

Direct intranasal application of the solid phase of ImmunoCAP® increases nasal specific immunoglobulin E detection in local allergic rhinitis patients

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Background: The measurement of nasal specific IgE (NslgE) in local allergic rhinitis (LAR) patients is challenging and shows variability. The objective of this work was to evaluate a minimally-invasive method of direct detection of NslgE in patients with LAR to *Dermatophagoides pteronyssinus* (DP) using an automated immunoassay.

Methods: Fifty patients participated (LAR, n = 14; allergic rhinitis (AR), n = 20; healthy controls [HC], n = 16). Detection of NslgE was performed by direct application of the solid phase of a commercial DP ImmunoCAP® test 24 hours after DP nasal provocation.

Results: There was no difference in the median volume of secretion absorbed by the solid phase of the ImmunoCAP test in the 3 studied groups ($p = 0.17$). According to receiver operating characteristic (ROC) curve analysis,

NslgE ≥ 0.1450 was the optimal cutoff point, obtaining in LAR patients 42.86% sensitivity with the highest specificity (100%), and 75% sensitivity and 100% specificity for AR.

Conclusion: This study demonstrates the detection of NslgE to DP in LAR by using a simple, commercial device with high specificity. © 2017 ARS-AAOA, LLC.

Key Words:
local; nasal; rhinitis; sensitivity; specific IgE

How to Cite this Article:

Campo P, del Carmen Plaza-Seron M, Eguiluz-Gracia I, et al. Direct intranasal application of the solid phase of ImmunoCAP® increases nasal specific immunoglobulin E detection in local allergic rhinitis patients. *Int Forum Allergy Rhinol.* 2017;XX:1-5.

Local allergic rhinitis (LAR) is a major differential diagnosis in patients featuring typical allergic rhinitis (AR) symptoms but negative skin-prick test (SPT) and non-detectable serum specific-immunoglobulin E (sIgE).¹ The immunopathology is unknown, but local production of

allergen-sIgE could be involved in a proportion of LAR individuals.^{2,3} Recent studies demonstrated that allergen-specific immunotherapy (AIT) induced significant clinical improvement in LAR subjects,⁴ so an accurate diagnosis is crucial to correctly manage these patients.

The nasal allergen provocation test (NAPT) is the gold standard for LAR diagnosis, but it is time-consuming and needs trained personnel. The measurement of specific IgE in nasal secretions has also been proposed for LAR diagnosis, but different studies in nasal lavage have reported a low sensitivity (22-25%).^{2,3} Moreover, the complex processing methods of the samples may affect the sensitivity, the variability of the results, and the time of patients evaluation.^{2,3,5,6}

The aim of this study was to evaluate a novel minimally-invasive method of direct detection of nasal sIgE (NslgE) in patients with LAR to *Dermatophagoides pteronyssinus* (DP) using a conventional automated immunoassay.

Patients and methods

Patients previously diagnosed with LAR (perennial symptoms typical of allergic rhinitis (itching, sneezing, nasal

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Funding sources for the study: Institute of Health "Carlos III" of the Ministry of Economy and Competitiveness (grants co-funded by European Regional Development Fund [ERDF]): Red de Reacciones Adversas a Alergenos y Farmacos RIRAAF RD07/0064, ARADyAL (Asma, Reacciones ADversas y ALérgicas) RD16/0006/0001, FIS PI12/00900, FIS PI14/00864, and Andalusian Regional Ministry Health grant PI-0346-2016.

Potential conflict of interest: None provided.

Received: 30 June 2017; Revised: 27 September 2017; Accepted: 20 October 2017

DOI: 10.1002/alr.22039

View this article online at wileyonlinelibrary.com.

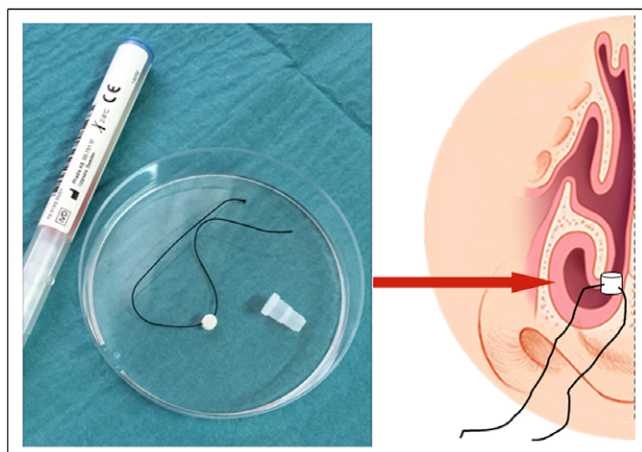


FIGURE 1. Material used for application of solid phases of ImmunoCAP® in nasal mucosa. Solid phases were attached with surgical silk to avoid aspiration.

blockage, and watery rhinorrhea), negative SPT to DP and all relevant aeroallergens in our area, negative serum sIgE to DP and other relevant aeroallergens in our area (<0.35 kU/L), and positive NAPT to DP (NAPT-DP) were recruited for the study between July 2016 and February 2017. From these patients, 3 had also local sensitization to *Alternaria* (negative SPT, <0.35 kU/L serum sIgE, and positive NAPT to *Alternaria*). As controls, a similar group of subjects with AR (perennial allergic rhinitis symptoms, positive SPT to DP, positive serum sIgE to DP >0.35 kU/L, and positive NAPT-DP) and healthy controls (HC) (no rhinitis symptoms, negative SPT to DP and all relevant aeroallergens in our area, negative serum sIgE to DP, and negative NAPT-DP) were also included. These patients were all well-characterized and did not have any exclusion criteria for the study (pregnancy/breastfeeding, immunological diseases, chronic rhinosinusitis with/without nasal polypsis [nasal endoscopy and/or computed tomography scan], respiratory infections in the previous 4 weeks, and treatment with corticosteroids or antihistamines in the previous 3 weeks). The local ethical committee approved the study and patients signed an informed consent.

Before the NAPT-DP was performed, nasal hyperreactivity (NHR) was ruled out by applying 2 puffs per nostril (100 μ L) of 0.9% saline using a metered spray. After 10 minutes, Volume 2–6 was measured by acoustic rhinometry, and if the value decreased $<10\%$ compared to baseline, the NRH was excluded. Later on, all patients underwent a NAPT with 100 μ L per nostril of DP extract (4 μ g/mL of *Der p 1*; Leti Laboratories, Madrid, Spain) using the same method. After the administration of the allergen, responses were evaluated by acoustic rhinometry (Vol 2–6) and symptoms score at 15 minutes, 60 minutes, and 24 hours.^{2,3} Measurement of NsIgE was performed 24 hours after NAPT by direct application of the solid phase of a commercial ImmunoCAP® to DP (Thermo Fisher Scientific, Waltham, MA) in the lower turbinate of each

nostril for 10 minutes. This time point was selected based on previous studies showing that sIgE reaches its maximum peak 24 hours after allergen exposure.³ The solid phase was applied using forceps and attached with a surgical silk to avoid aspiration (Fig. 1).

Once the solid phases were removed from both nostrils, they were weighed individually into 1.5-mL Eppendorf® tubes using a metered balance (Sartorius CPA225D, Göttingen, Germany).⁷ The maximum volume that the solid phase can take up was tested by soaking 10 units in saline for 10 minutes and weighing them afterward.

The solid phases were in contact with the nasal mucosa and secretions for 10 minutes, and the union of NsIgE to the DP antigens attached to the solid phase took place in situ before any manipulation. After the solid phases had been removed and weighed, they were incubated for 2 hours at 37°C mimicking the incubation step in the commercial ImmunoCAP immunoassay. Solid phases were afterward treated with dithiothreitol (DTT) 10 mmol/L (Sigma-Aldrich, St. Louis, MO), gently stirred for 10 minutes, centrifuged 1500 rpm (239 g), and finally rinsed in saline. Previous studies from our group showed that the treatment with DTT does not affect the IgE binding to the allergen attached to the solid phase of ImmunoCAP.^{2,3} After processing, solid phases were analyzed following the manufacturer's instructions, and to replace the serum sample, 20 μ L of saline was added to the UNICAP 100 (Thermo Fisher Scientific, Portage, MI, USA) machine at the corresponding step. The blank measurement of the ImmunoCAP was determined by processing non-incubated solid phases.

Comparisons of results were made using nonparametric tests for nonrelated samples (Mann-Whitney *U* test). Receiver operating characteristic (ROC) curves were performed to obtain the optimal cutoff point of the NsIgE value. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Outcomes were compared with NAPT since it is the gold standard for diagnosis. Statistics were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Results

A total of 50 subjects were recruited for the study (LAR, $n = 14$; AR, $n = 20$; HC, $n = 16$). Clinical and demographic characteristics of the subjects are shown in Table 1. Median total IgE serum levels (IU/mL) were significantly higher in AR (333 IU/L; range, 144–764) compared to LAR (32 IU/L; range, 11.50–63), and HC (20 IU/mL; range, 13–55.50), $p < 0.001$. As expected, only AR subjects had measurable levels of serum DP-sIgE (37.15 kU/L; range, 7.72–100 kU/L).

The experiments with saline showed that solid phases had a median capacity of absorption of 14.63 mg (range, 13.24–16.53 mg). There was no difference in the median amount of secretion absorbed by the solid phase in the 3 study groups (LAR, 15.11 mg (range, 7.65–20 mg); AR, 14.86 mg (range, 11.07–20.67 mg); HC, 10.39 mg (range, 8.95–13 mg), $p = 0.1711$). Because in nasal ImmunoCAP the

TABLE 1. Clinical and demographic characteristics of the three studied groups

Characteristic	LAR (n = 14)	AR (n = 20)	HC (n = 16)	LAR vs HC (p)	AR vs HC (p)	LAR vs AR (p)
Age (years), median (range)	32 (21.75–52.00)	30 (20.25–35.00)	36.50 (28.50–49.25)	0.394	0.022	0.233
Female (%)	64.29	55	31.25	0.141	0.191	0.728
FHA (%)	21.43	45	31.25	0.689	0.501	0.275
Nonsmokers (%)	83.33	73.68	81.25	1.000	0.700	0.676
Years of disease, median (range)	7 (5–10)	4.50 (2.75–11.25)	NA	1.000	1.000	0.535
Conjunctivitis (%)	65.29	57.89	6.25	0.001	0.002	1.000
Asthma (%)	42.86	21.05	6.25	0.031	0.347	0.257
Serum total IgE, median (range) IU/ml	32.00 (11.50–63.00)	333.00 (144.00–764.00)	20.00 (13.00–55.50)	0.789	0.001	<0.001
Serum slgE, median (range) KU/L	<0.35	37.15 (7.72–100.00)	<0.35	1.000	<0.001	<0.001

AR = allergic rhinitis; FHA = family history of atopy; HC = healthy controls; IgE = immunoglobulin E; LAR = local allergic rhinitis; NA = not applicable; slgE = specific immunoglobulin E.

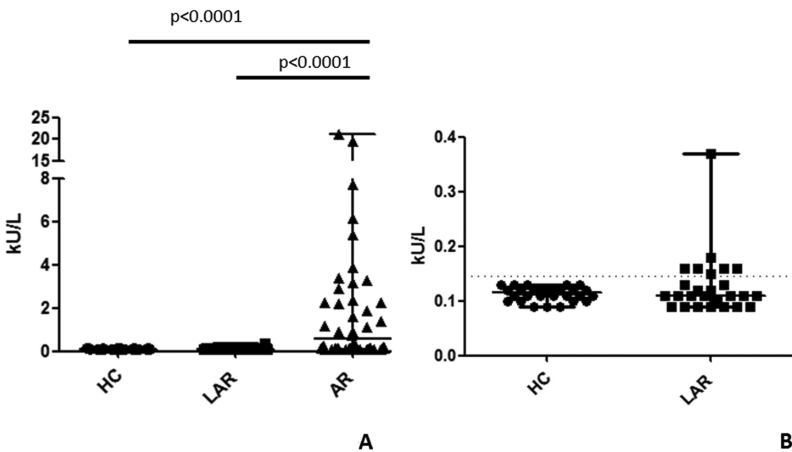


FIGURE 2. (A) Values of NslgE (kU/L) measured in the 3 studied groups (LAR, AR, and HC). (B) Values of NslgE (kU/L) measured in LAR vs HC. The cutoff point of 0.145 kU/L is marked as a horizontal dotted line. AR = allergic rhinitis; HC = healthy controls; LAR = local allergic rhinitis; NslgE = nasal specific immunoglobulin E.

binding of sIgE to the allergen takes place in situ, and thus is not affected by the volume taken up by the solid phase, the concentration of NslgE does not need to be normalized.

The average blank detection of non-incubated solid phases was 0.1 kU/L (data not shown). Sample analysis showed that the highest median level of NslgE was observed in the AR group (0.565 kU/L; range, 0.120-2.328 kU/L) compared to LAR and HC ($p \leq 0.0001$) (Fig. 2A). The median level of NslgE to DP for LAR was 0.110 kU/L (range, 0.090-0.152 kU/L), with no significant differences with the HC group (median 0.115 kU/L; range, 0.102-0.120 kU/L). Importantly, all HC values were below the positivity cutoff point (Fig. 2B).

ROC curves were calculated using the values obtained 24 hours post-NAPT. According to ROC analysis, a level of NslgE ≥ 0.1450 was the optimum cutoff point for discriminating between patients (both LAR and AR) and healthy controls (Fig. 3A,B). The use of this cutoff point in LAR individuals obtained a sensitivity of 42.86%, specificity of

100%, PPV of 100%, and NPV of 66.67%. The area under the curve (AUC) was 0.664 ($p = 0.079$) (Fig. 3A). For AR subjects, this cutoff point obtained 75% sensitivity, 100% specificity, PPV of 100%, and NPV of 80%. The AUC was = 0.870 ($p < 0.001$) (Fig. 3B). There were no significant differences in the clinical characteristics of the LAR patients displaying positive vs negative NslgE results ($p > 0.05$) (Table 2).

Discussion

This study demonstrates that a commercial, automated device which does not require a complex sample processing is able to detect DP-specific IgE in nasal secretions of LAR patients. The solid phase of ImmunoCAP reaches the maximum level of absorption after 10 minutes of incubation, even in the control group, and no differences among the study groups were observed. Therefore, the weighing of the samples or any data normalization is not necessary,

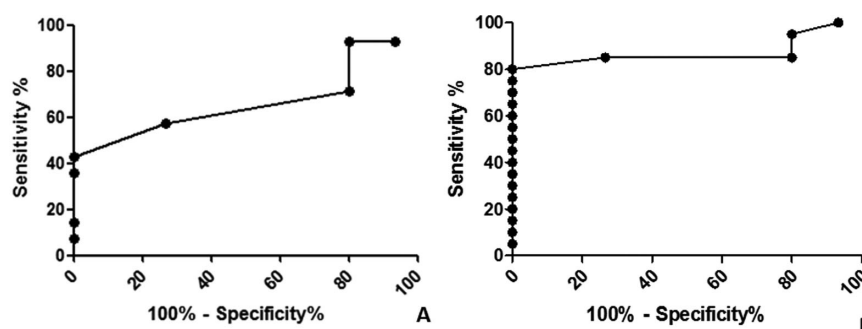


FIGURE 3. ROC curves calculated using the values obtained 24 hours post-NAPT. (A) ROC in LAR patients. (B) ROC in AR patients. AR = allergic rhinitis; LAR = local allergic rhinitis; ROC = receiver operating characteristic.

TABLE 2. Clinical and demographic characteristics of local allergic rhinitis individuals with positive vs negative nasal sIgE determination

	Age (years) median (range)	Female (%)	FHA (%)	Nonsmokers (%)	Years of disease median (range)	Conjunctivitis (%)	Asthma (%)	Serum total IgE median (range) IU/ml
(+) Nasal sIgE (n = 6)	34 (22.50–53.00)	66.67	33	66.67	8 (6–10)	66.67	33.33	24 (11.50–54.50)
(–) Nasal sIgE (n = 8)	25 (20.00–49.50)	62.50	0	100	5 (4–11)	62.50	62.50	46.50 (11.75–67.75)
<i>p</i> value	0.641	1.000	0.258	0.470	0.653	1.000	0.580	0.699

FHA = family history of atopy; IgE = immunoglobulin E; sIgE = specific immunoglobulin E.

which greatly simplifies the technique, and facilitates its performance in the clinical routine. Moreover, the binding of sIgE to the antigen proteins starts in the nasal cavity, so there is no manipulation of the sample at this crucial stage. There may be some limitations of the technique in case of severe septal deviation or severe turbinal hypertrophy, but in general the test is easy to perform and did not cause any bleeding or complications besides minimal discomfort during the manipulation.

The cutoff point for this study was chosen based on its high specificity for confirming LAR diagnosis when compared to the NAPT. The sensitivity of the test to detect NsIgE to DP in LAR (42.86%) was superior to those reported for nasal lavage using the Greiff/Grünberg and Nalerio method (22%).^{2,3} These techniques are troublesome for the patient, more difficult to perform, and require sample manipulation before the immunoassay. Moreover, the sampling of epithelial and subepithelial tissue from inferior turbinates showed promising results for detecting NsIgE in allergic patients, although these techniques are more invasive and difficult to perform.^{8,9} These and other studies showed that the nasal mucosa harbors a significant proportion of sIgE in AR patients, and that most of it is leaked

to nasal secretions.^{10,11} In this regard, several investigators have demonstrated that nasal secretions are suitable for IgE measurements with the advantage of an easier collection by noninvasive methods.^{2–7,10}

The higher number of subjects with NsIgE in the AR group is in agreement with our previous results,^{2,3} and with the well-defined role of IgE in that disease.¹⁰ Data showed that NsIgE levels are lower in LAR subjects, causing the differences in sensitivity with AR. Although LAR pathophysiology is mostly unknown, and there is still no evidence if other mechanisms besides IgE (eg, free light chains) could play a role,¹² some LAR patients display NsIgE^{2,3} and positive basophil activation test responses.¹³ Moreover, AIT induced both clinical improvement and immunological responses in a significant proportion of LAR individuals.⁴

Conclusion

This study presents a new method to measure NsIgE to DP in LAR subjects that displays a higher sensitivity than previously described techniques. 🌐

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