Original Article

Antigen specific quantification of sulfidoleukotrienes in patients allergic to Betalactam antibiotics

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Abstract. *Background:* After *in vitro* allergen-specific stimulation, basophils become activated and release sulfidoleukotrienes LTC4, LTD4 and LTE4. This can be detected by means of the CAST assay. We assessed the positivity criteria and the reliability of antigen-specific sulfidoleukotriene production (CAST) in the *in vitro* diagnosis of betalactam (BL) allergic patients.
Material and Methods: We studied a sample of 67 patients (age 48.94± 15.76 years) who had presented with anaphylaxis or urticaria-angioedema within the first 60 minutes after administration of Amoxicillin (54/67), Penicillin G (7/67), Cefuroxime (5/67) or Cefazoline (1/67). All of them had a positive skin test to at least one of the antigenic determinants of Penicillin. As control group 30 adults with negative skin tests who tolerated BL were included. All of them underwent skin tests, oral provocation tests, specific IgE (CAP-FEIA, Pharmacia) and CAST.
Results: Positivity criteria were established by means of ROC curves: a sLT release induced by Betalactams of at least 100 pg/ml and greater than or equal to 3 times the basal value. The overall sensitivity of CAST is 47.7% and specificity 83.3%. Sensitivity of specific IgE is 37.8% and specificity 83.3%.
Conclusions: We have established validated positivity criteria for the CAST technique in patients allergic to Betalactams. This technique is a useful *in vitro* diagnostic method in patients with IgE-mediated allergy to Betalactam antibiotics.

Key words: Betalactam, Allergy, Flow-cytometric *in vitro* diagnosis, CAST

Introduction

Immediate hypersensitivity reactions to betalactam antibiotics are the most frequent adverse reactions to drugs mediated by an immunological mechanism [1], with a prevalence that varies markedly (1-10%) [2].

Immediate allergic reactions to drugs are caused by an IgE mediated mechanism that recognises the drug, its metabolites or some of its galenic preparation components [3]. When drug allergens bind to specific IgE present on the mast cells' and basophils' surface, these cells are activated, produce a quick release of histamine and C4 leukotrienes [4], as well as other vasoactive inflammatory mediators [5,6].

The diagnostic methods to study subjects with immediate allergic reaction include clinical history and *in vivo* and *in vitro* diagnostic tests [1, 7]. Skin tests with Betalactam antibiotics and their derivatives have been, since the early seventies, the main diagnostic tool for the assessment of patients with IgE-mediated allergic reactions [3,5,8-13]. Nevertheless, it must be pointed out that systemic reactions induced by skin tests,

although rare, represent a sizeable risk (0.5% to 17%), especially in patients with a history of anaphylaxis [11,14-21]. Also, the sensitivity of skin tests decreases markedly with time [10,22-27], a reason why the number of subjects who require challenge tests tends to increase [11,21].

In vitro tests are a useful alternative for the evaluation of subjects with immediate reactions to Betalactams, since we can find cases with positive *in vitro* tests and negative skin tests [28,29]. These cases represent 10-13% of the subjects diagnosed as allergic [21,30]. The fact that there are very few safe, reliable, validated and correctly standardised methods as diagnostic tools for this kind of reactions, motivates the search for other useful techniques. The ideal method for the diagnosis of drug allergy would be, in our opinion, an *in vitro* technique with high sensitivity and specificity, which could substitute the *in vivo* tests and entail no risk for the patient's health.

In this study we analyse the diagnostic reliability of the antigen specific sulfidoleukotriene (sLT) determination (CAST assay) compared to the antigen-specific IgE determination (CAP assay) in patients allergic to Betalactams.

Material and Methods

A sample of 67 adult patients, 28 males and 39 females (mean age 48.94 ± 15.76 years) from the University Clinic of Navarra and the Basurto Hospital of Bilbao was selected. All the patients had presented with an immediate allergic reaction after administration of Betalactam antibiotics. Ten patients were atopic. Forty-seven had had anaphylactic reactions and twenty urticaria within one hour after administration of the drug. As controls we included 30 adult patients presumedly non allergic to Betalactams, eleven males and nineteen females, with a mean age of 44.66 ± 14.97 years. Eight of them were atopic as judged from history and positive skin tests to ubiquitous allergens. Thirty percent (nine) of the subjects from this group disclosed a history of immediate allergic reactions to Betalactams (urticaria, angioedema) but allergy to Betalactam was considered unlikely. All of them had negative skin tests to Betalactams and their clinical tolerance was checked by oral provocation and reprovocation one month later with the Betalactam involved (penicillin G 1000 IU subcutaneous administration; penicillin G 10000 IU intramuscular administration; penicillin G 100000 IU intramuscular administration; amoxicillin 100 mg, oral administration; amoxicillin 250 mg oral administration; amoxicillin 500 mg oral administration). Allergy to other drugs was ruled out in all controls. The inclusion criteria were: history of anaphylaxis and/or urticaria/angioedema immediately (less than one hour) after Betalactam administration and positive skin test to at least one of Betalactams tested (BPO-PPL, MDM, penicillinG, ampicillin, amoxicillin). In 54 cases, the reaction was induced by amoxicillin or amoxicillin-clavulanic acid, in 7 cases by penicillin G, in 5 cases by cefuroxime and in one case by cefazoline (n=67).

We considered the subjects to have suffered anaphylaxis if they had presented two or more or the following symptoms: palmoplantar and/or systemic pruritus, generalised erythema and/or angioedema on one or several areas of the body, vomiting, diarrhea and/or stomach ache, dysphonia, difficulty in breathing, speaking or swallowing, marked cardiovascular symptoms (hypotension) and/or loss of consciousness.

All patients underwent skin tests and antigen specific sLT and IgE determinations. In order to avoid bias, the three diagnostic tests were performed by different persons, all of them ignoring the results of the other tests.

All subjects included in the study were informed previously and signed the corresponding written consent. The study was approved by the Ethics Committee from both hospitals.

In vivo skin tests

Skin tests were performed by the prick technique followed by intradermal tests if necessary.

The extracts used were benzylpenicilloil-polylysine (BPO-PLL) (5×10^{-5} mol/L, Allergopharma, Hamburg, Germany), minor determinants mixture (MDM) (2×10^{-2} mol/L, Allergopharma, Hamburg, Germany), sodium penicillin G (BPN)(1000 UI/mL; Level Laboratories, Barcelona, Spain), ampicillin (AMP) (20 mg/mL, Antibiotics SA, León, Spain), and amoxicillin (AX) (20 mg/mL, Beecham, Toledo, Spain). As negative control, a saline solution was used (NaCl at 0.9%) and as positive control, histamine dihydrochloride at the concentration of 10 mg/mL in the prick technique, and of 1 mg/mL in the intradermal technique.

In the prick technique, papules greater than 3 mm compared with the negative control were considered positive, in the intradermal technique an increase of 5-10 mm over the negative control.

Antigen-specific sulfidoleukotriene (sLT) determination

The assay measures the amount of sulfidoleukotrienes (LTC_4, LTD_4 y LTE_4) produced by blood basophils after *in vitro* stimulation by allergen. Six mL of peripheral venous blood are collected in ACD tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) which are centrifuged for 10 minutes at 4ºC, at 550 g. Cells from the buffy coat layer are collected and centrifuged again at 4ºC for ten minutes at 550 g. The supernatant is then

decanted and the cells resuspended and mixed by gentle shaking with 500 ml of HEPES calcium buffer denominated stimulation buffer (HEPES 20 mM, NaCl 130 mM, KCl 5 mM, $CaCl_2$ 7 mM, $MgCl_2$ 3,5 mM, HSA 1 mg/mL, pH 7.4), containing IL 3 (2 ng/ml) and 10 ml of heparin (5000 UI/ml, ROVI, Madrid, Spain).

In a polystyrene microtitre plate (Greiner Microlon, Zaragoza, Spain) we first add to the corresponding wells 50 ml of Betalactam preparations for intravenous clinical use at the final concentrations of BPN (2 and 0.5 mg/ml), AMP 1.2 and 0.3 mg/ml), AX (1.2 and 0.3 mg/ml), MDM (0.5 and 0.25 mg/ml) and PPL (25 and 12.5 micrograms/ml), as well as cefuroxime (1.2 and 0.3 mg/ml) and cefazoline (1.2 and 0.3 mg/ml) for the patients who had reported a reaction to these drugs.

These drug concentrations were considered optimal in the light of previous studies. The drug solutions were freshly prepared daily. As positive control we used a monoclonal anti-IgE antireceptor antibody (Bühlmann, Allschwil, Switzerland) at a final concentration of 1 µl/ml. In order to evaluate basal values without stimulation, we added 50 ml of stimulation buffer alone in another well.

Fifty µl of the patient's cell suspension were then placed in each well; the plate was gently shaken and covered with an adhesive plastic sheet. The plates were incubated at 37ºC for 40 minutes. The reaction was then stopped with 100 µl of HEPES buffer, pH 7.3 without calcium and magnesium but containing EDTA (HEPES 20 mM, NaCl 133 mM, KCl 5 mM, EDTA 0.27 mM), denominated washing buffer. Soon afterwards, the plates were centrifuged for 5 minutes at 4ºC at 1000 *g*.

Finally, 100 µl of the supernatant was collected and frozen at -20ºC for less than a month until analysed by ELISA (Enzyme-Linked ImmunoSorbent Assay), following the manufacturers' instructions (CAST-ELISA, Bühlmann Laboratories, Allschwil, Switzerland) [31].

Antigen specific IgE determination

In vitro antigen specific IgE determinations by enzyme immune analysis CAP-FEIA (Pharmacia, Uppsala, Sweden), were performed in all cases for penicillin G, penicillin V, ampicillin and amoxicillin. For those patients who had a history of reaction to cephalosporins we also tested Cefaclor. Specific IgE values equal to or greater than 0.35 kU/l were considered positive.

Statistical analysis

The data were analysed through the statistical program SPSS 10.0.

Comparison between qualitative variables was done by χ^2 test with Yates correlation when necessary. For all statistical analyses $p<0.05$ was considered significant.

In order to establish the positivity criteria of antigen specific sLT determination, we applied ROC curves and chose as cut-off point the value with the highest specificity, since allergy to betalactam antibiotics is a low prevalence pathology [32].

In order to determine the inner validity of our series of patients, sensitivity and specificity were assessed for both *in vitro* techniques. Positive and negative predictive values were calculated assuming a 1% prevalence in the population tested, as well as the likelihood ratios for antigen specific sLT and IgE determinations.

Results

In Tables 1 to 3, we show the patients'clinical characteristics and the results of the diagnostic tests according to the drug culprit of the allergic reaction, amoxicillin, penicillins or cephalosporins.

sLT determinations against Betalactam antibiotics were considered positive, according to ROC curves, when sLT release was greater than 100 pg/mL, and a stimulation index in respect of the basal value equal to or greater than 3. Using these two positivity criteria enables us on the one hand to discount small unspecific releases of sLT, and on the other hand, to require a sufficient stimulus compared with basal release to affirm specific stimulation. Besides, by requiring two positivity criteria, the specificity of the test increases. We considered a test positive if one or both concentrations of the tested antigens fulfilled the positivity criteria. Analyzed in this way and using the five Betalactam allergens given, the overall sensitivity of the technique was 47.7% (32/67) and the specificity 83.3% (25/30). The positive predictive value, considering the prevalence of the disease (1%) [32] was 8.14%, and the negative predictive value was 98.09%. The positive likelihood ratio for the test was 2.8 and the negative one 0.6.

Amoxicillin is the antigen that offers the greatest sensitivity (24.6%) in respect of the other BL reagents tested (penicillin 18.96%, ampicillin 20%, PPL 16.05%, MDM 15.79%) although the differences are not statistically significant.

Concerning antigen specific IgE, we observed an overall sensitivity of 37.8% and a specificity of 83.3%. The positive predictive value for antigen specific IgE is 6.38%, and the negative predictive value 97.7%. The positive likelihood ratio is 2.2 and the negative one 0.75. When comparing the sensitivity and specificity of antigen specific IgE for the various penicillin antigens, penicillin G is the antigen with the highest specificity (96.6%), whereas Penicillin V shows the highest sensitivity (26.4%).

In patients and controls, we found no statistically significant differences in sensitivity or specificity for any of both *in vitro* techniques between atopic and non atopic subjects, or between males and females. In the

Table 1. Parameters studied in patients with reaction to Amoxicillin.

Patient Sex	**Symptoms**	**IgE** kU/l	**Specific IgE P,PV,A,Ax Class**	**sLT** pg/ml	**Skin tests**
1-male	U	53	0 – 0 – 0 - 0	Negative	MDM (ID)
2-female	An	279*	0 – 0 – 0 - 0	P (216.3)	P (ID)
3-female	An	61	3 – 3 – 3 - 3	Negative	Ax (ST)
4-male	An	245	1 – 2 – 1 - 2	Negative	P, Ax (ID)
5-female	An	353	0 – 0 – 0 - 0	Negative	P, Ax (ID)
6-male	An	83	1 – 1 – 2 - 0	Negative	A, Ax (ST)
7-female	An	102	0 – 0 – 0 - 0	P (100.1)	Ax (ID)
8-female	U	421*	0 – 0 – 0 - 0	Negative	MDM (ID)
9-female	U	59	2 – 3 – 2 - 2	Negative	MDM (ST)
10-male	An	ND	ND	A (763); Ax(995)	A, Ax (ST)
11-male	U	12	0 – 0 – 0 - 0	A (298.3)	A, Ax (ID)
12-male	An	522	4 – 5 – 3 - 5	P (175.3); PPL (189)	A, Ax (ID)
13-male	U	145	0 – 0 – 0 - 0	MDM (9327)	P, A, Ax, MDM (ID)
14-female	An	54	0 – 0 – 0 - 0	Negative	A, Ax (ID)
15-female	An	40	0 – 0 – 0 - 0	Negative	Ax (ST)
16-female	An	185	0 – 0 – 0 - 0	Negative	MDM (ST)
17-male	An	126	0 – 0 – 0 - 0	A (189); Ax (141.1)	Ax (ST)
18-female	An	6	0 – 0 – 0 - 0	Negative	Ax (ID)
19-female	An	35	0 – 0 – 0 - 0	Negative	Ax (ID)
20-female	An	44	0 – 0 – 0 - 0	P (107); Ax (107)	A, Ax (ID)
21-female	An	31	1 – 1 – 1 - 1	Ax (346)	Ax (ID)
22-female	An	377	1 – 0 – 1 - 0	Negative	Ax (ID)
23-female	An	1099	5 – 5 – 5 - 5	PPL (1412.4)	A, PPL, MDM (ST)
24-female	An	666*	1 – 0 – 0 - 0	Negative	A, Ax, MDM (ID)
25-female	An	17	0 – 0 – 0 - 0	A (161.6); PPL (195.8)	Ax (ID)
26-female	An	54	0 – 0 – 0 - 0	Negative	Ax (ST)
27-female	An	98*	0 – 0 – 0 - 1	Negative	Ax (ID)
28-female	An	37	3 – 2 – 3 - 3	P (134.3); MDM (236.8)	P, A, Ax, MDM (ST)
29-female	An	33	0 – 0 – 0 - 1	Negative	Ax (ID)
30-female	An	73	2 – 2 – 2 - 2	P (530);PPL (1220)	Ax (ST)
31-male	An	42	4 – 3 – 2 - 2	P(1330);A(743);Ax(605); PPL(879); MDM (695)	A, Ax (ID)
32-male	U	140	3 – 3 – 3 - 2	Negative	Ax (ID)

33-female	An	26*	0 – 0 – 0 - 0	P(216);A(1819);Ax(1654); PPL(784);MDM(907)	P, A, Ax (ID)
34-male	U	530	0 – 0 – 0 - 0	Negative	P, A, Ax, MDM(ID)
35-female	U	129	0 – 0 – 0 - 0	A (186);Ax (126); MDM (104)	Ax (ST)
36-male	An	59	0 – 0 – 0 - 0	Negative	Ax (ST)
37-female	An	304	0 – 0 – 0 - 0	Negative	A, Ax (ID)
38-male	U	183	0 – 0 – 0 - 0	P (388);PPL (882)	Ax, PPL (ST)
39-female	U	456*	0 – 0 – 0 - 0	MDM (1873)	A, Ax, MDM (ID)
40-male	An	142	0 – 0 – 0 - 0	Ax (111)	A, Ax (ST)
41-female	An	377	0 – 0 – 0 - 0	Negative	Ax (ID)
42-female	U	142	0 – 0 – 0 - 0	Negative	P (ID)
43-male	U	803	0 – 0 – 0 - 0	Ax (23,5); PPL (20); MDM (13.8)	Ax (ST)
44-male	An	191	0 – 0 – 2 - 2	Negative	Ax (ST)
45-male	U	276	0 – 0 – 0 - 0	Negative	Ax (ST)
46-male	U	1048	0 – 1 – 0 - 0	Negative	A, Ax (ST)
47-female	An	57	0 – 0 – 0 - 0	Ax (3434)	Ax (ST)
48-male	U	38	0 – 0 – 0 - 0	Negative	A, Ax (ST)
49-male	An	60	0 – 0 – 0 - 0	Negative	Ax (ST)
50-male	U	7	0 – 0 – 0 - 0	A (407);Ax (1810)	Ax (ID)
51-female	An	907*	0 – 0 – 0 - 0	Negative	Ax (ST)
52-female	U	34	0 – 0 – 0 - 0	A (866);Ax (1049)	Ax (ST)
53-female	An	160	2 – 2 – 1 - 1	Ax (154)	Ax, PPL (ID)
54-male	An	739*	0 – 0 – 0 - 0	P (193); A (177); Ax (1062); MDM (168)	Ax (ST)

An: Anaphylaxis
U: Urticaria
sLT: sulfidoleukotrienes
ST: skin prick test
ID: Intradermal test
ND: Not done
* Atopy

P: Penicillin G
PV: Penicillin V
A: Ampicillin
Ax: Amoxicillin
PPL: Penicilloyl-polylysine
MDM: Minor Penicillin Determinants Mixture

Table 2. Parameters studied in patients with reaction to Penicillin.

Patient Sex	**Symptoms**	**IgE** kU/l	**Specific IgE P, PV, A, Ax** Class	**sLT** pg/ml	**Skin Tests**
55-male	An	82	0 – 0 – 0 - 0	Negative	PPL (ID)
56-male	An	164	0 – 0 – 0 - 0	P (168.4) MDM (346.2)	MDM (ID)
57-female	An	20	1 – 1– 0 - 0	Negative	PPL (ID)
58-male	An	211	2 – 2 – 1 - 0	**A (240)** PPL (4380)	MDM (ID)
59-female	An	ND	0 – 0 – 0 - 0	**A (160)**	Ax (ST)
60-male	An	90	2 – 0 – 0 - 0	A (3155.3)	PPL (ST)
61-female	An	2380*	2 – 1 – 1 - 0	Negative	Ax, PPL, MDM (ID)

Table 3. Parameters studied in patients with reaction to cephalosporins.

Patient Sex	**Symptoms**	**IgE** kU/l	**Specific IgE P,PV,A,Ax** Class	**sLT** pg/ml	**Skin tests**
62-male	An	417	0 – 0 – 0 - 0	Negative	P (ID)
63-female	An	554*	2 – 2 – 1 - 0	Negative	Cefu (ST)
64-female	U	304	1 – 1– 0 - 0	Cefu (510)	PPL (ID)
65-male	An	287	1 – 0 – 0 - 0	P(168); PPL(114); MDM (107); Cefu (189)	Cefu (ID)
66-female	U	301	0 – 0 – 0 - 0	Cefu (265)	MDM (ID)
67-female	U	ND	0 – 0 – 0 - 0	Negative	Cefu (ID)

An: Anaphylaxis
U: Urticaria
sLT: sulfidoleukotrienes
ST: skin prick test
ID: Intradermal test
ND: Not done
* Atopy

Cefu: Cefuroxime
P: Penicillin G
PV: Penicillin V
A: Ampicillin
Ax: Amoxicillin
PPL: Penicilloyl-polylysine
MDM: Minor Penicillin Determinants Mixture

group of patients neither did we find statistically significant differences regarding either the drug involved in the reaction or the symptoms presented.

The median time elapsed between the clinical reaction after betalactam administration and performance of the allergological tests was two months (interquartile range 1-11.3 months). There were no statistically significant differences in the results obtained with a time lapse between the clinical reaction and the allergologic study of more or less six months and of more or less twelve months.

In the six patients allergic to cephalosporins, antigen specific sLT determination to this drug detected half of the patients while none of them could be detected with the antigen specific IgE test to Cefaclor.

In the whole group of patients allergic to Betalactams, the combination of both techniques detected 68.7% (46 patients). Antigen specific sLT determination detected 21 patients (31.3%), antigen specific IgE 14 patients (20.9%), and both techniques 11 patients (16.4%). Both techniques were negative in 21 patients (31.3%).

Among the controls, 21 were negative in both techniques (70%). Each of the techniques detected five considered false positives (16.6%), with one of them false positive in both techniques.

Discussion

In several publications, the antigen specific sLT determination (CAST) has been suggested as a useful *in vitro* diagnostic technique in allergic diseases [31, 33-35]. Although some groups have reported some positive results with sLT with Betalactams and other drugs [34,36,37], there are apparently no previous studies encompassing such a large number of cases, establishing the optimal cut off points or values by ROC curves, and determining the sensitivity and specificity of the technique. The validation of a technique requires a careful selection of patients and of the control group. In respect of the control group, we included here a heterogeneous population of subjects in order not to obtain an artificially increased specificity by selecting only healthy controls, i.e. patients with no previous history of reaction to betalactam antibiotics compatible with an IgE-mediated reaction. In none of the CAST studies on Betalactam allergic patients published hitherto had the controls presented a history of reactions suggestive of immediate-type allergic reactions to Betalactams. This would mean that the specificity values could be increased in those studies because the population included in the control group is not heterogeneous.

In the present study, we have established by ROC curves positivity criteria as sLT release to Betalactams by greater than 100 pg/mL with respect to basal values and a stimulation index equal to or greater than 3. Requiring these two conditions for positivity, the specificity of the technique reaches values enabling to consider this test as a useful complement in the daily practice. This technique, using five Betalactam reagents, offers a sensitivity of 47.7% and a specificity of 83.3%. We found no significant differences between the two concentrations of Betalactam antigens tested. Nevertheless we consider useful to use both antigenic concentrations since it provides a higher sensitivity while maintaining high specificity values.

If we compare the sensitivity of the antigen specific sLT determination obtained with various Betalactam antigens, amoxicillin yields the highest sensitivity. This could be due to the fact that amoxicillin is the drug most frequently involved in the clinical allergic reactions included in this study (80.6%), as in other publications [11,17,38]. Amoxicillin is also the Betalactam antigen showing the highest percentage of positive skin tests (73.1%). On the other hand, the antigen specific IgE determination (CAP) seems to have a lower sensitivity in patients with amoxicillin-induced allergic reactions [11,30,39,40], as also shown in this study.

In this study, 26.6% of the patients were atopic but we found no statistically significant differences between the atopic and non atopic groups indicating that the atopic condition does not influence drug-induced sLT production.

The results of antigen specific IgE determinations are difficult to interpret in patients with total IgE values over 1000 kU/l, because all of them show antigen specific IgE positive to some allergen [41]. In our study, there are three such cases but two of them were only specif IgE positive to some Betalactam. In other instances, this phenomenon could increase the number of false positives, e.g. in patients with negative skin tests but positive Betalactam-specific IgE and elevated levels of total IgE.

We did not observe a statistically significant influence of the time elapsed between the clinical allergic reaction and the performance of the allergologic study; considering the results obtained before or after six months or before or after twelve months since the clinical allergic reaction. We found this interesting, as the time elapsed since the clinical reaction, up to twelve months, does not seem to reduce the usefulness of the CAST test, in contrast to what has been reported for Betalactam-specific IgE determination.

The only *in vitro* technique widely used nowadays in the diagnosis of immediate allergy to betalactam antibiotics is indeed the detection of antigen specific IgE antibodies (CAP). In our opinion, antigen specific IgE determinations present some diagnostic limitations in drug allergy, since these IgE levels do not correlate with cellular responses against the same allergenic stimulus. Besides, antigen specific IgE determinations can only be performed with commercially available antigens whereas cellular techniques, such as CAST, can

be performed with any drug antigens, as long as they are soluble in media suitable for cells. The sensitivity of antigen specific IgE determination (CAP) to Betalactam antibiotics is not very high [18,21], ranging from 86% in some selected groups [30,42] to 20-30% in other studies [43,44], with a specificity around 83-97% [30,42]. In our study, we found lower sensitivity values for antigen specific IgE to betalactams than in the other technique studied, namely CAST: 37.8% against 47.7%, the specificity being similar in both techniques (83.3%).

In drug allergy studies using antigen specific determination of sLT, other authors found specificity values ranging from 79 to 100%, with sensitivity values ranging from 21 to 43%. The only study performed with betalactams, by Lebel et al [34], found relatively high specificity values (79%).

Antigen specific determination of sLT (CAST) shows sensitivity and specificity values similar if not higher than antigen specific IgE determination (CAP). It can therefore be considered a useful additional technique that can give information on the diagnosis of allergy to betalactams, since a number of patients negative with CAP are nevertheless positive with CAST. This is particularly the case in patients allergic to Amoxicillin, which is nowadays the most frequently used antibiotic. A large number of patients seem to react exclusively to the lateral chain of Amoxicillin [7,12,15,19,39,45,46]. The combination of antigen specific sLT determination and antigen specific IgE determination enables to detect 68.6% of the patients, but reduces specificity to 70%.

Further work on new *in vitro* diagnostic techniques in drug allergy, with the aim to achieve high specificity but better sensitivity is still warranted.

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References

1. De Weck AL. Penicillins and Cephalosporins. In: De Weck AL, Bondgaard H, editors. Allergic reactions to drugs. Berlin: Springer-Verlag, 1983: 423-64.
2. Patterson R, De Swarte R, Greenberger P, Grammer L et al. Drug allergy and protocols for management of drug allergies. Allergy Proc 1994 Sept-Oct; 15: 239-64.
3. Weiss ME, Adkinson NF. Immediate hypersensitivity reactions to penicillin and related antibiotics. Clin Allergy 1988 Nov;18(6):515-40.
4. Schroeder JT, Kagey-Sobotka A, Lichtenstein LM. The role of the basophil in allergic inflammation. Allergy. 1995 Jun;50(6):463-72.
5. Blanca M, Mayorga C, Torres MJ, Warrington R, Romano A, Demoly P, Silviu-Dan F, Moya M, Fernandez J, Juarez C. Side-chain-specific reactions to betalactams: 14 years later. Clin Exp Allergy. 2002 Feb;32(2):192-7.
6. Moreno F, Blanca M, Fernandez J, Ferrer A, Mayorga C, del Cano A, Aguilar F, Juarez C, Garcia J. Determination of inflammatory markers in allergic reactions to drugs. Allergy Proc 1995 May-Jun;16(3):119-22.
7. Blanca M, Perez E, Garcia J, Miranda A, Fernandez J, Vega JM, Terrados S, Avila M, Martin A, Suau R. Anaphylaxis to amoxycillin but good tolerance for benzyl penicillin. In vivo and in vitro studies of specific IgE antibodies. Allergy 1988 Oct;43(7):508-10.
8. Boger WP, Sherman WB, Schiller IW. Allergic reactions to penicillin. A panel discussion. J Allergy 24: 283 (1953).
9. Tuft L, Gregory DC, Gregory I. Evaluation of skin testing methods employed in the diagnosis of penicillin allergy. Am J Med Sci 230: 370 (1955).
10. Green GR, Rosenblum AH, Sweet LC. Evaluation of penicillin hypersensitivity: value of clinical history and skin testing with penicilloyl-polylysine and penicillin G. A comparative prospective study of the penicillyn study group of the American Academy of Allergy. J Allergy Clin Immunol 1977 Dec;60(6):339-45.
11. Blanca M, Vega JM, Garcia J, Carmona MJ, Terados S, Avila MJ, Miranda A, Juarez C. Allergy to penicillin with good tolerance to other penicillins; study of the incidence in subjects allergic to beta-lactams. Clin Exp Allergy 1990 Sep;20(5):475-81.
12. Silviu-Dan F, McPhillips S, Warrington RJ. The frequency of skin test reactions to side-chain penicillin determinants. J Allergy Clin Immunol 1993 Mar;91(3):694-701.
13. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. Allergy. 2002 Jan;57(1):45-51.
14. Coleman WP, Swineford O Jr. Penicillin hypersensitivity: a brief review and report of an extreme case. Virginia Med Monthly 83: 6, 1956.
15. Van Dellen RG. Skin testing for penicillin allergy. J Allergy Clin Immunol 1981 Sep;68(3):169-70.
16. Sullivan TJ. Penicillin Allergy. In: Lichtenstein LM, Fauci A, eds. Current Therapy in Allergy. St Louis: CV Mosby, 1985: 57-61.
17. Vega JM, Blanca M, Garcia JJ, Carmona MJ, Miranda A, Perez-Estrada M, Fernandez S, Acebes JM, Terrados S. Immediate allergic reactions to amoxicillin. Allergy 1994 May;49(5):317-22.
18. Blanca M, Garcia J, Vega JM, Miranda A, Carmona MJ, Mayorga C, Moreno F, Juarez C. Anaphylaxis to penicillins after non-therapeutic exposure: an immunological investigation. Clin Exp Allergy. 1996 Mar;26(3):335-40.
19. Romano A, Blanca M, Mayorga C, Venuti A, Gasbarrini G. Immediate hypersensitivity to penicillins. Studies on Italian subjects. Allergy 1997 Jan;52(1):89-93.
20. Torres MJ, Mayorga C, Pamies R, Rodriquez JL, Juarez C, Romano A, Blanca M. Immunologic response to different determinants of benzylpenicillin, amoxicillin, and ampicillin. Comparison between urticaria and anaphylactic shock. Allergy 1999 Sep;54(9):936-43.
21. Torres MJ, Romano A, Mayorga C, Moya MC, Guzman AE, Reche M, Juarez C, Blanca M. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. Allergy 2001 Sep;56(9):850-6.
22. Kraft D, Wide L. Clinical patterns and results of radioallergosorbent test (RAST) and skin tests in penicillin allergy. Br J Dermatol 1976 Jun;94(6):593-601.
23. Chandra RK, Joglekar SA, Tomas E. Penicillin allergy: anti-penicillin IgE antibodies and immediate hypersensitivity skin reactions employing major and minor determinants of penicillin. Arch Dis Child. 1980 Nov;55(11):857-60.

24. Sullivan TJ, Wedner HJ, Shatz GS, Yecies LD, Parker CW. Skin testing to detect penicillin allergy. J Allergy Clin Immunol 1981 Sep;68(3):171-80.
25. Gadde J, Spence M, Wheeler B, Adkinson NF Jr. Clinical experience with penicillin skin testing in a large inner-city STD clinic. JAMA 1993 Nov 24;270(20):2456-63.
26. Patriarca G, Schiavino D, Nucera E, Milani A. Positive allergological tests may turn negative with no further exposure to the specific allergen: a long-term, prospective, follow-up study in patients allergic to penicillin. J Investig Allergol Clin Immunol. 1996 May-Jun;6(3):162-5.
27. Blanca M, Torres MJ, Garcia JJ, Romano A, Mayorga C, de Ramon E, Vega JM, Miranda A, Juarez C. Natural evolution of skin test sensitivity in patients allergic to beta-lactam . J Allergy Clin Immunol 1999 May;103(5 Pt 1):918-24.
28. Jarisch R, Roth A, Boltz A, Sandor I. Diagnosis of penicillin allergy by means of Phadebas RAST penicilloyl G and V and skin tests. Clin Allergy 1981 Mar;11(2):155-60.
29. Torres MJ, Mayorga C, Cornejo-Garcia JA, Romano A, Blanca M. IgE antibodies to penicillin in skin test negative patients. Allergy. 2002 Oct;57(10):965.
30. Blanca M, Mayorga C, Torres MJ, Reche M, Moya MC, Rodriguez JL, Romano A, Juarez C. Clinical evaluation of Pharmacia CAP System RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. Allergy 2001 Sep;56(9):862-70.
31. De Weck AL. ACI News, 1993; 5: 9-15.
32. Dreborg S, Backman A, Basomba A. Skin tests used in type I allergy testing. Allergy 1989; 44 (suppl. 10): 1-59.
33. Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. Ann Allergy Asthma Immunol 1999 Jan;82(1):33-40.
34. Lebel B, Messaad D, Kvedariene V, Rongier M, Bousquet J, Demoly P. Cysteinyl-leukotriene release test (CAST) in the diagnosis of immediate drug reactions. Allergy 2001 Jul;56(7):688-92.
35. Furukawa K, Tengler R, de Weck AL, Maly FE. Simplified sulfidoleukotriene ELISA using LTD4-conjugated phosphatase for the study of allergen-induced leukotriene generation by isolated mononuclear cells and diluted whole blood. J Investig Allergol Clin Immunol. 1994 May-Jun;4(3):110-5.
36. De Weck AL, Sanz ML. Flow Cytometric Cellular Allergen Stimulation Test (FAST/FLOW-CAST). Technical and Clinical Evaluation of a New Diagnostic Test in Allergy and Pseudo-Allergy. ACI International 2002; 14:204-215.
37. May A., Weber A., Gall H., Kaufmann R., Zollner T.M. Means of increasing sensitivity of an in vitro diagnostic test for aspirin intolerance. Clin Exp Allergy, 1999; 29: 1402-1411).
38. Audicana M, Bernaola G, Urrutia I, Echechipia S, Gastaminza G, Munoz D, Fernandez E, Fernandez de Corres L. Allergic reactions to betalactams: studies in a group of patients allergic to penicillin and evaluation of cross-reactivity with cephalosporin. Allergy 1994 Feb;49(2):108-13.
39. Mata E, Guéant JL, Moneret-Vautrin DA. Clinical evaluation of in vitro leukocyte histamine release in allergy to muscle relaxant drugs. Allergy 1992; 47: 471-476.
40. Sastre J, Quijano LD, Novalbos A, Hernandez G, Cuesta J, de las Heras M, Lluch M, Fernandez M. Clinical cross-reactivity between amoxicillin and cephadroxil in patients allergic to amoxicillin and with good tolerance of penicillin. Allergy 1996 Jun;51(6):383-86.
41. Sanz ML, Prieto I, García BE, Oehling A. Diagnostic reliability considerations of specific IgE determination. J Invest Allergol Clin Immunol, May-June 1996; vol 6 (3): 152-161.
42. Garcia JJ, Blanca M, Moreno F, Vega JM, Mayorga C, Fernandez J, Juarez C, Romano A, de Ramon E. Determination of IgE antibodies to the benzylpenicilloyl determinant: a comparison of the sensitivity and specificity of three radio allergo sorbent test methods. J Clin Lab Anal 1997;11(5):251-7.
43. Romano A, Di Fonso M, Papa G, Pietrantonio F, Federico F, Fabrizi G, Venuti A. Evaluation of adverse cutaneous reactions to aminopenicillins with emphasis on those manifested by maculopapular rashes. Allergy 1995 Feb;50(2):113-8.
44. Sanz ML, Gamboa PM, Antepara I, Uasuf C, Vila L, Garcia-Aviles C, Chazot M, De Weck AL. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. Clin Exp Allergy 2002 Feb;32(2):277-86.
45. Blanca M, Vega JM, Garcia J, Miranda A, Carmona MJ, Juarez C, Terrados S, Fernandez J. New aspects of allergic reactions to betalactams: crossreactions and unique specificities. Clin Exp Allergy 1994 May;24(5):407-15.
46. Baldo BA. Penicillins and cephalosporins as allergens-structural aspects of recognition and cross-reactions. Clin Exp Allergy 1999 Jun;29(6):744-9.

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