

Original article

Determining the negative predictive value of provocation tests with beta-lactams

Background: The beta-lactam allergic work-up is mostly standardized. However, the negative predictive value of drug provocation tests is not yet well established. **Method:** A historical-prospective multicentre cohort study was conducted in four centres (one in France, one in Portugal, two in Italy) to assess the negative predictive value of provocation tests with beta-lactams in patients initially tested for a suspicion of drug allergy/hypersensitivity. Patients were contacted at least 6 months after the work-up, between 2003 and 2007. A new allergic work-up was proposed to reacting patients.

Results: Among the 457 patients included, 365 (79.9%) were followed up (159 [79.1%] from France, 153 [82.7%] from Italy and 53 [74.6%] from Portugal). Only 118 (25.8%) were re-exposed to the negatively tested beta-lactam. Nine (7.6%) reported a non-immediate (occurring more than 1 h after drug administration) reaction: five urticaria, three exanthema and one undefined cutaneous reaction. None were severe. Only four accepted a re-challenge, negative in two cases and positive in the two others. The negative predictive value was 94.1% (89.8–98.3) (111 out of 118 patients).

Conclusion: Although the negative predictive value of drug provocation tests may not be 100%, none of the false negative patients experienced a life-threatening reaction. This should reassure doctors who might hesitate to prescribe beta-lactams, even in patients with negative allergic work-ups.

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Provocation tests with beta-lactams play an important role in the diagnosis of hypersensitivity reactions to these antibiotics (1–3). In particular, drug provocation tests are included in both of the diagnostic algorithms of the European Network for Drug Allergy (ENDA) for evaluating immediate and non-immediate hypersensitivity reactions to beta-lactams (2, 3). Immediate reactions are those occurring within the first hour after the last drug administration and are manifested clinically by urticaria, angioedema, rhinitis, bronchospasm and anaphylactic shock. Non-immediate reactions occur more than one

hour after the last drug administration. The main non-immediate reactions are maculopapular eruptions and delayed-appearing urticaria/angioedema.

Indications of provocation tests with beta-lactams are extensive for the European Academy of Allergology and Clinical Immunology – ENDA group (2–4) and more restricted for the American Academy of Allergy Asthma and Immunology (5). Recently, ENDA simplified the protocol of provocation tests in patients with immediate reactions to beta-lactams by reducing the number of doses (6). In addition, the ENDA questionnaire (7) allows

patients with a potential beta-lactam allergy to be identified, and recommendations on drug provocation tests are available (4).

Though not well established, the negative predictive value is important for both the patient and the physician. The patient has to know whether a reaction can occur after taking a drug, which was tested negatively. It is now well known that adverse drug reaction and especially drug allergy/hypersensitivity are sources of anxiety and led to an avoidance of the drug. On the other hand, the physician has to know whether she or he can safely prescribe a drug with previous negative allergy work-up.

Thus, a historical-prospective multicentre cohort study was conducted in four centres to assess the negative predictive value of provocation tests with beta-lactams in patients initially tested for a suspicion of drug allergy/hypersensitivity.

Patients and methods

This multicentre study was conducted in four allergy units: Hôpital Arnaud de Villeneuve (Montpellier, France), Complesso Integrato Columbus (Rome, Italy), Oasi Maria Santissima (Troina, Italy) and Hospital São João (Porto, Portugal). It included patients, without any age restriction, with a clinical history suggestive of beta-lactam allergy and a negative allergy work-up, including challenges with the suspected beta-lactams. Patients who had experienced severe reactions, such as toxic epidermal necrolysis, Stevens–Johnson syndrome, acute generalized exanthematous pustulosis, hypersensitivity syndrome or drug reaction with eosinophilia and systemic symptoms, blood alterations, nephritis, pneumonitis, hepatitis, and vasculitis, were not involved in the study, as the provocation test is contraindicated in such patients (3, 4).

The French centre included all of the patients tested between January 2003 and December 2004, the Italian centres those assessed between December 1995 and May 2007, and the Portuguese centre those evaluated between January 2000 and June 2007. All patients were followed up for at least 6 months after the *initial* allergy work-up to obtain information on any reactions to the suspected beta-lactams found negative in allergy work-ups or to compounds of the same class.

Questionnaires and data collection

All centres used a similar questionnaire, which can be summarized as follows:

- Did the patient take the previously suspected beta-lactam or one of the same class since she or he underwent allergy tests with negative results?
- If yes, did a reaction occur, and if so, what kind?
- If no, did the patient not need the drug, and did the doctor prescribe a drug of another family?
- If an alternative drug was prescribed, what was the reason?

In patients reporting reactions to beta-lactams found to be negative in the first allergy work-up, a re-evaluation including repeated challenges was proposed. After the

latter work-up, the patient was classified as a true negative (absence of drug allergy) or as a false negative (true drug allergy with an initial negative work-up). When the patient refused the re-evaluation, she or he was classified as a false negative, even if the clinical presentation was suspicious (no clear and objective reaction compatible with the previous episode).

With regard to the French centre, the questionnaire was first sent to the patients through mail. A reminder was sent after 3 months. Finally, if the patient did not respond, a phone call was made to increase the number of participating patients. The Italian centres directly contacted the patients by phone, and they answered the questions by phone. The Portuguese centre contacted patients either by mail or by phone. If those contacted by mail did not answer, a phone call was made after 1 month.

Drug allergy initial work-up

The allergy work-up design was similar for all centres. The standardized ENDA questionnaire on drug allergy (7) was first filled in. According to the ENDA protocols for immediate (2) and non-immediate (3) reactions, patients with a compatible history of beta-lactam allergy underwent allergy work-ups and, in the case of negative results, provocation tests with the suspected drug. Before testing, patients signed a written informed consent. Local ethical committees agreed with the study design and protocols. The allergologic work-ups for patients evaluated before the date of publication of the ENDA guidelines were carefully reviewed. All of them were compliant with such guidelines.

Skin tests (prick and intradermal) were performed as previously described (8–10) with the classical penicillin reagents: penicilloyl polylysine (PPL, Allergopharma, Reinbeck, Germany), minor determinants mixture (MDM: benzylpenicillin and sodium benzylpenicilloate, Allergopharma) and benzylpenicillin (penicillin G). Semi-synthetic penicillins (amoxicillin and ampicillin) were also systematically tested, as well as any other suspected beta-lactam. Because Allergopharma ceased the production of penicillin reagents, the Italian and Portuguese centres (as from 2004 and July 2005 respectively) used Diater S.A. reagents (DAP, Madrid, Spain): PPL and MDM (benzylpenicillin, sodium benzylpenicilloate, and benzylpenicilloic acid). The procedure ended when a positive skin test was found.

In the Italian centres, PPL, MDM and benzylpenicillin were tested on the first day, and amoxicillin, ampicillin and any other suspected beta-lactam on the second. The French and Portuguese centres performed all of the skin tests on the same day (2).

In patients with an unknown chronology or with a non-immediate reaction, a late reading of skin tests was performed after 24–48 h. Positive controls for prick tests were carried out with histamine at 10 mg/ml. As a

negative control for prick and intradermal tests, normal saline solution was used.

In accordance with the ENDA recommendations (2, 3, 6), in the case of negative results of skin tests and serum-specific IgE assays (when available, not performed in the French centre), provocation tests with the suspected beta-lactams were performed under strict hospital surveillance (11). Provocation tests consisted of administering increasing doses of the suspected drug up to the full therapeutic dose or until symptoms of a drug reaction occurred. Administration was performed by a physician with full resuscitation back-up. Patients were asked to contact the physician whether a reactions occurred in the days following the provocation test to identify delayed reactions. Patients with histories of anaphylactic reactions had intravenous catheters in place during the entire test. Patients had not been taking any antihistamines or other drugs that could have affected skin tests or drug provocations. Patients on beta-blockers were requested to ask their cardiologist whether they could stop taking the drug 2 days before the provocation tests.

Detection of serum-specific IgE

In the Italian and Portuguese centres, blood samples were collected when patients with immediate reactions were evaluated with skin testing, and sera were stored at minus 20°C until assayed.

Assays for serum-specific IgE to penicilloyl G, penicilloyl V, ampicilloyl, amoxicilloyl and cefaclor (CAP-FEIA) were performed according to the manufacturer's instructions with UniCAP™ (Phadia, Uppsala, Sweden). A positive result (i.e. detectable specific IgE antibodies) was defined as a value ≥ 0.35 kU/l. When positive, an alternative beta-lactam was selected and tested (skin tests, serum-specific IgE and challenge).

Statistical analysis

Sample characteristics and results of the questionnaires are expressed in frequency and percent for qualitative data and median, 25–75th percentiles for quantitative ones. Comparisons were made between subjects who answered the questions and those who did not using chi-square tests or the Wilcoxon Mann and Whitney test, depending on the data. The negative predictive value with its 95% confident interval was computed. The analysis was conducted under SAS, SAS Institute, Cary, NC, USA.

Results

Overall, 457 patients negatively tested (*in vitro* and *in vivo* tests) were included in the study: 201 (44.0%) from the French centre, 185 (40.5%) from the Italian centres and 71 (15.5%) from the Portuguese one. Clinical character-

Table 1. Patients' characteristics and clinical presentation of the previous reaction

	France	Italy	Portugal*
N	201	185	71
Sex: female (%)	131 (65.2%)	134 (72.4%)	35 (66.0%)
Atopics	95 (47.3%)	12 (6.5%)	7 (13.2%)
Clinical presentation			
Anaphylaxis/anaphylactic shock	24 (12.0%)	17 (9.2%)	10 (18.9%)
Urticaria/angioedema	105 (52.2%)	64 (34.6%)	25 (47.2%)
Exanthema	55 (27.4%)	24 (13%)	7 (13.2%)
Other/unknown cutaneous	4 (2.0%)	57 (30.8%)	—
Other reaction	13 (6.5%)	23 (12.4%)	11 (20.8%)
Reaction type			
<1 h	24 (11.9%)	27 (14.6%)	16 (30.2%)
1–6 h	23 (11.4%)	63 (34.1%)	—
6–24 h	17 (8.5%)	17 (9.2%)	—
>24 h	83 (41.3%)	44 (23.8%)	26 (49.1%)
Unknown	54 (26.9%)	34 (18.4%)	11 (20.8%)
Last drug involved and tested			
Amoxicillin	95 (47.3%)	86 (46.5%)	33 (62.3%)
Ampicillin	6 (3%)	22 (11.9%)	—
Benzylpenicillin	2 (1%)	2 (1.1%)	19 (35.8%)
Phenoxymethylpenicillin	37 (18.4%)	—	—
Cephalosporin 1 generation	18 (9%)	12 (6.5%)	—
Cephalosporin 2 generation	15 (7.5%)	13 (7.0%)	—
Cephalosporin 3 generation	16 (8%)	22 (11.9%)	1 (1.9%)
Other beta-lactams/unknown	12 (6%)	28 (15.1%)	—

Data expressed in frequency and per cent.

*Non-responder data not available for the Portuguese centre. Only patients who responded to the survey are presented.

istics (Table 1) varied among centres and drug classes, even if trends were observed.

Clinical manifestations

Cutaneous reactions, specifically urticaria, angioedema and macular/maculopapular exanthema, were the most frequently reported reactions by patients (at least 75% of them). About 10% of patients had experienced an anaphylactic reaction, which was diagnosed according

Table 2. Beta-lactam follow-up and clinical presentation of reacting patients

	France	Italy	Portugal
Response rate	159 (79.1%)	153 (82.7%)	53 (74.6%)
Follow-up duration	14.8 (9.4–21.1)	48.0 (46.4–48.1)	36.7 (19.6–56.2)
Took the drug	51 (32.1%)	47 (30.7%)	20 (37.7%)
Same drug	25 (49%)	44 (93.6%)	20 (100%)
Same family	26 (51%)	3 (6.4%)	—
Reacted	3	2	4
Urticaria	2	1	2
Exanthema	—	1	2
Undefined	1	—	—
cutaneous reaction			
Non immediate (>6 h)	3	2	4

Data expressed in frequency and per cent or median and 25–75th percentiles.

to the clinical criteria proposed by Sampson et al. (12). Non-immediate reactions were the most common manifestations, especially in the French and Portuguese centres. Amoxicillin represented around one half of the suspected drugs and was the most commonly involved beta-lactam. Thereafter, phenoxymethylpenicillin was the second most commonly involved beta-lactam in the French centre, ampicillin in the Italian ones and benzylpenicillin in the Portuguese one.

Follow-up

Among the 457 patients included in the present study, 365 (79.9%) answered the questionnaire: 159 (79.1%) from the French centre, 153 (82.7%) from the Italian centres and 53 (74.6%) from the Portuguese one (Table 2).

Patients who answered the questions were representative of the study population. There were no major differences in sex ratio, clinical manifestations, delay between the drug administration and the clinical reaction, and the follow-up duration (respectively $P = 0.16$, 0.75 , 0.68 and 0.70 for the French centre and $P = 0.67$, 0.40 , 0.21 and 0.01 for the Italian ones). Comparison was not allowed for Portuguese centre (local ethical reason: not allowed to use data without patient permission).

Among the 118 (32.3%) who took the drug that was previously found negative in allergy/hypersensitive work-up or another related beta-lactam than the one initially tested, nine (7.6%) reported a reaction: three from the French centre, two from the Italian ones and four from the Portuguese one.

Clinical manifestations of reacting patients (Table 2)

With regard to the French centre, three patients reacted to the suspected beta-lactams found to be negative in the diagnostic work-up (amoxicillin in two subjects and ceftriaxone in one). The clinical manifestations were urticaria (in two patients) and an undefined cutaneous reaction (in one). Reactions occurred at least 6 h after the first drug administration. Only one of these subjects accepted an allergy re-evaluation, including the repeated provocation test, which was negative. After this test, a full therapeutic course with amoxicillin was tolerated by the patient.

Two patients from the Italian centres reacted to amoxicillin (the beta-lactam responsible for the previous reaction), experiencing a non-immediate urticaria and an exanthema, respectively. However, none of them agreed to undergo an allergy re-evaluation.

Finally, four patients from the Portuguese centre experienced hypersensitivity reactions to amoxicillin, the drug responsible for the previous ones. All patients had non-immediate (delayed) reactions: two urticarias, one palmar erythema and the remaining one a maculopapular exanthema. Three of them accepted allergy re-evalua-

tions, including repeated challenges: two reacted more than one hour after a full dose and a half dose of amoxicillin, respectively.

Negative predictive value

Among the 118 subjects, nine reported a reaction, and seven were classified as allergic: the five who refused re-evaluations plus the two who reacted to repeated challenges. Therefore, the negative predictive value of provocation tests was 94.1% (89.8–98.3).

Additional information – qualitative analysis

Patients were asked several questions to find out why they took or did not take the beta-lactam found negative in the allergy work-up, or one of the same class. It turned out that in many cases, the drug was not needed. In some cases, the patient took a structurally unrelated alternative because the physician feared a new reaction.

Discussion

The present study includes 457 patients who reported hypersensitivity reactions to beta-lactams and displayed negative results in allergy tests, including challenges. Three hundred and sixty-five (79.9%) of them were contacted for at least 6 month after the initial allergy work-up. Among the 118 patients (25.8%) who took the previously suspected beta-lactam or one of the same class, only 9 (7.6%) experienced a reaction. However, two of them displayed negative results in allergy re-evaluations, which included repeated challenges. Therefore, the negative predictive value after such re-evaluations was 94.1% (89.8–98.3) (111 out of 118 patients).

One limitation of the present study is the fact that it was performed in three different countries at three different periods. Moreover, the allergy work-ups were not completely identical. Specifically, the French centre did not perform serum-specific IgE assays in patients with immediate reactions and after 2004 the Italian and Portuguese centres used Diater penicillin reagents. However, in two recent studies (13, 14), such reagents proved to be a reliable and safe alternative to the Allergopen ones, with a very similar sensitivity. In addition, there were differences with regard to how patients were contacted. For instance, depending on the countries, patients were contacted by phone or by both mail and phone. These limitations may have been increased by the fact that patients involved in the study did not have the exactly same characteristics (depending on the countries). However, the latter limitation appears to be insignificant. Results were very similar in all the countries.

Another limitation is a selection bias. Not all the patients could be contacted (e.g. change of address or telephone number), and some of those contacted did not

answer. However, this bias probably did not affect the results, because the characteristics of the responding population were similar to the ones of the initial population.

Some could argue that the present study was a 're-sensitization study'. However, for different reasons this was not the case. First, in many re-sensitization studies, diagnosis was only assessed by skin tests and challenges were not performed twice restricting the impact of the findings. Second, even if challenges were performed twice, the exposition was limited to the dose and the use of a single use of the beta-lactam. In the present study, the full course of the treatment was studied. Thirdly, some authors assumed that potential cofactors such as fever or infection are involved and needed to express a hypersensitive reaction to drug. The present study being a 'real-life' study, cofactors were present during the treatment course, which is not the case in re-sensitization studies (only 'healthy' patients being tested).

Five of the nine patients who reacted refused allergy re-evaluations. The fact that such patients were classified as allergic probably entailed an overestimation of sensitive patients and, therefore, lowered the negative predictive value of provocation tests. This consideration is reinforced by the fact that only one half (two out four) of the patients re-challenged expressed a reaction. It is also important to note that in many cases, the reaction presented by the patients and so-called 'allergy' was in fact a reaction induced by the infection itself (first cause of maculopapular eruption in context of infection). Moreover, in some cases the second reaction was slightly different from the initial one, without being more severe. Finally, many patients who reacted did not contact their physician. It was therefore difficult to assess the 'real' reaction.

This negative predictive value is with accordance to a previous study performed by Macy et al. (15), even if the

study design was different. Among 568 subjects with a suspicion of allergic reaction to penicillin and a negative work-up, 71 reactions were reported in 65 (11.4%) subjects. This negative predictive value (88.6%) was probably underestimated since the allergic work-up was restricted to skin tests. It is now well known that some patients who experienced either immediate or non-immediate reactions to beta-lactams can present positive challenges with negative skin tests (16–20). Specifically, in a study by Torres et al. (16), 49 (16.9%) of 290 patients with immediate reactions to penicillins displayed negative results in both skin tests and serum-specific IgE assays and reacted to challenges. More recently, the same group demonstrated that the sensitivity of skin testing is low in both adults and children reporting non-immediate reactions to beta-lactams (19, 20). Indeed, challenges were required in addition to skin tests to establish a diagnosis of hypersensitivity in 20 of 22 adults (19) and 19 of 20 children (20).

It is interesting to note that the number of patients who took the previously suspected beta-lactam or one of the same class was low (118 out of the 365 who answered the questions, 32.3%). This was partly because the fact that both patients and physicians were afraid of a new reaction and preferred to avoid the drug. This entails another selection bias. However, no link was observed between the drug avoidance and the initial clinical reaction type.

Finally, among the patients who reacted, none experienced a manifestation more severe than the previous one.

In conclusion, although the negative predictive value of drug provocation tests is not 100%, it is quite high (94.1%), and none of the false negative patients experienced a life-threatening reaction. Therefore, we hope that this study – in spite of its limitations – will be useful in reassuring doctors who otherwise might hesitate to prescribe beta-lactams suspected of having caused adverse reactions and found negative in allergy work-ups.

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