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PPL and MDM skin test: New test kit is helpful in detecting immediate-type allergy to beta-lactams

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Key words
- drug allergy
- beta-lactam
- penicillin
- allergy test

Summary

Background: The diagnosis of PPL (major determinant) and MDM (minor determinant) sensitization as relevant allergens in beta-lactam allergy has been recently hampered by withdrawal from the market of formerly available test kits. We investigated a new PPL/MDM test kit in the work-up of beta-lactam allergy.
Patients and Methods: 15 patients with history of beta-lactam allergy were investigated for specific IgE and received patch, skin prick (SPT) and intracutaneous tests (ICT; immediate and late readings) using the relevant beta-lactams. In addition the new test kit was used for parallel SPT and ICT.
Results: 14 women and 1 man (16–73 years) with immediate (n=7), delayed (n=7) or unclear (n = 1) reactions to beta-lactams 8–300 months previously (penicillin G/V n = 3, aminopenicillins n = 7, cephalosporins n = 4, unknown n = 2) were tested. In patients with immediate type reactions, n = 2 had specific IgE, n = 4 reacted to the new test kit (n = 3 MDM, all of whom reacted exclusively to this test, n = 1 PPL). Two patients with non-immediate reactions reacted to other beta-lactams.
Conclusions: Our data show that the new test kit may be helpful in detecting patients with immediate type allergy to beta-lactams. Without this test, in those three patients reacting exclusively to MDM, and oral provocation test would have been necessary to clarify their allergy. Data from larger groups of patients are needed to determine the sensitivity and specificity of this test kit.

Introduction

Due to their favorable risk-benefit ratios, beta-lactams are still antibiotics of first choice for many infections such as for syphilis and during pregnancy. Among beta-lactams, amoxicillin is presently the most frequently prescribed in Europe. At the same time, beta-lactams are among the antibiotics most frequently causing intolerance reactions. Depending on the time interval between intake and appearance of signs and symptoms, intolerance reactions are classified as immediate or delayed reactions. Up to ten percent of the German population reports an allergy to penicillin. Among treated patients an immediate reaction in form of urticaria/angioedema can be expected in 0.2 %, anaphylaxis in 0.0004 to 0.015 % and most commonly delayed reactions in 1–4 % [1–3].

When beta-lactam allergy is suspected, it is usually possible to use an alternative antibiotic. If urgent reasons exist for choosing beta-lactam antibiotics, one turns to allergy diagnostics for clarification. This can occur in patients with polyvalent antibiotic intolerances, during pregnancy when it may be difficult to administer other antibiotics or when diseases such as neurosyphilis or neuroborreliosis urgently demand beta-lactams. A step-by-step diagnostic approach is recommended, with *in vivo* tests only following *in vitro* tests [1–3].

Beta-lactams are small molecules that serve as haptens. They can cause IgE-mediated immediate reactions and delayed reactions often mediated by T

cells. These reactions can, in principle, be caused by all beta-lactams: penicillins, cephalosporins, carbapenems and monobactams (Figure 1) [1, 4].
Frequently prescribed aminopenicillins more commonly lead to delayed reactions, while penicillin G and penicillin V tend to cause immediate reactions [1, 2, 5]. Not only immunological reactions but also non-immune mechanisms such as parainfectious effects, drug interactions and direct effects of beta-lactams on vascular endothelia can cause symptoms. In rare, severe bullous skin reactions, in addition to immunological and apoptotic mechanisms, possibly genetic variations in drug metabolism may play an important role [1].
Well-known allergenic structures are derived from the instable beta-lactam ring on the one hand, from side chains on the other, and rarely from the second ring (e.g. thiazolidine ring). From the beta-lactam ring the major allergen known as benzylpenicilloyl poly-L-lysin (PPL) as well as a series of minor allergens (e.g. penilloate, penicilloate, penicillin, penicanyl) also known as minor determinant mix (MDM) are derived [1, 6, 7]. In diagnosing beta-lactam allergy it is recommended to test these major and minor allergens in particular [1, 6–9]. In Germany a commercially available test kit was withdrawn from the market on economic grounds. A company in Spain has been working on a replacement (Figure 2) which we studied in the diagnostics of beta-lactam allergy.

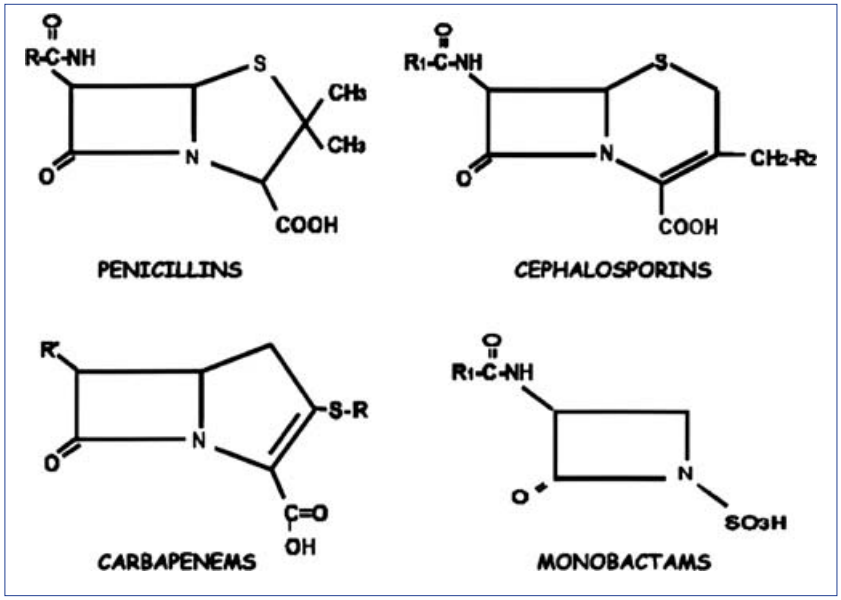

Figure 1: History of adverse reactions to beta-lactams and results of allergy tests (n. d. – not done, Ax – amoxicillin, Amp – ampicillin, Pen. – penicillin).

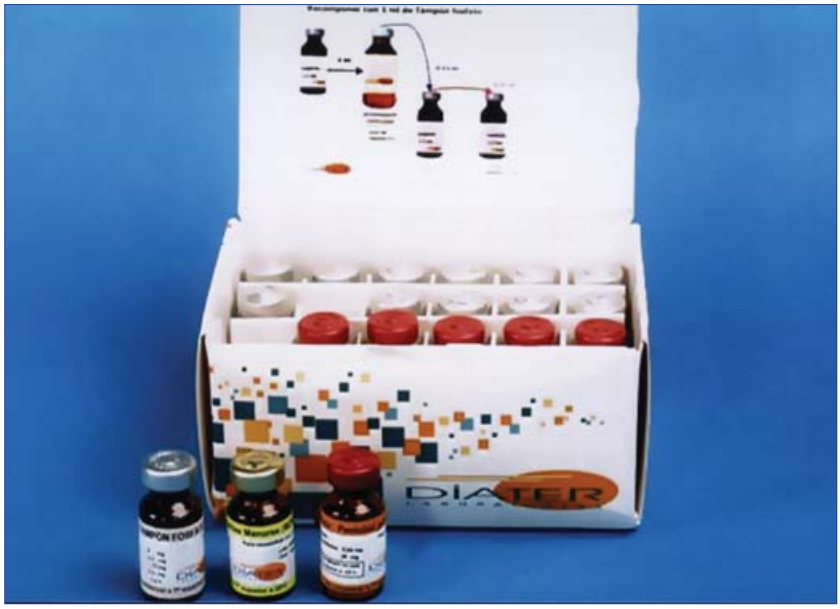

Figure 2: PPL and MDM reactivity in skin tests is reported more often in patients with immediate reactions compared to those with delayed reactions.

Patients and methods

Patients presenting at our Department of Dermatology, Venereology and Allergy between January and June 2006 to clarify a possible beta-lactam allergy underwent the following step-by-step work-up:
1. *History* especially concerning the clinical picture of the intolerance reaction (time point, possible classification into immediate or delayed reaction, administered drug),
2. *In vitro* diagnostics with determination or total IgE and specific IgE to penicillin G, penicillin V, amoxicillin and ampicillin and with appropriate history also to cephalosporins, as well as serum tryptase in cases of anaphylactic immediate reactions (CAP-FEIA, Sweden Diagnostic, Freiburg,Germany),
3. *Patch testing* (epicutaneous test, ECT) with commercially available drugs containing penicillin G, penicillin V, amoxicillin and ampicillin and with appropriate history suspected cephalosporins applied according to the recommendations for patch testing [13, 15]. Interpretation was done according to the guidelines of the German Contact Dermatitis Research Group,
4. *Prick and intracutaneous testing (ICT)* with reading after 15 minutes and again after 24–48 h for delayed reactions using aqueous solutions of the above mentioned medications; in ICT only sterile aqueous solutions of penicillin G and ampicillin diluted 1:10 in 0.9 % NaCl should be employed. Parallel to testing with these substances, tests were performed with PPL and MDM of the test kit of the firm DIATER (Madrid, Spain, diater@diater.com). This kit contains a vial PPL with 0.04 mg benzylpenicilloyl poly-L-lysin (PPL) + 20 mg mannitol, a vial MDM with 0.5 mg each of benzylpenicillin, benzylpenilloate and ben-

Table 1: History of adverse reactions to beta-lactams and results of allergy tests (n. d. – not done, Ax – amoxicillin, Amp – ampicillin, Pen. – penicillin).

No.	Age in years	Months since reaction	Suspected drug	Spec. IgE	ECT	Prick test (reading)	ICT (reading)
Immediate reactions							
1	62 ♀	6	Amp	Amp	n. d.	–	**PPL** (after 15 min.)
2	39 ♀	14	Ceftriaxone	–	–	–	PG, Ceftriaxone, **MDM** (after 15 min.)
3	38 ♀	5	Cephalexin	–	–	–	**MDM** (after 15 min.)
4	38 ♀	60	PenG	–	n. d.	–	
5	26 ♀	5	PenV	PenG PenV Amp Ax	–	–	
6	16 ♀	4	Cefaclor	–	–	**MDM** (after 15 min.)	
7	58 ♀	12	Ax	–	–	–	
Delayed reactions							
8	27 ♀	8	Ax	–	Ax	–	Ax (after 24–48 h)
9	43 ♀	12	Ax	–	–	–	–
10	58 ♀	25	Amp	–	–	–	–
11	47 ♀	36	PenV	–	–	–	–
12	73 ♀	60	?	–	–	–	–
13	62 ♀	36	Ax	–	–	–	–
14	72 ♀	8	Ax Ceftriaxone	–	–	Ax Ceftriaxone (after 24–48 h)	–
Unclear reaction							
15	62 ♀	300	?	–	–	–	–

zylpenicilloate + 20 mg mannitol as well as a solvent containing sodium chloride, potassium dihydrogen phosphate, disodium hydrogen – 2- hydroxy phosphate, potassium chloride and aqua p. infusion. Testing was performed according to the recommendation of the manufacturer in an escalating algorithm: first prick test with PPL pure, then ICT with a 1:10 dilution and finally pure. MDM was tested correspondingly. The following results were considered positive: immediate reading after 15 minutes: prick test with wheal ≥ 3 mm, ICT with increase of wheal size ≥ 3 mm in comparison to the initial wheal after application, in the delayed reading after 24 and 48 h: palpable infiltrate > 5 mm. When the prick test was positive, ICT was not performed.

5. *Oral exposition* of an alternative antibiotic was recommended to all patients with beta-lactam allergy. All patients gave written consent for *in vivo* and *in vitro* testing.

6. Statistics. The median was calculated for the age as well as the latent period between clinical reaction and testing. Using the Mann-Whitney-Wilcoxon-U test, the latent period was compared between the following patient groups: 1) Immediate vs. delayed reaction, and 2) Skin test-positive vs. skin test-negative.

Results

Fifteen patients (14 ♀,1 ♂, age 16–73 years, median 39) were evaluated accord-

Table 2: PPL and MDM reactivity in skin tests is reported more often in patients with immediate reactions compared to those with delayed reactions.

Author	Type of reaction	n	Skin test positive	PPL positive	MDM positive	Ax. pos.	Amp pos.
Gadde et al. 1993 [16]	All Immediate/ anaphylactic Immediate/ urticaria Delayed/ exanthema	776 Not stated Not stated Not stated	7.1 % 17.3 % 12.4 % 4 %			n. d.	n. d.
Walker et al. 2000 [3]	All Immediate Delayed	149 73 76	28.2 % 26 % 30 %	28.2 % 26 % 30 %		n. d.	n. d.
Torres et al. 2001 [21]	Immediate Anaphylaxis Urticaria	290 206 54	70 %	22 %	21 %	43 %	31 %
Romano et al. 2002 [10]	Delayed	259	39 %	9 %	3 %	39 %	39 %
Torres et al. 2004 [17]	Delayed	20	100 %	0 %	0 %	100 %	100 %
Kalogeromitros et al. 2004 [18]	All	18	72.2 %	72.2 %		n. d.	n. d.
Bousquet et al. 2005 [8]	All	829	16.5 %	16.5 % (only PPL 4.4 % only MDM 6.6 % only PPL + MDM 3.7 %)		n. d.	n. d.

ing to the protocol depicted above (Table 1). Seven immediate and seven delayed reactions were identified; one reaction occurring a very long time ago (pat. 15) could not be definitively be classified. The immediate reactions consisted of urticaria combined with respiratory distress in two patients (pat. 2, 3) and in a further case with respiratory distress and hypotension (pat. 1). Among the delayed reactions maculopapular exanthemas (n = 5, pat. 8–12), erythroderma (pat. 14) and a bullous fixed drug eruption of the oral mucosa (pat. 13) were recorded. The interval between the clinical intolerance reaction and testing ranged between 5–300 months, with the median for immediate reactions clearly less than for delayed reactions (5 vs 36 months; $p<0.05$). Patients with a positive skin test had a clearly shorter latent period than those with a negative skin test (median 7 vs 36 months, $p<0.03$). The administered beta-lactams were penicillin G (n = 1), penicillin V (n = 2), ampicillin (n = 2), amoxicillin (n = 5) and cephalosporin (n = 4), with no reliable data for patients 12 and 15. Average total IgE was 143 kU/l (43–1043 kU/l). Specific IgE was found in patients 1 and 5 with immediate reactions in one case towards the suspected ampicillin and one towards penicillin V, penicillin G, ampicillin and amoxicillin following a reaction towards penicillin V. Tryptase levels were in the normal range in all patients.

Patch testing was positive for amoxicillin in patient 8, who reported an immediate reaction against this drug. In prick testing one positive immediate reaction to MDM was observed in a patient with an immediate reaction after intake of cefaclor (pat. 6) and interestingly towards both amoxicillin and ceftriaxone in a patient with immediate reaction after combined administration (pat. 14). ICT in the reading after 15 minutes was positive for PPL (n = 1) in patient 1 after ampicillin administration, for MDM (n = 2) in patients 2 and 3 after cephalosporin administration, with patient 3 also reacting to penicillin G and cefaclor, as well as for amoxicillin in patient 8 with a delayed reaction in the

delayed reading. No patient displayed a systemic intolerance reaction during testing with the new test kit.
A total of six of our 15 patients (40 %) displayed a positive skin test with 4/7 patients (58 %) with immediate reactions being positive, but no patient with an unclear or delayed reaction tested positive with the new test kit. Oral exposition, usually with an alternative antibiotic, was up to now performed in 6/15 patients and well-tolerated. One patient (pat 14) accidentally received amoxicillin again, after erythroderma was not attributed to this antibiotic, and developed the same skin reaction.

Conclusions

In our study seven of fifteen tested patients reacted positively with five having a history of immediate and two a history of delayed reactions and the interval since the reaction was a maximum of 14 months, with longer intervals in all patients with negative tests. This is compatible with the data of other groups, which show that the greatest likelihood of positive tests to identify the responsible drug exists in an interval three weeks to three months after the intolerance reaction, In immediate reactions towards penicillins test reactivity profoundly decreases > 2 years after the last clinical reaction, while after delayed reactions, test reactivity can remain constant for > 6 years [1, 5, 10].
We could identify specific IgE antibodies towards beta-lactams in two of seven patients with immediate reaction, of these only one had a positive skin test (towards PPL). The sensitivity of IgE measurements *in vitro* is estimated at between 38 and 57 %; specificity is reported to be 87 to 100 % [1, 11, 12].
According to current recommendations, *in vivo* testing consists of skin testing, in immediate reactions in form of prick test and ICT, in delayed reactions as patch test (ECT) as well as prick test/ICT with delayed reading [1, 11, 13–15]. Skin testing is reported to be particularly promising for maculopapular, pustular or fixed eruption. Skin testing is frequently negative for bullous exanthemas as well as for delayed urticaria [13].
The relevance of testing with PPL and MDM – as allergens deriving from the beta-lactam ring – has been shown worldwide in quite divergent patient collectives (Table 2). Of tested patients, between 4 and 72 % had positive skin tests for PPL and/or MDM. The highest rate of positive test reaction occurred in patients with immediate reaction, but these allergens do seem to play a role in at least some patients with delayed reactions [8, 9, 11, 15–18]. The differing rates of sensitization in the various studies can be explained by the differing prescribing and consumption habits of beta-lactam antibiotics in different countries. Sensitization rates change in the course of time, as shown in a Spanish collective with sensitization rates towards PPL and MDM among tested patients: about 77 % in 1990, 42 % in 2000 and 22 % in 2005 [9]. In a collective of German patients (n = 149) a sensitization rate towards PPL and/or MDM of 28.2 % was found [3]. The specificity of testing with PPL and MDM is estimated at 97–99 % - particularly for immediate reactions [1, 4]. In one study on 59 patients with immediate reactions towards penicillins it could also be shown that positivity for PPL in prick tests or ICT is frequent in patients with severe anaphylactic reactions [19]. For delayed reactions, PPL and MDM seem to play only a minor role in comparison to side chain antigens (particularly in aminopenicillins) [5, 7].
All these studies were performed with a test kit no longer commercially available, usually ALLERGOPEN® (0.035 mg PPL + 20 mg mannitol, MDM 0.6 mg sodium benzylpenicillin, 0.5 mg sodium benzylpenicilloate + 20 mg mannitol; Allergopharma, Reinbek, Germany) which were used as the worldwide standard for testing with PPL and MDM. In the USA PRE-PEN® as a PPL product (0.2 ml in a concentration of 6.0′105 M benzylpenicilloyl polylysine; Rivex Pharma, USA) was available. Both products were removed from the market 2004 on economic grounds.
A diagnostic void thus resulted, especially for immediate reactions. This was emphasized in a recently published paper by Bousquet et al. [8]. In a retrospective analysis of 825 patients with immediate and delayed reactions, about 30 % reacted towards PPL and MDM, with most patients also reacting to the beta-lactams contested. In 20 cases, patients reacted exclusively to PPL and MDM, thus confirming beta-lactam allergy, with six patients reacting to PPL, nine to MDM and five to both determinants. Even if this was only 2.4 % of the total patient population, the authors concluded that these patients may have unnecessarily had to undergo oral exposition in order to clarify their allergy had not MDM and PPL been available. Torres and Blanca [9] have also recently called for the re-introduction of commercially available PPL and MDM test kits.
After Vernuri et al. 2004 [20] suggested the possibility of replacing PPL, a commercial PPL and MDM test kit (Diater, Madrid, Spain) has recently become available in Europe; its usefulness is object of our study. With regards to PPL, the concentration of 0.04 mg is somewhat higher than in ALLERGOPEN®, with regards to MDM the concentrations of benzylpenicillin and benzylpenicilloate are identical and with benzylpenilloate a further minor determinant is tested.
In our study, six of 15 patients (40 %) displayed positive skin test reactions with four patients (27 %) reacting to the allergens of the new test kit. All four patients had presented to diagnose an immediate reaction. Interestingly, MDM (n = 3) as minor allergen was positive more often than PPL as major allergen (n = 1), with all patients positive for MDM having reacted towards cephalosporins, the patient with positive PPL towards ampicillin. Our patients thus reflect altered prescribing habits with more frequent us of aminopenicillins and cephalosporins than in the past.
The relevance of testing with PPL and MDM was shown in our study for patients with immediate but not those with delayed reactions, of whom none reacted to these determinants. As three of four patients showed positive reactions only towards the new test kit and serology and skin testing with the suspected drug were negative, use of the new test kit filled a diagnostic void. Further, none of our patients developed systemic intolerance to the PPL and MDM test kit, while in one study on 290 patients with immediate reaction this was observed in a total of 11 % of cases with amoxicillin being involved in 50 %, PPL in 29 %, MDM in 15 % (ALLERGOPEN®) and ampicillin in 6 % [21].
The low rate of positive tests in patients with delayed reaction might be explained by the longer time interval since the incident in comparison to the group with a history of immediate reactions. That skin testing can be prudent in patients with delayed reactions is shown by positive patch (ECT) and ICT in one patient towards amoxicillin and a positive prick test in the delayed reading

towards amoxicillin and ceftriaxone. It has been suggested that intracutaneous testing with a delayed reading possesses greater sensitivity but less specificity than patch testing in patients with delayed reactions. The fact that patients with delayed reactions are rarely positive for PPL and MDM might also be due to the fact that such reactions are usually due to aminopenicillins and are possibly caused by side chains [5].

That negative *in vitro* and skin testing cannot rule out a penicillin allergy was shown in a study on 330 patients, where almost 15 % with a history of an immediate reaction did react in exposition to a beta-lactam antibiotic despite negative skin tests [22]. Perhaps these patients were sensitized to minor allergens not routinely tested or to side chain allergens. When a beta-lactam allergy must urgently be excluded, negative skin testing should always be followed by an exposition [14].

We always recommended such an exposition with an alternative drug to our patients and, up to now, carried it out in six patients with good tolerability. One patient (pat. 14) subsequently accidentally received the suspected antibiotic (amoxicillin) again and developed the same delayed reaction in the form of erythroderma, which attested to the relevance of the skin test.

In summary, our study shows that the new PPL and MDM test kit can be helpful in the diagnostic work-up of patients with immediate reactions towards beta-lactam antibiotics. The diagnostic void left after the disappearance of previous test kits might now be filled. A direct comparison between the new not officially available kit and the older product can unfortunately no longer be realized due to unavailability of the latter. Observations on larger groups are needed for exact determinations of sensitivity, specificity and tolerability of the new test kit. Approval of this test kit for use in Germany appears desirable.

Abbreviations

Amp – ampicillin
AP – aminopenicillin
Ax – amoxicillin
ECT – epicutaneous or patch test
ICT – intracutaneous test
MDM – minor determinant mix
PPL – penicilloyl polylysine <<<

Conflict of interest

None.

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