

Short communication

Benzylpenicillin skin testing is still important in diagnosing immediate hypersensitivity reactions to penicillins

Background: The fact that both Hollister-Stier and Allergopharma ceased the production of penicilloyl-polylysine (PPL) and minor determinant mixture (MDM) in 2004 is severely hampering the diagnosis of β -lactam hypersensitivity and may produce negative consequences.

Objective: To assess the contribution of skin testing with benzylpenicillin to the diagnosis of immunoglobulin E-mediated hypersensitivity to penicillins, in order to determine how much such testing could compensate for PPL and MDM unavailability.

Methods: We selected patients with histories of immediate reactions to penicillins and positive results to skin tests for one or more penicillin reagents (PPL, MDM, or benzylpenicillin), one or more semi-synthetic penicillins (ampicillin, amoxicillin, or piperacillin), or both.

Results: A total of 300 patients were selected, 105 in the French center and 195 in the Italian centers. Amoxicillin and ampicillin were the main responsible drugs. The most common clinical manifestation was anaphylaxis. The reagents most frequently positive to skin tests were amoxicillin (188, 62.7%), ampicillin (151, 50.3%), and benzylpenicillin (111, 37.0%). Among the 300 subjects, 113 (37.7%) were positive only to semi-synthetic penicillins, 109 (36.3%) to both semi-synthetic penicillins and the classic penicillin reagents, and 78 (26.0%) only to the latter. In the last group, 64 (21.3% of the 300 subjects) were positive only to PPL and/or MDM and 14 (4.7%) to benzylpenicillin, of whom 8 (2.7%) were positive only to the latter.

Conclusions: Skin testing with benzylpenicillin can partially compensate for PPL and MDM unavailability. Moreover, it can slightly increase the allergologic workup's sensitivity and therefore reduce the number of potentially dangerous challenges.

**A. Romano^{1,2}, L. Bousquet-Rouanet³,
M. Viola¹, F. Gaeta¹, P. Demoly^{3,4},
P.-J. Bousquet^{3,5}**

¹Department of Internal Medicine and Geriatrics, UCSC-Allergy Unit, Complesso Integrato Columbus, Rome, Italy; ²IRCCS Oasi Maria S.S., Troina, Italy;

³Exploration des Allergies, Hôpital Arnaud de Villeneuve, Montpellier Cedex 5, France; ⁴INSERM, U657, Montpellier, France; ⁵Département Biostatistique, Epidemiologie Clinique, Santé Publique et Information Médicale, Hôpital Carémeau, Nîmes Cedex 9, France

Key words: benzylpenicillin; Drug Allergy and Hypersensitivity Database; hypersensitivity; penicillins; skin tests.

Prof. Antonino Romano
Unità di Allergologia
Complesso Integrato Columbus
Via G. Moscati, 31
00168 Rome
Italy

Accepted for publication 18 July 2008

Skin tests are the most readily available diagnostic tools and continue to be essential for evaluating the subjects with hypersensitivity reactions to penicillins. Indeed, in such patients, the clinical history is not reliable (1, 2). In both the American practice parameters (3) and the European Network for Drug Allergy (ENDA) position paper (4), skin testing with penicilloyl-polylysine (PPL) and minor determinant mixture (MDM) represents the first-line method for evaluating immediate (i.e. occurring within 1 h after the last drug administration) reactions to β -lactams. Recent studies have emphasized the importance of skin testing with PPL and MDM in diagnosing β -lactam hypersensitivity (5–7). However, the fact that

both Hollister-Stier and Allergopharma ceased the production of penicillin reagents in 2004 is severely hampering the diagnosis of β -lactam hypersensitivity and may produce negative consequences. In effect, a diagnostic approach based only on the clinical history might erroneously classify many patients suspected of being sensitive to β -lactams, but who could have a good tolerance after an appropriate allergologic workup. On the other hand, a protocol, which does not include skin tests with PPL and MDM, might not diagnose many cases of β -lactam hypersensitivity. In two recent series of subjects with hypersensitivity reactions to β -lactams (5, 6), 14.7% and 47.0% of positive cases would have been missed, respectively, if PPL and MDM had not been used in evaluating them.

In this cohort study, using information from our database, we assessed the contribution of skin testing with benzylpenicillin to the diagnosis of immunoglobulin E

Abbreviations: DAHD, Drug Allergy and Hypersensitivity Database; ENDA, European Network for Drug Allergy; IgE, immunoglobulin E; MDM, minor determinant mixture; PPL, penicilloyl-polylysine.

(IgE)-mediated hypersensitivity to penicillins, in order to determine how much such testing could compensate for PPL and MDM unavailability.

Patients and methods

Patients

Subjects were recruited prospectively from a large outpatient population with histories of immediate reactions to at least one penicillin confirmed by positive skin tests. This population was evaluated between September 1996 and April 2007 in the allergy units of Complesso Integrato Columbus (Rome, Italy), Oasi Maria Santissima (Troina, Italy), and Hôpital Arnaud de Villeneuve (Montpellier, France). This historical-prospective cohort study is based on a part of a very large cohort: the Drug Allergy and Hypersensitivity Database (DAHD), which uses the File Maker Pro™ (Filemaker, Santa Clara, CA, USA) and is available on-line. More generally, the DAHD collects information on suspected drug hypersensitivity reactions and the related allergologic examinations.

Skin tests

All subjects were prick and intradermal tested with the classic penicillin reagents: PPL (Allergopharma, Reinbeck, Germany), MDM (Allergopharma), and benzylpenicillin. The final concentrations were, respectively, 5×10^{-5} mM/l, 2×10^{-2} mM/l, and 10 000 IU/ml (Italian centers) and 25 000 IU/ml (French center). Because Allergopharma ceased the production of penicillin reagents, from July 2005 the Italian centers used Diater S.A. (Madrid, Spain) reagents: PPL (final concentration: 1.07×10^{-2} mM/l) and MDM (benzylpenicillin, sodium benzylpenicilloate, and benzylpenicilloic acid; final concentration: 1.5 mM/l). In the French center, these reagents being not available, the study was therefore stopped earlier than in the Italian centers. Semi-synthetic penicillins (ampicillin and amoxicillin) were also skin tested in all patients. The final concentrations were 20 mg/ml 0.9% NaCl in the Italian centers and 25 mg/ml in the French center. Piperacillin was routinely used only in the Italian centers at a final concentration of 20 mg/ml 0.9% NaCl (Fig. 1).

In the Italian centers, PPL, MDM, and benzylpenicillin were tested on the first day and amoxicillin, ampicillin, and piperacillin on the second day. In the French center, all skin tests were performed on the same day.

All of the above reagents were initially tested on the volar forearm skin by the prick method and the reactions were considered positive when a wheal larger than 3 mm in diameter with surrounding erythema was present 20 min later. When prick tests were negative, 0.02 ml of the reagent solution was injected intradermally on the volar forearm skin. Readings were made 20 min after injections. Results were considered positive when larger than 3 mm increase of the initial wheal diameter accompanied by erythema was present (8). Positive controls for prick tests were performed with histamine at 10 mg/ml. As a negative control for prick and intradermal tests, 0.9% NaCl was used.

The concentrations of piperacillin and benzylpenicillin, as well as the final concentrations of Diater PPL and MDM used for skin testing in the Italian centers, have been validated in previous studies

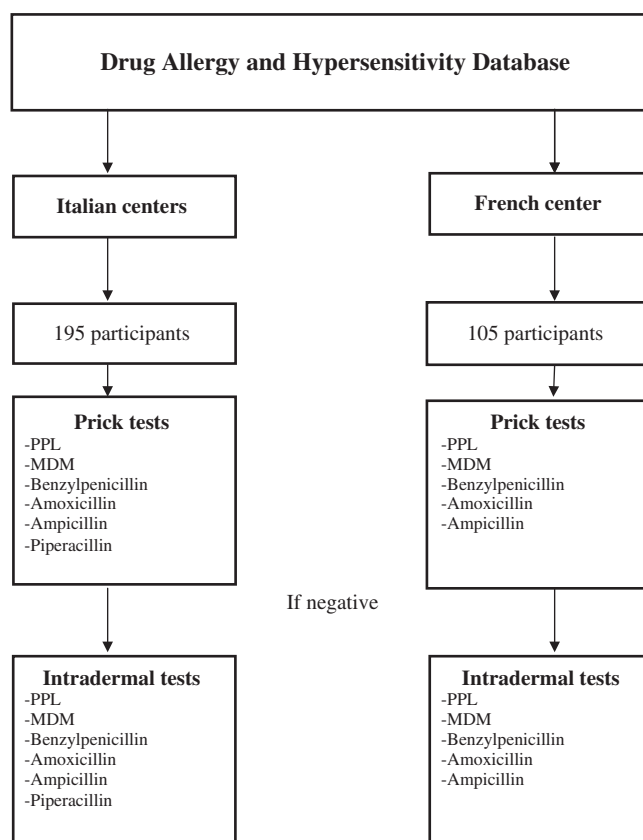


Figure 1. Study design.

of ours (9–11). The highest benzylpenicillin concentration used in the French center was nonirritant in a control group of 40 healthy subjects, 20 of whom had previously tolerated benzylpenicillin.

Statistical analysis

Qualitative variables, such as symptoms and proportion of positive tests, were described using frequencies and percentages. Age and the time interval between the last adverse reaction and testing were reported as median and 25th–75th percentiles. Comparison between centers was performed using the chi-squared test or Wilcoxon–Mann–Whitney test.

Results

A total of 300 patients had at least one immediate positive skin test (prick and/or intradermal reaction), 105 (35.0%) in the French center and 195 (65.0%) in the Italian centers (Table 1). Among them, 103 (34.3%) were male patients, 40 (13.3%) and 114 (38.0%) were asthmatic and atopic (having at least one positive skin test to the most common aeroallergens) respectively, and the median age was 44.0 (32.0–58.0). There was no difference between centers concerning the sex ratio [38 (36.2%) males vs 65 (33.3%), $P = 0.58$], but asthmatics [28 (26.7%) vs 12

Table 1. Clinical characteristics of the patients assessed, broken down by center

	French center	Italian centers	Overall
<i>n</i>	105	195	300
Sex (male, %)	38 (36.2)	65 (33.3)	103 (34.3)
Age (years)	39.0 (24.0–51.0)	47.0 (35.0–61.0)	44.0 (32.0–58.0)
Time-interval*	22.3 (8.3–186.3)	5.0 (2.0–24.0)	8.4 (2.0–48.0)
Asthma (%)	28 (26.7)	12 (6.2)	40 (13.3)
Atopy (%)	71 (67.6)	43 (22.2)	114 (38)
Type of reaction (%)			
Anaphylaxis	35 (33.3)	121 (62.1)	156 (52)
Urticaria/angioedema	38 (36.2)	73 (37.4)	111 (37)
Other/unknown reaction	32 (30.5)	1 (0.5)	33 (11)
Responsible drug (%)			
Amoxicillin	51 (48.6)	90 (46.2)	141 (47)
Benzylpenicillin	0	19 (9.7)	19 (6.3)
Ampicillin	7 (6.7)	70 (35.9)	77 (25.7)
Piperacillin	1 (1)	16 (8.2)	17 (5.7)
Other/unknown penicillin	46 (43.8)	0	46 (15.3)

*Time (months) elapsed between last adverse reaction and current allergologic examination.

(6.2%), $P < 0.001$] and atopics [71 (67.6%) vs 43 (22.2%), $P < 0.001$] were more frequent in the French center. Subjects were older in the Italian centers [47.0 (35.0–61.0) vs 39.0 (24.0–51.0), $P < 0.001$].

Amoxicillin and ampicillin were the main responsible drugs. The most common clinical manifestation was anaphylaxis (156, 52.0%), which was diagnosed according to the clinical criteria proposed by Sampson et al. (12), and was more frequent in the Italian centers [121 (62.1%) vs 35 (33.3%), $P = 0.001$]. Many French patients did not recall the type of reaction or the name of the responsible penicillin. The time interval between the last adverse reaction and testing was shorter in the Italian centers [5.0 (2.0–24.0) vs 22.3 (8.3–186.3) months, $P < 0.001$] (Table 1).

Among the 300 subjects, 78 (26.0%) were positive only to the classic penicillin reagents (PPL, MDM, and benzylpenicillin), 109 (36.3%) to both the classic penicillin reagents and semi-synthetic penicillins, and 113 (37.7%) only to the latter. Overall, 222 (74.0%) of the 300 subjects were positive to at least one semi-synthetic penicillin (amoxicillin, ampicillin, or piperacillin), and 187 (62.3%) were positive to the classic penicillin reagents. The reagents most frequently positive to skin tests were amoxicillin (188, 62.7%), ampicillin (151, 50.3%), and benzylpenicillin (111, 37.0%). The rate of subjects with positive skin tests to ampicillin was significantly higher in the Italian centers, while that of subjects who displayed positive skin test responses only to amoxicillin was significantly higher in the French center (Table 2).

As far as subjects positive only to the classic penicillin reagents are concerned, 64 (21.3% of the 300 subjects) were positive only to PPL and/or MDM and 14 (4.7%) to benzylpenicillin, of whom 8 (2.7%) were positive only to the latter (Tables 2 and 3). These eight patients reacted

Table 2. Positive results to skin tests

	French center	Italian centers	Overall	<i>P</i> -value
Positive skin tests				
PPL	30 (28.6)	52 (26.7)	82 (27.3)	0.72
MDM	37 (35.2)	63 (32.3)	100 (33.3)	0.61
Benzylpenicillin	31 (29.5)	80 (41.0)	111 (37.0)	0.05
Amoxicillin	76 (72.4)	112 (57.4)	188 (62.7)	0.01
Ampicillin	39 (37.1)	112 (57.4)	151 (50.3)	<0.001
Piperacillin*	2 (1.9)	64 (32.8)	66 (22.0)	
One or more semi-synthetic penicillins	79 (75.2)	143 (73.3)	222 (74.0)	0.72
One or more classic penicillin reagents	62 (59.0)	125 (64.1)	187 (62.3)	0.40
Subjects positive only to the classic penicillin reagents	26 (24.8)	52 (26.7)	78 (26.0)	0.72
Positive only to PPL and/or MDM	23 (21.9)	41 (21.0)	64 (21.3)	0.86
Positive to benzylpenicillin	3 (2.9)	11 (5.7)	14 (4.7)	0.28
Of whom monosensitive to benzylpenicillin	1 (1)	7 (3.6)	8 (2.7)	0.27
Subjects positive only to semi-synthetic penicillins	43 (41.0)	70 (35.9)	113 (37.7)	0.39
Of whom positive only to amoxicillin	23 (21.9)	14 (7.2)	37 (12.3)	<0.001
Of whom positive only to ampicillin	2 (1.9)	8 (4.1)	10 (3.3)	0.26
Of whom positive only to piperacillin	1 (1)	10 (5.1)	11 (3.7)	0.10
Subjects positive to both the classic penicillin reagents and semi-synthetic penicillins	36 (34.3)	73 (37.4)	109 (36.3)	0.59
Of whom positive to benzylpenicillin	28 (26.7)	69 (35.4)	97 (32.3)	0.15

Values given in parenthesis are percentages.

Semi-synthetic penicillins: amoxicillin, ampicillin and piperacillin. Classic penicillin reagents: penicilloyl-polylysine (PPL) and minor determinant mixture (MDM), and benzylpenicillin.

*Piperacillin was not systematically tested in the French center.

Table 3. Patients positive only to benzylpenicillin

Sex	Asthma	Atopy	Age (years)	Time interval*	Type of reaction	Responsible drug
F	No	No	62	2	Anaphylaxis	Ampicillin
F	No	No	37	8	Anaphylaxis	Amoxicillin
F	No	No	75	300	Anaphylaxis	Ampicillin
F	No	No	75	420	Anaphylaxis	Benzylpenicillin
F	Yes	Yes	55	459	Other reaction	Benzylpenicillin
F	No	No	69	2	Urticaria/angioedema	Ampicillin
M	No	No	30	3	Urticaria/angioedema	Amoxicillin
F	No	No	37	37	Urticaria/angioedema	Ampicillin

*Time (months) elapsed between last adverse reaction and current allergic examination.

to the highest concentrations used in the centers: 25 000 IU/ml (=42.09 mM/l) in the French center and 10 000 IU/ml in the Italian centers (=16.84 mM/l).

Among the 111 subjects positive to benzylpenicillin, 30 (27.0%), 62 (55.9%), 90 (81.1%), 83 (74.8%), and 39 (35.1%) were also positive to PPL, MDM, amoxicillin, ampicillin, and piperacillin, respectively. Of these 111 patients, 31 (27.9%) came from the French center, and 80 (72.1%) from the Italian centers. Only 15 (13.5%) were asthmatic and 30 (27.0%) atopic.

Discussion

We studied a total of 300 subjects with histories of immediate reactions to penicillins and positive skin tests to at least one penicillin reagent. Comparison between the centers shows some differences in asthma and atopy frequencies explainable by the differences in the center populations: unlike the Italian centers, the French center is located in a pulmonary department and focused on respiratory allergies. The difference in the time interval between the last adverse reaction and the allergologic examination (longer in the French center) and the unavailability of pertinent medical records may well explain why numerous French patients did not recall the type of reaction or the name of the responsible penicillin. In any case, such patients reported reactions to the penicillin class and thus were tested.

The fact that we did not precisely follow the ENDA diagnostic algorithms for evaluating immediate reactions to β -lactams (4) might be considered a limitation of the present study. In effect, these algorithms advise performing serum specific IgE assays first and then, in case of negative results, skin testing with PPL, MDM, and the suspected drug. However, the primary objective of our study was to assess the contribution of skin testing with benzylpenicillin and semi-synthetic penicillins to the diagnosis of IgE-mediated reactions to penicillins, in order to determine how much such testing, especially with benzylpenicillin, could compensate for PPL and MDM unavailability. We therefore skin tested all our subjects with a fixed panel of reagents, the classic penicillins and some semi-synthetic penicillins (three in the Italian centers, and two in the French center).

As observed in the previous studies of ours concerning subjects with IgE-mediated hypersensitivity to penicillins (9, 11), a high percentage (74%) of the 300 subjects of the present study displayed positive responses to skin tests with semi-synthetic penicillins. Thus, skin testing with the latter alone could have diagnosed an immediate hypersensitivity in 222 subjects, about half of whom (113, 50.9%) were negative to the classic penicillin reagents, while 55 (48.7%) of the latter subjects were positive to at least two semi-synthetic penicillins (Table 2).

The fact that the great majority of the French patients who were able to report the name of the responsible penicillin had reacted to amoxicillin, while about half of the Italian patients had reacted to amoxicillin, more than one-third to ampicillin, and the rest to benzylpenicillin or piperacillin (Table 1) can explain the lower rate of the French subjects with positive skin tests to ampicillin, and the higher rate of such subjects with positive skin test responses to amoxicillin, including those with positive results only to the latter (Table 2).

As far as the 187 subjects (62.3%) positive to the classic penicillin reagents are concerned, 78 (26.0% of the 300 subjects) were positive only to them and 109 also to semi-synthetic penicillins. Of the former 78 subjects, 64 were positive only to PPL and/or MDM, eight only to benzylpenicillin, and six to benzylpenicillin and PPL and/or MDM. Therefore, there was only a small overlap between the patients reacting to benzylpenicillin and those reacting to PPL and/or MDM. In any case, skin testing with the classic penicillin reagents, particularly PPL and MDM, remains essential in the diagnosis of immediate reactions to penicillins. In effect, 64 (21.3%) of the 300 positive subjects would have been missed if PPL and MDM had not been used in the diagnostic protocol.

As previously stated, the final concentrations of Diater PPL and MDM used for skin testing in the Italian centers have been validated in a previous study of ours (10), which compared the performance of PPL and MDM from Allergopharma (Allergopen) with that of Diater (DAP), proving that DAP reagents are a reliable alternative to Allergopen ones. Therefore, even though the final concentrations of the DAP reagents used in the Italian centers were higher than those recommended in the ENDA position paper (4), false-positive results to skin testing with these reagents are highly unlikely.

In the eight patients (2.7% of the 300) monosensitive to benzylpenicillin, negative results in skin testing with MDM, which contains benzylpenicillin, could be explained by the fact that the final concentrations of benzylpenicillin used are higher than those of the Allergopharma and Diater MDM, i.e. 1.68 and 1.40 mM/l, respectively.

In conclusion, skin tests with benzylpenicillin can compensate to a certain extent for PPL and MDM unavailability. Moreover, it can slightly increase the sensitivity of the allergologic workup for evaluating subjects with immediate reactions to penicillins and therefore reduce the number of potentially dangerous challenges. In effect, our study proves that a small percentage of such subjects can present negative results to skin tests with PPL and MDM and positive results to skin tests with benzylpenicillin. Therefore, even though benzylpenicillin cannot fully replace PPL and MDM, it is advisable to include benzylpenicillin among the reagents of the allergologic workup for evaluating subjects with immediate reactions to penicillins.

References

1. Solensky R, Earl HS, Gruchalla RS. Penicillin allergy: prevalence of vague history in skin-test positive patients. *Ann Allergy Asthma Immunol* 2000; **85**:195–199.
2. Wong BBL, Keith PK, Wasserman S. Clinical history as a predictor of penicillin skin test outcome. *Ann Allergy Asthma Immunol* 2006; **97**:169–174.
3. Executive summary of disease management of drug hypersensitivity: a practice parameter. Joint Task Force on Practice Parameters, the American Academy of Allergy, Asthma and Immunology, the American Academy of Allergy, Asthma and Immunology, and the Joint Council of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1999; **83**:665–700.
4. Torres MJ, Blanca M, Fernandez J, Romano A, de Weck A, Aberer W et al. Diagnosis of immediate reactions to beta-lactam antibiotics. *Allergy* 2003; **58**:961–972.
5. Bousquet PJ, Co-Minh HB, Arnoux B, Daures JP, Demoly P. Importance of mixture of minor determinants and benzylpenicilloyl poly-L-lysine skin testing in the diagnosis of β -lactam allergy [Letter]. *J Allergy Clin Immunol* 2005; **115**:1314–1316.
6. Matheu V, Pérez-Rodríguez E, Sánchez-Machin I, de la Torre F, García-Robaina JC. Major and minor determinants are high-performance skin tests in β -lactam allergy diagnosis [Letter]. *J Allergy Clin Immunol* 2005; **116**:1167–1168.
7. Torres MJ, Blanca M. The contribution of major and minor determinants from benzylpenicillin to the diagnosis of immediate allergy to β -lactams [Letter]. *J Allergy Clin Immunol* 2006; **117**:220–221.
8. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002; **57**:45–51.
9. Romano A, Guéant-Rodriguez RM, Viola M, Pettinato R, Guéant JL. Cross-reactivity and tolerability of cephalosporins in patients with immediate hypersensitivity to penicillins. *Ann Intern Med* 2004; **141**:16–22.
10. Romano A, Viola M, Bousquet JP, Gaeta F, Valluzzi R, Caruso C et al. A comparison of the performance of two penicillin reagent kits in the diagnosis of β -lactam hypersensitivity. *Allergy* 2007; **62**:53–58.
11. Romano A, Viola M, Guéant-Rodriguez RM, Gaeta F, Valluzzi R, Guéant JL. Tolerability of meropenem in patients with IgE-mediated hypersensitivity to penicillins. *Ann Intern Med* 2007; **146**:266–269.
12. Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A et al. Second symposium on the definition and management of anaphylaxis: Summary report – Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006; **117**:391–397.