

Review article

Diagnosis of nonimmediate reactions to β -lactam antibiotics

Nonimmediate manifestations (i.e. occurring more than 1 h after drug administration), particularly maculopapular and urticarial eruptions, are common during β -lactam treatment. The mechanisms involved in most nonimmediate reactions seem to be heterogeneous and are not yet completely understood. However, clinical and immunohistological studies, as well as analysis of drug-specific T-cell clones obtained from the circulating blood and the skin, suggest that a type-IV (cell-mediated) pathogenic mechanism may be involved in some nonimmediate reactions such as maculopapular or bullous rashes and acute generalized exanthematous pustulosis. In the diagnostic work-up, the patient's history is fundamental; patch testing is useful, together with delayed-reading intradermal testing. The latter appears to be somewhat more sensitive than patch testing, but also less specific. In case of negative allergologic tests, consideration should be given to provocation tests, and the careful administration of the suspect agents. With regard to *in vitro* tests, the lymphocyte transformation test may contribute to the identification of the responsible drug. Under the aegis of the European Academy of Allergology and Clinical Immunology (EAACI) interest group on drug hypersensitivity and the European Network for Drug Allergy (ENDA), in this review we describe the general guidelines for evaluating subjects with nonimmediate reactions to β -lactams.

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β -Lactams, particularly penicillins, may cause several kinds of allergic reactions, which have been classified as immediate, accelerated, or delayed according to the time interval between the last drug administration and their onset (1). This terminology may be simplified by classifying them as immediate or nonimmediate reactions. The former occur within the first hour after drug administration and are manifested clinically by urticaria and/or angioedema, rhinitis, bronchospasm, and anaphylactic shock (2, 3). Nonimmediate reactions are those occurring more than 1 h after drug administration. The main nonimmediate reactions are maculopapular or 'morbilliform' exanthems, particularly during treatment with amoxicillin or ampicillin (4–6). In addition, β -lactams can elicit delayed-appearing urticaria/angioedema, exfoliative dermatitis, acute generalized exanthematous pustulosis (AGEP), more severe bullous exanthems like the Stevens-Johnson syndrome (SJS) and toxic epidermal

necrosis (TEN) (2, 3). Furthermore, blood alterations may occur and certain β -lactams can even cause interstitial nephritis, pneumonitis, hepatitis and/or vasculitis with or without signs of serum sickness (Table 1). The combination of skin eruptions, visceral involvement, blood alteration, fever, and lymphadenopathy is termed drug-induced hypersensitivity syndrome or drug rash (or reaction) with eosinophilia and systemic symptoms (DRESS) (7).

Immediate reactions involving IgE antibodies have been extensively studied, but the mechanisms involved in nonimmediate reactions seem to be heterogeneous (8–17).

Recent data indicate that allergic maculopapular exanthems are T-cell-mediated diseases, whereby cytotoxic and IL-5-producing, drug-specific CD4 T cells migrate into the skin and kill keratinocytes presenting the drug on MHC-class II molecules, in a perforin-dependent way (Table 1). Both the high IL-5 production

Table 1. Pathogenic mechanisms and clinical symptoms of nonimmediate reactions to β -lactams [adapted from Ref. (17)]

Extended Coombs and Gell classification	Type of immune response†	Pathologic characteristics	Clinical symptoms†
Type II	IgG and FcR*‡	FcR-dependent cell destruction	Blood cell dyscrasia
Type III	IgG and complement or FcR*‡	Immunocomplex deposition	Vasculitis
Type IVa	Th1(IFN- γ)*	Monocyte activation	Eczema
Type IVb	Th2 (IL-5 and IL-4)*	Eosinophilic inflammation	Maculopapular exanthema, bullous exanthema
Type IVc	CTL (perforin and granzyme B; FasL)*	CD4- or CD8-mediated killing of cells (i.e. keratinocytes)	Maculopapular exanthema, bullous exanthema, pustular exanthema
Type IVd	T cells (IL-8/CXCL8)*	Neutrophil recruitment and activation	Pustular exanthema

* CTL, cytotoxic T cells; FasL, Fas ligand; FcR, Fc receptor; IFN, interferon; Ig, immunoglobulin; IL, interleukin; Th, T helper.

† Only major reactions are shown. In maculopapular exanthema, type IVb and IVc reactions may occur simultaneously; in pustular exanthema, type IVb, IVc, and IVd may occur simultaneously; and in bullous exanthema, type IVc with IVb and/or IVa can occur simultaneously. Most frequently, one type is clinically dominant (type IVc in maculopapular and bullous exanthema, type IVd in pustular exanthema).

‡ In addition, T-cell help in Ig-production/switch by some cytokines (e.g. IL-4, IL-5, IFN- γ).

and local chemokine production (RANTES, eotaxin etc.) contribute to eosinophilia (8–17).

In AGEP, T cells (and keratinocytes) secrete large amounts of IL-8 (CXCL8), which explains the typical neutrophilia (18) (Table 1).

In bullous skin diseases, drug-specific CD8 T cells play a more important role (Table 1). They are present in larger amounts in the affected lesions. They may be isolated from the blood, the affected skin and patch test lesions; they are cytotoxic and may also kill keratinocytes (14, 19).

During most of these drug-induced hypersensitivity reactions, increased percentages of circulating cutaneous lymphocyte-associated antigen (CLA)-positive T cells and a higher percentage of the T-cell activation marker human leucocyte antigen (HLA)-DR may be found on circulating CD4 and CD8 T cells (12, 20).

In this review, we describe the general ENDA/EAACI guidelines for evaluating and diagnosing subjects with suspected nonimmediate allergic reactions to β -lactams.

Clinical evaluation

The diagnosis of nonimmediate reactions to drugs requires: (a) identification of the disease as a drug-hypersensitivity reaction. This is sometimes difficult, because of the heterogeneity of the clinical manifestations, which can be quite similar to the symptoms of infectious or autoimmune diseases. Furthermore, these reactions may actually be facilitated by a concomitant viral infection by the human immunodeficiency virus, cytomegalovirus, human herpes virus 6 or Epstein-Barr virus (21). However, most drug allergies have a rather typical exanthem and sometimes an eosinophilia; (b) identification of the suspect drug. Frequently, the patient has been exposed to different drugs that are known to be possible elicitors of drug allergic reactions.

In any case, the patient's history is essential for confirming the diagnosis. The ENDA questionnaire could be helpful (22).

Table 2. Common nonimmediate reactions that may be assessed by skin tests, patch tests, and LTT [adapted from Ref. (53); for LTT see Ref. (66)]

Acute generalized exanthematous pustulosis*
Contact dermatitis
DRESS* (drug rash with eosinophilia and systemic symptoms or severe hypersensitivity syndrome with cutaneous involvement)
Erythema multiforme*†
Exanthematous or maculopapular drug eruption
Fixed drug eruption‡
Stevens-Johnson syndrome*†
Toxic epidermal necrolysis*†
Delayed appearing urticaria and/or angioedema†

* Patch tests (and/or LTT) should be used as the first line of investigation.

† Allergologic tests are often negative.

‡ Patch tests should be performed in the previously affected area.

The common nonimmediate hypersensitivity reactions that may be assessed by *in vivo* and *in vitro* tests are listed in Table 2.

Skin tests

There are three classical methods for performing skin tests: prick, intradermal and patch. For presumably IgE-mediated hypersensitivity reactions, skin tests are performed by prick, and if responses are negative then intradermal tests are carried out.

Since 1966 there have been studies suggesting that delayed-reading intradermal tests can be an effective way of diagnosing nonimmediate allergic reactions to β -lactams, such as maculopapular exanthems, urticaria and/or angioedema, TEN, erythroderma, erythema multiforme and generalized eczema (1, 15, 23–43). In some cases of maculopapular exanthems, tested skin has been biopsied and found to present histological features similar to those of the original lesion with cell infiltrates like those in delayed hypersensitivity skin reactions of the tuberculin type (9, 23, 29, 33, 43), while in others, challenges with suspect β -lactams have confirmed

delayed intradermal test positivity (15, 24, 25, 27–33, 37, 40).

Patch test positivity has been reported in nonimmediate cutaneous reactions to systemically administered β -lactams (14, 18, 26–28, 30–32, 34–37, 39–52). These manifestations include all those described above as well as others like AGEP (18, 36, 47, 48), flexural exanthem (50) and IgA bullous dermatosis (51).

Of particular importance is the finding that elution of T cells from patch test lesions from two patients with bullous reactions to β -lactams revealed that about one-third of the infiltrating T cells were drug specific (52).

Furthermore, many studies with patients who have developed delayed hypersensitivity reactions to β -lactams (26–28, 30–32, 34, 36, 37, 40–43) indicate that various conditions are associated with late responses (some hours after injection) to both intradermal and patch testing.

Prick and intradermal testing

The following commercially available preparations for prick and intradermal testing are suggested: (a) penicilloylpolylysine (PPL) (Allergopen, Reinbeck, Germany). A 1 : 10 dilution of the original solution in 0.9% NaCl should be used initially. When results are negative, testing should be repeated with the undiluted solution (5×10^{-5} mmol/l); (b) minor determinant mixture (MDM) (Allergopen), initially diluted 1 : 100 in 0.9% NaCl. Tests should be repeated with the undiluted solution (2×10^{-2} mmol/l) when initial results are negative; (c) benzylpenicillin (BP), diluted in 0.9% NaCl and administered at concentrations of 100 IU/ml and 10 000 IU/ml; (d) ampicillin (AM) and amoxicillin (AX), both at concentrations of 1–2 and 20–25 mg/ml, after dilution in 0.9% NaCl.

Any other suspect penicillins (at concentrations of 1 and 20 mg/ml, after dilution in 0.9% NaCl) or cephalosporins (at a concentration of 2 mg/ml in 0.9% NaCl) should also be used. The haptens have to be freshly reconstituted; normal saline should be used as a negative control.

Patch testing

Patch tests should be administered with (a) BP, (b) AM, (c) AX and any suspect penicillins and/or cephalosporins following the recommendations of Brockow et al. (53). A concentration of 5% in petrolatum was found to be suitable; higher concentrations are possible (and still not irritating), but are not more sensitive.

For skin tests with drugs unavailable in a parenteral preparation (e.g. only tablets), it should be ascertained whether they can be solubilized. Then, they should be ground in a mortar, diluted with 0.9% NaCl (for prick and patch testing) or added to petrolatum (for patch testing). The drugs should be weighed and the concentration of the active ingredient should be determined and

expressed in mg drug/ml vehicle. For intradermal tests, sterile solutions are obligatory (53).

Cross-reactivity. In delayed allergy to aminopenicillins, both the β -lactam core structure and the whole molecule (core structure and the amino-benzyl group of the side chain) are recognized by T cells (54, 55). However, the amino-benzyl group plays a predominant role: more than 70% of patients with such hypersensitivity display negative responses to BP in intradermal and/or patch tests (32, 33, 37, 42, 46). This means that alterations of side chains affect recognition, but also that the same side chains presented by cephalosporin core structures are not recognized. Thus, cross-reactivity between cephalosporins and penicillins seems to be very rare for T-cell reactions.

Scoring. Prick test and intradermal test readings should be carried out after 20 min, to assess any immediate responses (53, 56).

Readings of delayed reactions are important for intradermal tests: they should be performed after 48 and 72 h. As time intervals between testing and positive responses may vary, patients should be advised to return to show any positive responses occurring after the 72-h reading.

Late reactions to intradermal testing should be documented by the diameter of the erythema and the papulation/infiltrate, as well as a morphological description (erythematous swelling, erythematous infiltrate, only erythema, eczema with papulation with or without vesicles). Any infiltrated erythema with a diameter > 5 mm should be considered a positive reaction. For later comparison and research purposes, photodocumentation and, if possible, histology may be recommended (53).

Patch test readings should be made according to the European Environmental and Contact Dermatitis Research Group patch test classification (57), 15 min after removal of the strips and 24 h later.

Evolution of skin testing over time

There are findings with β -lactams, particularly aminopenicillins, showing that delayed hypersensitivity may be a long-lasting condition, which does not appear to be influenced by the time interval between the last adverse reaction and allergologic testing (32, 37, 42). This differs from the immediate type, which tends to disappear with time if the culprit drug is avoided (58–61).

Sensitivity and specificity

As provocation tests in patients with positive skin tests are lacking because of ethical reasons, evaluation of the sensitivity and specificity of such tests is limited. To establish specificity, subjects tolerating β -lactams can be tested with the tolerated drug. The negative predictive value of delayed-reading intradermal tests and patch tests

seems to be good, as most test-negative nonimmediate reactors tolerate the drug.

Only few studies have been carried out with large samples of subjects previously classified as nonimmediate reactors to β -lactams (37, 42, 46). de Haan et al. (46) assessed 64 patients with late (beginning 24 h or more after the start of therapy) reactions to aminopenicillins, and patch testing with BP was positive in 9.3% of them, while that with AM and AX in 35.9%. In a more recent study, which evaluated 241 subjects with nonimmediate reactions to aminopenicillins, patch tests with BP were positive in 7.5% of them, while AM and AX elicited positive reactions in 37.3% (42). Delayed-reading intradermal tests with penicillin determinants (MDM and BP) were positive in 12%, while that with AM and AX in 39%. However, considering only the 166 subjects with aminopenicillin-associated maculopapular exanthems, patch tests and delayed-reading intradermal tests with AM and AX were positive in 52.4 and 54.2%, respectively. Moreover, all but one of the 64 subjects who were negative to the allergologic tests tolerated provocation tests with the suspect aminopenicillin, indicating that most of the results were not falsely negative (42). Although patients with positive tests were not challenged in this study, it suggests that these tests can reduce the number of patients falsely labelled as 'penicillin allergic'.

In the same study, the specificity of patch tests and delayed-reading intradermal tests with penicillin determinants as well as with AM and AX was 100%: all 30 healthy subjects who had previously been treated with one or more of these penicillins showed negative results (42).

Regarding other β -lactams, such as cephalosporins, no large studies and no definitive data about skin test sensitivity are available.

Generally, intradermal testing appears to be somewhat more sensitive than patch testing (27, 28, 30, 36–38, 41–43). In some studies, subjects with delayed intradermal test positivity and patch test negativity were challenged with positive responses in six of nine patients (30, 37, 42).

False-negative results in drug patch tests may be due to insufficient penetration of reagents into the epidermis and/or to the fact that the concentration used is too low.

Patch tests appear to be more specific than delayed-reading intradermal tests, because some patients with positive delayed responses to intradermal tests were negative in challenge tests (24, 25, 37, 42), while all 33 reported subjects who displayed patch test positivity and were challenged with the positive drug reacted to the challenge (31, 32, 40, 49, 62).

***In vitro* tests: lymphocyte transformation test**

The lymphocyte transformation test (LTT) measures the proliferative response of T cells to the drug under *in vitro*

conditions (63–66). It requires live cells (the anti-coagulated blood should be handled within 24–36 h) and is rather cumbersome, lasting about 1 week, as it relies on the measurement of the proliferation of drug-specific T cells in a cell culture system with various variables (composition of circulating and isolated mononuclear cells, availability of optimal serum supplements for the cell culture, etc). Some researchers assessed 78 patients with adverse reactions to penicillins using LTT and found a sensitivity of 74% – higher than that of skin tests (62%) – and a specificity of 85% (63). Other studies confirmed the sensitivity of this test in the diagnosis of β -lactam allergy (64, 65).

Provocation tests

Although it is sometimes contested because of ethical considerations, in many instances the provocation or challenge is, at present, the best tool for confirming a causal relationship between drug administration and nonimmediate adverse reactions. It relies on the careful administration of the suspect agent (67).

In some studies, cases of β -lactam-associated cutaneous eruptions – displaying negative results in allergologic evaluations – have been challenged with suspect drugs; most subjects tolerated provocation tests (27, 32–34, 37, 42, 65, 68). However, there were also patients who react to the provocation test with nonimmediate cutaneous eruptions, particularly maculopapular and urticarial (27, 33, 42, 65). Specifically, in the study by Luque et al. (65), which assessed 19 patients with nonimmediate reactions to β -lactams, 12 subjects displaying negative responses to intradermal tests reacted to the challenge.

In evaluating nonimmediate reactions to β -lactams, subjects who are negative in all the above tests could, in addition, undergo provocation tests with the suspect antibiotic. One-hundredth of the therapeutic dose can be administered as an initial dose. In cases with negative results, 3 days to 1 week later (depending on the time interval between drug intake and adverse reaction), a dose of one-tenth should be given and, if the result is again negative, after the same time interval chosen before, a full dose. As skin test positive subjects react to the drug (32), this provocation test protocol is not *per se* desensitizing. The test should take place in a specialized centre under careful clinical observation, with particular regard to skin symptoms. All patients should be reviewed in the unit at the time of an adverse response and skin lesions should be carefully described. The time elapsed between the last dose and the onset of symptoms should also be noted. It is also important to record the therapy administered as well as the duration of the symptoms.

In the case of severe reactions, such as the hypersensitivity syndrome, SJS, AGEP or TEN, the drug provocation test must be avoided (67).

High-risk patients

For evaluating patients who have suffered severe skin reactions (e.g. TEN, severe bullous exanthems, AGEP, SJS) or systemic reactions (e.g. DRESS), patch tests should be used as the first line of investigation; in case of positive results, intradermal testing may be avoided. In case of patch test negativity, for intradermal testing, the drug should be initially tested with the highest dilution (53). Provocation tests must not be performed (67).

Diagnostic evaluation of children

In children, the percentage of β -lactam-associated non-immediate skin eruptions, particularly maculopapular exanthems, that actually represent allergic phenomena is significantly lower than in adults. In fact, in children such manifestations are thought to be mainly caused by the infection itself (69). In any case, a diagnostic protocol which includes delayed-reading intradermal tests and patch tests can be helpful in clarifying the nature of many

of these reactions. If warranted, residual doubt can be eliminated with provocation tests (34).

Diagnostic algorithm

In Fig. 1, we propose an algorithm for *in vivo* allergologic evaluation of nonimmediate reactions to β -lactams, which combines skin tests and patch tests with a common panel of reagents – including penicillin determinants (PPL, MDM, and BP) and the two most used aminopenicillins (AM and AX) – as well as the suspect β -lactam; in selected cases, provocation tests with the latter are also suggested.

Defining selective hypersensitivity

In case of positive tests to determined, β -lactams and in particular situations, such as cystic fibrosis, exclusion of the whole group may result in more harm than benefit because of the potential adverse consequences of

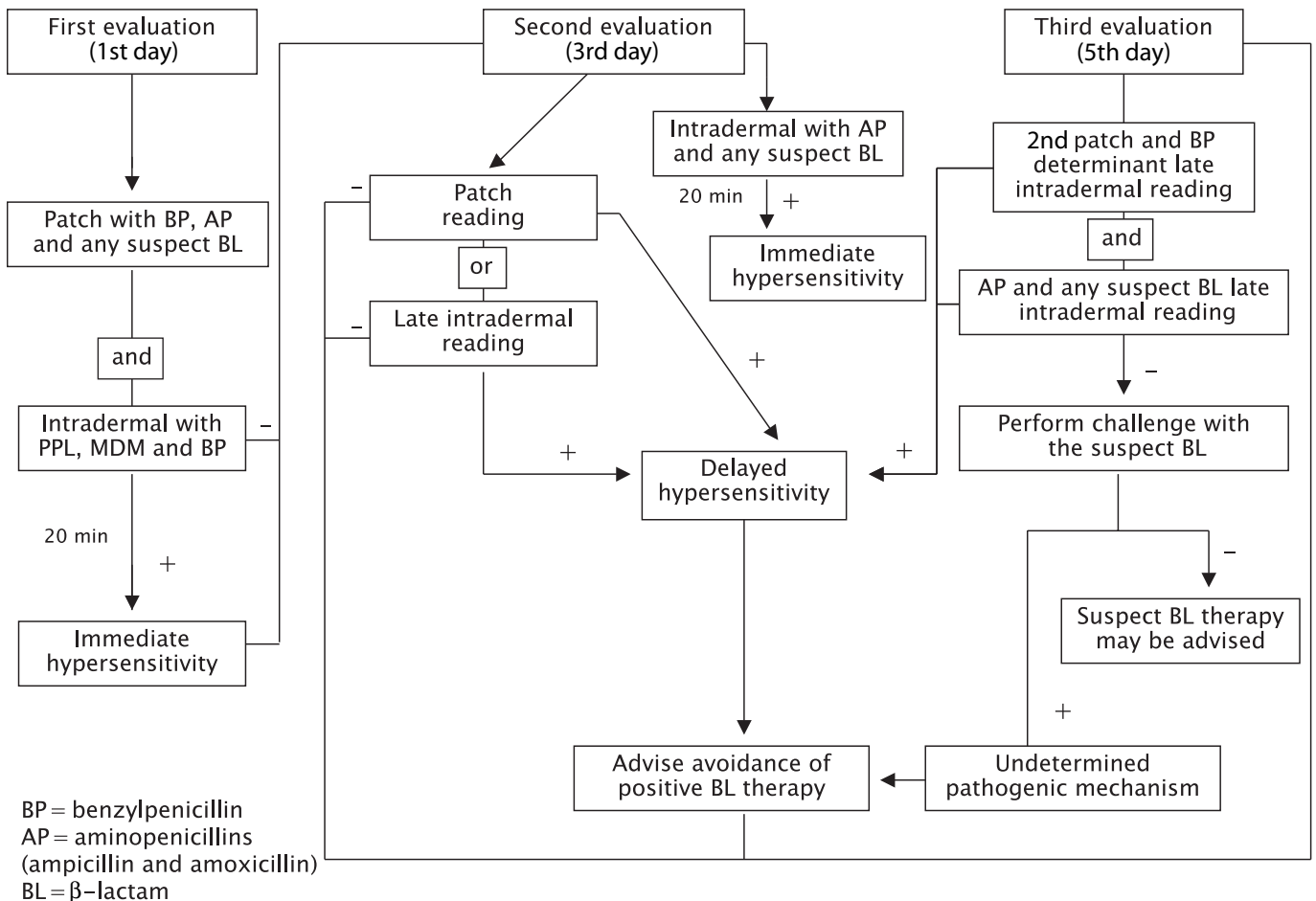


Figure 1. Algorithm for *in vivo* allergologic work-up of nonimmediate reactions to β -lactams.

untreated infections or the choice of another, more toxic drug. Tolerability of any other negative β -lactam may be confirmed by administering an initial dose of one-tenth and, in case of a negative result, after 3 days to 1 week, a full dose.

Conclusion

Patch tests, delayed-reading intradermal tests and LTT are useful tools in evaluating nonimmediate reactions to

β -lactams, particularly maculopapular, pustular, or bullous exanthems, with or without involvement of internal organs. Intradermal testing appears to be somewhat more sensitive, but less specific, than patch testing. In case of positive reactions, β -lactam therapy should be avoided. Patients with mild symptoms, but negative allergologic work up, can be subjected to provocation tests.

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Appendix

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