved diagnosis or molecular allergy diagnosis, purified HDM allergen molecules are available for identification of HDM-sensitized subjects.⁴ However, the

TABLE I. Serologic characteristics of patients with HDM allergy from cohorts 1 and 2

Cohort 1 (n = 27)	Minimum-maximum		Mean	
Total IgE (kU _A /L)	42.9-3425.0		576.5	
CAP extract (kU _A /L)	0.7-117.0		29.0	
CAP allergen (kU _A /L)	1.1-201.6		43.6	
ISAC (ISU)	Prevalence (n)	Percentage	Minimum-maximum of positive	Mean of positive
Clinically important <i>Dermatophagoides pteronyssinus</i> allergens				
nDer p 1	23	85.2	0.3-11.3	2.4
rDer p 2	22	81.5	0.3-52.4	16.7
rDer p 5	14	51.9	0.4-30.0	5.0
rDer p 7	7	25.9	0.3-39.1	6.0
rDer p 21	13	48.1	0.3-8.9	2.8
rDer p 23	19	70.4	0.3-24.8	4.7
Other <i>D pteronyssinus</i> allergens				
rDer p 4	4	14.8	0.3-1.5	0.7
rDer p 10	4	14.8	0.3-0.3	0.3
rDer p 11	0	0.0	—	—
rDer p 14	0	0.0	—	—
rDer p 15	0	0.0	—	—
rDer p 18	5	18.5	0.8-6.4	2.2
clone 16	3	11.1	1.1-2.3	1.7
Cohort 2 (n = 26)	Minimum-maximum		Mean	
Total IgE (kU _A /L)	18.0-2104.0		261.2	
CAP extract (kU _A /L)	1.5-42.1		14.1	
CAP allergen (kU _A /L)	0.2-117.4		27.8	
ISAC (ISU)	Prevalence (n)	Percentage	Minimum-maximum of positive	Mean of positive
Clinically important <i>D pteronyssinus</i> allergens				
nDer p 1	17	65.4	0.4-9.9	3.7
rDer p 2	24	92.3	0.4-81.6	19.3
rDer p 5	9	34.6	0.3-38.5	11.8
rDer p 7	9	34.6	0.3-26.6	7.6
rDer p 21	8	30.8	0.4-27.7	6.9
rDer p 23	19	73.1	0.4-27.7	8.4
Other <i>D pteronyssinus</i> allergens				
rDer p 4	0	0.0	—	—
rDer p 10	2	7.7	0.3-2.7	2.0
rDer p 11	0	0.0	—	—
rDer p 14	0	0.0	—	—
rDer p 15	0	0.0	—	—
rDer p 18	0	0.0	—	—
clone 16	4	15.4	0.3-3.9	1.8

ISU, ISAC standardized units.

panel of HDM allergen molecules comprising the majority of HDM-specific IgE epitopes needs to be defined.

To identify the most important HDM allergens, we have established IgE reactivity profiles to a panel of 13 HDM allergen molecules using ImmunoCAP ISAC technology⁵ in 2 cohorts of patients with HDM allergy. Table E1 in this article's Online Repository at www.jacionline.org describes the characteristics and demographics of the patient population. One cohort (ie, cohort 1) comprising 27 patients with a doctor's diagnosis of HDM sensitization (see Table E1) and a second cohort (ie, cohort 2) of 26 patients who also had symptoms of rhinitis on controlled HDM allergen exposure were obtained from the Vienna Challenge Chamber (see the Methods section and Table E1 in this article's Online Repository at www.jacionline.org). Six (ie, Der p 1, 2, 5, 7, 21, and 23) of the 13 tested HDM allergen

molecules were identified as relevant allergens by using ImmunoCAP ISAC, according to prevalence of IgE recognition and allergen-specific IgE levels (Table I), and testing with these allergens confirmed IgE sensitization to HDM in each of the tested patients (see Fig E1 in this article's Online Repository at www.jacionline.org). Results obtained for different methods of IgE detection (ie, RAST-based dot blot, ImmunoCAP ISAC, and ELISA) were highly correlated, yielding best results for ImmunoCAP ISAC and the highly sensitive RAST-based assay (see Table E2 in this article's Online Repository at www.jacionline.org).

We then prepared molecular ImmunoCAPs containing the 6 important HDM allergen molecules (ie, Der p 1, 2, 5, 7, 21, and 23) by using Streptavidin ImmunoCAP technology (o212 ImmunoCAP; Thermo Fisher Scientific/Phadia, Uppsala,

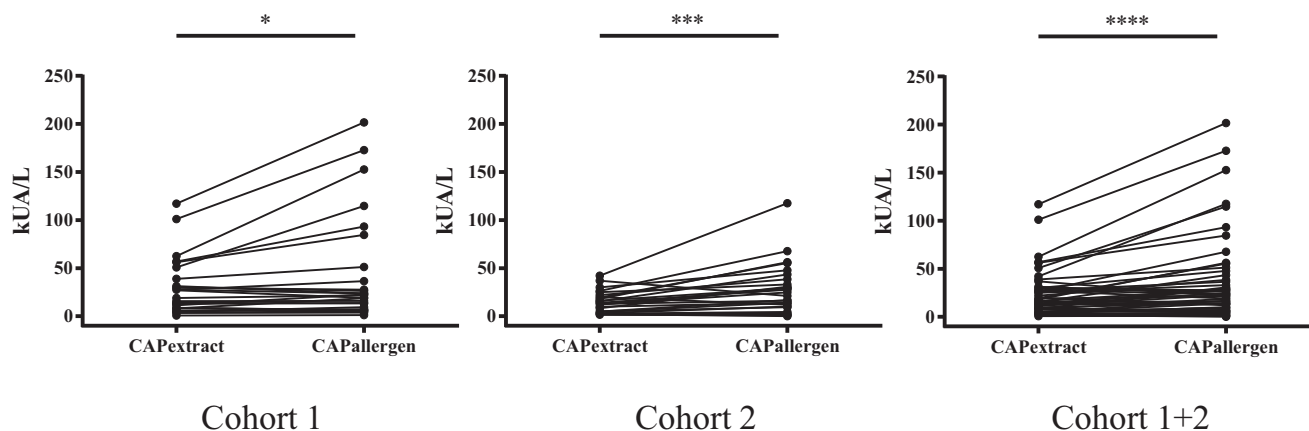


FIG 1. Comparison of allergen-specific IgE levels measured with commercial HDM ImmunoCAPs and molecular ImmunoCAPs for sera from patients with HDM allergy from cohort 1, cohort 2, and cohort 1 plus cohort 2. Significant differences of IgE levels are indicated as follows: * $P < .05$, *** $P < .001$, and **** $P < .0001$.

Sweden) and compared them with the commercial allergen extract-based ImmunoCAP (d1) and the ImmunoCAP ISAC for quantification of HDM-specific IgE levels (see Fig E2 in this article's Online Repository at www.jacionline.org). The best correlation was found between the extract-based ImmunoCAP and molecular ImmunoCAP containing a mix of Der p 1, 2, 5, 7, 21, and 23 (CAP allergen) ($r = 0.95$ and $P < .0001$ in cohort 1 and $r = 0.82$ and $P < .0001$ in cohort 2) (see Figs E2 and E3 in this article's Online Repository at www.jacionline.org). Interestingly, HDM-specific IgE levels determined with the molecular ImmunoCAP were considerably greater than those measured with the allergen extract-based ImmunoCAP as follows: cohort 1, 43.6 kUA/L (1.1-201.6 kUA/L) versus 29 kUA/L (0.7-117.0 kUA/L); cohort 2, 27.8 kUA/L (0.2-117.4 kUA/L) versus 14.1 kUA/L (1.5-42.1 kUA/L; Table I; Fig 1: cohort 1, $P = .0136$; cohort 2, $P = .0009$; and cohort 1 plus cohort 2, $P < .0001$). All patients were given a diagnosis with the molecular ImmunoCAP (see Table E3 in this article's Online Repository at www.jacionline.org). IgE levels measured with the molecular ImmunoCAP were considerably greater (mean, 97%) in 39 (74%) of the 53 patients than those measured with the allergen extract (see Table E3). Only in a few patients (14/53 [26%]) were slightly greater HDM-specific IgE levels (mean, 29%) measured with the extract-based ImmunoCAP (see Table E3).

There are several possible explanations for this finding. First, it is possible that certain allergens are underrepresented in natural allergen extracts, and as a consequence, allergen-specific IgE levels measured for patients with IgE sensitization to the underrepresented components will be low. Second, natural allergen extracts contain large quantities of nonallergenic proteins in addition to the allergens, which can then preferentially couple to the solid phase used for immobilization of the allergens, resulting in underestimation of allergen-specific IgE levels. Third, both of the first 2 factors might play a role. Therefore we performed a more detailed analysis. We grouped sera according to HDM allergen-specific IgE levels in sera containing levels of greater than 20 kUA/L and sera containing levels of less than 20 kUA/L and found that significantly greater HDM allergen-specific IgE levels were measured with the molecular

ImmunoCAP versus the extract-based ImmunoCAP in patients with HDM allergen-specific IgE levels of greater than 20 kUA/L compared with those with HDM-specific IgE levels of less than 20 kUA/L, indicating that important allergens can be coupled in quantities that are too low in the allergen extract-based ImmunoCAPs (see Fig E4, A, in this article's Online Repository at www.jacionline.org).

We then analyzed patients with IgE reactivity to 1 to 3 HDM allergens of the panel Der p 1, 2, 5, 7, 21, and 23 and those with IgE reactivity to 4 to 6 of the allergens (see Fig E4, B). In both groups HDM-specific IgE levels were greater when measured with the molecular ImmunoCAP, especially in the group with IgE reactivity to 4 to 6 allergens, but there was no significant difference in the group recognizing only 1 to 3 allergens (see Fig E4, B).

Finally, we grouped the patients according to the presence or absence of IgE specific for the 6 important allergens (see Fig E4, C-H). We found that Der p 2, Der p 5, Der p 21, and Der p 23 seemed to be underrepresented in natural allergen extracts because specific IgE levels measured with the molecular ImmunoCAPs were greater for patients with specific IgE to these allergens compared with those in patients without specific IgE to these allergens (see Fig E4). For Der p 5, this difference was even statistically significant (see Fig E4, E). Therefore it seems that the factors mentioned above, underrepresentation of certain allergens and/or competition by nonallergenic proteins, are responsible for IgE levels measured with HDM allergen extract-based ImmunoCAPs being lower than those measured with molecular ImmunoCAPs.

Our finding might be of great clinical relevance because the accurate measurement of allergen-specific IgE levels is important. Allergen-specific IgE levels inform about allergen exposure and whether measures, such as allergen avoidance, have been successful; they are important as thresholds for certain symptoms such as rhinitis versus asthma; and they might help predict severe allergic reactions.⁶

Among the limited number of patients tested in our study, we did not obtain evidence that greater HDM-specific IgE levels are associated with certain manifestations of HDM allergy, but we have evidence that threshold IgE levels can be established for

symptomatic and silent IgE sensitization to HDM (S. Vrtala, unpublished). Therefore the question is how the measurement of HDM-specific IgE levels could be improved. One possibility would be to add recombinant allergen molecules to natural HDM allergen extracts, as has been done previously for other complex allergen sources.⁷ The other possibility would be to prepare molecular ImmunoCAPs that contain the most important allergens, as shown in our study. The advantage of such molecular ImmunoCAPs would be that cross-reactive allergens, which are also present in other unrelated allergen sources, can be omitted so that it is possible to identify patients with a genuine sensitization to the given allergen source with molecular tests. Such molecular tests are very useful not only because they have high specificity and sensitivity for detection of IgE sensitization but also because they facilitate the accurate prescription of allergen-specific immunotherapy.⁸ Therefore we suggest replacing allergen extract-based tests for the detection of HDM sensitization with molecular tests that are highly reproducible and not dependent on varying qualities of allergen extracts.

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In vivo imaging reveals increased eosinophil uptake in the lungs of obese asthmatic patients



To The Editor:

Eosinophils play an important pathogenic role in pulmonary and systemic conditions, including eosinophilic asthma and eosinophilic granulomatosis with polyangiitis.^{1,2} Although progress has been made in understanding the mechanisms responsible for the activation of these cells, existing biomarkers of eosinophilic inflammation are indirect and/or invasive and do not always correlate with tissue eosinophilia. Hence, there is a need to develop noninvasive biomarkers of tissue eosinophilia. We have previously demonstrated the capacity of single photon emission computed tomography (SPECT) to quantify neutrophil uptake into the lungs of patients with chronic obstructive pulmonary disease.³ We sought to determine whether this methodology could be used to quantify eosinophil kinetics and pulmonary uptake, which may differ among diseases characterized by eosinophilic inflammation. In particular, the role of the eosinophil in asthma with obesity, a distinct asthma endotype associated with increased severity,⁴ is controversial. We hypothesized that injection of radiolabeled eosinophils, coupled with SPECT/CT, would reveal changes in eosinophil kinetics in patients compared with healthy volunteers.

To determine the initial distribution of eosinophils, and to ensure that the reinjected cells had not been activated, planar gamma camera imaging was performed following injection of technetium-99m-labeled eosinophils. These scans were performed in healthy volunteers, patients with asthma, and patients with focal eosinophilic inflammation (Fig 1, A; see Tables E1 and E2 in this article's Online Repository at www.jacionline.org). The initial transit of radiolabeled eosinophils through the lung was similar in all subjects (Fig 1, B and C). We generated a "first-pass" transit curve to calculate the time taken for the initial bolus of eosinophils to transit from the right ventricle across the pulmonary circulation (see Fig E1 in this article's Online Repository at www.jacionline.org). This value was constant across all study groups (Fig E1, B). The 45-minute blood recovery values (Fig 1, D) did not differ between the study groups and, importantly, were comparable to published levels for unmanipulated radiolabeled eosinophils from healthy volunteers, indicating that the reinjected eosinophils were nonactivated.⁵ As predicted,^{5,6} circulating eosinophils accumulated rapidly within the liver and spleen, most likely within known marginated intravascular pools (Fig 1, E-G).

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METHODS

Sera from patients with HDM allergy

Sera from patients with HDM allergy (cohort 1, $n = 27$; cohort 2, $n = 26$) were obtained in 2 centers in Vienna (Department of Pathophysiology and Allergy Research, Medical University of Vienna, and Vienna Challenge Chamber), Austria. Patients had a positive case history and perennial airway symptoms indicative of HDM allergy (ie, rhinitis, asthma, or both). IgE sensitization to HDM was confirmed by means of detection of specific IgE antibodies to *Dermatophagoides pteronyssinus* by using ImmunoCAP (d1 ImmunoCAP; Thermo Fisher Scientific/Phadia). Sera from both cohorts were also tested for IgE reactivity to a panel of 176 microarrayed allergen molecules by using ImmunoCAP ISAC technology (Thermo Fisher Scientific/Phadia).^{E1} Patients from cohort 2 underwent controlled HDM allergen exposure in the Vienna Challenge Chamber and had a Total Nasal Symptom Score of greater than 6.^{E2} For control purposes, serum from at least 1 nonallergic subject was included in all analyses. Table E1 provides a demographic and clinical characterization of the patients from cohorts 1 and 2. Serologic characteristics are summarized in Table 1. Patients' sera were anonymously analyzed for allergen-specific IgE reactivity with approval from the Ethics Committee of the Medical University of Vienna (EK 1641/2014) after written informed consent was obtained.

Allergen molecules

Natural purified Der p 1 was obtained from Professor W. R. Thomas.^{E3} rDer p 2, rDer p 5, rDer p 21, and rDer p 23 were expressed in *Escherichia coli* strain BL21-Gold (DE3; Thermo Fisher Scientific/Agilent Technologies, Santa Clara, Calif) and purified, as previously described.^{E4-E6} rDer p 7 was expressed in *E coli* BL21-Gold (DE3) and purified by means of hydrophobic interaction chromatography and hydroxyapatite chromatography. Endotoxins were removed from purified rDer p 5, 7, 21, and 23 by using immobilized polymyxin resins (Affi-Prep Polymyxin Matrix; Bio-Rad, Austin, Tex), and endotoxin from rDer p 2 was removed by using the Endotoxin Removal Kit (ProteoSpin Endotoxin Removal Maxi Kit; Norgen Biotek, Ontario, Canada). Endotoxin levels were measured with the Limulus-Amebocyte-Lysate assay (Lonza, Basel, Switzerland). Endotoxin contents of Der p 2, 5, 7, 21, and 23 were less than 250 endotoxin units/mL, and the endotoxin content of Der p 1 was 1300 endotoxin units/mL. The other HDM allergens on the chip were purified and characterized, as previously described.^{E1}

IgE ELISA and the RAST-based IgE dot blot assay were performed, as previously described.^{E7,E8}

ImmunoCAP ISAC chip assay and ImmunoCAP assays

A customized microarray based on ImmunoCAP ISAC chip technology was produced by Phadia Austria GmbH (Vienna, Austria).^{E1} Detection of IgE binding was performed as described, and levels of IgE antibodies were shown in ISAC standardized units, with a cutoff of 0.3 ISAC standardized units.^{E1}

The quantification of allergen-specific IgE antibodies to *D pteronyssinus* allergen extract (Allergen d1) and of total IgE was performed by using ImmunoCAP technology, according to the manufacturer's instructions, on an ImmunoCAP 100 instrument (Thermo Fisher Scientific/Phadia). Streptavidin ImmunoCAPs (o212 ImmunoCAP, Thermo Fisher Scientific/Phadia) were used to prepare ImmunoCAPs containing a mix of Der p 1, 2, 5, 7, 21, and 23, as previously described.^{E9} For this purpose, the allergens were dialyzed against a buffer containing 0.1 mol/L sodium bicarbonate and 1 mol/L sodium chloride and biotinylated with a 5 times molar surplus of

biotinamidohehexanoyl-6-aminohehexanoic acid N-hydroxysuccinimide ester (B3295; Sigma-Aldrich, St Louis, Mo) at pH 8.0. After 2 hours of incubation at room temperature, allergens were dialyzed against PBS to remove unbound biotin. Different amounts of biotinylated allergens were tested (1, 0.5, and 0.1 μg), showing that 0.1 μg of biotinylated allergen was sufficient for IgE detection (ie, reaching the plateau for IgE binding). Accordingly, 0.1 μg of each of the biotinylated allergens (Der p 1, 2, 5, 7, 21, and 23) were mixed (in 50 μL of PBS) and coupled to streptavidin CAPs for 30 minutes at room temperature. Measurement of specific IgE levels in sera from patients with HDM allergy was performed according to the manufacturer's instructions on an ImmunoCAP 100 (Thermo Fisher Scientific/Phadia). Allergen-specific IgE levels of greater than 0.1 kU_A/L were considered positive.

Statistical analysis

Correlation between different IgE-binding assays was determined by calculating the Spearman correlation coefficient (r) for patients from the 2 cohorts. The comparison of allergen-specific IgE levels measured with the commercial allergen extract-based *D pteronyssinus* ImmunoCAPs and molecular ImmunoCAPs containing a mix of the 6 allergens (Der p 1, 2, 5, 7, 21, and 23) was analyzed by using the Student t test for patients from both cohorts and patients who had been stratified according to (1) IgE levels (ie, >20 kU_A/L or <20 kU_A/L) measured with the molecular ImmunoCAP and (2) IgE reactivity to 1 to 3 or 4 to 6 of the relevant allergens and (3) for patients with and without IgE reactivity to each of the 6 allergens. A P value of less than .05 was considered statistically significant.

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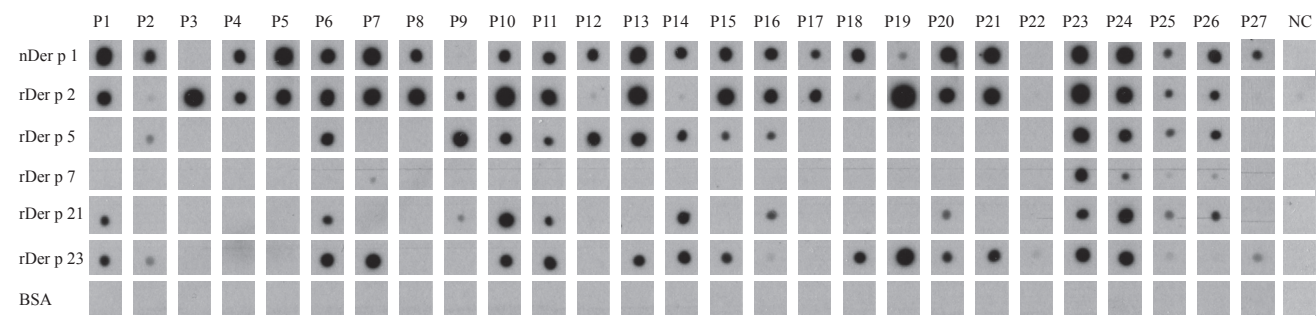
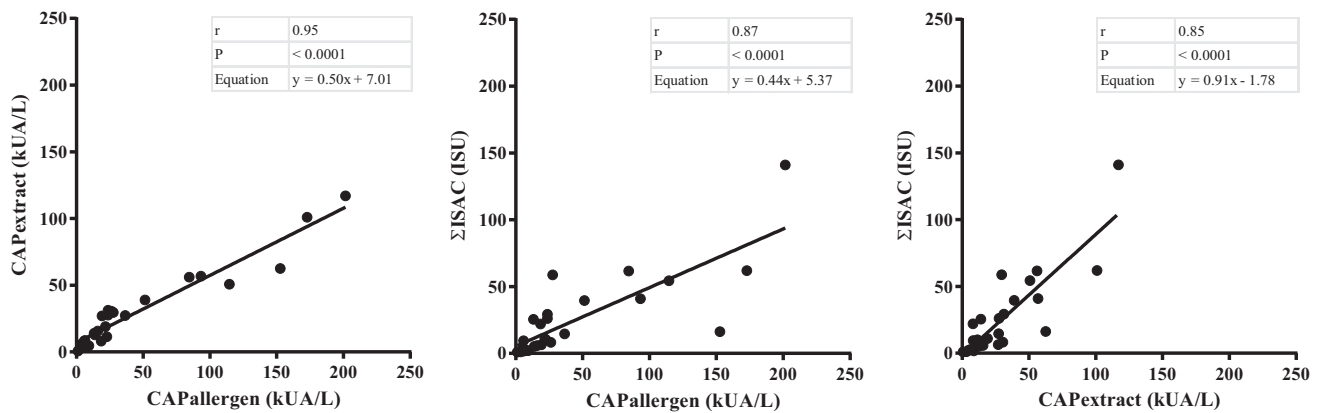


FIG E1. Qualitative determination of IgE reactivity to HDM allergen molecules determined by using RAST-based dot blotting. Dot-blotted HDM allergens and BSA were tested for IgE reactivity with sera from 27 patients with mite allergy (cohort 1, P1–P27; [Table E1](#), left column) and serum from a nonallergic subject (NC).

Cohort 1



Cohort 2

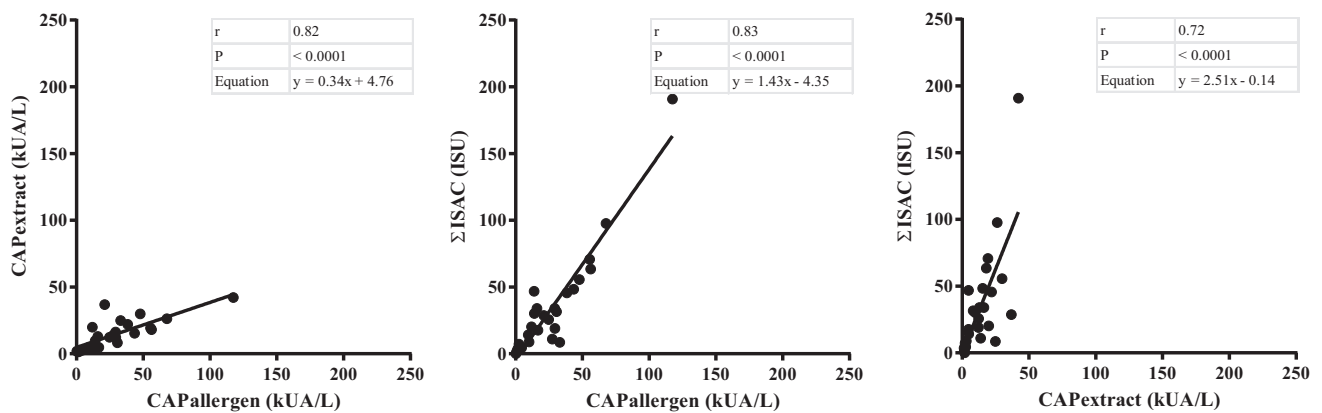


FIG E2. Correlations of IgE levels to HDM allergen extract and HDM allergen molecules measured by using different assays. IgE levels to HDM allergen extract-based ImmunoCAPs (CAPextract) to a molecular ImmunoCAP containing Der p 1, 2, 5, 7, 21, and 23 (CAPallergen) or to ImmunoCAP ISAC chips (Σ ISAC) were measured in sera from patients with HDM allergy (top, cohort 1; bottom, cohort 2) and correlated with each other. Correlation coefficients (r), regression equations, and statistical significances (P values) are shown for each correlation.

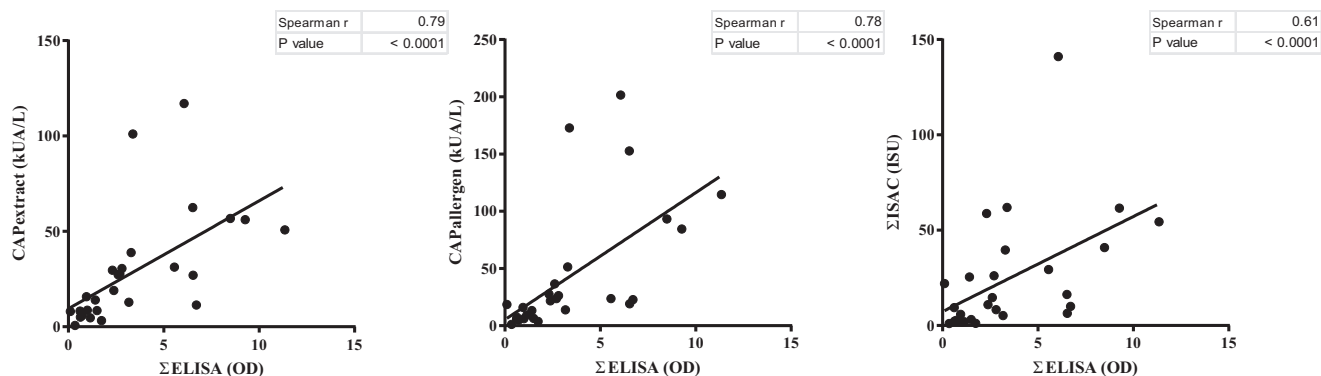


FIG E3. Correlations of IgE levels to HDM allergen extract and HDM allergen molecules measured by using different assays. IgE levels to HDM allergen extracts (CAP extract) or to a mix of allergen molecules (Der p 1, 2, 5, 7, 21, and 23) bound to ImmunoCAP (CAP allergen) or ImmunoCAP ISAC chips ($\Sigma ISAC$) or measured by means of ELISA ($\Sigma ELISA$) were determined in sera from patients with HDM allergy of cohort 1. Correlation coefficients (r) and statistical significances (P values) are shown for each correlation.

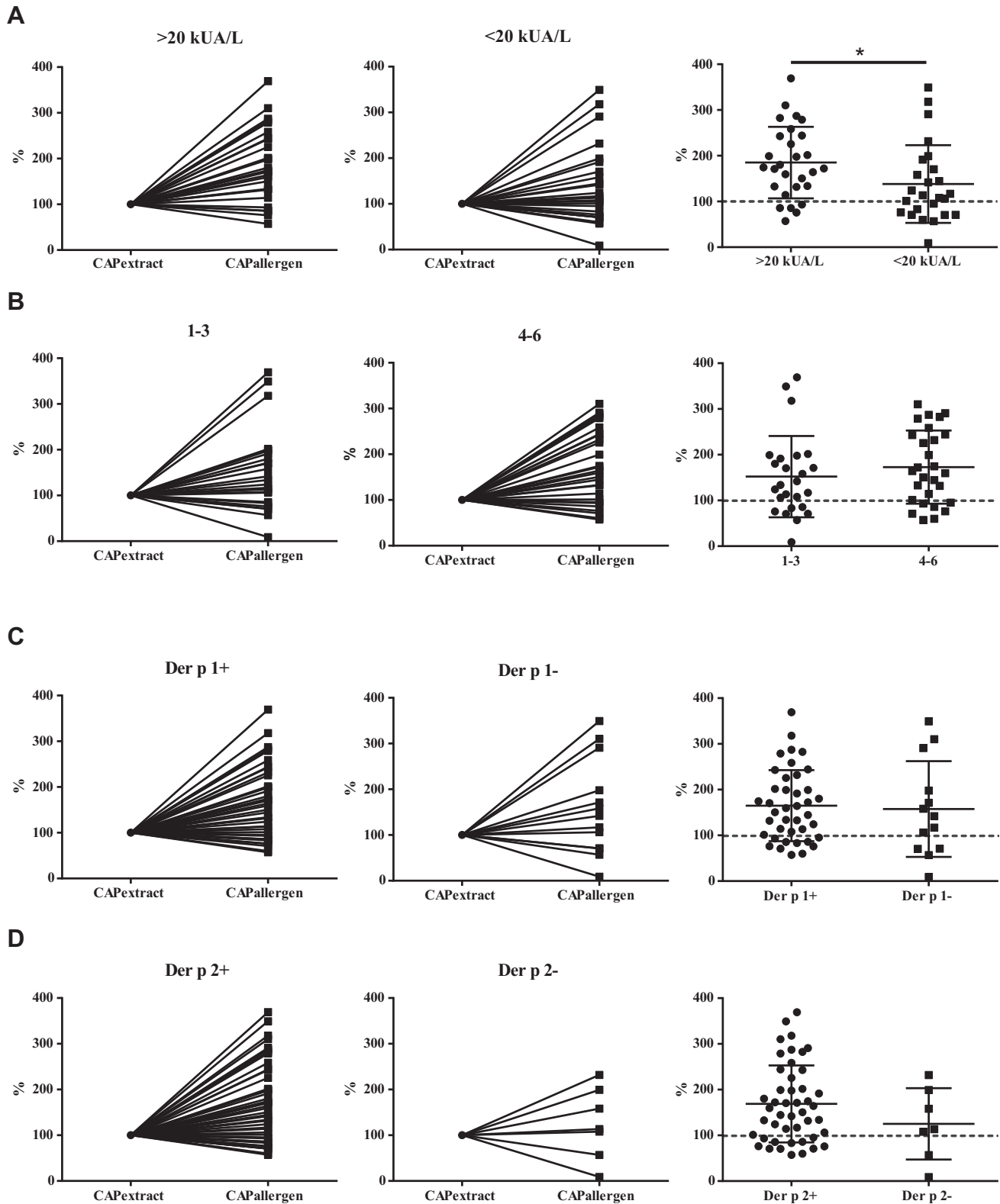
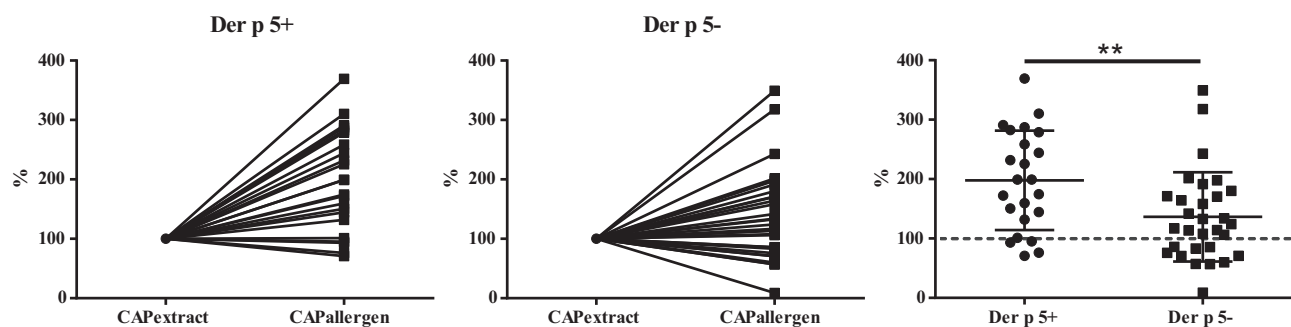
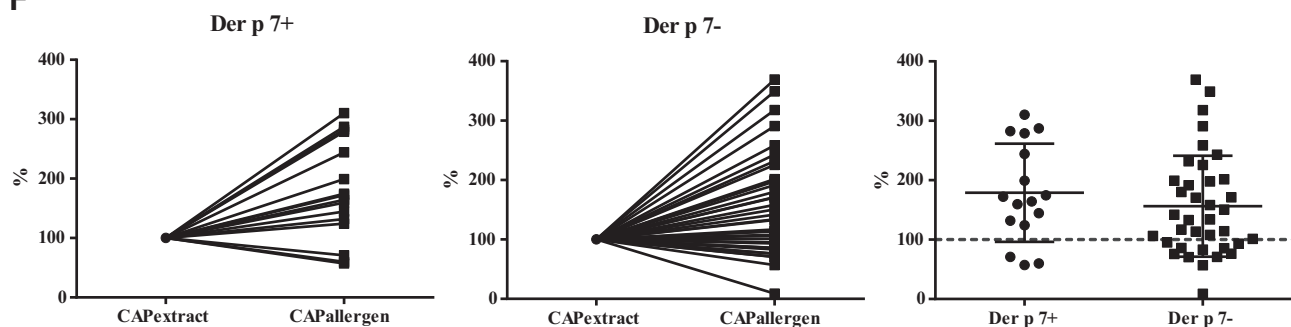


FIG E4. Percentage differences in allergen-specific IgE levels measured with the molecular ImmunoCAP compared with the allergen extract-based ImmunoCAP for patients with HDM-specific IgE levels of greater than 20 kUA/L and less than 20 kUA/L (**A**) or IgE reactivity to 1 to 3 HDM allergens or IgE reactivity to 4 to 6 of the allergens (**B**) and in patients with and without IgE reactivity to each of the 6 allergens: Der p 1 (**C**), Der p 2 (**D**), Der p 5 (**E**), Der p 7 (**F**), Der p 21 (**G**), and Der p 23 (**H**). * $P < .05$ and ** $P < .01$.

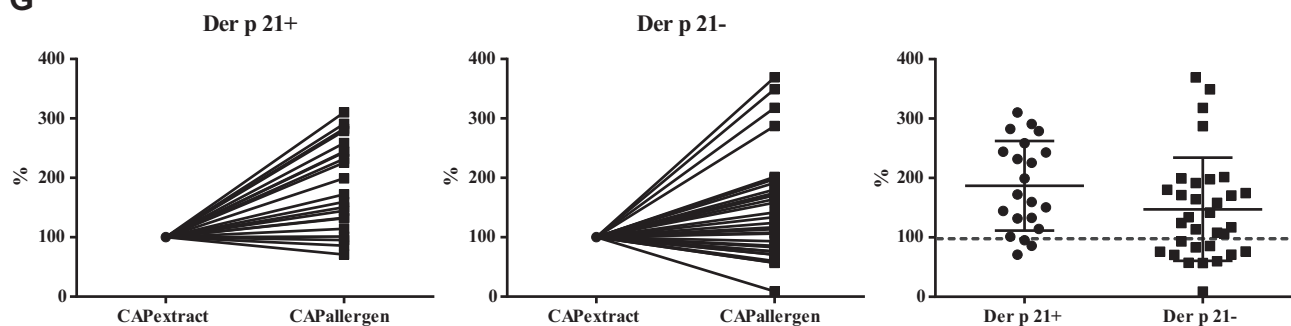
E



F



G



H

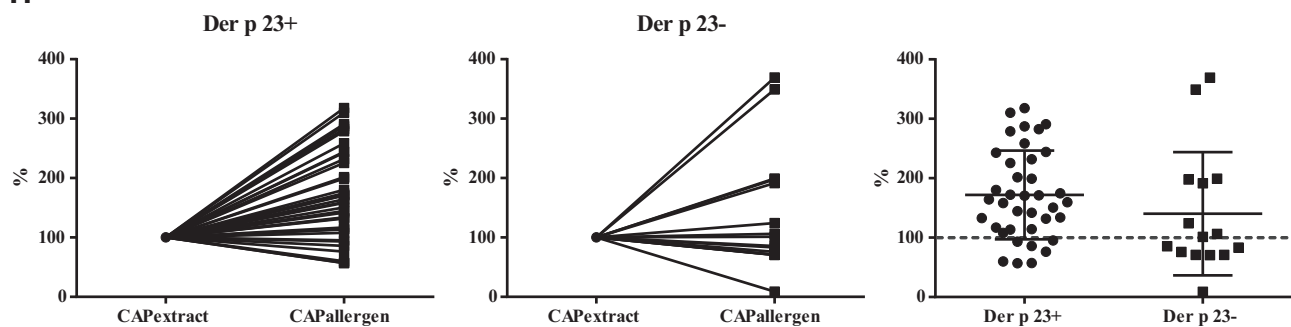


FIG E4. (Continued).

TABLE E1. Demographic and clinical characterization of patients with HDM allergy

Cohort 1 (n = 27)		Cohort 2 (n = 26)	
Age (y)		Age (y)	
Mean (minimum-maximum)	26.5 (19-36)	Mean (minimum-maximum)	25.7 (18-44)
Sex (%)		Sex (%)	
Male	33.3	Male	53.9
Female	66.7	Female	46.1
Symptoms (%)		Symptoms (%)	
Airway	100.0	Airway	100.0
Bronchial asthma	16.7	Bronchial asthma	15.4
Rhinitis	95.8	Rhinitis	100.0
Skin	16.7	Skin	30.8
Immunotherapy (%)		Immunotherapy (%)	
HDM	11.1	HDM	3.8
Plant	7.4	Plant	7.6
Other IgE sensitizations (%)		Other IgE sensitizations (%)	
Grass pollen	59.3	Grass pollen	80.8
Tree pollen	37.0	Tree pollen	61.5
Cat	44.4	Cat	57.7
Dog	25.9	Dog	23.1
Horse	11.1	Horse	0.0
Mouse	14.8	Mouse	0.0
Apple	37.0	Apple	34.6
Olive	25.9	Olive	26.9
Peach	11.1	Peach	30.8
Kiwi	7.4	Kiwi	23.1
Wheat	3.7	Wheat	3.8
Soy	3.7	Soy	11.5
Milk	7.4	Milk	0.0
Shrimp	0.0	Shrimp	11.5
<i>Anisakis</i> species	0.0	<i>Anisakis</i> species	19.2
Peanut	29.6	Peanut	38.5
Walnut	14.8	Walnut	19.2
Almond	11.1	Almond	11.5
Hazelnut	22.2	Hazelnut	23.1
Cashew	3.7	Cashew	0.0
Wasp	14.8	Wasp	38.5
Cockroach	0.0	Cockroach	3.8
Latex	22.2	Latex	19.2
Mold	22.2	Mold	15.4

Cohort 1 (n = 27) was used for comparison of different methods for measuring allergen-specific IgE levels and for quantification of HDM-specific IgE levels, and cohort 2 (n = 26) was used for quantification of HDM-specific IgE levels.

TABLE E2. Numbers and percentages of patients with HDM allergy of cohort 1 with IgE reactivity to important HDM allergen molecules determined by using different assays

	No.	Percentage
Der p 1		
ELISA	21	77.78
ISAC	23	85.19
Dot blot	24	88.89
Der p 2		
ELISA	17	62.96
ISAC	22	81.48
Dot blot	21	77.78
Der p 5		
ELISA	14	51.85
ISAC	14	51.85
Dot blot	14	51.85
Der p 7		
ELISA	5	18.52
ISAC	7	25.93
Dot blot	5	18.52
Der p 21		
ELISA	12	44.44
ISAC	13	48.15
Dot blot	12	44.44
Der p 23		
ELISA	13	48.15
ISAC	19	70.37
Dot blot	19	70.37

TABLE E3. Quantification of HDM allergen-specific IgE levels of the patients with HDM allergy (cohorts 1 and 2) measured with allergen extract-based ImmunoCAPs and molecular ImmunoCAPs

Cohort 1 (n = 27)	CAP extract (kU _A /L)	CAP allergen (kU _A /L)	Cohort 2 (n = 26)	CAP extract (kU _A /L)	CAP allergen (kU _A /L)
P1	19.0	21.7	P1	12.9	16.0
P2	8.5	6.5	P2	22.0	38.4
P3	8.2	5.8	P3	2.6	4.4
P4	8.7	6.2	P4	4.8	13.9
P5	27.6	23.6	P5	42.1	117.4
P6	11.4	22.7	P6	2.2	2.6
P7	56.8	93.3	P7	4.9	9.4
P8	31.3	23.7	P8	26.2	67.7
P9	27.0	19.1	P9	13.9	27.5
P10	50.8	114.6	P10	24.9	33.1
P11	56.1	84.5	P11	19.9	11.9
P12	4.7	9.3	P12	29.9	47.7
P13	38.9	51.3	P13	16.2	29.2
P14	8.0	18.6	P14	12.1	29.4
P15	29.6	27.6	P15	1.9	0.2
P16	14.0	13.3	P16	1.5	1.6
P17	5.9	4.9	P17	4.8	16.7
P18	12.8	13.8	P18	19.3	55.4
P19	101.0	172.8	P19	12.3	24.8
P20	30.5	26.2	P20	3.2	10.0
P21	27.3	36.5	P21	8.3	30.6
P22	0.7	1.1	P22	9.8	13.9
P23	117.0	201.6	P23	36.9	21.1
P24	62.5	152.7	P24	1.5	0.9
P25	5.0	7.3	P25	15.4	43.5
P26	15.7	15.9	P26	18.1	56.2
P27	3.3	3.7			