

## The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams

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### Summary

**Background** Basophil activation by allergens, including drugs, has been used to determine sensitivity and to study IgE recognition and cross-reactivity.

**Objective** We sought to determine the sensitivity and specificity of a basophil activation test (Basotest) in patients with immediate allergic reactions to betalactams, with a later comparison between patients who were selective (those recognizing the culprit drug excluding benzylpenicillin (BP)) and cross-reactors (those recognizing several penicillin determinants including BP).

**Methods** Basotest to different haptens was performed in 70 patients with immediate allergic reactions to betalactams, classified into three groups: (A) skin test positive independently of CAP/RAST immunoassay value, (B) skin test negative and CAP/RAST positive, and (C) skin test and CAP/RAST negative but drug provocation test positive. Basotest was carried out by flow cytometry following the manufacturer's instructions using different betalactam determinants and results expressed as a stimulation index.

**Results** Of the 70 patients, 34 (48.6%) were positive to Basotest (sensitivity: 48.6%), 31 (44.3%) to CAP/RAST and 46 (65.7%) to either one or the other. Considering the different groups, Basotest was positive in 50.9% of patients in Group A, 60% in Group B and 14.3% in Group C. The specificity was 91.3%. Positivity to the haptens was 28.6% to amoxicillin (AX), 21.7% to BP, 20% to benzylpenicilloyl-poly-L-lysine, 12.5% to ampicillin and 2.2% to minor determinant mixture. In patients with cephalosporin reactions, Basotest to the culprit cephalosporin was positive in 77.7%. There were differences between the two reactor groups in the sensitivity of Basotest (selective to AX = 50%, cross-reactors = 28.6%;  $\chi^2 = 10.809$ ,  $P = 0.004$ ) and in the CAP/RAST (selective to AX = 28.6%, cross-reactors = 61.9%;  $\chi^2 = 8.944$ ,  $P = 0.011$ ).

**Conclusions** The sensitivity of Basotest is similar to immunoassays (CAP/RAST). Sensitivity is improved when used in combination. Although further studies are required, Basotest results for cephalosporin allergy seem very promising. This technique does not help differentiate between selective reactors and cross-reactors.

**Keywords** allergic reactions, basophils, betalactams, diagnosis, IgE, immediate, *in vitro* tests

Submitted 11 December 2003; revised 25 June 2004; accepted 2 August 2004

### Introduction

IgE-mediated reactions to betalactams are well known and much information exists concerning reactions to common determinants [1, 2], the side-chain of amoxicillin (AX) [3], cephalosporins [4], and to a lesser extent, other betalactams [5–7]. However, the diagnosis of these is now more difficult than before, and up to 30% of patients will not be diagnosed in spite of a correct clinical history, skin testing and *in vitro* assays [8]. Furthermore, results differ from country to country [3, 9], probably because of different patterns of consumption [10] and also, although to a lesser extent, to

different diagnostic protocols [11]. Additionally, the appearance of systemic reactions is not uncommon during skin testing with betalactams, mostly when the maximum concentrations recommended are used [8]. The most widely available *in vitro* method is the CAP immunoassay that, in our experience, detects specific IgE antibodies to benzylpenicillin (BP) and/or AX in just 50% of patients allergic to these drugs [12]. No validated series are available for the CAP assay to cephalosporins. It is therefore reasonable to look for alternative *in vitro* tests for the diagnosis of immediate allergic reactions to betalactams.

Recently, a new technology based on the expression of different activation markers in the basophil after *in vitro* stimulation with several allergens and haptens, including betalactams, has been proposed as a complementary method for *in vitro* specific IgE determination [13–16]. In this paper,

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we analyse in detail a basophil activation test (Basotest) in a group of patients with immediate allergic reactions to betalactams, including cephalosporins, diagnosed by skin testing, immunoassays or drug provocation test (DPT). An additional issue, not considered in previous reports, was to establish how the basophils from patients with cross- or selective immediate reactions to betalactams, respond *in vitro* to different derivatives. The data presented in this manuscript provide additional information concerning Basotest for the diagnosis of immediate reactions to betalactams.

## Patients and methods

### Patients

Subjects, evaluated from 2001 to 2003, with an immediate allergic reaction to betalactams were included. The diagnostic procedure was basically as described in the European Network for Drug Allergy (ENDA) protocol [11], although skin testing, CAP/RAST and Basotest were carried out in all cases in order to make comparative studies. Two clinical categories were established: anaphylaxis and urticaria [8]. Patients were also classified into three groups according to the results of the diagnostic protocol: (A) those with skin test positive whatever the CAP/RAST results, (B) those with skin test negative and CAP/RAST positive, and (C) those with both skin test and CAP/RAST negative and DPT positive.

Subjects were further classified into two groups: cross-reactors when they recognized major and minor determinants of BP by skin testing, CAP/RAST or DPT; and selective reactors when, though negative to determinants of BP, they recognized aminopenicillins or cephalosporins [8].

Forty skin-test-negative patients with good tolerance to betalactams were used as negative controls. The study was approved by the institutional review boards, and informed consent for the diagnostic procedures was obtained from patients and controls.

### Skin test

Skin testing was carried out as previously described [8], using 0.02 mL of solution prepared daily. The reagents were benzylpenicilloyl-poly-L-lysine (BPO-PLL) (Allergopharma Merck, Reinbeck, Germany) ( $5 \times 10^{-5}$  M), minor determinant mixture (MDM) (Allergopharma Merck) ( $2 \times 10^{-2}$  M), AX (SB Smithkline Beecham, Madrid, Spain) (20 mg/mL) and ampicillin (AMP) (Normon, Madrid, Spain) (20 mg/mL) and culprit cephalosporins: cefuroxime (curoxima, Glaxo-Wellcome, Madrid, Spain) and cefaclor (ceclor, Lilly, Madrid, Spain) (2 mg/mL). In the skin prick tests, a weal larger than 2 mm with a negative response to the control saline was considered positive. In the intradermal tests the weal area was marked initially and 20 min after testing, and an increase in diameter greater than 3 mm was considered positive [3, 11].

### *In vitro specific immunoglobulin E antibody determination*

This was performed by CAP-FEIA system (Pharmacia diagnostics, Uppsala, Sweden), using c1 (benzylpenicilloyl, BPO) and c6 (amoxicilloyl, AXO) following the manufac-

turer's instructions; briefly, the betalactams, covalently coupled to ImmunoCAP, react with the specific IgE in the patient's serum sample. After washing away non-specific IgE, enzyme-labelled antibodies against IgE were added to form a complex. In a second step, after incubation and washing, an incubation with a developing agent was carried out. After stopping the reaction, the fluorescence of the elute was measured in a FluoroCount 96. The results were obtained by direct comparison with standards run in parallel, considering a value  $\geq 0.35$  kUA/L as positive [12].

Because CAP is not available for cephalosporin studies, the RAST using cefuroxime and cefaclor conjugated to PLL (Sigma, St Louis, MO, USA) in the solid phase was carried out as described [4, 7]. Briefly, 30  $\mu$ L of the patient's sera was incubated with the cellulose disc with different cephalosporin-PLL conjugates. After wash radiolabelled anti-IgE antibody (Pharmacia diagnostics) was then added and incubated overnight. The discs were then washed and their radioactivity measured in a gamma counter, Cobra II auto-gamma (Packard BioScience Company, Frankfurt, Germany). Results were calculated as a percentage of the maximum. Samples were considered positive if they were higher than 2.5% of label uptake, which was the mean + 2SD of the negative control group.

### Drug provocation test

Single-blind placebo-controlled DPT was carried out for diagnosis in those patients with skin test and CAP/RAST negative and also for classifying patients as selective- or cross-reacting, as described [3, 11, 17]. In the first week, after completion of skin testing with BPO-PLL and MDM, we began with the parenteral administration of 1 mL of BP ( $10^4$  IU/mL), followed by 1 mL at  $10^5$  IU/mL and completing with 1 mL of  $10^6$  IU/mL if good tolerance was established at the previous doses. Similarly, if the skin tests with AMP and AX undertaken the following week were negative, AX was given orally at the following doses: 5, 50, 100 and 500 mg with a 1 h interval between each. If the culprit drug was a cephalosporin, an additional week was necessary for evaluation.

### *Basophil activation by flow cytometry*

Basotest (Orpegen Pharma, Heidelberg, Germany) was used following the manufacturer's recommendations. Briefly, 100  $\mu$ L of heparinized whole blood was aliquoted per test and 20  $\mu$ L of stimulation buffer was added and incubated for 10 min at 37 °C in a water bath. After this, 100  $\mu$ L of the washing solution was added to the negative control tube, 100  $\mu$ L of the fMLP (chemotactic peptide *N*-Formyl-Met-Leu-Phe) to the positive control tube and 100  $\mu$ L of two drug concentrations to the different samples. The concentrations used were: BPO-PLL at 0.025 and 0.005 mg/mL; MDM at 0.5 and 0.1 mg/mL; BP at 2 and 0.4 mg/mL; AX, AMP and cephalosporins at 1.2 and 0.25 mg/mL. These optimal concentrations were chosen on the basis of dose-response curves and cytotoxicity studies. Concentrations higher than those described above were cytotoxic and concentrations below all those indicated for each hapten decrease sensitivity. The samples were incubated for 20 min at 37 °C in a water bath. The degranulation was stopped by incubating the

samples on ice for 5 min and then 20 µL of staining reagent containing two monoclonal antibodies, anti-IgE PE and anti-gp53 FITC (gp53 is a glycoprotein expressed on activated basophils), was added to each tube and incubated for 20 min in an ice bath covered to prevent exposure to light. Then, 2 mL of pre-warmed lysing solution was added and incubated for 10 min at room temperature. After centrifuging and washing, the cells were analyzed in a flow cytometer by acquiring at least 1000 basophils per sample. Results were considered as positive when the stimulation index (SI), calculated as the ratio between the percentage of degranulated basophils with the different haptens and the negative control, was  $\geq 2$  to at least one of the dilutions mentioned above. When the percentage of spontaneously activated basophils was less than 2.5%, we required an additional condition, i.e. that the percentage of basophils activated after contact with the antigen should be equal to or greater than 5%, as previously described [18].

### Statistical studies

Comparisons for quantitative variables without normal distribution were carried out by the Mann–Whitney and Kruskal–Wallis tests and between qualitative variables by  $\chi^2$ . correlation between quantitative variables was carried out by Spearman's rank analysis. All reported *P*-values represented with two-tailed tests, with values  $\leq 0.05$  were considered statistically significant. The statistical analysis was performed using the SPSS program version 9.0.

## Results

### *Classification of patients according to skin tests, immunoassays and drug provocation test*

The 70 patients were classified into three groups: (A) skin test positive ( $N = 53$ ); (B) skin test negative and CAP/RAST positive ( $N = 10$ ); (C) skin test and CAP/RAST negative but DPT positive ( $N = 7$ ). The mean interval between the reaction and the study was 7.78 months (range: 1–24) with no significant differences between the three groups. In Group A, 45 (84.9%) patients were skin test positive to AX, 19 (35.8%) to AMP, six (11.3%) to MDM, six (11.3%) to BPO-PLL and five just to cephalosporin. Further details are shown in Table 1.

Based on skin testing, CAP/RAST and DPT patients were classified as cross-reactors ( $N = 21$ ) or as selective reactors ( $N = 49$ ). Of these 49 patients, 43 were selective to AX and seven to cephalosporins, 13 tolerated BP in the DPT and in the others, BP was not administered.

### *Basotest in the three groups of patients with allergic reactions to betalactams*

Thirty-four of the 70 patients evaluated were positive to at least one determinant giving a sensitivity of 48.6% (Table 2); the specificity was 91.3%. Figure 1 shows one example (case 57) of the flow cytometry results. Comparisons between these groups indicate that 27 out of 53 (50.9%) were positive in Group A, six out of ten (60%) in Group B and one out of seven (14.3%) in Group C. Differences between Groups A

and B were not significant. It is important to mention that the percentage of patients with a positive Basotest in Group C seemed abnormally low, but no statistical comparisons could be made because of the low number of cases. The percentages of positivity to the different haptens were 28.6% to AX, 21.7% to BP, 20% to BPO-PLL, 12.5% to AMP and 2.2% to MDM. In those patients with allergic reactions to cephalosporin, Basotest to the culprit drug was positive in 77.7%. Most of the patients who were positive to BPO-PLL were also positive to BP, except for one who was only positive to BP and two who were only positive to BPO-PLL. All the patients who were positive to AMP were also positive to AX (Table 2).

Although Basotest was positive in 34 (48.6%) patients and CAP/RAST in 31 (44.3%), a total of 46 patients were positive to one or the other of the two tests, increasing the sensitivity to 65.7%. The percentage of patients detected by Basotest was greater than by CAP/RAST, although not significantly so ( $\chi^2 = 0.948$ ;  $P = 0.480$ ), even in Group A where 50.9% was Basotest positive and 39.6% CAP positive ( $\chi^2 = 0.151$ ;  $P = 0.697$ ). These comparisons between Basotest and CAP/RAST could not be made in Groups B and C because a positive or negative CAP/RAST result was an inclusion criteria. There were no significant differences in the sensitivity of either test in patients with anaphylaxis compared with urticaria (Basotest:  $\chi^2 = 0.255$ ,  $P = 0.880$ ; CAP/RAST:  $\chi^2 = 0.188$ ,  $P = 0.910$ ). There was no correlation between the Basotest SI and specific IgE levels measured by CAP/RAST to any of the haptens tested or between either of these two methods and time interval.

### *Basotest in selective and cross-reactive groups*

Considering Basotest or CAP/RAST as positive when results were positive to at least one determinant assayed, we compared the sensitivity of both methods in selective and cross-reacting patients. In the 21 patients with cross-reactions, we found that six were Basotest positive and 13 CAP/RAST positive and in the 42 selective reactors to AX, 21 were Basotest positive and 12 CAP/RAST positive. Statistical comparison showed differences in the sensitivity of Basotest between patients selective to AX (50%) and cross-reactors (28.6%) ( $\chi^2 = 10.809$ ;  $P = 0.004$ ) and, in a reverse direction, in the sensitivity of CAP/RAST (selective to AX = 28.6%, cross-reactors = 61.9%,  $\chi^2 = 8.944$ ;  $P = 0.011$ ). In the seven patients who were selective reactors to cephalosporins, we found a Basotest positive in 100% of the patients and a positive CAP/RAST to at least one determinant in 66.7%.

Further evaluation of the 42 patients with selective reactions to AX showed that 12 were Basotest positive to AX and negative to BP determinants, six were positive to BP determinants alone and three to both. If we consider a positive result only in those with positivity to the culprit determinant, then the sensitivity decreased to 35.7%. Furthermore, it is important to note that although these patients were selective reactors to AX, 43% with a positive Basotest also recognized BP determinant and 57% recognized only AX. Although this technique with the five determinants was not performed in all patients, only in 32, we found the same results as for the whole group. For example, in the 23 selective reactors to AX, where the five haptens were

**Table 1.** Clinical characteristics of the 70 patients studied classified in three groups: Group A with skin test positive, Group B with skin test negative and CAP/RAST positive, and Group C with skin test and CAP/RAST negative and DPT positive

| Patient | Group | Sex | Age | Drug | Interval (months) | Reaction    | Skin test             | DPT    | CAP/RAST |      |      |
|---------|-------|-----|-----|------|-------------------|-------------|-----------------------|--------|----------|------|------|
|         |       |     |     |      |                   |             |                       |        | BPO      | AX   | CEP  |
| 1       | A     | F   | 68  | AX   | 7                 | Urticaria   | AX                    | ND     | 3.65     | 0    | ND   |
| 2       | A     | F   | 19  | AX   | 4                 | Anaphylaxis | AX                    | ND     | 5.71     | 6.59 | ND   |
| 3       | A     | M   | 56  | AX   | 4                 | Anaphylaxis | AX                    | ND     | 1.2      | 0    | ND   |
| 4       | A     | M   | 47  | AX   | 4                 | Anaphylaxis | BPO-PLL, AX, AMP      | ND     | 26.7     | 14.2 | ND   |
| 5       | A     | M   | 55  | AX   | 21                | Urticaria   | BPO-PLL, MDM, AX, AMP | ND     | 0        | 0    | ND   |
| 6       | A     | F   | 25  | AX   | 24                | Anaphylaxis | BPO-PLL, MDM, AX, AMP | ND     | 0        | 0    | ND   |
| 7       | A     | M   | 43  | CL   | 3                 | Urticaria   | MDM                   | ND     | 4.88     | 0    | ND   |
| 8       | A     | M   | 50  | AX   | 2                 | Anaphylaxis | MDM, AX               | ND     | 0        | 0    | ND   |
| 9       | A     | M   | 39  | AX   | 6                 | Anaphylaxis | AX                    | ND     | 0        | 10.1 | ND   |
| 10      | A     | F   | 36  | AX   | 21                | Anaphylaxis | BPO-PLL, MDM, AX      | ND     | 0        | 0    | ND   |
| 11      | A     | F   | 52  | PENI | 10                | Urticaria   | BPO-PLL, MDM          | ND     | 0        | 0    | ND   |
| 12      | A     | M   | 22  | AX   | 1                 | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 13      | A     | M   | 52  | PENI | 24                | Urticaria   | AX, AMP               | BP (–) | 0        | 0    | ND   |
| 14      | A     | F   | 55  | AX   | 7                 | Anaphylaxis | AX                    | ND     | 0        | 0    | ND   |
| 15      | A     | F   | 53  | AX   | 23                | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 16      | A     | F   | 57  | AX   | 1                 | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 17      | A     | F   | 22  | AX   | 12                | Urticaria   | AX                    | BP (–) | 0        | 0    | ND   |
| 18      | A     | F   | 54  | PENI | 11                | Urticaria   | AX, AMP               | ND     | 0        | 0    | ND   |
| 19      | A     | F   | 64  | AX   | 4                 | Anaphylaxis | AX                    | ND     | 1.18     | 0    | ND   |
| 20      | A     | M   | 65  | AX   | 7                 | Urticaria   | BPO-PLL               | ND     | 19.71    | 0    | ND   |
| 21      | A     | M   | 62  | AX   | 18                | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 22      | A     | F   | 21  | AX   | 4                 | Anaphylaxis | AX                    | BP (–) | 0        | 0    | ND   |
| 23      | A     | F   | 38  | AX   | 12                | Anaphylaxis | AX                    | BP (–) | 0        | 0    | ND   |
| 24      | A     | M   | 49  | AX   | 6                 | Anaphylaxis | AX                    | ND     | 0        | 0    | ND   |
| 25      | A     | M   | 33  | AX   | 7                 | Anaphylaxis | AX                    | ND     | 0        | 0    | ND   |
| 26      | A     | M   | 38  | AX   | 7                 | Anaphylaxis | AX                    | ND     | 0        | 0.76 | ND   |
| 27      | A     | F   | 31  | AX   | 18                | Anaphylaxis | AX                    | BP (–) | 0        | 1.7  | ND   |
| 28      | A     | M   | 37  | AX   | 3                 | Anaphylaxis | AX                    | ND     | 0        | 0    | ND   |
| 29      | A     | F   | 43  | AX   | 16                | Anaphylaxis | AX                    | ND     | 0        | 3.85 | ND   |
| 30      | A     | M   | 55  | AX   | 5                 | Urticaria   | AX                    | ND     | 0        | 29.8 | ND   |
| 31      | A     | M   | 59  | AX   | 16                | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 32      | A     | M   | 40  | AX   | 4                 | Anaphylaxis | AX                    | BP (–) | 0        | 0    | ND   |
| 33      | A     | F   | 19  | AX   | 14                | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 34      | A     | F   | 30  | AX   | 9                 | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 35      | A     | F   | 32  | AX   | 5                 | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 36      | A     | F   | 41  | AX   | 1                 | Anaphylaxis | AX, AMP               | ND     | 0.91     | 0.28 | ND   |
| 37      | A     | F   | 23  | AX   | 8                 | Urticaria   | AX                    | ND     | 0        | 0    | ND   |
| 38      | A     | F   | 33  | AX   | 4                 | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 39      | A     | F   | 36  | AX   | 2                 | Anaphylaxis | AX                    | ND     | 0        | 0    | ND   |
| 40      | A     | M   | 27  | AX   | 4                 | Urticaria   | AX                    | ND     | 0        | 0    | ND   |
| 41      | A     | F   | 22  | CU   | 2                 | Anaphylaxis | Cefuroxime            | ND     | 0        | 0    | 5.37 |
| 42      | A     | M   | 40  | AX   | 1                 | Anaphylaxis | AX, AMP               | ND     | 0        | 2.07 | ND   |
| 43      | A     | F   | 43  | CO   | 5                 | Urticaria   | Cefaclor              | ND     | 0        | 0    | 2.94 |
| 44      | A     | F   | 35  | CU   | 9                 | Anaphylaxis | Cefuroxime            | BP (–) | 0        | 0    | ND   |
| 45      | A     | F   | 56  | CU   | 1                 | Anaphylaxis | Cefuroxime            | ND     | 0        | 0    | 0.43 |
| 46      | A     | F   | 36  | AX   | 4                 | Anaphylaxis | AX                    | ND     | 0        | 0.9  | ND   |
| 47      | A     | M   | 37  | AX   | 1                 | Anaphylaxis | AX                    | ND     | 0        | 0.78 | ND   |
| 48      | A     | M   | 66  | CU   | 2                 | Anaphylaxis | Cefuroxime            | ND     | 0        | 0    | 1.35 |
| 49      | A     | F   | 36  | AX   | 7                 | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 50      | A     | M   | 47  | AX   | 3                 | Anaphylaxis | AX                    | ND     | 0        | 0    | ND   |
| 51      | A     | F   | 36  | AX   | 6                 | Anaphylaxis | AX, AMP               | BP (–) | 0        | 0    | ND   |
| 52      | A     | F   | 51  | AX   | 6                 | Anaphylaxis | AX                    | ND     | 0.86     | 0.75 | ND   |
| 53      | A     | F   | 26  | AX   | 7                 | Anaphylaxis | AX, AMP               | ND     | 0        | 0    | ND   |
| 54      | B     | M   | 55  | AX   | 8                 | Urticaria   | Negative              | ND     | 27.3     | 6.39 | ND   |
| 55      | B     | M   | 65  | AX   | 11                | Anaphylaxis | Negative              | ND     | 3.29     | 5.13 | ND   |
| 56      | B     | M   | 53  | AX   | 21                | Urticaria   | Negative              | ND     | 0        | 3.54 | ND   |
| 57      | B     | F   | 39  | CO   | 6                 | Anaphylaxis | Negative              | ND     | 0        | 0    | 6.99 |
| 58      | B     | F   | 29  | CO   | 6                 | Anaphylaxis | Negative              | ND     | 0        | 0    | 7.13 |

Table 1. (Contd.)

| Patient | Group | Sex | Age | Drug | Interval (months) | Reaction    | Skin test | DPT           | CAP/RAST |      |      |
|---------|-------|-----|-----|------|-------------------|-------------|-----------|---------------|----------|------|------|
|         |       |     |     |      |                   |             |           |               | BPO      | AX   | CEP  |
| 59      | B     | M   | 50  | AX   | 1                 | Anaphylaxis | Negative  | ND            | 2.87     | 2.3  | ND   |
| 60      | B     | F   | 22  | CU   | 2                 | Anaphylaxis | Negative  | ND            | 0.29     | 0.41 | 5.37 |
| 61      | B     | M   | 34  | AX   | 3                 | Anaphylaxis | Negative  | ND            | 0        | 1.05 | ND   |
| 62      | B     | M   | 25  | AX   | 4                 | Anaphylaxis | Negative  | BP (-)        | 0        | 1.27 | ND   |
| 63      | B     | M   | 34  | AX   | 5                 | Anaphylaxis | Negative  | ND            | 0        | 0.62 | ND   |
| 64      | C     | F   | 60  | AX   | 13                | Urticaria   | Negative  | BP (+)        | 0        | 0    | ND   |
| 65      | C     | F   | 54  | BP   | 7                 | Anaphylaxis | Negative  | BP (+)        | 0        | 0    | ND   |
| 66      | C     | F   | 43  | AX   | 12                | Urticaria   | Negative  | BP (-)/AX (+) | 0        | 0    | ND   |
| 67      | C     | M   | 31  | AX   | 6                 | Anaphylaxis | Negative  | BP (-)/AX (+) | 0        | 0    | ND   |
| 68      | C     | F   | 39  | AX   | 13                | Urticaria   | Negative  | BP (-)/AX (+) | 0        | 0    | ND   |
| 69      | C     | M   | 29  | AX   | 6                 | Anaphylaxis | Negative  | BP (+)        | 0        | 0    | ND   |
| 70      | C     | M   | 39  | AX   | 8                 | Anaphylaxis | Negative  | BP (-)/AX (+) | 0        | 0    | ND   |

DPT, drug provocation test; BPO-PLL, benzylpenicilloyl-poly-L-Lysine; MDM, minor determinant mixture; AX, amoxicillin; PENI, unrecalled penicillin; AMP, ampicillin; BP, benzylpenicillin; CL, cefalexin; CU, cefuroxime; CO, cefaclor; CEP, cephalosporin; ND, not done.

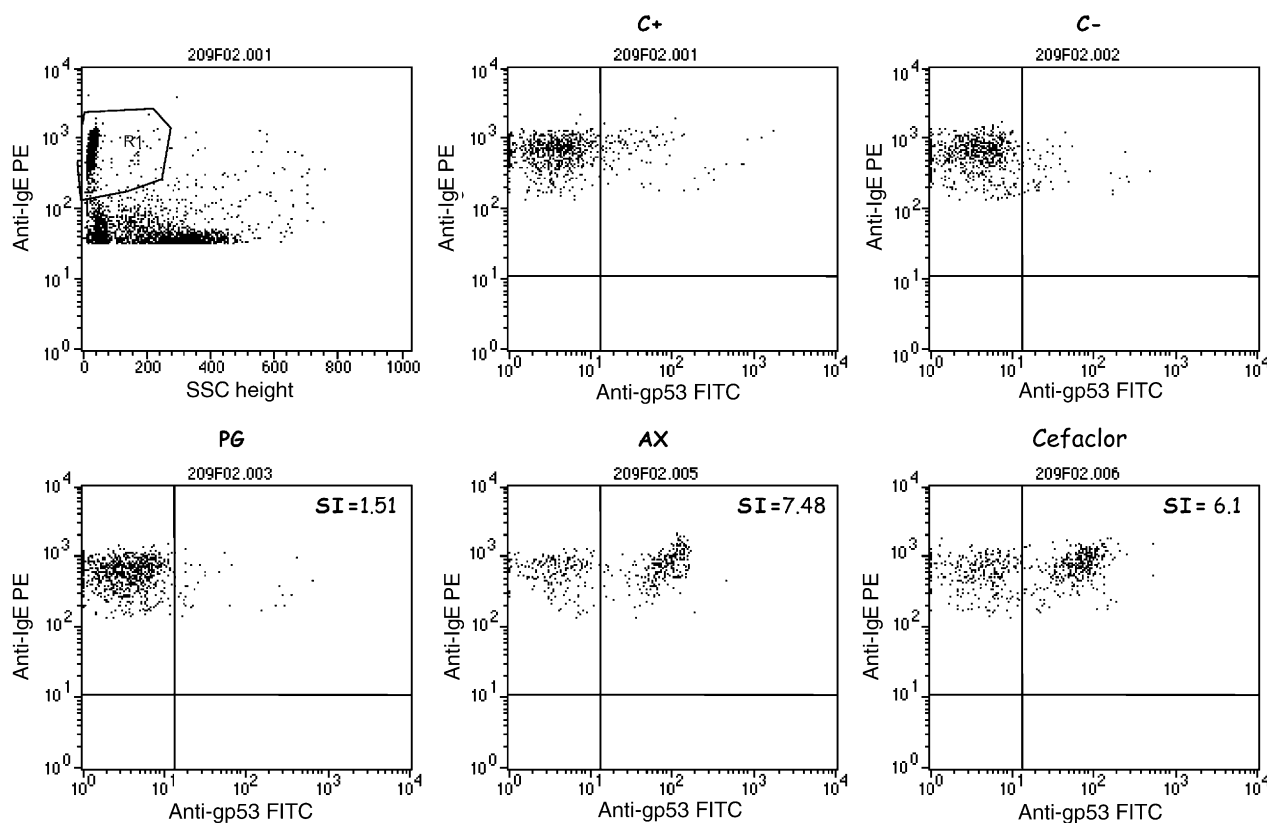


Fig. 1. Dot plot of case 57, with Basotest positive to amoxicillin and cefaclor.

analysed, 41.6% reacted to BP determinants and 58.3% only to AX determinants. In order to evaluate whether the recognition to BP determinants, although positive, was lower in the selective reactors to AX we compared the mean values of Basotest to BP and CAP to BPO in the cross- and selective reactors to AX. We found that although in the CAP there were significant differences between cross- and selective reactors ( $P < 0.001$ ), these differences were not observed in Basotest (Fig. 2). Similar results were found with cephalosporin selective reactors (data not shown).

## Discussion

The Basotest is an *in vitro* diagnostic technique that has received increasing attention, particularly regarding the diagnosis of allergic drug reactions [19, 20]. Based on previous studies, the purpose of our work was to determine in more detail the diagnostic value of this technique using a more precise classification of patients with immediate allergic reactions to betalactams [18–20]. For this reason, we established three well-differentiated groups from which

**Table 2.** Basotest results expressed as a stimulation index (SI) in the three groups evaluated

| Patient | Selectivity    | Basotest    |             |              |             |             |              |
|---------|----------------|-------------|-------------|--------------|-------------|-------------|--------------|
|         |                | BPO-PLL     | MDM         | BP           | AX          | AMP         | CEP          |
| 1       | Cross-reactive | 1.57        | ND          | 1.19         | <b>5.01</b> | ND          | ND           |
| 2       | Cross-reactive | 1.12        | ND          | 0.96         | 1.11        | 1.03        | ND           |
| 3       | Cross-reactive | 0.5         | ND          | 0.9          | 0.79        | 0           | ND           |
| 4       | Cross-reactive | ND          | 0           | <b>5.74</b>  | <b>7.9</b>  | ND          | ND           |
| 5       | Cross-reactive | 0           | ND          | 1.19         | 1.12        | 0.5         | ND           |
| 6       | Cross-reactive | 1.24        | ND          | 1.18         | 1.12        | 0.27        | ND           |
| 7       | Cross-reactive | ND          | 0           | 0.59         | 0.22        | 0.18        | ND           |
| 8       | Cross-reactive | <b>5.97</b> | 1.07        | <b>6.31</b>  | <b>9.08</b> | ND          | ND           |
| 9       | Selective      | 0.74        | 0           | 1.96         | 1.61        | 1.02        | ND           |
| 10      | Cross-reactive | ND          | ND          | 0.26         | 0.89        | 0.24        | ND           |
| 11      | Cross-reactive | 0.12        | ND          | 0.59         | 0.93        | ND          | ND           |
| 12      | Selective      | <b>2.59</b> | ND          | <b>2.41</b>  | 0.7         | ND          | ND           |
| 13      | Selective      | ND          | 0.5         | 1.16         | 1.17        | 0.18        | ND           |
| 14      | Selective      | 0.87        | 0.97        | ND           | <b>2.54</b> | <b>2.03</b> | ND           |
| 15      | Selective      | ND          | ND          | 0.87         | 0.67        | 0.97        | ND           |
| 16      | Selective      | <b>7.28</b> | ND          | <b>9.71</b>  | <b>2.42</b> | ND          | ND           |
| 17      | Selective      | ND          | ND          | 0.82         | 0.61        | 0.17        | ND           |
| 18      | Selective      | 0.57        | ND          | 0.53         | 0.29        | 0.50        | ND           |
| 19      | Cross-reactive | 0.24        | 0.85        | 0.91         | 0.57        | 0.33        | ND           |
| 20      | Cross-reactive | 1.35        | 0.97        | 1.56         | 1.6         | 0.82        | ND           |
| 21      | Selective      | 0.6         | 0.33        | 0.08         | 0.25        | ND          | ND           |
| 22      | Selective      | 0.3         | 0.63        | 0.94         | 0.51        | 0.43        | ND           |
| 23      | Selective      | 0.24        | 0.69        | 0.82         | 0.3         | 0.27        | ND           |
| 24      | Selective      | <b>6.54</b> | 1.58        | <b>8.46</b>  | 0.86        | 0.12        | ND           |
| 25      | Selective      | 0.15        | 0.24        | 1.2          | 1.09        | 0.9         | ND           |
| 26      | Selective      | 1.18        | ND          | 1.41         | <b>8.71</b> | <b>3.5</b>  | ND           |
| 27      | Selective      | <b>2.78</b> | 0.37        | <b>2.48</b>  | 0.82        | 0           | ND           |
| 28      | Selective      | <b>4.28</b> | ND          | <b>5.51</b>  | <b>4.97</b> | <b>2.8</b>  | ND           |
| 29      | Selective      | <b>2.05</b> | 1.58        | <b>2.26</b>  | 1.86        | 0.12        | ND           |
| 30      | Selective      | 1.05        | 0           | 1.32         | <b>3.02</b> | 0.3         | ND           |
| 31      | Selective      | 0.24        | 0.27        | 1.77         | 1.16        | 0.47        | ND           |
| 32      | Selective      | 1.52        | ND          | 1.31         | 1.18        | 0.18        | ND           |
| 33      | Selective      | 1.12        | ND          | 0.96         | <b>2.47</b> | 0.97        | ND           |
| 34      | Selective      | 1.38        | 1.02        | 1.07         | 1.06        | 0           | ND           |
| 35      | Selective      | 0.6         | 0.3         | 0.59         | <b>7.14</b> | <b>5.1</b>  | ND           |
| 36      | Cross-reactive | 0.98        | ND          | 0.9          | 0.82        | 0.77        | ND           |
| 37      | Selective      | 1.21        | 0.81        | 0.86         | <b>2.46</b> | 0           | ND           |
| 38      | Selective      | 0.36        | 0.7         | 0.65         | <b>9.54</b> | 0           | ND           |
| 39      | Selective      | 1.41        | 1.77        | 1.02         | 1.29        | 0           | ND           |
| 40      | Selective      | 1.14        | ND          | 1.23         | <b>2.57</b> | 1.29        | ND           |
| 41      | Selective      | <b>2.16</b> | ND          | <b>2.57</b>  | 1.21        | ND          | <b>3.15</b>  |
| 42      | Selective      | <b>2.36</b> | <b>2.11</b> | <b>2.48</b>  | <b>3.37</b> | 1.76        | ND           |
| 43      | Selective      | <b>2.03</b> | 1.58        | <b>2.03</b>  | 1.06        | 0.18        | <b>10.64</b> |
| 44      | Selective      | 1.01        | 0.3         | 0.64         | 0.6         | 0.3         | <b>3.31</b>  |
| 45      | Selective      | 1.29        | 0.26        | <b>4.28</b>  | 0.86        | 0.24        | 1.23         |
| 46      | Selective      | <b>2.18</b> | 0           | 1.61         | 0.69        | ND          | ND           |
| 47      | Selective      | 1.01        | 1.64        | 1.21         | <b>2.64</b> | 0           | <b>2.95</b>  |
| 48      | Selective      | 0.96        | 0.85        | 0.85         | 1.12        | ND          | <b>2.48</b>  |
| 49      | Selective      | 0.57        | 0.69        | 1.81         | 1.14        | 0.78        | ND           |
| 50      | Selective      | 1.07        | 0.27        | 1.45         | <b>2.42</b> | 1.52        | ND           |
| 51      | Selective      | <b>2.23</b> | 0.3         | 0.72         | 1.26        | 1.7         | ND           |
| 52      | Cross-reactive | 1.39        | ND          | 1.45         | 1.84        | 0           | ND           |
| 53      | Selective      | 0.91        | ND          | 1.76         | 1.6         | 0           | ND           |
| 54      | Cross-reactive | ND          | ND          | <b>19.13</b> | 1.19        | ND          | ND           |
| 55      | Cross-reactive | ND          | 0.87        | <b>4.46</b>  | 1.91        | 1.2         | ND           |
| 56      | Selective      | 1.2         | 0.57        | 0.84         | 0.86        | ND          | ND           |
| 57      | Selective      | 0           | 0           | 1.51         | <b>7.48</b> | <b>5.4</b>  | <b>6.1</b>   |
| 58      | Selective      | 0.24        | ND          | 1.33         | 1.05        | 1.01        | <b>3.40</b>  |

**Table 2.** (Contd.)

| Patient | Selectivity    | Basotest |      |             |             |             |      |
|---------|----------------|----------|------|-------------|-------------|-------------|------|
|         |                | BPO-PLL  | MDM  | BP          | AX          | AMP         | CEP  |
| 59      | Cross-reactive | 1.35     | 1.23 | 1.67        | 1.18        | 1.3         | ND   |
| 60      | Cross-reactive | 0.94     | 1.06 | 0.88        | 0.75        | 0           | 0.58 |
| 61      | Selective      | 0.83     | 0.74 | 0.68        | <b>3.6</b>  | <b>3.22</b> | ND   |
| 62      | Selective      | 0.76     | 1.01 | 0.58        | 0.62        | 0.89        | ND   |
| 63      | Selective      | 0.55     | ND   | 0.28        | <b>2.4</b>  | 0.51        | ND   |
| 64      | Cross-reactive | ND       | ND   | <b>8.96</b> | <b>2.31</b> | <b>2.24</b> | ND   |
| 65      | Cross-reactive | 0        | 0.02 | 0.3         | 0.29        | ND          | ND   |
| 66      | Selective      | 0.15     | 0.18 | 0.74        | 0.77        | 0.19        | ND   |
| 67      | Selective      | 1.03     | 0.24 | 1           | 0.83        | 0           | ND   |
| 68      | Selective      | ND       | 0.33 | 1.07        | 0.24        | 0.3         | ND   |
| 69      | Cross-reactive | 0.42     | 0.81 | 0.38        | 0.13        | 0.58        | ND   |
| 70      | Selective      | 1.2      | 0.97 | 0.6         | 1.1         | ND          | ND   |

BPO-PLL, benzylpenicilloyl-poly-L-Lysine; MDM, minor determinant mixture; BP, benzylpenicillin; AX, amoxicillin; AMP, ampicillin; CEP, cephalosporin; ND, not done. The Basotest results shown in bold are considered to be positive.

consistent data have been reported in the past [8]. Thus, 53 patients were skin test positive to at least one hapten tested (Group A), 10 were skin test negative and positive IgE *in vitro* (Group B) and seven required DPT to establish the diagnosis of drug allergy (Group C). These figures are representative of those usually found in our daily practice [8, 17] with the inclusion of a group with selective responses to cephalosporins [4]. In order to make comparisons, the interval between the performance of the study and the occurrence of the reaction was similar for all groups, being lower than or equal to 24 months with no statistical differences [21].

The overall sensitivity of Basotest was 48.6% with a specificity of 93%, which is in general agreement with previous reported data [18]. If we consider the sensitivity for each individual group, this was increased to 59% for Group A and to 60% for Group B; in Group C, it decreased to 14.3%. Although the sensitivity of the Basotest in Group C was low, no definitive conclusions can be drawn because of the low number of cases in this group. However, the fact that both the skin test and CAP/RAST were also negative in these patients suggests that the hapten generated *in vivo* is not easily identified and not formed *in vitro* or that all these methods still have limitations in sensitivity.

Considering the total number, a positive CAP/RAST (44.4%) was not always paralleled by a positive Basotest (48.6%) and vice versa; thus, sensitivity increases to 65.7% if we consider positive results in both methods. Our past experience indicates that up to 10% of persons with side-chain-specific reactions may develop systemic symptoms during skin testing, although this has decreased by using serial dilutions in both prick and intradermal testing in those patients with anaphylaxis [8]. However, the possibility still exists of a systemic reaction, especially when the maximum concentrations recommended are used to establish the diagnosis [11]. A combination of two *in vitro* approaches, therefore, may be a useful alternative, at least for high-risk patients such as those with hypertension,  $\beta$  blocking therapy or other conditions.

Although we used a complete panel of penicillin determinants, free or conjugated, we found that BP, AX and

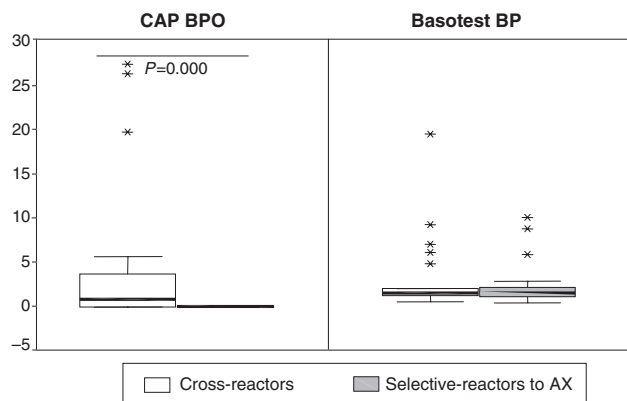


**Fig. 2.** Comparison of the results of CAP to benzylpenicilloyl (BPO) and Basotest to benzylpenicillin (BP) between cross-reactors and selective reactors to amoxicillin (AX).

cephalosporin were the most important haptens for the Basotest, which implies that the use of these can save money and resources.

An important objective in our work was to study the concept of cross-reactivity and selective response between different penicillins, mainly BP vs. AX, with further extension to cephalosporins [11]. Selective response is mainly a clinical concept applied to subjects with skin test and/or CAP/RAST positive and/or DPT to AX with skin test negative to penicillin determinants (BPO-PLL and MDM) and good tolerance to BP. Some selective cases can often show a very high CAP/RAST to AX and a weak positivity to BPO but still have good tolerance to BP. We have never yet detected selective responders to AX with positive CAP/RAST to BPO and negative to AX. Therefore, RAST has been considered as a good complement for classifying selective responders [3, 12, 22]. In the Basotest assay, however, a certain number of cases responded mainly to BP with a lower or negative response to AX, although they had been diagnosed clinically as selective responders to AX. This is one of the reasons why the Basotest was more sensitive in the selective group (57.1%) and the immunoassay in the cross-reactive patients (61.9%) (Fig. 2). This contrasts with results found with other drugs, where a good correlation between Basotest and the skin response has been found [23]. Several possible explanations exist for this. First, these patients have specific IgE that recognize BP and which can be detected at the Basotest level, but for technical reasons only the side chain of AX is detected by CAP/RAST. This seems unlikely because these patients are skin test negative to BP and have good tolerance to this drug, whereas they respond to AX by skin testing or DPT. Another explanation, suggested above, could be that in the Basotest assay, a BP conjugate is generated that is not formed in the skin testing or DPT. These hypotheses are currently under investigation by our group.

The sensitivity for the cephalosporin selective group was 77%. Although a larger group is needed, these figures look promising for drugs where no facilities for skin testing are available, when the insolubility of some betalactams does not enable skin testing at the concentration recommended for other betalactams, and in countries where no approval has been given for *in vivo* testing with medications. Thus,

Basotest appears to be a good approach for evaluating subjects with an IgE response to cephalosporins.

Summarizing, we can conclude that Basotest is a technique that can be used in the diagnosis of immediate allergic reactions to betalactams. It has a similar sensitivity to immunoassays, although the combination of both improves sensitivity. It does not, however, seem to be adequate for studying cross-reactivities. Nevertheless, for immediate allergic reactions to cephalosporins this test has great sensitivity, although further studies with more patients are required.

## Acknowledgements

We thank Ian Johnstone for help with the final English language version of this paper. This work has been supported by grants from the Comunidad de Madrid (0.8.3/0026/2001), Fundación de la Sociedad Española de Alergología e Inmunología Clínica and Fondo de Investigación Sanitaria (PIO31165).

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