

Original article

Clinical evaluation of Pharmacia CAP SystemTM RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy

Background: The diagnosis of IgE-mediated immediate reactions to penicillins can be supported by *in vivo* or *in vitro* tests using classical benzylpenicillin determinants. The wide variety of β -lactams and the description of new specificities requires a re-evaluation of the different tests available. The objective was to evaluate the diagnostic capacity of Pharmacia CAP SystemTM RAST FEIA amoxicilloyl c6 (AXO) and benzylpenicilloyl c1 (BPO) in patients with a documented IgE-mediated penicillin allergy.

Methods: We studied 129 patients in five groups. Groups 1, 2, and 3 had developed an immediate reaction after penicillin treatment. Group 1 ($n=19$) were skin test positive to amoxicillin (AX) and/or BPO and/or minor determinant mixture (MDM); group 2 ($n=29$) were skin test positive to AX but negative to BPO and MDM; and group 3 ($n=26$) were skin test negative to all determinants, the diagnosis being confirmed by a previous repetitive history or controlled administration. Two control groups, one with nonimmediate reactions – group 4 ($n=25$) – and one with good tolerance to penicillin – group 5 ($n=30$) – were included. All samples were analyzed *in vitro* for AXO and BPO, and the results compared to the *in vivo* diagnosis.

Results: AX was the drug most often involved. In group 1, 53% were *in vitro* positive for AXO and 68% for BPO, but 74% had at least one positive test result. In group 2, only 10% had a positive *in vitro* test to BPO compared to 41% to AXO. In group 3, 42% had positive BPO and/or AXO *in vitro* tests. In the control groups 4 and 5, the negative *in vitro* results for AXO were 96% and 100%, and for BPO 100% and 97%, respectively. A positive correlation between specific IgE levels and the time interval from the reaction to the evaluation was found only for group 3.

Conclusions: This *in vitro* assay is beneficial for evaluating subjects allergic to β -lactams. It is necessary to test for specific IgE to AXO in addition to BPO in patients with immediate allergic reactions after AX. The combination of *in vivo* and *in vitro* tests for estimating IgE antibodies to penicillins is important because of the existence of patients with a positive history but negative skin test.

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Allergic reactions to penicillin are the most common cause of immunologic drug reactions. Both IgE-mediated and T-cell-dependent responses have been reported after the administration of β -lactams (1), the IgE-mediated reactions being the most frequent and best characterized (2). Subjects develop IgE antibodies which recognize the structure formed by a combination of the penicillin molecule plus a protein carrier (3). In studies in both man and animals, penicilloyl has long been recognized as the major antigenic determinant (4). Soon after the introduction of benzylpenicillin (BP), a number of other β -lactams were developed, resulting in the presence of a wide number of chemical structures that are quite different from those initially used (5). This means that populations are exposed to various haptens

that can be recognized by the immunologic system (2, 3). Furthermore, the influence of these chemical structures varies over time because of the different patterns of consumption. BP, the drug that generates the major determinant benzylpenicilloyl (BPO), is now no longer the most often used β -lactam. These changes have influenced not only the specificity of antibody (3, 6) but also the pattern of allergic responses (7).

The diagnostic methods for evaluating subjects with an immediate allergic reaction include an adequate clinical history plus the performance of *in vivo* and/or *in vitro* tests (2). Soon after the description of allergic reactions to penicillin, the development of a penicillin-polylysine conjugate helped contribute to the diagnosis of these cases (9). Later, the addition of the so-called

minor determinants (MDM) improved the skin testing (2, 8, 9). However, there is limited access to MDM, since they have not been licensed in all countries and they can only be used for research purposes (8–10); therefore, the pattern of responses in different populations cannot always be determined. Recently, several groups have reported an increase in cases of anaphylaxis over urticaria, so that skin testing in these patients may involve additional risk (7, 11–13).

Another approach in evaluating allergic reactions to penicillins is *in vitro* quantitation of specific IgE antibodies to the culprit drug (14, 15). These specific IgE antibodies were detected in a population of allergic patients taking penicillin V in 1979 (16). Additional reports of selective reactions to other penicillins and cephalosporins have since been published (17–21). The most relevant and best-studied selective response and further evidence of blood IgE-antibody-positive results but negative skin tests has been observed in Spain with amoxicillin (AX) (11, 22, 23).

The objective of the study was to evaluate the diagnostic advantages of *in vitro* testing for specific IgE antibodies with the Pharmacia CAP System[™], in a group of patients allergic to penicillins according to clinical history and *in vivo* tests. Results indicate that *in vitro* testing is beneficial, especially in patients positive in clinical history but skin test negative.

Material and methods

Patient and control selection

Subjects who developed an immediate reaction after the administration of a penicillin derivative were included in the study. Two clinical entities were defined: anaphylaxis and urticaria. The criterion for considering a reaction as anaphylaxis was the presence of several of the following symptoms: systemic pruritus, generalized erythema, angioedema in one or more parts of the body, dysphonia, difficulty in breathing, abdominal cramps, and hypotension. The criterion for urticaria was whether the immediate manifestations were limited exclusively to the skin and consisted of maculopapulae at various sites of the body, whether or not associated with angioedema.

Three groups of patients were considered, based on the skin test reactivity to the penicillin determinants:

Group 1. This group comprised subjects with an immediate reaction to BP and/or AX. The skin test had to be positive to BPO and AX independently of positivity to the other determinants MDM and ampicillin (AMP). This group comprised 19 subjects, six women (32%) and 13 men (68%). The mean age was 47.5 years, range: 19–69 (Tables 1 and 2).

Group 2. This group comprised subjects who, after having an immediate reaction to an AX derivative, were skin test positive to AX determinants and negative to BPO and MDM, and had good tolerance to BP. Details of the characterization of this group are reported elsewhere (11, 12). There were 29 subjects, 17 (59%) women and 12 (41%) men. The mean age was 35.1 years, range: 21–54 (Tables 1 and 2).

Group 3. This group comprised subjects with an immediate reaction to penicillin or AX who were skin test negative to all penicillin derivatives used in the study. These included:

- 1) patients who had experienced at least two or more episodes of immediate response to a penicillin derivative
- 2) patients who developed systemic symptoms compatible with anaphylaxis after skin testing in spite of being skin test negative
- 3) patients who had a positive challenge after penicillin administration, and who were skin test negative.

A total of 26 patients, 14 women (53.8%) and 12 men (46.2%), were included in this group. The mean age was 43.8 years, range: 23–64 (Table 3).

Two control groups were included:

Group 4. This group comprised patients with a clinically documented non-IgE-mediated reaction to penicillin. Subjects who developed maculopapular or exanthemic reactions with an interval greater than 6 h and usually within 24–48 h after taking the drug were included in this group. In all these cases, confirmation of penicillin allergy was established by controlled administration. Immediate skin tests to BPO, MDM, AX, and AMP had to be negative. The group included 22 (88%) women and three (12%) men. The mean age was 40.0 years, range: 17–71.

Group 5. This group comprised 30 subjects with no history of allergic reaction to β -lactams; a negative skin test to BPO, MDM, AX, and AMP; and good tolerance to BP and AX. There were 18 (60%) women and 12 (40%) men. The mean age was 39.7 years, range: 14–65.

Skin tests

These were carried out as previously reported (11, 12), with 0.02 ml of solution. The reagents were benzylpenicilloyl polylysine (BPO) (Allergopharma Merck, Reinbek, Germany) (5×10^{-5} M), MDM (Allergopharma) (2×10^{-2} M), AX (SB SmithKline Beecham, Madrid, Spain) (20 mg/ml), and AMP (Antibiotic, León, Spain) (20 mg/ml). The concentrations shown were the maximum used. Skin tests to BPO and MDM were made on one day and to AX and AMP 1 week later. In order to determine whether systemic symptoms were induced by each particular hapten, we initiated the skin test the first day with BPO beginning with prick testing at the concentration of 5×10^{-5} M and then intradermal testing following the procedure described elsewhere (11, 12). In all cases, the concentration was a 10-fold lower dilution; if the test was negative, we proceeded to the next higher concentration. After 1 h, we proceeded to test the MDM in the same way. One week later, we proceeded to the skin testing with AX and AMP. We began with AMP at 20 mg/ml by prick, followed by intradermal administration at 2 mg/ml and then 20 mg/ml. We then performed the AX tests. The sequence was scheduled in this way because of the high incidence expected of selective responses to AX, thereby placing the most common hapten last for testing in order to complete the whole study. Responses were classified as positive or negative, as previously described (24). In the skin prick tests, a wheal larger than 2 mm with a negative response to the control saline was considered positive. In the intradermal tests, the wheal area was marked initially and 20 min after testing, and an increase in diameter greater than 3 mm was considered positive.

Sera collection

Samples were obtained from the patients at the moment they were evaluated in the clinical outpatient department before the skin test procedure, and were frozen at -20°C until use. All samples were run in parallel for the *in vitro* test.

Table 1. Clinical characteristics of subjects who responded to penicillin and AX (groups 1 and 2)

	Patient no.	Age (years)	Sex	Diagnosis	Drug involved	Interval (days)
Group 1	1	32	M	Anaphylaxis	AX	550
	2	55	M	Anaphylaxis	AX	128
	3	34	F	Urticaria	AX	73
	4	49	M	Anaphylaxis	AX	15
	5	43	F	Urticaria	AX	92
	6	37	M	Anaphylaxis	AX	80
	7	37	M	Anaphylaxis	AX	324
	8	59	M	Anaphylaxis	AX	69
	9	61	M	Anaphylaxis	PG	746
	10	52	F	Anaphylaxis	AX	716
	11	54	M	Anaphylaxis	AX	3
	12	47	F	Anaphylaxis	AX	5
	13	64	M	Anaphylaxis	AX	156
	14	50	M	Anaphylaxis	AX	52
	15	65	M	Anaphylaxis	AX	40
	16	69	M	Anaphylaxis	AX	5
	17	36	M	Urticaria	AX	102
	18	19	F	Anaphylaxis	AX	57
	19	40	F	Anaphylaxis	AX	7
Group 2	20	32	M	Anaphylaxis	AX	788
	21	54	F	Anaphylaxis	AX	11
	22	21	F	Urticaria	AX	134
	23	31	F	Urticaria	AX	22
					AX plus	
	24	36	F	Anaphylaxis	clavulanic acid	20
	25	31	F	Urticaria	AX	348
	26	21	M	Urticaria	AX	454
	27	37	F	Urticaria	AX	212
	28	29	F	Anaphylaxis	AX	58
	29	40	F	Anaphylaxis	AX	15
					AX plus	
	30	39	F	Urticaria	BP (benzathine)	99
	31	40	F	Urticaria	AX	73
	32	45	M	Urticaria	AX	533
	33	51	F	Anaphylaxis	AX	2
	34	33	F	Anaphylaxis	AX	27
	35	33	F	Anaphylaxis	AX	57
	36	30	M	Anaphylaxis	AX	399
	37	33	M	Anaphylaxis	AX	52
	38	27	F	Anaphylaxis	AX	681
	39	46	M	Anaphylaxis	AX	128
	40	32	M	Anaphylaxis	AX	189
	41	44	M	Anaphylaxis	AX	8
	42	35	F	Anaphylaxis	AX	4
	43	35	F	Urticaria	AX	25
	44	31	F	Anaphylaxis	AX	28
	45	24	M	Anaphylaxis	AX	10
	46	37	M	Anaphylaxis	AX	16
	47	30	M	Anaphylaxis	AX	98
	48	42	M	Urticaria	AX	155

Controlled administration

In order to establish the involvement of the penicillin in the induction of the reaction in skin-test-negative subjects, a controlled challenge was done in those in whom only one episode occurred. Those patients who developed systemic symptoms after skin testing were also considered positive and hence not given the drug. Basically, this was carried out as previously described (11, 12) with some modifications. In the first week, after completion of skin testing with BPO and MDM, we began with the parental administration of 1 ml of BP (10^4 IU/ml), followed by 1 ml at 10^5 IU/ml, and finishing with 1 ml of 10^6 IU/ml if good tolerance was established at

Table 2. Skin test and serum IgE antibody results in patients from groups 1 and 2

	Patient no.	Skin test				Serum IgE antibodies (kU _A /l)	
		BPO	MDM	AX	AMP	BPO	AXO
Group 1	1	+	+	+	+	<0.35	<0.35
	2	+	+	+	+	<0.35	<0.35
	3	+	—	+	+	<0.35	<0.35
	4	+	—	+	+	<0.35	<0.35
	5	+	+	+	+	<0.35	3.6
	6	+	+	+	+	<0.35	<0.35
	7	+	+	+	+	0.5	<0.35
	8	+	+	+	+	0.5	<0.35
	9	+	+	+	+	1.4	<0.35
	10	+	—	+	+	1.6	1.1
	11	+	+	+	+	2.6	3.1
	12	+	—	+	—	2.8	3.7
	13	+	+	+	+	9.1	7.8
	14	+	—	+	—	9.9	11.1
	15	+	+	+	+	16.8	<0.35
	16	+	—	+	+	17.4	3.4
	17	+	—	+	+	21.7	33.7
	18	+	+	+	+	36.4	23.2
	19	+	+	+	+	>100	>100
Group 2	20	—	—	+	+	<0.35	<0.35
	21	—	—	+	—	<0.35	<0.35
	22	—	—	+	—	<0.35	<0.35
	23	—	—	+	—	<0.35	<0.35
	24	—	—	+	+	<0.35	<0.35
	25	—	—	+	+	<0.35	<0.35
	26	—	—	+	+	<0.35	<0.35
	27	—	—	+	—	<0.35	<0.35
	28	—	—	+	—	<0.35	<0.35
	29	—	—	+	+	<0.35	<0.35
	30	—	—	+	—	<0.35	<0.35
	31	—	—	+	+	<0.35	<0.35
	32	—	—	+	+	<0.35	<0.35
	33	—	—	+	+	<0.35	<0.35
	34	—	—	+	—	<0.35	<0.35
	35	—	—	+	+	<0.35	<0.35
	36	—	—	+	+	<0.35	<0.35
	37	—	—	+	—	<0.35	0.8
	38	—	—	+	+	<0.35	0.8
	39	—	—	+	—	<0.35	0.9
	40	—	—	+	+	<0.35	1.1
	41	—	—	+	—	0.6	1.8
	42	—	—	+	—	<0.35	2.0
	43	—	—	+	+	<0.35	2.2
	44	—	—	+	+	<0.35	2.5
	45	—	—	+	—	<0.35	2.7
	46	—	—	+	+	<0.35	2.9
	47	—	—	+	—	2.1	2.9
	48	—	—	+	+	0.4	5.8

the previous doses. Similarly, if skin tests with AMP and AX the next week were negative, AX was given by the oral route at the following doses: 5, 50, 100 and 500 mg with a 1-h interval between each.

Specific IgE antibody determination: Pharmacia CAP System

The Pharmacia CAP System FEIA is an *in vitro* test system, based on ImmunoCAP technology, for determination of circulating specific IgE antibodies. The drug of interest, covalently coupled to ImmunoCAP, reacts with the specific IgE in the patient's serum specimen. After nonspecific IgE is washed away, enzyme-labeled antibodies against IgE are added to form a complex. After

Table 3. Clinical characteristics and drug involved in patients with negative skin test to penicillin determinants and results of *in vitro* assay to serum IgE antibodies to BPO and AXO in patients from group 3

Patient no.	Age (years)	Sex	Diagnosis	Drug involved	Interval (days)	Serum IgE antibodies (kU _A /l)	
						BPO	AXO
49	46	F	Anaphylaxis	AX	65	<0.35	0.5
50	47	M	Anaphylaxis	AX	143	<0.35	<0.35
51	63	F	Urticaria	AX	545	<0.35	<0.35
52	32	F	Urticaria	BP	587	<0.35	0.5
53	41	M	Urticaria	AX	95	<0.35	<0.35
54	43	M	Anaphylaxis	AX	55	<0.35	<0.35
55	50	F	Anaphylaxis	CLX	55	<0.35	<0.35
56	44	M	Urticaria	AX	92	<0.35	<0.35
57	49	M	Anaphylaxis	BP	53	<0.35	0.4
58	38	M	Anaphylaxis	BP	5	<0.35	<0.35
59	35	F	Anaphylaxis	AX	42	<0.35	<0.35
60	56	F	Anaphylaxis	AX	95	<0.35	<0.35
61	30	F	Anaphylaxis	AX	32	<0.35	<0.35
62	44	F	Urticaria	AX	67	<0.35	<0.35
63	59	F	Anaphylaxis	BP	113	<0.35	<0.35
64	25	M	Urticaria	AX	22	<0.35	<0.35
65	43	M	Urticaria	AX	62	<0.35	<0.35
66	32	F	Anaphylaxis	AX	116	<0.35	<0.35
67	64	M	Anaphylaxis	AX	29	0.5	<0.35
68	23	F	Urticaria	AMP	619	0.7	0.4
69	34	F	Urticaria	AX	206	1.1	1.1
70	38	F	Urticaria	AX	559	1.1	2.7
71	63	F	Urticaria	AMP	544	1.2	0.7
72	53	M	Anaphylaxis	AX	5618	1.4	1.8
73	38	M	Urticaria	AX	940	2.1	0.9
74	50	M	Anaphylaxis	BP	691	13.3	2.9

incubation, unbound enzyme anti-IgE is washed away, and the bound complex is then incubated with a developing agent. When the reaction is stopped, the fluorescence of the elute is measured by FluoroCount 96. For classification of test results, the fluorescence of patient samples is compared directly by fluorescence for standards run in parallel. The Pharmacia CAP System specific IgE measuring range is 0.35–100 kU_A/l, with a cutoff value of ≥ 0.35 kU_A/l for positive test results and <0.35 kU_A/l for negative test results.

Statistical studies

For each patient group, a descriptive analysis of demographic characteristics was made, and the arithmetic mean, age range, and sex distribution were calculated. The frequency of positive and negative results for Pharmacia CAP System RAST FEIA c1 (BPO) and c6 (AXO) was also calculated.

The time interval means for groups 1, 2, and 3 were compared by the Levene test. The relationship of the time interval between the occurrence of the reaction and the sera collection and the specific IgE values was studied by Spearman rank correlation. These variables were transformed into categoric variables for chi-square analysis. Time interval and blood IgE antibody values were classified into two categories

- 1) for time interval, less or greater than 60 days
- 2) for the Pharmacia CAP System RAST FEIA c1 (BPO) and c6 (AXO) as positive (≥ 0.35 kU_A/l) or negative (<0.35 kU_A/l).

The sensitivity of the CAP test was calculated by clinical history, skin tests, and/or controlled administration as the reference methods in groups 1, 2, and 3, and the specificity was calculated in the negative controls (groups 4 and 5).

Results

Clinical characteristics of subjects

This was a study based on clinical and immunologic data of patients from a population that formed part of a large cohort of allergic patients in Spain. A total of 129 patients were included in the study, of whom 74 (groups 1, 2, and 3) developed an immediate reaction after penicillin treatment. Patients in groups 1 and 2 were skin test positive to at least one penicillin determinant, as described in Material and methods. Group 3 comprised 26 patients with a positive history but negative skin tests. Patients in group 4 had developed a nonimmediate reaction after the penicillin intake, and subjects in group 5 had shown good tolerance to penicillin and no symptoms after the drug intake. The most common drug involved in the adverse reactions in all five groups was AX (87.8%), followed by BP (8.1%), AMP (2.7%), and cloxacillin (1.3%).

The demographic and clinical characteristics of the patients were as follows.

Group 1. In 18 (95%) patients, AX was the drug involved, and in one (5%) it was BP. Anaphylaxis occurred in 16 (84%) patients and urticaria in three (16%). The mean interval between the occurrence of the reaction and sera collection was 136 ± 44 days (Table 1).

Table 4. Clinical data in patients from group 3

Patient no.	Clinical characteristics
Patients who developed systemic reaction after skin testing	
52	Systemic reaction with skin test, pruritus, and facial angioedema with 2 mg i.d. of AX
58	Maculopapulae after skin test with MDM (2×10^{-2} M)
59	Systemic pruritus and dysphonia after skin testing with AX (2 mg/ml)
62	Systemic pruritus after i.d. testing with AX (20 mg/ml)
65	Systemic pruritus and eyelid angioedema after i.d. testing with AX (2 mg/ml)
70	Regional urticaria after skin testing with AX (2 mg/ml)
73	Systemic pruritus and hypotension after skin testing with BPO (5×10^{-5} M)
Patients with negative skin test and positive challenge	
53	Maculopapulae in abdomen and legs with 5 mg of AX
54	Hypotension, systemic pruritus, and generalized erythema with 5 mg of AX
55	Maculopapulae in abdomen and chest after administration of 100 mg of PV
56	Pruritus in hands, legs, and eye, and angioedema with 15 mg of AMP
57	Maculopapulae in abdomen, legs, and chest with 100 IU of BP
60	Systemic pruritus, erythema, abdominal cramps, and hypotension with 5 mg of AX
63	Systemic pruritus and facial angioedema with 1000 IU of BP
64	Systemic pruritus and facial erythema with 5 mg of AX
66	Pruritus in palms and soles plus hypotension after 5 mg of oral AX
68	Systemic pruritus, facial angioedema, and maculopapulae in abdomen with 5 mg of AX

Group 2. AX was involved in 27 cases (93%), AX plus clavulanic acid in one (3%), and AX-BP (benzathine) in one (3%). Anaphylaxis occurred in 19 (66%) patients and urticaria in 10 (34%). The mean interval between the occurrence of the reaction and sera collection for the study was 160 ± 41 days (Table 1).

Group 3. AX was the drug involved in 18 patients (69%), BP in five (19%), AMP in two (8%), and cloxacillin in one (4%). Of these patients, 14 (54%) had anaphylaxis and 12 (46%) urticaria. The mean interval between the occurrence of the reaction and sera collection in this group was 440 ± 214 days (Table 3).

Group 4. The drug most frequently involved was AX in 84% of patients, followed by BP in three (12%), and AMP in one (4%).

Group 5. This group comprised subjects who tolerated AX. The intake of β -lactams was AX (85%), BP (11%), and AMP (1%).

In vivo and in vitro studies

Patients in group 1 had positive skin test results to at least BPO and AX (Table 2). Twelve patients (63%) had a positive skin test to all penicillin determinants tested, and the remainder were positive to BPO and AX; of the latter, 17 were positive to AMP (89%) and 12 to MDM (63%). The immunoassay to BPO was positive in 13 (68%) patients while that to AXO was positive in 10 (53%) patients. The immunoassay was positive to AXO and negative to BPO in only one patient (5%), and four

patients (21%) were positive only to BPO. In nine patients (47%), the immunoassay was positive to both haptens.

The skin test results of group 2 are shown in Table 2. Skin tests were positive to AX and negative to BPO and MDM; 16 (55%) of the 29 patients were also positive to AMP. With AX, eight (27%) patients were positive by prick test, and the remainder were positive by intradermal test. Two of the 16 patients positive to AMP were positive by prick test and the remainder by intradermal test. In all cases, skin tests were negative to BPO and MDM, and the skin response to AX was stronger than to AMP. The results of specific serum IgE antibodies are also presented in Table 2, showing 12 (41%) patients positive to AXO. Three (10%) patients were also positive to BPO.

Patients in group 3 had negative skin test results. The details of controlled administration and systemic symptoms after skin testing are presented in Table 4. Nine patients developed a clinical reaction on two or more occasions and therefore were considered positive (nos. 49, 50, 51, 61, 67, 71, 72, and 74 of Table 3). Seven of the 26 patients (27%) developed systemic symptoms after skin testing. Except for patients 58 and 73, who responded at the maximum dose of determinants used, in the remaining patients the concentration that induced the reaction was lower than the maximum. Interestingly, in patient 73, the responsible hapten was the major determinant BPO.

The second part of Table 4 shows the 10 (38%) patients with negative test results to penicillin determinants who developed a positive response after controlled administration of the drug. The symptoms were variable and hypotension was present only in patients 54 and 66. In the remaining patients, the symptoms

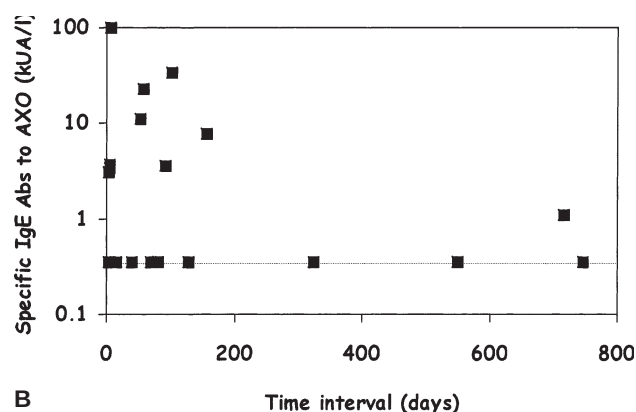
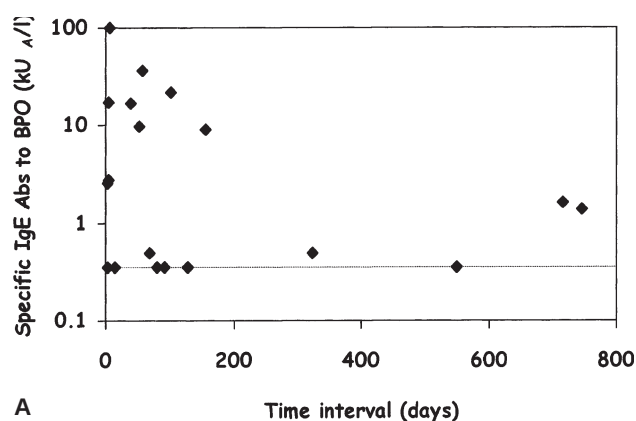


Figure 1. A) Serum specific IgE antibodies to BPO in group 1 in relation to time interval between serum collection and occurrence of reaction. B) Serum specific IgE antibodies to AXO in group 1 in relation to time interval between serum collection and occurrence of reaction.

were of minor intensity. The dose that induced the response was variable, but in no instance was the full dose required to induce the symptoms.

The results of the Pharmacia CAP System for group 3 are presented in Table 3. Positive results to BPO were found in eight patients (31%) and to AXO in 10 (38%). Combined positivity to either one or the other was exhibited by 11 (42%). With one exception, all patients that were positive to BPO were also positive to AXO.

Statistical studies

Although the time interval means in groups 1 and 2

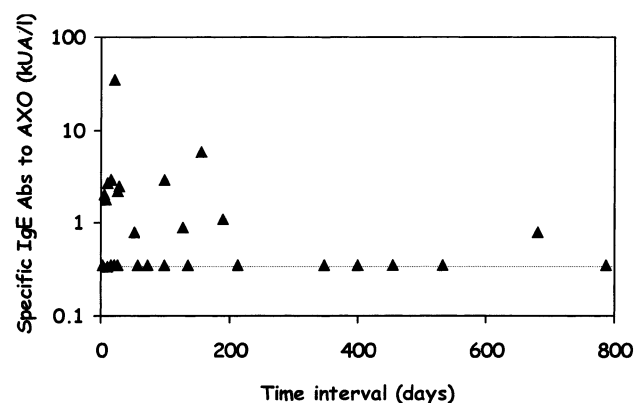


Figure 2. Serum specific IgE antibodies to AXO in group 2 in relation to time interval between serum collection and occurrence of reaction.

were shorter than those found in group 3, the Levene test showed that these differences were not statistically significant. The relationship between specific IgE measurements and the time interval between occurrence of the reaction and the sera collection was studied with Spearman's rank correlation. The correlations obtained were low and nonsignificant. In group 1, for BPO, it was $r=0.30$ ($P=0.22$) and, for AXO, $r=0.16$ ($P=0.52$); in group 2, for BPO, it was $r=0.06$ ($P=0.77$) and, for AXO, $r=0.22$ ($P=0.26$). Plots of these data are presented in Figs. 1A and 1B for group 1, and Fig. 2 (AXO only).

Moreover, when these data were transformed into categorical variables, chi-square analysis showed no significant association between a time interval of less than or greater than 60 days with a specific IgE level lower than or greater than $0.35 \text{ kU}_A/\text{l}$. In group 3, however, the study of the relationship between time interval and specific IgE levels showed significant correlations for both BPO ($r=0.3$; $P=0.047$) and AXO ($r=0.4$; $P=0.003$).

The numbers of positive and negative *in vitro* test results are presented in Tables 5 and 6. The proportion of positive results in group 1 was 68% for BPO and 53% for AXO. However, for positivity to either AXO or BPO, the test results rose to 74%. In group 2, which reacted specifically to AXO, only 10% had a positive *in vitro* test result to BPO compared to 41% with a positive *in vitro* test result to AXO. In group 3, which had a negative skin test but positive penicillin allergy diagnosis, 42% showed

Table 5. Number of positive and negative *in vitro* test results to BPO and AXO in five groups studied

Group	Serum specific IgE to BPO		Serum specific IgE to AXO	
	Positive	Negative	Positive	Negative
1	13 (68%)	6 (32%)	10 (53%)	9 (47%)
2	3 (10%)	26 (90%)	12 (41%)	17 (59%)
3	8 (31%)	18 (69%)	10 (38%)	16 (62%)
4	0 (0%)	25 (100%)	1 (4%)	24 (96%)
5	1 (3%)	29 (97%)	0 (0%)	30 (100%)

Table 6. Sensitivity in positive group (on left) and specificity in negative group (on right). Positive group comprised 1, 2, and 3 groups according to clinical history, skin tests, and/or controlled administration of drug

Hapten	Positive	False negative	Sensitivity	Total	Negative	False positive	Specificity	Total
BPO	24	50	32%	74	54	1	98%	55
AXO	32	42	43%	74	54	1	98%	55
BPO + AXO	37	37	50%	74	53	2	96%	55

positive BPO and/or AXO test results. The proportions of negative test results for AXO in the control groups 4 and 5 were 96% and 100%, respectively, compared to 100% and 97% for BPO. Table 6 shows the sensitivity and specificity of the *in vitro* test considering the contribution of a single hapten (BPO or AXO) or the combination of both.

Discussion

The use of *in vivo* and *in vitro* tests in the diagnosis of patients allergic to β -lactams needs to be adapted to the present situation in which the pattern of consumption of these antibiotics has changed, and BP is no longer the drug most frequently prescribed (6, 25), having been replaced mainly by AX (6, 22, 23, 25–27) and, to a lesser extent, by the cephalosporins (28, 29). We therefore used a well-established *in vitro* method to evaluate the presence of IgE antibodies in a defined allergic population in which AX was the drug most commonly inducing the immediate reaction. The advantage of using the Pharmacia CAP System is that it is a nonisotopic immunoassay system, well validated with classical allergens. Since the relevant drug that induced the reactions in the groups studied was AX, the Pharmacia CAP System assay employed used both AXO and BPO for the validation.

The purpose of this work was to study the IgE response to BP and AX in two groups defined by clinical history and positive skin tests, and a third, skin-test-negative group with positive history confirmed by either controlled administration or repeated reactions to the penicillin derivatives. The reason for including this third group in the Pharmacia CAP System validation was the evidence of allergic but skin-test-negative subjects (11, 12, 30, 31). In all these groups, the most relevant drug involved in the reaction was AX. For the assays, the positive and negative study groups had to be defined. The definition of a positive control for these studies is complex because controlled administration cannot be used as a reference standard due to the risk of inducing a systemic response. Therefore, the criteria considered for defining the positive study groups was a history of a clearly anaphylactic or urticaria reaction and skin test positivity to AX and at least one other penicillin determinant in group 1, and

positivity to only AX in group 2. In group 3, subjects had to have had several episodes of an immediate reaction to AX or BP or confirmation by controlled administration. In order to assess the clinical specificity of the test, we chose two further groups, one with a non-IgE-mediated allergic response (group 4) and another with good tolerance to AX and other β -lactams (group 5). The proportion of positive *in vitro* test results in group 1 suggested that in this group cross-reactivity existed between the penicillin determinants. These results also demonstrated the good agreement between the *in vitro* and skin tests. The results of group 2, with specific reactions to AX, confirmed that this was a selective group in whom AXO was the relevant determinant.

Although we chose very well-defined groups for the study, the sensitivity of IgE *in vitro* testing was not as high as with classical allergens, where values over 90% have been described (34). However, the specificity in our groups was high and similar to that of other protein allergens (34, 35). If we consider the combination of both haptens, the sensitivity increased to 74% in group 1, but there was no change (41%) in group 2. This indicates that reactions in 26% of the patients in group 1 and 59% of those in group 2 were not confirmed by *in vitro* testing. The explanation for this could be the interval between the occurrence of the reaction and the serum collection, defined as time interval, or that an inadequate hapten carrier conjugate was used. Since there was no relation between the time interval and *in vitro* assay values, it is difficult to establish the cause of the false negatives, but the existence of patients, with a negative *in vitro* assay with a short time interval indicates that other causes need to be considered. However, in a previous study, we found an association between IgE antibody response in the *in vivo* test and the time interval, a finding which seems surprising and contradictory (36) – although similar data have been found by others (31) – but which can possibly be explained by nontherapeutic exposure to penicillins (31, 37). If we consider together the data of the three positive groups, and combine the results of the *in vivo* test to BPO and the *in vitro* test to AXO, the number of positive patients rose from 19 (25.6%) to 41 (55.4%) out of a total of 74 patients studied.

In conclusion, the results suggest that the Pharmacia CAP System RAST FEIA *in vitro* assay may be used to

evaluate subjects allergic to β -lactams. It demonstrates the necessity of testing for specific IgE to AXO, in addition to BPO, in patients who have had an immediate allergic reaction after treatment with AX. Since there are some indications that anaphylaxis predominates over urticaria in allergic reactions to β -lactams (7), the ability to use an *in vitro* test is a good alternative means to detect positive patients. Furthermore, because of the possibility of finding patients with a positive history but

negative skin test, the combination of *in vivo* and *in vitro* tests to estimate IgE antibodies to penicillins is a useful approach.

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