Molecular diagnosis in house dust mite allergic patients suggests clinical relevance of Der p 23 in asthmatic children

Short title: Molecular diagnosis in dust mite allergic children.

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Abstract

Background: Sensitization patterns to dust mites depend on the geographic area and are important in clinical practice. However, the role of molecular diagnosis is not currently defined. We sought to characterize a pediatric population focusing on sensitization to different mite species and major mite components, to assess the clinical relevance of sensitization to allergenic components in our practice.

Methods: Consecutive children with respiratory allergy sensitized to house dust mites by skin prick test were recruited. Specific IgE to nDer p 1, rDer p 2, rDer p 23 by ImmunoCAP, and sIgE by ImmunoCAP-ISAC microarray was determined. Subjects were followed-up for three years.

Results: A total of 276 children were recruited. Sensitization was 86.6% to nDer p 1, 79.3% to rDer p 2 and 75.8% to rDer p 23. *Lepidoglyphus* was the most common storage mite detected by SPT. Twenty-six patients (9.4%) were not sensitized to Der p 1 and Der p 2; noteworthy, 14 of them (53.8%) had positive IgE binding to Der p 23.

Asthmatic patients, and especially those with a persistent moderate and severe phenotype, recognized more often the three major allergens.

Conclusions: Most of our population with mite allergy is sensitized to Der p 1, Der p 2 and Der p 23 major allergens; however, 5% were sensitized only to Der p 23 of the allergens evaluated. Sensitization to Der p 23 should be considered in the diagnosis and treatment of mite allergy, especially in patients with moderate and severe asthma because it may worsen the clinical phenotype.

Keywords: Asthma, Component resolved diagnosis, Der p 1, Der p 2, Der p 10, Der p 23, House dust mites, Storage mites, Tropomyosin.

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Resumen

Antecedentes: El perfil de sensibilización a los ácaros del polvo depende del área geográfica y es importante en la práctica clínica. Sin embargo, el papel del diagnóstico molecular no ha sido del todo definido. Nuestro objetivo fue la caracterización del perfil de sensibilización de una población pediátrica a diferentes especies de ácaros; y evaluar la sensibilización a componentes alergénicos y su relevancia en nuestra práctica clínica.

Métodos. Se reclutaron de forma consecutiva pacientes con alergia respiratoria y sensibilización a ácaros del polvo doméstico mediante pruebas cutáneas. Se determinó la IgE específica por ImmunoCAP a nDer p 1, rDer p 2, rDer p 23 y la sIgE mediante el microarray de ImmunoCAP ISAC. Los pacientes fueron evaluados durante tres años según práctica cínica habitual.

Resultados. Se reclutaron un total de 276 niños. La sensibilización fue de 86,6% a nDer p 1, 79,3% a rDer p 2 y 75,8% a rDer p 23. *Lepidoglyphus* fue el ácaro de almacén más común. Un total de veintiséis pacientes (9,4%) no estaban sensibilizados a Der p 1 ni Der p 2; cabe destacar que 14 de ellos (53,8%) presentaban IgE positiva a Der p 23. Los pacientes con asma, y en especial los de fenotipo persistente moderado y grave, reconocieron con mayor frecuencia los tres alérgenos mayores.

Conclusiones: La mayoría de nuestra población pediátrica con alergia a ácaros está sensibilizada a los alérgenos mayores Der p 1, Der p 2 y Der p 23; sin embargo, un 5% estaba sensibilizado sólo a Der p 23. La sensibilización a Der p 23 debe considerarse en el diagnóstico y tratamiento de la alergia a ácaros, especialmente en pacientes con asma persistente moderada y grave.

Palabras clave: Asma, Diagnóstico por componentes, Der p 1, Der p 2, Der p 10, Der p 23, Ácaros del polvo doméstico, Ácaros de almacenamiento, Tropomiosina.

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Introduction

Dust mites are the leading cause of respiratory allergy worldwide [1, 2]. However, sensitization patterns to house dust mites (HDM) and storage mites (SM) vary depending on the geographic area [3-8]. In children, HDM are the earliest respiratory cause of sensitization [2].

More than 30 allergens of *Dermatophagoides pteronyssinus* have been identified and sequenced until now [9]. Traditionally the "major'' allergens are Der p 1 and Der p 2 [7-10]. However, Der p 23 has recently been identified as another HDM major allergen [11]. Nonetheless little is currently known about its role in sensitization and its clinical relevance [12-18].

The efficacy of immunotherapy in patients sensitized to the major allergens of group 1 and 2 has been thoroughly demonstrated. However, in patients sensitized to other allergens, such as Der p 23 the indication and efficacy of immunotherapy warrants further studies [4, 11, 15]. Overall, molecular diagnosis may play a role in mite allergy [14-18], but its exact utility in clinical practice has not been fully defined yet.

We sought to characterize a pediatric Mediterranean population, focusing on the prevalence of sensitization to different mite species (both HDM and SM) and major components Der p 1, Der p 2 and Der p 23. Furthermore, we sought to assess the clinical relevance of sensitization to Der p 23 and the usefulness of commercially available allergenic components in the diagnostic work-up of mite allergy.

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Methods

Study population and study design

Consecutive children (3-18 years-old), with clinical manifestations of respiratory allergy, from the Pediatric Allergy and Clinical Immunology Department of Hospital Sant Joan de Déu (Barcelona, Spain) sensitized to HDM *Dermatophagoides pteronyssinus* (DP) and/or *Dermatophagoides farinae* (DF), diagnosed by skin prick test (SPT), were recruited over a period of one year. Patients included were classified according to GINA (Global Initiative for Asthma) and ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines [19, 20]. Pharmacologic treatment and allergen-specific immunotherapy was prescribed following the personal medical criteria of each specialist in charge of the patient, in the event that clinical criteria were fulfilled.

Individuals under treatment with allergen-specific immunotherapy before the study began were excluded. All subjects that were under treatment were followed for a least three years as regular clinical practice.

We considered patients without clinical response to immunotherapy those patients with uncontrolled asthma according to GINA guideline: daytime symptoms, nocturnal waking due to asthma, rescue medication needed for symptoms relief and any activity limitation, after at least 3 years of treatment with immunotherapy and pharmacological treatment at the highest steps of the guideline.

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Skin Prick Test

All patients were SPT with biologically standardized extracts including DP, DF and *Euroglyphus maynei* (100 HEP/mL), *cypress pollen, olive tree pollen, grass mix pollen*, Alternaria*, cat and dog epithelium* (30 HEP/mL), and with a battery of standardized extracts of SM, including *Acarus siro, Chortoglyphus arcuatus, Glycyphagus domesticus, Lepidoglyphus destructor, Tyrophagus putrescentiae* (all 30 HEP/mL), and *Blomia tropicalis* (100 HEP/mL). Patients were also tested with purified tropomyosin from shrimp extract (50 µg/mL) [21].

All the SPT extracts were from LETI SL and were performed following EAACI guidelines [22]. A positive response was defined as the presence of a wheal with diameter ≥3 mm. Histamine chloride 10 mg/mL was used as positive control.

Serum Specific IgE

Serum specific IgE (sIgE) to nDer p 1, rDer p 2, rDer p 10, was determined in all patients (n=276) by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden) in accordance with manufacturer´s specifications. sIgE to rDer p 23 was investigated afterwards, when commercially available, in the 265 samples with sufficient volume left. Results over 0.35 kU_A/L were considered positive.

sIgE to a panel of 112 allergens was also evaluated by ImmunoCAP ISAC microarray (ThermoFisher Scientific) in all patients. Results were expressed as ISAC standardized units (ISU) and a cut-off of 0.3 ISU was considered.

The study was approved by hospital ethics committee and signed informed consent was obtained from all patients (PIC-108-12).

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Statistical analysis

Normal distribution of variables was investigated using the Komolgonov-Smirnov test. Variables with normal and asymmetric distribution were expressed as mean ± standard deviation (SD) and as median (interquartile range 25%-75%; IQ) respectively.

Categoric variables were expressed as percentages and analyzed using the Chi-square test. Spearman's correlation was used to identify linear relationships between the techniques used for molecular diagnosis. Differences were considered statistically significant if $P<0.05$. Statistical analysis was performed using the IBM SPSS statistical software package (v.18).

Results

Two hundred and seventy-six children who met the inclusion criteria of respiratory allergy and sensitization to DF and/or DP by SPT were prospectively and consecutively recruited. Sixty-nine percent of these patients were exclusively sensitized to HDM. Clinical characteristics of the patients are summarized in **table 1**.

Sensitization profile

Sensitization to mite extracts by *Skin pricks tests and p*revalence of sensitization to mite's allergens by *in vitro* tests is summarized in **tables 2 and 3**, respectively. By ImmunoCAP, 90.6% (250/276) of patients were found to be sensitized to either nDer p1 and/or rDer p 2. Sensitization to rDer p 23 was 75.8% (201/265). Of these 265 patients, 5.3% (n=14) were sensitized to rDer p 23, but not to nDer p 1 or rDer p 2, without present-

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ing a different clinical phenotype compared to those sensitized also to Der p 1 and / or Der p 2 **(table 4).**

The prevalence of sensitization to Der p 1, Der p 2 and Der p 23 increased with age (figure 1).

A correlation between sIgE detected by ImmunoCAP and microarray for values of sIgE for Der p 1 (r=0.87; p<0.0001), for Der p 2 (r=0.87; p<0.0001) and for Der p 10 (r=0.88; p<0.0001) was observed, (figure 2).

Sensitizations and clinical implications

Major allergens were more often recognized by asthmatics patients. The prevalence of sensitization by ImmunoCAP to individual HDM allergens nDer p 1 (88.8% vs 75.5%), rDer p 2 (83.3% vs 70.5%) and rDer p 23 (79% vs 67%) differed significantly between children with and without asthma (P=0.003, P=0.01 and P=0.02, respectively). Furthermore, patients with moderate and severe persistent asthma according to GINA criteria were more frequently sensitized to Der p 23 than patients with intermittent and mild persistent forms of asthma (75.8% vs 90%, P=0.008). Among those patients completing ITE for 3 years, 11 did not show a clinical response to allergen-specific immunotherapy, since they maintained uncontrolled asthma. All of them were sensitized to Der p 23 **(table 5).**

Considering those patients sensitized to Der p 23 but not to Der p 1 and Der p 2 (n=14), only 3 had been prescribed immunotherapy, even though 50% had asthma and 71.4% per-

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sistent rhinitis. Of these three patients, two did not respond to treatment after 3 years **(table 4).**

Comparison between SPT and molecular diagnosis

All patients with sensitization to Der p 1 or Der p 2 had a positive SPT to DP and 97% had a positive SPT to DF. Likewise, all patients with selective sensitization to rDer p 23 had a positive SPT to DP while only 50% had a positive SPT to DF (P<0.002).

Sensitization to tropomyosin by ImmunoCAP was higher than by SPT (9.8% vs 6.5%). SPT for tropomyosin was positive for the two patients who had a positive specific IgE to rDer p 10 without sensitization to nDer p 1, rDer p 2 and rDer p 23.

Sensitization to *Lepidoglyphus* and *Blomia* significantly differed when detected by SPT versus microarray (47.8% vs 23.2% and 30.8% vs 15.6%, respectively; P=0.03, P=0.02). All patients sensitized to *Blomia* by SPT were also sensitized to major components of *Dermatophagoides*. Regarding patients sensitized to *Lepidoglyphus*, only one patient had reactivity to rLep d 2 without sensitization to Der p 1, Der p 2, and Der p 23.

Discussion

This study describes the sensitization profile to different HDM and SM species as sensitization to major allergens of a pediatric population with respiratory allergy disease. Moreover, it illustrates the clinical relevance of sensitization to Der p 23. For the first time in children, sensitization to Der p 23 has been linked to persistent moderate/severe asthma. To date, the diagnostic value of molecular diagnosis complementing SPT has not been fully

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established. Our study proposes criteria for the real-life use of molecular diagnosis in patients with respiratory allergy and positive SPT to mites. As expected, Der p 1 and/or Der p 2 were recognized by most of the patients (90.6%), and Der p 23, recently identified as a HDM major allergen [7,8,11,12,13,14,15,18,23,24,25], was recognized by 75.8% of HDM allergic patients, similar to other Mediterranean series [10,12,13,14,24,26]. Remarkably, in 9.4% of our patients, no sensitization to the major allergens Der p 1 and Der p 2 was detected. It is important to note that 56% of these patients were sensitized to Der p 23.

Interestingly, sensitization to group 1 was more prevalent than to group 2 major allergens. In our patients, prevalence of asthma was almost 70% (63% persistent asthma), and Der p 1 has been related to the development and severity of asthma in childhood [20, 23]. Our study shows a greater recognition of Der p 1, Der p 2 and Der p 23 in asthmatic patients compared to rhinitis, which agrees with other investigations [9, 11, 13, 15]. An important finding in our work was the significantly higher recognition of Der p 23 in patients with persistent moderate/severe asthma.

Furthermore, most patients with sensitization to Der p 23, but not to Der p 1 and Der p 2, had not received immunotherapy even when they could have potentially benefited from this.

Moreover, the eleven patients that did not respond to immunotherapy were all sensitized to Der p 23.

Therefore, Der p 23 was an important component that should be included in the diagnosis of patients with respiratory allergy as well as a possible tool for improving the clinical course of our patients.

In our study, tropomyosin sensitization was 9.8%, similar to another study with children from a Mediterranean area [20]. As expected, most children sensitized to Der p 10 were

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also sensitized to major allergens tested. Only two (0.7%) had a sensitization to Der p 10 without sensitization to Der p1, Der p 2 and Der p 23, which confirms that Der p 10 represents a minor allergen in respiratory allergy to mites [19-22]. These children had a positive SPT to tropomyosin. Thus, the inclusion of purified shrimp tropomyosin in the diagnostic panel of respiratory allergy could be relevant. Moreover, Resch et al [10] recently concluded that Der p 10-positive patients reacted to other mite allergens rDer p 5, rDer p 7 and rDer p 21. Therefore, these patients with SPT to tropomyosin positive could be interesting candidates for further molecular diagnosis.

Finally, in our study we observed a high sensitization rate to SM allergens; a limitation of our study was to study SM sensitization in patients selected based on positive SPT to HDM. We therefore missed those patients of selectively sensitization to SM, although in our area exclusive SM sensitization has been previously described as low (0.8%) [6]. *Lepidoglyphus*, was considered the predominant SM species in Europe, followed by *Blomia*. Prevalence of sensitization to Lep d 2 and Blo t 5 was lower than sensitization detected with the corresponding extracts by SPT. This discrepancy may indicate sensitization to other components, not represented in the molecular diagnosis platform used. Therefore, determination of sensitization to both the whole extract and the major allergens Lep d 2 and Blo t 5 prior prescription of allergen specific immunotherapy to SM is important.

It is also remarkable that all children sensitized to *Lepidoglyphus*, except one, and all sensitized to *Blomia* were also sensitized to the major allergens of DP and DF. This is in accordance with previous works [4, 25] that defend that sensitization to SM is related to HDM exposure.

In conclusion, this study confirms the importance of molecular components in aiding further diagnosis in mite allergic patients. We provide evidence for the importance of major

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allergens in patients with respiratory allergy to mites and the implications for clinical practice of Der p 23, as it is more prevalent in persistent moderate/severe asthma in children. The high frequency of sensitization to Der p 23 detected suggests the need to include it in the diagnostic and therapeutic allergen preparations. Further studies are required to consolidate these observations.

The authors declare that no foundings were received for the present study.

Each author has participated sufficiently in the work and were agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest

The following authors involved: Jiménez-Feijoo R, Pascal M, Domínguez O, Lózano J, Riggioni C, Álvaro M, Piquert M, Machinena A, Folque M, Dias M and Ana M Plaza declare that they have no conflict of interest. Two authors, Moya R. and Carnés J. were at the time of the study and are currently employed by LETI laboratories; however, there are no direct conflicts with the data presented in this study.

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TABLES

	N (%)
Patients recruited	276
Age (years SD/range)	10.32±6.7 /3-18
Males	85%
Allergic rhinoconjunctivitis	237 (85.9%)
Persistent	89 (37.5%)
Intermitent	148 (62.5%)
Asthma	191 (69.2%)
Intermitent	69 (36%)
Persistent	122 (63%)
Mild n (%)	49 (25%)
Moderate n (%)	67 (35%)
Severe n (%)	6 (3%)
Immunotherapy Prescription	182 (66%)
Completed 3 years	140 (51%)
Responders	129 (47%)
Non-responders	11 (4%)
Food allergy	32 (11.6%)
Shellfish allergy	4 (1.4%)

Table 1. Characteristics of the patient population. Data are presented as number (percentage) of patients with regard to the total (N=276).

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Skin prick test	**N (%)**
Dermatophagoides pteronyssinus	269 (97.5)
Dermatophagoides farinae	257 (93.1)
Euroglyphus maynei	252 (91.3)
Lepidoglyphus destructor	132 (47.8)
Acarus siro	132 (47.8)
Chortoglyphus arcuatus	126 (45.6)
Blomia tropicalis	85 (30.8)
Glycyphagus domesticus	56 (20.3)
Tropomyosin	18 (6.5)

Table 2. Prevalence of sensitization to mites by skin prick test.
Data are presented as number (percentage) of patients with regard to the total (N=276).

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ImmunoCAP	N (%)	Median kU_A/L (IQ)
nDer p 1	239 (86.6)	33.7 (8.3-83.9)
rDer p 2	219 (79.3)	42.5 (18.6-96.1)
rDer p 23	201 (75.8)	6.6 (3.1-16.1)
rDer p 10	27 (9.8)	3.6 (1.2-8.5)
Microarray	N (%)	Median ISU (IQ)
nDer p 1	208 (75.4)	12 (5.1-23.0)
rDer p 2	219 (79.3)	22 (9.0-43.0)
nDer f 1	207 (75.0)	12 (5.2-26.0)
rDer f 2	215 (77.9)	31 (15.0-61.0)
rLep d 2	64 (23.2)	1.95 (0.7-4.7)
rBlo t 5	43 (15.6)	1.7 (0.7-4.9)
rDer p 10	26 (9.4)	10.5 (2.9-28.5)

Table 3. Prevalence of sensitization to mite's components by *in vitro* tests.
Data are presented as number (percentage) of patients with regard to the total (N=276), except for rDer p 23 (N=265).

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	Sensitized to Der p 23 with Der p 1 ± Der p 2	Sensitized to Der p 1± Der p 2 without sensitization to Der p 23	Sensitized to Der p 23 without sensitization to Der p 1 ± Der p 2	P value
N	183	79	14	
Age (years) medium ± SD	10±2	10± 5	11± 3	0.37
SPT DP Median mm^2 (25-75)	25 (10-45)	23 (12-35)	27 (18-55.5)	0.53
SPT DF Median mm^2 (25-75)	32 (12-32)	36 (37-46)	30 (18-48)	0.16
Asthma	132 (70%)	36(45.5%)	7 (50%)	<0.0001
Inttermitent	36 (27%)	25(69.5%)	3(43%).	0.2
Mild	32(24.5%)	5(14%)	1(14%)	0.06
moderate	58(44%)	6(16.5%)	3(43%)	<0.0001
severe	6 (4.5%)	0 (0%)	0(0%)	0.69
Rhinitis	150 (82%)	27 (69%)	10 (71%)	0.73

Table 4. Comparison of the clinical data of patients sensitized to Der p 23 without sensitization to Der p1 and/or Der p 2, and those sensitized to Der p 1 or Der p 2 without sensitization to Der p 23. Data are presented as number (percentage). SPT=Skin prick test, DP= *D. pteronyssinus*, DF= *D. farinae.*

	Responders to immunotherapy	Non - responders to immunotherapy	P value
N (%)	129/140(92%)	11/140(8%)	
Age (years)±SD	10±3	15±2	0.53
Sensitization to nDer p 1	116(90%)	11(100%)	0.56
Sensitization to rDer p 2	109(87.2%)	11(100%)	0.46
Sensitization to rDer p 23	99(79.2%)	10(91%)	0.24
Sensitized to nDer p 1 ± rDer p 2 with sensitization rDer p 23	12(9.3%)	1(9%)	0.12
Sensitized to rDer p 23 without sensitization nDer p 1 ± rDer p 2	1(0.7%)	2(18%)	
IgE to nDer p 1 kU_A/L (25-75)	34(10-93)	34(1.5-100)	0.73
IgE to rDer p 2 kU_A/L (25-75)	38(0.5-98.5)	24(0.5-92)	0.36
IgE to rDer p 23 kU_A/L (25-75)	6(1-14)	11(3-22)	0.16

Table 5. Comparison of the clinical data of patients with clinical response to immunotherapy with regard to the patients without response to immunotherapy. Data are presented as number (percentage) N=140.

FIGURES

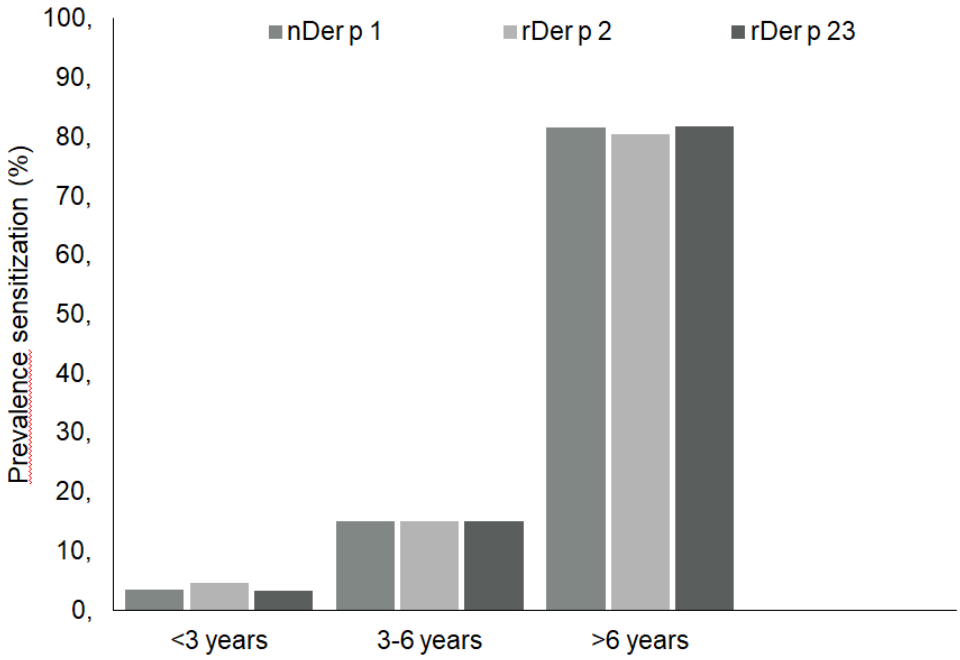

Figure 1. Prevalence of sensitization to nDer p 1, rDer p 2, and rDer p 23 by age groups (detected by ImmunoCAP.

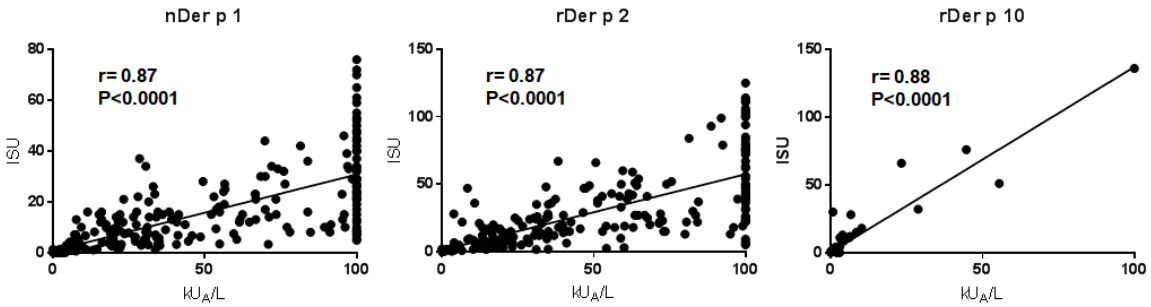

Figure 2. Correlation between specific IgE levels against nDer p 1, rDer p2 and rDer p 10 determined by ImmunoCAP (kUA/L) or ISAC (ISU).