

ORIGINAL ARTICLE

Influence of total serum IgE levels on the *in vitro* detection of β -lactams-specific IgE antibodies

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Summary

Background Allergic reactions to β -lactams are a frequent cause of adverse drug reactions; the diagnosis is based on history, clinical examination, skin testing (prick and intradermal) and demonstration of serum-specific IgE antibodies (Abs).

Objective We compared the diagnostic performance of the Phadia CAP system for the detection of IgE to β -lactams carried out using the new test with cut-off limits of 0.10 kUA/L and the old test with cut-off limits of 0.35 kUA/L for positive results; subsequently, we analysed the effect of total serum IgE values and of atopic phenotype on the diagnostic performance of the tests.

Methods The study comprised a total of 34 patients with a history of immediate adverse reactions to β -lactams, which were confirmed by positive skin testing, and 115 control subjects with tolerance to β -lactams over the last year. The Phadia CAP System was used for the determination of serum total and specific IgE Abs towards penicilloyl G (c1), penicilloyl V (c2), ampicilloyl (c5) and amoxicilloyl (c6). The overall diagnostic performance was assessed as a diagnostic odds ratio (DOR).

Results The new test showed a higher sensitivity (85% vs. 44%) than the old test and a lower specificity (54% vs. 80%) but the overall diagnostic performance was poor (DOR 6.78 vs. 3.16, $P = 0.333$) in both tests. The total IgE value influences the DOR of both tests; DOR was better for values under 200 kU/L [DOR = 66; 95% confidence interval (CI): 11.3–384.1] or 500 kU/L (DOR = 45.7; 95% CI: 5.3–394.4) for the new and old tests, respectively.

Conclusions The reduction in the positive cut off value has not significantly improved the overall diagnostic performance of the β -lactams-specific IgE assay. Because of the influence of serum total IgE on the detection of β -lactam-specific IgE Abs, the combination of both tests is mandatory in the *in vitro* diagnostic approach of β -lactam allergy.

Keywords β -lactam allergy, drug reactions, *in vitro* tests, total serum IgE antibodies

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Introduction

Allergic reactions to penicillins, cephalosporins and derivatives (β -lactams) are the most frequent cause of adverse drug reactions (ADRs) mediated by specific immunologic mechanisms [1]. The diagnosis of ADRs remains a major problem in clinical practice. Indeed, the diagnosis of immediate and/or delayed hypersensitivity reactions to a specific drug is quite difficult and mainly based on the history, the exact definition of clinical manifestations and,

when available, on positive skin prick/intradermal testing and the demonstration of serum-specific IgE antibodies (Abs) [2]. Interpretation of the results, however, has to be made with caution, because a negative result could be related to the lack of optimal sensitivity of the tests and/or the involvement of cell-mediated mechanisms, while a positive result shows a humoral response to the drug even though it does not prove its direct involvement in clinical symptoms. On the other hand, *in vivo* tests such as an oral challenge may cause severe reactions, particularly in patients with previous systemic events, but these tests can rule out an incriminated culprit drug and otherwise

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confirm whether the response is immediate (urticaria and anaphylaxis) or delayed (maculo-papular exanthema). The detection of serum drug-specific IgE performed using the CAP system method (Phadia) is important due to its safety and accessibility. However, it is well known that its sensitivity is low and not sufficient for the diagnosis of ADRs to β -lactams, which needs to be associated with positive skin testing and challenge tests [2]. Recently, the value of 0.10 kUA/L has come to be considered the positive cut-off limit of the Phadia CAP system for the detection of allergen-specific IgE Abs, also including IgE towards β -lactams. In the present study, we compared the diagnostic performance of the tests for the detection of IgE Abs to β -lactams performed with the new (cut-off limit 0.10 kUA/L) and the old (cut-off limit 0.35 kUA/L) Phadia CAP system. In addition, we investigated the effect of total serum IgE values on the diagnostic performance of the tests.

Materials and methods

Subjects

A total of 61 consecutive patients who were referred to our Immunoallergy Department, with a history of suspected immediate ADR to β -lactams in the past year, were enrolled in the study. Evaluation of the atopic phenotype was based on history, clinical symptoms and positive skin prick tests (SPT) for common allergens. A patient was diagnosed as having a positive diagnosis of β -lactam allergy (ADR+ patients) if he or she had a history of reactivity supported by skin testing positivity. Ten patients, who had experienced severe reactions, refused to allow *in vivo* tests; thus, they were excluded from further analysis. Seventeen out of the remaining 51 patients displayed negative skin testing; thus, they were also excluded from the study. Seventeen out of the remaining 34 patients were atopic (50%) and amoxicillin was the most frequent (85.3%) culprit drug. We also enrolled a negative control group (ADR- patients) of 115 subjects, 77 atopic and 38 non-atopic, who showed good tolerance to β -lactams administration in the last year and displayed negative skin testing. Table 1 summarizes the key characteristics of ADR+ and ADR- patients.

Detection of serum-specific immunoglobulin E antibodies

CAP System FEIA (Phadia, Uppsala, Sweden) is an *in vitro* test system based on ImmunoCap technology and used for the determination of circulating specific IgE Abs. Sera obtained from all patients were analysed for IgE towards the hapten c1 (penicilloyl G), c2 (penicilloyl V), c5 (ampicilloyl) and c6 (amoxicilloyl) according to the manufacturer's instructions. Two available commercial tests were performed (herein called the old and the new CAP

Table 1. Clinical characteristics of analysed patients

	ADR+	ADR-
Number	34	115
Gender (M/F)	11/23	50/65
Age (year, range)	18-67	18-58
Allergic sensitization* (%)	17 (50)	77 (66.9)
Total serum IgE	554 $\pm$ 139	782 $\pm$ 127
Symptoms (%)		-
Anaphylaxis [†]	13 (38)	
Urticaria/angioedema	21 (62)	
Culprit drug (%)		-
Amoxicillin	29 (85.3)	
Ampicillin	2 (5.9)	
Other penicillins	3 (8.8)	
Time delay (days)	87 $\pm$ 10.2	

*Confirmed by a positive skin test to inhalant and/or food allergens.

[†]Patients who displayed a severe reaction affecting at least two organs with bronchospasm and/or hypotension.

Total serum IgE was detected as described in 'Materials and methods', values are reported as mean $\pm$ SE.

ADR, adverse drug reaction.

System) characterized by different cut-off values of positivity (0.35 and 0.10 kUA/L, respectively). The methodology is the same for both tests [3], but the new CAP system is characterized by a different standard curve with a lower detection limit (0.10 kU/L). Serum samples were considered positive when one or more hapten positivities occurred.

Total immunoglobulin E antibody detection

Total serum IgE Abs detection was performed by the Phadia CAP system immunoassay according to the manufacturer's instructions.

Skin testing

Skin testing was carried out as described previously with 0.02 mL of solutions [2]. The reagents were penicilloyl-polylysine (5×10^{-5} M, Diater Laboratories, Spain), minor determinant mixture (2×10^{-2} M, Diater Laboratories, Madrid, Spain), amoxicilline (20 mg/mL, GlaxoSmithKline, Beecham S.A., Belgium) and ampicilline (20 mg/mL, Pfizer Italia, Rome). In both prick and intradermal testing, a minimum weal area of 3 mm diameter, or an increase of area > 3 mm, was considered the positive response compared with the negative response to the saline control.

Statistical analysis

Sensitivity and specificity data were obtained from the cross-tabulation of positive and negative test results in the whole sample, and in subgroups of variables of interest. The overall diagnostic performance of the new and old

tests was assessed as the diagnostic odds ratio (DOR), which is a single indicator of performance [4]. The diagnostic accuracy study is based on the use of positive (LR+) and negative (LR-) likelihood ratios in clinical decision models [5, 6]. Quartiles of total serum IgE levels were created for an exploratory analysis. Cut-offs were based on this analysis as well as on the graphical display of sensitivity and specificity at different levels of total IgE, obtained in a logistic regression model [7] in which total IgE were log-transformed to take into account a non-linear and geometric relationship between diagnostic performance and IgE values. These analyses were based on STATA 10.0 software (Statacorp, College Station, TX, USA). When required, t and χ^2 tests were used to analyse differences between groups of patients and $P < 0.05$ value was considered significant.

Results

Diagnostic performance of new vs. old CAP system tests

We evaluated circulating IgE Abs towards β -lactams using two available commercial tests (old and new CAP System) in 34 patients suffering from immediate ADR to β -lactams, in which the diagnosis was confirmed by positive skin testing. The results are reported in Table 2.

We compared the performance characteristics of the new CAP system with a cut-off value of 0.10 kUA/L for positive results with the old CAP system with a cut-off value of 0.35 kUA/L. As summarized in Fig. 1a, the new test showed a higher sensitivity [85%, 95% confidence interval (CI): 69–95%] than the old test (44%, 95% CI: 27–62%). As a consequence, the new test yielded 14 more

Table 2. CAP-FEIA results in patients with ADRs to β -lactams

	β -L	Clinical manifestation	Delay (days)	Old CAP				New CAP				Skin testing
				c1	c2	c5	c6	c1	c2	c5	c6	
1	AX	AN	45	+	+	+	–	+	+	+	–	+
2	AX	AN	15	+	+	+	+	+	+	+	+	+
3	AX	UA	52	+	+	+	+	+	+	+	+	+
4	AX	UA	180	–	+	–	+	+	+	+	+	+
5	AX	UA	86	+	+	+	–	+	+	+	+	+
6	PEN	AN	181	+	+	+	–	+	+	+	+	+
7	AX	UA	72	–	–	+	–	–	–	+	–	+
8	AX	UA	76	+	+	+	+	+	+	+	+	+
9	PEN	UA	123	–	–	+	–	+	–	+	+	+
10	PEN	AN	72	–	–	–	+	–	–	–	+	+
11	AX	UA	52	–	+	–	–	+	+	+	+	+
12	AX	UA	205	–	+	–	–	–	+	+	–	+
13	AX	UA	63	+	+	+	–	+	+	+	–	+
14	AX	AN	20	+	+	+	+	+	+	+	+	+
15	AX	AN	47	–	–	–	–	–	–	–	–	+
16	AX	UA	78	–	–	–	–	–	–	–	–	+
17	AX	AN	64	–	–	–	–	–	–	–	–	+
18	AX	AN	147	–	–	–	–	–	+	+	–	+
19	AX	UA	30	–	–	–	–	–	+	+	–	+
20	AMP	UA	78	–	–	–	–	+	+	+	–	+
21	AX	AN	46	–	–	–	–	–	+	+	–	+
22	AX	UA	45	–	–	–	–	–	+	+	+	+
23	AX	AN	210	–	–	–	–	–	–	–	–	+
24	AX	AN	32	–	–	–	–	–	–	–	–	+
25	AX	AN	156	–	–	–	–	–	–	+	–	+
26	AX	UA	55	–	–	–	–	–	+	+	–	+
27	AMP	UA	74	–	–	–	–	–	–	+	+	+
28	AX	UA	52	–	–	–	–	–	+	+	–	+
29	AX	UA	82	–	–	–	–	+	+	+	–	+
30	AX	UA	184	–	–	–	–	–	–	+	–	+
31	AX	UA	215	–	–	–	–	–	–	–	+	+
32	AX	UA	39	+	–	+	–	+	–	+	–	+
33	AX	UA	32	–	–	–	–	–	–	+	–	+
34	AX	AN	51	–	–	–	–	–	–	+	–	+

β -L, β -lactams; c1, penicilloyl G; c2, penicilloyl V; c5, ampicilloyl; c6, amoxicilloyl; AX, amoxicillin; AMP, ampicillin, PEN, penicillin, AN, anaphylaxis; UA, urticaria/angioedema; ADR, adverse drug reaction.

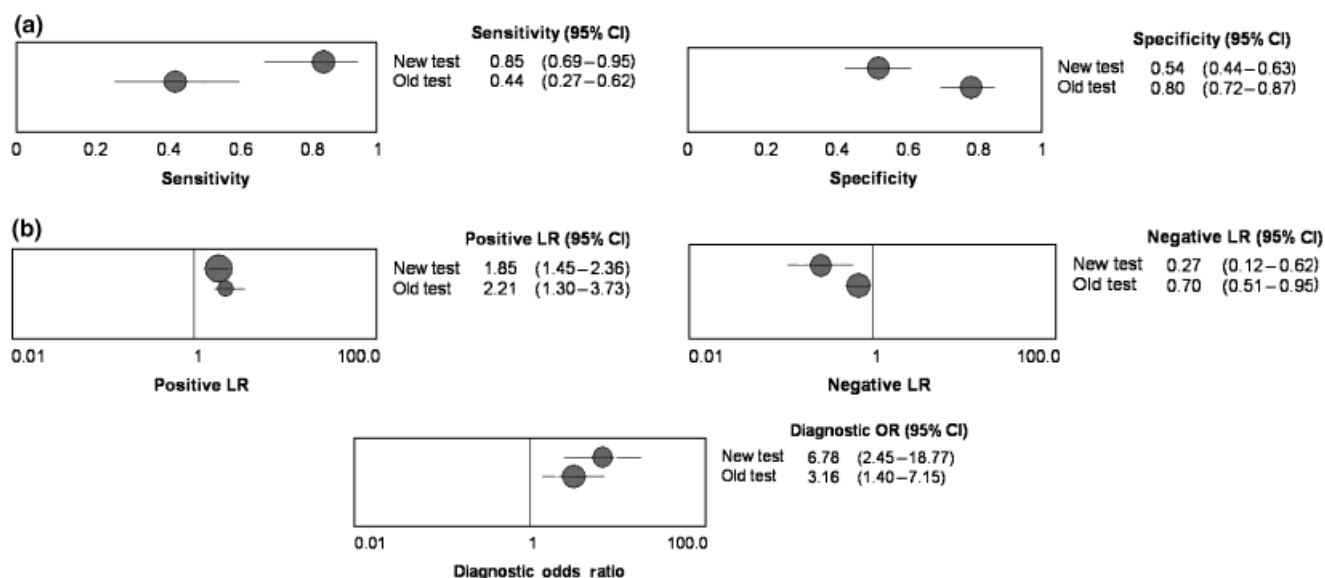


Fig. 1. Diagnostic performance of new and old CAP system tests for β -lactam allergy. (a) The new test shows a higher sensitivity but a lower specificity than the old test. (b) The diagnostic odd ratio (DOR) is below 10 for both the new and old test, showing a low overall diagnostic performance in the whole sample.

Table 3. Sensitivity and specificity of the new and old test according to total IgE serum levels

Total IgE quartiles (n, 149)		< 58 (n, 37)		58–193 (n, 37)		193–722 (n, 37)		> 722 (n, 38)	
Reaction		Yes	No	Yes	No	Yes	No	Yes	No
New test	Positive (n, 82)	4	0	6	1	10	23	9	29
	Negative (n, 67)	1	32	4	26	0	4	0	0
		Se: 80%	Sp: 100%	Se: 60%	Sp: 96%	Se: 100%	Sp: 15%	Se: 100%	Sp: 0%
Old test	Positive (n, 39)	3	0	3	0	4	3	6	20
	Negative (n, 110)	2	32	7	27	6	24	3	9
Se: 40%		Se: 60%	Sp: 100%	Se: 30%	Sp: 100%	Se: 40%	Sp: 88%	Se: 66%	Sp: 31%

Se, sensitivity; Sp, specificity.

positive patients than the old test. However, the introduction of the lower cut-off value (0.10 kUA/L) significantly decreased the specificity of the new test (54%, 95% CI: 44–63) compared with the old test (80%, 95% CI: 72–87) (Fig. 1a).

The overall diagnostic performance of both the new and the old tests was assessed as DOR, which was obtained from a positive (LR+) and a negative (LR–) likelihood ratio; on assessing the whole sample, the performance was poor (Fig. 1b). The DOR of the new test (6.78) was slightly better than the old test (3.16), but there was no difference between the two DORs in repeated measure regression models ($P = 0.333$), and both were below 25, the value above which a test is considered useful in most clinical fields.

Diagnostic performance of both new and old CAP system tests according to atopy and total immunoglobulin E serum levels

Because most of the ADR– subjects with a positivity of β -lactam IgE Abs showed an atopic phenotype (new test:

63% atopic vs. 18.4% non-atopic; old test: 27.2% atopic vs. 2.6% non-atopic), this condition could be considered a potential modifier of the diagnostic performance of the tests. We asked whether the presence of atopy, but particularly the total IgE serum values, could affect the performance characteristics of the new and old tests. To this end, we explored the relationship between total IgE and the test performance using quartiles of total IgE serum levels. Table 3 summarized the sensitivity and specificity of the old and new tests according to the total IgE serum levels. Both tests were highly specific when the total IgE value was below the median value (193 kU/L), but the new test was more sensitive. For values above the median, the new test was highly sensitive but had a very low specificity, whereas the old test was slightly less sensitive but showed a higher specificity (Table 3).

Figure 2 presents the sensitivity and specificity for the new and old tests as obtained from the regression model described in 'Materials and methods'. As shown in the figure, the performance of the new test was maximized below 200 kU/L of the total IgE levels, because the

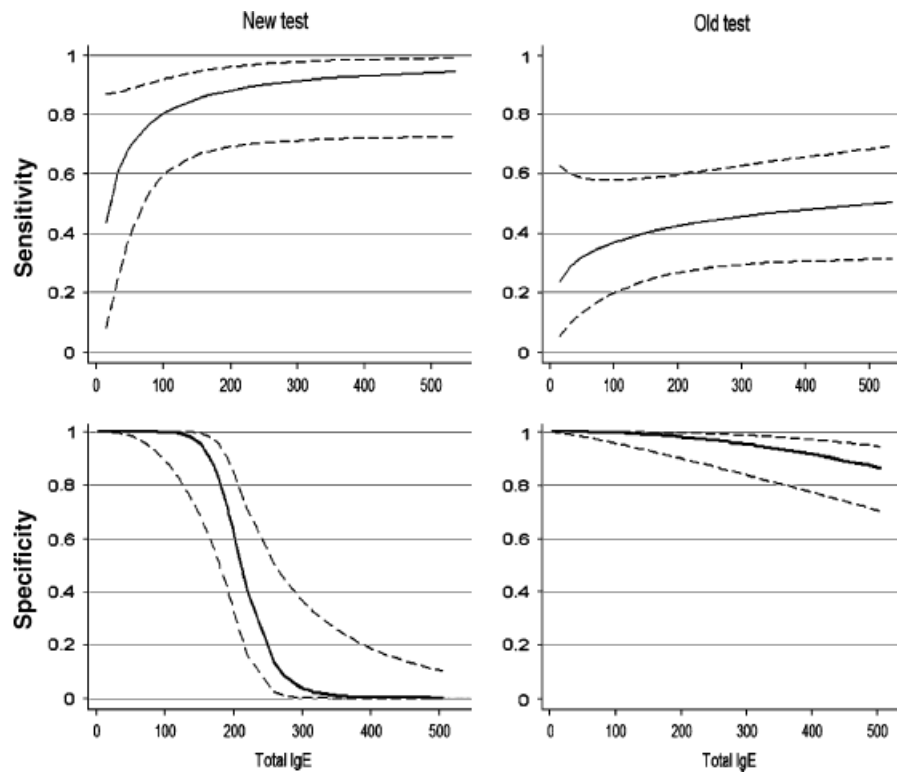


Fig. 2. Curves of sensitivity and specificity at different levels of total serum IgE for both the new and the old CAP test. The sensitivity and specificity of the new and old test in relation to the total IgE serum values are shown. Data are obtained from the regression model described in 'Materials and methods' section. Solid line, point estimate; dashed line, 95% confidence limits.

specificity declined after this value. The sensitivity of the old test continued to increase up to 500 kU/L while maintaining an acceptable specificity. Therefore, these cut-offs were chosen for further analyses on the interaction of the IgE value and diagnostic performance.

Finally, the performance of the old and new tests was compared using repeated-measure logistic regression according to Siadat et al. [7].

Figure 3 shows the indexes of diagnostic performance according to the total IgE values as defined above. The DOR was better for values of 200 kU/L or less (DOR = 66; 95% CI: 11.3–384.1) as compared with values of > 200 kU/L (DOR = 1.8, 95% CI: 0.1–39.2). The LR+ was 21.3 (95% CI: 5.2–86.7) for patients with positive tests and a total IgE value below 200 kU/L. The corresponding values for the old test were 27.7 (95% CI: 2.95–260.7) at 200 kU/L or less, and 2.7 (1.0–7.0) at > 200 kU/L, with a relative DOR of 1.8 (95% CI: 0.6–5.1) that did not yield a statistically significant value (P -value = 0.29, data not shown). Using a total IgE cut-off of 500 kU/L, the DOR was 45.7 (95% CI: 5.3–394.4) below the threshold and 0.39 (95% CI: 0.1–1.5) above it. The use of higher total IgE value cut-off values did not improve the performance of the old test. The LR+ was 29.5 (95% CI: 3.9–233.1) below 500 kU/L. Again, the uncertainty of this estimate (95% CI limits) is considerable due to the small number of cases at

low IgE values. Lastly, the absence of atopy improved the diagnostic performance of the new test (DOR: 39.7 vs. 4.3, P = 0.052) as well as the old test (25.9 vs. 2.2, P = 0.050) with borderline statistical significance.

Discussion

The diagnostic methods for evaluating subjects with allergic reactions to β -lactams include clinical history as well as *in vivo* and/or *in vitro* tests [1]. β -lactam-specific IgE Abs detection is of particular interest in the diagnosis of patients with suspected ADRs to β -lactams for safety reasons and clinical significance [8]. However, one of the major topics of discussion concerns the sensitivity and specificity of the IgE test assays, taking into account that β -lactam-specific Abs are usually lower than IgE towards inhalant allergens. Recent data indicated that the specificity for the CAP system ranged from 85.7% to 100% but the sensitivity ranged from 0% to 25% depending on the initial clinical symptoms [9]. Several studies have shown that the IgE-mediated response to β -lactams decreases with time [10]. In this context, the availability of a new CAP system test for the detection of β -lactam-specific IgE Abs with the cut-off level of 0.10 kUA/L is important from the clinical point of view.

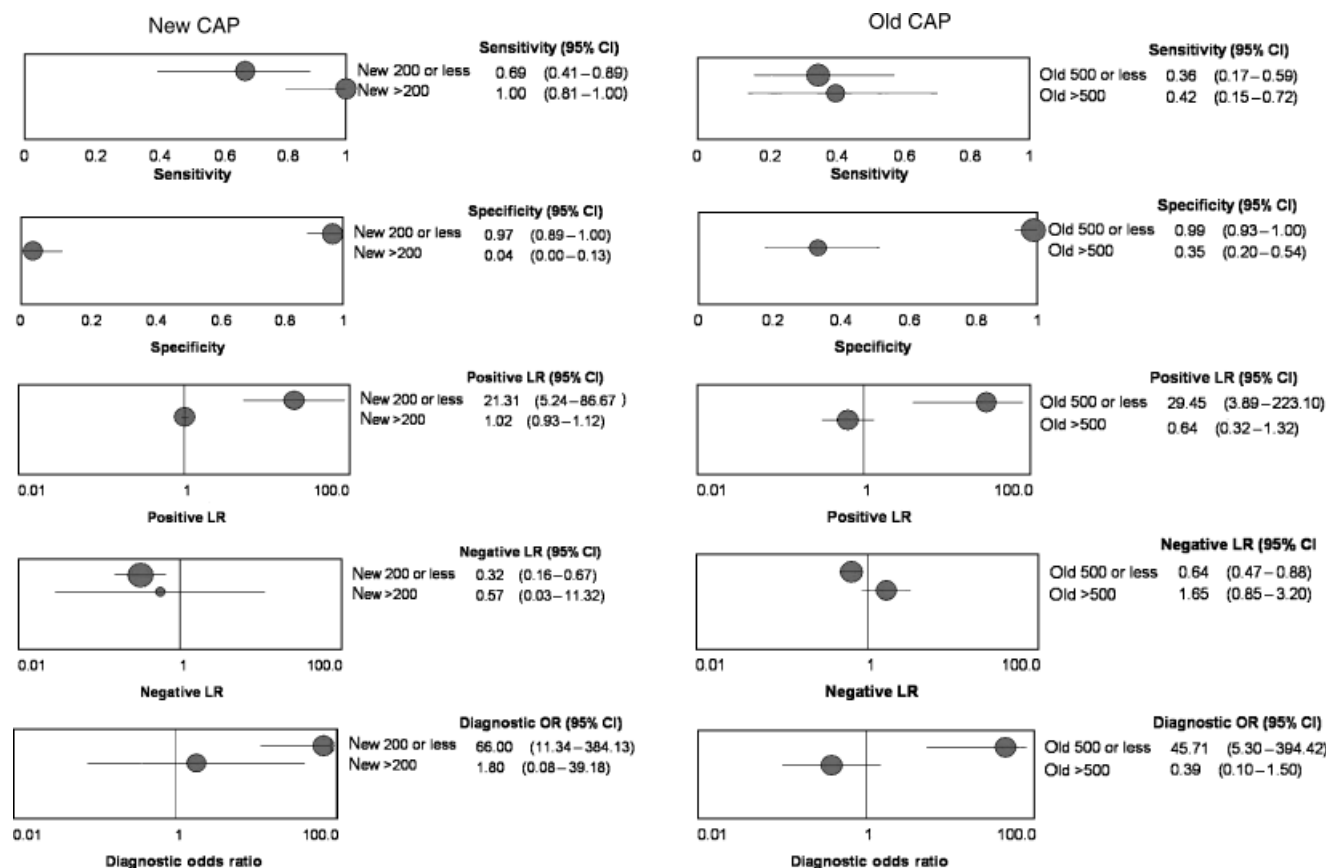


Fig. 3. Diagnostic performance of new and old CAP system tests according to total serum IgE values. Cut-off values for new (200 kU/L) and old (500 kU/L) tests were chosen based on exploratory analysis as described in 'Materials and methods'. The sensitivity, specificity, positive likelihood ratio (LR) and finally the diagnostic odds ratio (DOR) were strongly affected by high levels of total IgE above the reported cut-off value for both new and old tests.

The present study provided evidence of a significant increase of sensitivity using this new test, but with a strong parallel impairment concerning specificity. The fact that the new test is more sensitive but less specific than the old one must be considered a threshold effect. The predictive value of the tests is particularly critical in patients with β -lactam allergy, considering the clinical problems related to *in vivo* tests. Both the new and the old tests were found to perform better in the negative than in the positive prediction of ADR to β -lactams. Indeed, all ADR– patients showing positive IgE Abs were currently taking β -lactams with good tolerance. In addition, as expected, all ADR– patients gave negative results at skin testing (data not shown). Thus, we hypothesized that the result of the tests, and particularly their specificity, was affected by some additional factors such as total serum IgE levels.

Our results showed that total serum IgE values influence the diagnostic performance of both tests, as demonstrated by the fact that the DOR breaks down for total IgE values above 200 or 500 kU/L for new and old CAP system tests, respectively. Of note, values of DOR over 25 and 100 suggest a good and a very good performance, respectively.

The negative influence of high levels of total IgE in the serum can be proved by the correlation between total and hapten-specific IgE serum levels in ADR+ and ADR– patients. Indeed, we observed a direct correlation between total and specific IgE values for each single hapten in ADR– subjects, but not in ADR+ patients (data not shown).

These data suggest that the evaluation of IgE serum levels is useful for the diagnosis of allergy to β -lactams in patients with total IgE values below a given threshold (200 kU/L for the new test and 500 kU/L for the old test), thanks to a high specificity and despite the fact that the sensitivity was moderately low. A more realistic method of presenting the results of a diagnostic accuracy study is usually based on the use of LR+ and LR– in clinical decision models [5, 6]. Good and very good values of LR+ are 5 and 10, respectively, and the corresponding values of LR– are their reciprocal 0.2 and 0.1. As an example, an LR+ of 21, as observed in our study using the new test in patients with low total IgE levels (< 200 kU/L), increases the pre-test probability of a reaction from 5%, the prevalence in our outpatients attending the clinic for drug-allergic reactions, to a post-test probability of about 53%

in those who showed test positivity. However, the uncertainty regarding this estimate is very high and we recommend that further studies are performed before applying these diagnostic criteria to clinical practice. An additional limitation of the study is its case-control design, which typically tends to increase the estimates of diagnostic performance because cases with doubtful target condition are typically excluded. We then suggest that further studies must be performed with a cohort study design in order to include a much larger sample of consecutive patients presenting the full spectrum of the reactions to β -lactams, particularly cases with suspect allergic reactions. This is expected to provide a more realistic estimate of the diagnostic performance and also more consistent statistical estimates.

The false-positive results observed with high IgE serum levels may be due to several factors including high stickiness with hapten-bound substrates and the presence of low levels of anti-IgE auto-Abs, sometimes found in severe atopic disorders [11].

Because of cross-reactivity, IgE usually react to different haptens, thus influencing the results of immunoassays [12]. The correlation between the number of hapten positivity and the history of ADR may be important in order to reduce the influence of total IgE levels on the specificity of the test. Some preliminary data showed that the proportion of patients with a single positivity is higher in ADR+ than in ADR- subjects (unpublished data). This could suggest that, independent of the total IgE levels, the presence of a single hapten positivity in the full-blown panel could really correlate with a sensitization towards β -lactams. Moreover, unpublished data from our group suggest that the absolute levels of specific IgE (at least for penicillin G) correlate with the history of ADR.

We also assessed the diagnostic performance of the positivity to each β -lactam for the new test using a repeated measure logistic regression model and adjusting for the IgE level as a dichotomous variable. In this model, the diagnostic performance of positivity to penicilloyl V was higher compared with penicilloyl G, ampicilloyl and amoxicilloyl (relative DOR: 2.7, 2.5 and 8.2, respectively), although only the last comparison yielded statistical significance (P -values: 0.285, 0.407 and 0.028, respectively). This scenario is still further complicated by the lack of a correlation between the culprit drug and the hapten positivity displayed. In fact, although in our experience amoxicillin was the most frequent culprit drug, only one patient displayed a single positivity to amoxicilloyl hapten in the *in vitro* assay. In our opinion a wider panel of patients is needed to obtain definitive data to determine how each antigenic determinant is relevant in this *in vitro* assay.

On the whole, the reduction of the positivity cut-off has not significantly improved the poor overall diagnostic performance of a β -lactam-specific IgE assay. However, because the performance characteristics significantly improve when the total IgE serum levels are below 200 kU/L, we suggest that both assays are performed in the *in vitro* diagnostic approach of β -lactam allergy.

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