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Article type : EAACI Position Paper

TITLE: Towards a more precise diagnosis of hypersensitivity to beta-lactams – an EAACI position paper

SHORT TITLE: Updated beta-lactam hypersensitivity diagnosis

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Total word count: 6320 words.

Conflict of interest: None of the authors have any conflict of interest, nor have they received any money for this study. Research is part of their daily activities. All authors had full access to all data and take responsibility for the integrity and accuracy of the data analysis.

KEYWORDS: Allergy, beta-lactams, cross-reactivity, diagnosis, guidelines, risk stratification.

ABBREVIATIONS: AGEP, acute generalized exanthematous pustulosis; BAT, basophil activation tests; BL, beta-lactams; BP-OL, benzylpenicilloyl-octa-L-lysine; CADR, cutaneous adverse drug reactions; DAIG, drug allergy interest group; DPT, drug provocation tests; DRESS, drug reaction (or rash) with eosinophilia and systemic symptoms; DTD, daily therapeutic dose; EAACI, European Academy of Allergy and Clinical Immunology; FDE, fixed drug eruption; IDT, intradermal tests; LTT, lymphocyte transformation tests; MD, minor determinants; MPE, maculopapular exanthemas; MSUD, maximum single unit dose; NPV, negative predictive value; PPL, benzylpenicilloyl-poly-L-lysine; PPV: positive predictive value, PT, patch tests; SJS,

Stevens-Johnson syndrome; SPT, skin prick tests; SsIgE, serum specific IgE assays; ST, skin tests; TEN, toxic epidermal necrolysis.

Abstract

A recent survey of the European Academy of Allergy and Clinical Immunology (EAACI) Drug Allergy Interest Group (DAIG) on how European allergy specialists deal with beta-lactam (BL) hypersensitivity demonstrated a significant heterogeneity in current practice, suggesting the need to review and update existing EAACI guidelines in order to make the diagnostic procedures as safe and accurate, but also as cost-effective, as possible. For this purpose, a bibliographic search on large studies regarding BL hypersensitivity diagnosis was performed by an EAACI task force, which reviewed and evaluated the literature data using the GRADE system for quality of evidence and strength of recommendation.

The updated guidelines provide a risk stratification in BL hypersensitivity according to index reaction(s), as well as an algorithmic approach, based on cross-reactivity studies, in patients with a suspicion of BL hypersensitivity and an immediate need for antibiotic therapy, when referral to an allergist is not feasible. Furthermore, the update addresses availability and concentrations of skin test (ST) reagents, ST and drug provocation test (DPT) protocols, as well as diagnostic algorithms and administration of alternative BL in allergic subjects. Specifically, distinct diagnostic algorithms are suggested depending on risk stratification of the patient into high and low risk based on the morphology and chronology of the reaction, immediate (i.e., occurring within 1 to 6 hours after the last administered dose) or nonimmediate (i.e., occurring more than 1 hour after the initial drug administration), and the reaction severity. Regarding the allergy workup, the main novelty of this document is the fact that in some low-risk nonimmediate reactions ST are not mandatory, especially in children. For DPT, further studies are necessary to provide data supporting the standardization of protocols, especially of those regarding nonimmediate reactions, for which there is currently no consensus.

INTRODUCTION

Beta-lactams (BL) are the first-choice antibiotics to treat the majority of bacterial infections. Among them, amoxicillin constitutes the most consumed antibiotic in Europe.[1] However, sometimes the therapeutic agent becomes the problem and antibiotic allergy is nowadays a worldwide health issue. Its diagnosis is complex, but generally easy for expert allergists. Nevertheless, it can be difficult when patients are referred after a long delay and when information about the nature of the symptoms and the suspected antibiotic is incomplete. Moreover, even a slight suspicion of BL allergy results in the use of alternative treatments that can be less effective, lead to prolonged treatments, be more toxic, more expensive, and contribute to the increase in bacterial resistance.[2,3]

The diagnostic approach and outcomes of investigations in patients with BL hypersensitivity are continuously changing due to the introduction of new BL and consequent changes in the pattern of consumption, which varies among European countries.[1,4,5] This influences the sensitivity of the tests, as well as the rate of cross-reactivity, and might also explain the different results found across Europe in studies performed according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines for diagnosing BL hypersensitivity.

Due to the low prevalence of true BL hypersensitivity and the drawbacks from using alternative treatments, all individuals with histories of hypersensitivity reactions to BL should undergo an allergy workup soon after such reactions, if possible, in order to establish a firm diagnosis.[6,7] At the same time, in most countries, there is an urgent need for reducing healthcare costs, which generally is incompatible with precision medicine. Therefore, determining the most safe, precise, and also cost-effective method(s) for confirming clinically significant IgE-mediated or T-cell-mediated BL hypersensitivity is becoming increasingly important.[8] In addition, a recent survey of the EAACI drug allergy interest group (DAIG) on how European allergy specialists deal with BL hypersensitivity[5] demonstrated a significant heterogeneity in current practice not only among countries, but also among centers belonging to the same country. All this suggests the need to review and update current EAACI guidelines and algorithms, as well as standardize test protocols for diagnosing BL hypersensitivity. The aim of the present document is to meet this need providing data and recommendations regarding the diagnosis of BL hypersensitivity based on published studies in order to make the diagnostic procedures as safe and accurate, but also as cost-effective, as possible. Notably, this paper is primarily focused on the therapeutic use of BL and does not specifically address the problem posed by antibiotic prophylaxis in subjects with a suspected allergy to BL.

METHODS

A bibliographic search was performed by an EAACI task force using electronic databases (MEDLINE and PubMed), electronic libraries (Science Direct, OVID), and a systematic review database (Cochrane library). Especially, publications since 2009 were considered. Keywords were allergy, beta-lactams, cross-reactivity, diagnosis, drug provocation tests, hypersensitivity, immediate reactions, *in vitro* tests, nonimmediate reactions, patch tests, skin tests, specific IgE, and T cells. The relevance of articles was evaluated by the authors based on title and abstract. Selected articles were then retrieved and analyzed. The submission of each author was discussed by the task force members, confirmed or amended by consensus of the group. Key statements on the quality of evidence and strength of recommendation were made using the GRADE system.[9] The quality of the evidence was rated high, if further research is very unlikely to change our confidence in the estimate of effect; moderate, if further research is likely to have a significant impact on our confidence in the estimate of effect and may change the estimate; low, if further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; and very low, if any estimate of effect is very uncertain. The strength of recommendation is strong, if clinicians are very certain that the benefits outweigh the risks; it is weak, if the benefits and risks are finely balanced, or appreciable uncertainty exists about the magnitude of the risk.

RİSK ASSESSMENT

In most patients with suspicion of BL allergy, the standard procedure is to avoid or stop the suspected drug and do the allergy workup later (high/strong). It is very important to decide whether to stop or to continue BL therapy, as often alternative antibiotics are less effective and associated with a higher level of predictable adverse reactions, such as gastrointestinal troubles (i.e., nausea, cramping, diarrhea) resulting from macrolides, tendon ruptures due to fluoroquinolones, and aminoglycoside ototoxicity (moderate/strong). Clues for diagnostic steps include morphology (characteristics) and chronology of the index reaction, risk factors for potential drug allergy, number of previous reactions to the same drug, treatment needed for the index reaction, and any antibiotics tolerated after this reaction. Regarding risk factors, it is advisable to consider both drug-related ones, such as type of treatment (e.g., intermittent, repeated, or uninterrupted) and administration route, and those that are patient-related, such as age, genetic characteristics, concomitant disease states (e.g., cystic fibrosis, EBV and HIV infections), and occupational activity.

The first step is to check if the clinical picture of the reaction is compatible with that of a drug allergy, or if an alternative diagnosis (e.g., viral exanthema) is more likely (moderate/strong).[10] By many patients and some health care workers any BL side effects, such as nausea, vomiting, and

headache, are incorrectly termed allergic. Also, some patients may have been re-exposed and tolerated the same drug since the suspected reaction; in these patients, the allergy label can be removed without testing. In all patients, it is compulsory to check for the danger signs (high/strong) (Table 1).

The next step is to assess the chronology of the reaction, differentiating immediate reactions from nonimmediate (often also called delayed) (moderate/strong).[11] The former typically occur within 1 hour, but may occur up to 6 hours, after the last administered dose, and are mostly associated with an IgE-mediated pathogenic mechanism. In fact, in immediate reactions to BL, the activation of the mast cell MRGPRX2 receptor does not play a role, unlike in immediate reactions to vancomycin and fluoroquinolones, which are the most commonly recognized mast-cell activators that cause non-IgE-mediated reactions to antibiotics.[12] Immediate reactions usually manifest as isolated symptoms, such as urticaria, angioedema, and bronchospasm, or as anaphylaxis (Table 2). Nonimmediate reactions may occur at any time from 1 hour after the initial drug administration, commonly after many days of treatment, and are often associated with a T-cell-dependent type of allergic mechanism.[11,13] Maculopapular exanthemas (MPE) and delayed urticaria are the most common clinical presentations of nonimmediate reactions (Table 2).

However, a classification restricted to the chronology may lead to overlaps. For example, a reaction occurring between 1 and 6 hours after the first (and only) dose would be classified as both immediate and nonimmediate. Therefore, to overcome this limit it is advisable to take into account both the chronology and the morphology of the reaction. Considering the peculiarity of most reactions (in Table 2, only urticaria is listed in both the immediate and the nonimmediate reactions), this approach would limit the number of possible overlaps. In the case of an urticarial reaction occurring after more than an hour but within 6 hours of the first dose of a BL, it is advisable to classify it as immediate.

If the characteristics of the reaction and its chronology are indicative of a hypersensitivity reaction, treatment should be stopped. In some cases (e.g., mild MPE), the BL could be continued (treating through) under careful supervision if, after an evaluation of the entire clinical picture, it appears that there are no clear danger signs (Table 1) and that the benefit outweighs the risk (low/weak).[14]

The management of patients with suspected BL hypersensitivity reactions depends on their risk profile, the accessibility of an Allergy Unit/Department, and the clinical indication for the suspected BL or an alternative one. Table 2 shows the risk stratification in BL allergy and management. Subjects who suffered severe reactions, or who have a high probability of experiencing a reaction more severe (e.g., anaphylaxis) than the index reaction (e.g., urticaria) in case of re-exposure to the culprit BL, are classified at high risk. Subjects who experienced not

serious reactions, or who have a low probability of experiencing a reaction more severe than the index reaction in case of re-exposure to the culprit drug, are classified at low risk. The former are at greater risk of having systemic reactions to allergy tests than the latter. Table 3 shows the algorithmic approach in patients with a suspicion of hypersensitivity to specified penicillins and/or cephalosporins and an immediate need for antibiotic therapy, when referral to an allergist is not feasible. In all cases, a non-BL alternative antibiotic can be given without restriction (high/strong). In case of a need for an alternative BL, structural similarities or identities between the culprit and the alternative drug should be avoided (high/strong) (Figure 1).[15,16]

DIAGNOSIS

The clinical history is not reliable as a diagnostic tool (high/strong) and predictive models, as well as clinical decision-making algorithms, based on the clinical history of patients with suspicions of allergic reactions to BL have proved to be unable to accurately differentiate between allergic and nonallergic individuals (moderate/strong).[17-19] Confirmation or exclusion of BL allergy is mainly based on skin tests (ST), patch tests (PT), and drug provocation tests (DPT) (high/strong). Clearly positive *in vitro* tests (e.g., serum specific IgE assays) can be useful for avoiding DPT, especially in subjects who experienced severe reactions like anaphylaxis (moderate/strong). However, in vitro test sensitivity may vary depending on various factors, such as the time interval between reaction and test, the severity of the reaction, and its chronology. Generally, in evaluating subjects with immediate reactions, in vitro tests are less sensitive than ST, while in assessing subjects with nonimmediate reactions, the reverse is true.[20]

Distinct diagnostic algorithms can be applied depending on risk stratification of the patient into high and low risk based on the morphology and chronology of the reaction, immediate or nonimmediate, and the reaction severity (Figures 2 and 3).

Skin tests and patch tests

Indications and timing for their performance

Immediate-reading ST, namely skin prick tests (SPT) and intradermal tests (IDT), are indicated for evaluating subjects with immediate reactions, whereas delayed-reading ST and/or PT are recommended for assessing patients with nonimmediate reactions.[21]

In immediate reactions, ST should be performed after a time interval of 3-6 weeks from the reaction (high/strong), which generally allows the resolution of clinical symptoms, as well as the clearance from the circulation of the incriminated drugs and anti-allergic medications. However, IgE-mediated skin reactivity to BL decreases with time;[22] therefore, it is recommended that ST should be performed as soon as possible after the aforementioned 3-6 weeks have elapsed (high/strong). Nevertheless, there is no upper limit where SPT may be considered inaccurate.

In nonimmediate reactions, it is recommended to perform ST at least 4 weeks after the disappearance of cutaneous adverse drug reactions (CADR) and discontinuation of systemic glucocorticoids or immunosuppressive drugs and, in the case of PT, 4 weeks after ultraviolet exposure on the skin area tested, and one week after discontinuation of topical glucocorticoids on the test site (high/strong).[21,23] In drug reaction (or rash) with eosinophilia and systemic symptoms (DRESS), PT must be carried out at least 6 months after the disappearance of the CADR and after verification by quantitative real-time polymerase chain reaction on serum of the absence of reactivation of viruses of the herpes group (i.e., HHV6, HHV7, EBV, and human cytomegalovirus) (high/strong).[24,25]

Reagents and concentrations

The panel of reagents for evaluating hypersensitivity reactions to BL by ST includes the classic penicillin reagents (i.e., major and minor benzylpenicillin determinants and benzylpenicillin), amoxicillin, and any other suspected BL (high/strong).

Benzylpenicilloyl-poly-L-lysine (PPL, Pre-Pen®, AllerQuest LLC, Plainville, CT, US) and benzylpenicilloyl-octa-L-lysine (BP-OL, 0.04 mg/mL, DAP®, Diater, Leganés, Madrid, Spain) are the commercially available major determinants. In Europe, the only commercially available minor determinant (MD) other than benzylpenicillin is sodium benzylpenilloate (MD, 0.5 mg/mL, DAP®). However, PPL, BP-OL, and MD are not available in all European countries.[5] The highest nonirritating concentrations recommended in both SPT and IDT[26,27] are shown on Table 4.

In immediate reactions, skin testing is recommended to be performed with the aforesaid panel of reagents at progressively increasing concentrations up to the highest nonirritating one (high/strong), whereas some studies[28,29] have demonstrated that ST with the major and minor determinants of benzylpenicillin are scarcely useful in nonimmediate reactions (moderate/strong).

SPT should be done with injectable solutions. However, if the BL concerned is not available in this form, SPT can be done with any form of commercialized BL (moderate/weak),[21] although results must be interpreted with caution because in this case the exact concentration of active drug is unknown. A positive control is done with histamine at 10 mg/mL or codeine phosphate. As a negative control, normal saline and/or any other solvent employed to dilute are used.

For IDT, sterile injectable solutions are obligatory (high/strong). Performing a positive control with histamine at 1 mg/mL is not mandatory if a positive control SPT is performed. As a negative control, normal saline and/or any other solvent employed to dilute are used.

For PT, previous European guidelines[30,31] suggest a BL concentration of 5% in petrolatum. Higher concentrations (up to 50% in petrolatum) have proved to be nonirritating,[32,33] but not more sensitive.[32] In some European countries, 11 BL diluted at 10% in petrolatum, and marketed by Chemotechnique (Velinge, Sweden) as ready-to-use products in syringe, are available.

When the active ingredient is available in pure form (e.g., lyophilized), it is recommended to dilute it at 10% in petrolatum (high/strong),[23] otherwise the powder contained in capsules, or obtained by removing the external layer of tablets with a scalpel and crushing them in a mortar, can be diluted at 20%[21] or 30% in petrolatum.[23,34]

Skin prick tests

ST generally represent the first-line method for evaluating immediate hypersensitivity reactions to BL (high/strong).[22,31,35] The suggested sequence of ST is as follows: (a) SPT (1/10 and the highest nonirritating concentrations) at intervals of 20 minutes, and if SPT are negative (b) IDT(1/100 of the highest nonirritating concentration, 1/10, and the highest nonirritating concentrations) at intervals of 20 minutes. The procedure is stopped when a positive ST is found.

In evaluating subjects who suffered severe anaphylactic reactions, starting concentrations of ST reagents should be at least 10^{-3} of the highest nonirritating ones in order to avoid systemic reactions (high/strong).[27,36] In low-risk patients (Table 2), the workup can be simplified performing SPT and IDT directly with the highest nonirritating concentrations.

Reactions to SPT are considered positive when the diameter of the wheal is at least 3 mm compared to a negative control and is surrounded by erythema, 20 minutes after the prick.[21,22]

SPT are of value for investigating immediate hypersensitivity reactions to BL (high/strong).[22,31] Seldom, late positive responses to SPT have been reported in MPE, DRESS, and acute generalized exanthematous pustulosis (AGEP).[24,25] A SPT has a delayed positive reaction when there is erythema and infiltration at the puncture site after 1 or 2 days.[21]

Intradermal tests

IDT are performed with the BL concerned after ensuring the negativity of SPT (high/strong). The recommended volume for injection is 0.02 mL. The diameter of the injection papule (wheal) should be measured immediately after injection (Wi). If the wheal is not round, the mean wheal diameter (mWi) [(D + d)/2, where D = the longest diameter of the wheal, and d = the longest diameter orthogonal to D] should be measured. Such diameters are then measured at 20 minutes. At that time, the IDT is considered positive if diameter of the wheal (W20/mW20) is at least 3 mm greater than that of the Wi/mWi and is surrounded by erythema that also has to be measured.[21,22]

In subjects with nonimmediate reactions, IDT can be positive on delayed readings (e.g., after 24, 48, or 72 hours). Any late responses to IDT should be documented by the diameter of the erythema and the infiltration, 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 greater than 5 mm should be considered a positive reaction.[30] Patients are advised to return to show any positive responses appearing within 1 week after IDT,

as well as to take pictures of positive or doubtful IDT. Delayed-reading IDT are of value in MPE, but can be omitted in palmar exfoliative exanthemas (high/strong), where they are usually negative.[37]

Patch tests

PT are a simple and safe diagnostic tool; in effect, systemic reactions to them are extremely rare.[25]

In evaluating patients who experienced severe cutaneous reactions, such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), DRESS, AGEP, bullous exanthemas, or MPE with systemic symptoms, as stated in previous European guidelines,[21,30,31] PT with the suspected BL should be used as the first line of investigation (i.e., prior to ST) (high/strong). In case of positive results of PT, ST should be avoided, whereas, in case of negative results, IDT can be performed, starting with a lower concentration of the drug concerned (e.g., 1 mg/mL for semi-synthetic penicillins) (moderate/strong).[24,38]

Regarding low-risk subjects (Table 2), PT are the method of choice in those with contact dermatitis; they are useful in MPE, flexural exanthemas, and, if done *in situ*, also in fixed drug eruptions (FDE) (high/strong).[25] PT are applied on the upper back using chambers, according to the methods used for contact dermatitis. When negative, they have to be complemented by IDT with delayed readings, which are more sensitive than PT.[25,30] In subjects with FDE, PT should be applied to the site of eruption (residual pigmented lesion).They are left for 2 days, then read at Day 2 (30 minutes after taking off the test's material) and Day 4 or 5. Reading result's criteria are identical to those used for contact allergy (i.e., negative, irritant, + to +++).[21]

In vitro *tests*

The main *in vitro* tests for evaluating immediate reactions to BL are the serum specific IgE assay (SsIgE) and the basophil activation test (BAT).[20,39-41] These tests are complementary to ST[22,31] because there are cases with clear-cut histories of immediate hypersensitivity reactions to BL, such as penicillins, cephalosporins, and clavulanic acid, which display negative ST and positive SsIgE or BAT.[42-47] Therefore, it is advisable to perform *in vitro* tests in addition to ST in high-risk patients in order to improve the sensitivity of the allergy workup and thus reduce the need for DPT (moderate/strong).

As previously reported for ST,[22] also the sensitivity of both SsIgE and BAT decreases with time;[48,49] therefore, it is recommended that they should be performed as soon as possible after the reaction (high/strong).[20] In subjects with histories of severe anaphylaxis, performing *in* vitro tests before ST may reduce the need for the latter, lessening the risk of systemic reactions (high/strong) (Figure 2).[22,31] In addition, in vitro tests can replace ST in cases where ST are

contraindicated, not appropriate, or not possible[20] (e.g., in pregnant women, in patients who are immunosuppressed or in an unstable clinical condition, in subjects with dermographism etc).

The most widely used commercial method is the fluorescent-enzyme-immunoassay (ImmunoCAP®, Thermo-Fisher, Uppsala, Sweden). However, it is available only for benzylpenicillin (penicillin G), penicillin V, amoxicillin, ampicillin, and cefaclor. Its sensitivity is rather low and variable (0-50%) and seems to correlate with the severity of the reaction.[42,50,51] Lowering the threshold from 0.35 to 0.1 kU/L increases the sensitivity, although it also reduces the specificity, particularly in subjects with total IgE >200 kU/L.[20] A study by Vultaggio et al[52] demonstrated that a specific IgE/total IgE ratio ≥0.002 increases the ImmunoCAP® specificity. However, false positive test results with penicillin ImmunoCAP®, as with ST, have been reported.[53,54] These false-positive results could make the ImmunoCAP a poor choice for the diagnosis of penicillin allergy.[54]

The BAT can be used in evaluating immediate reactions to BL,[20,39-41,45,50,55,56] especially to those, such as clavulanic acid[49,57-59] and cefazolin[60] for which no immunoassays are commercially available. However, 1-10% of false positive results with this test, as well as 5-10% of non-responder (to a positive control for the IgE-mediated releasability of basophils with anti-IgE or anti-FcεRI antibodies) have been observed.[39] This lack of basophil responsiveness has been attributed to a down regulation of cytoplasmic tyrosine kinase (Syk), which is involved in the intracellular signaling pathway of the FcεRI.[61,62]

The lymphocyte transformation test (LTT) and the enzyme-linked immunosorbent spot (ELISpot) assay, as well as cell markers and cytokine release can be used for evaluating nonimmediate reactions to BL.[20,39,63-65] The *in vitro* tests for assessing nonimmediate reactions, particularly the LTT, should be used in high-risk patients before deciding on further investigations (moderate/strong). However, they are still rather complex procedures, which require skilled personnel and specific experience.[66] Moreover, most *in vitro* tests for diagnosing nonimmediate reactions are not commercially available, and therefore they are not as standardized as those for immediate reactions.[20] For these reasons, they are not routinely used and thus have not been validated in large patient cohorts.[20] Nevertheless, it is advisable to use *in vitro* tests (where appropriate) alongside ST for a complete diagnosis of nonimmediate reactions.

Drug Provocation Tests

A DPT is the controlled administration of a suspected drug in order to confirm or rule out hypersensitivity.[11,67] In this document, the term graded challenge refers to the administration of an alternative BL with high probability of being tolerated.

DPT have been recommended by both the European guidelines[22,30,31,67,68] and the U.S. practice parameter[35] as a part of the BL allergy workup. However, only the European guidelines[22,30,31,67,68]

emphasized their role in the establishment of a firm diagnosis of BL hypersensitivity in selected cases presenting negative ST and reliable *in vitro* tests and suggested using different DPT protocols depending on the type of reaction, immediate or nonimmediate. The American document[35] recommended DPT only if the probability of hypersensitivity is low and the clinical scenario justifies the possible risk, e.g., there is no comparable alternative medication.[27]

During the last decade DPT with the suspected BL have been performed in series larger than 100 subjects involving either pediatric[69-86] or adult[29,58,87-99] populations or both,[18,100-109] highly enhancing our knowledge on the usefulness and safety of this diagnostic tool in BL allergy workup. This knowledge has contributed to achieving agreement on several areas of the general principles for DPT (high/strong) (Table 5).

However, DPT protocols varied widely among studies, including European ones, in terms of dose steps, time intervals between incremental doses, and days of dosing, as well as diagnostic criteria for a positive result. In effect, the recent European survey[5] demonstrated a significant heterogeneity in current practice, especially concerning DPT. Several factors contribute to these differences: chronology of the index reaction (immediate *versus* nonimmediate reactions), reaction severity (anaphylaxis *versus* urticaria/MPE), population involved (children *versus* adults), as well as the experience and resources available in clinics.

In the last decade, several investigators have performed DPT without previous ST in subjects, especially children, with mild MPE or delayed-appearing urticaria.[79,80,83,86,98,108,110,111] This approach has also been suggested by other researchers,[82,85,95,105,112-116] getting a general consensus, as well as the endorsement by the EAACI DAIG pediatric task force[117] and the British Society for Allergy and Clinical Immunology[68] for use in children. Also in our opinion, DPT without previous ST can be performed in children with mild MPE, as well as in subjects with palmar exfoliative exanthema, whereas DPT without previous ST are not recommended in low-risk adults with nonimmediate reactions other than palmar exfoliative exanthema. (Figure 3).

However, there are still two main areas where consensus is lacking: (i) the optimal doses and dosing schedule for DPT; and (ii) whether exposing subjects with nonimmediate reactions to a single daily therapeutic dose (DTD) or a maximum single unit dose (MSUD; i.e, one-day DPT) is sufficient to establish a firm diagnosis or whether prolonged DPT (i.e., with a duration of more than one day) are needed in these subjects.

(i) Standardization of dosing is lacking and several different dosing approaches exist. Some studies, which assessed either nonimmediate reactors[29] or both immediate and nonimmediate reactors,[71,73,103] performed DPT with the suspected BL using three steps or less: e.g., 1–10–100%,[73] 25–25–50%,71 10–100%,[29] or 100%[103] of the MSUD. In one study,[95] either titrated or nontitrated DPT were carried out, depending on the estimated risk of inducing a reaction on the

basis of severity of the index reaction and the patient's comorbidities. Titrated DPT were performed using three steps (i.e., 1–10–100% of the MSUD). Nontitrated DPT were done administering directly a MSUD. Another study identified eliciting thresholds based on a survival analysis and suggested the following steps for DPT when using 30-minute intervals: 5–15–30–50% of the DTD, with additional lower steps of 0.01%, 0.1%, and 1% for index reactions of anaphylaxis.[106]

Considering studies that evaluated large samples of subjects with immediate reactions[58,91,94,100,101] and the aforementioned study,[106] two different protocols might be used in these subjects (moderate/weak) (Table 6), according to their risk profile (Table 2).

Regarding subjects with nonimmediate reactions, in low-risk adults and children with moderate MPE who are negative to allergy tests, it is advisable to carry out DPT with the suspected BL (high/strong). In this regard, one-tenth of the MSUD should be administered initially and, if tolerated, a full dose 1 to 7 days after, depending on the time interval of the index reaction. If the patient requires therapy at the time of the challenge, the one-tenth dose (if tolerated) should be followed one hour later by a full dose. If a full dose is tolerated, a normal course of the drug can be administered. Nevertheless, according to some studies[77,84,86,95,97,98,103,107,108] and the opinion of some of the authors of this paper, nonimmediate reactors at low risk might undergo directly DPT with 100% of MSUD of the suspected BL.

Following a DPT, a nonimmediate reaction can be excluded if after the achievement of the therapeutic dose a time interval has elapsed equal to that of the index reaction without the appearance of symptoms. Patients are also advised to return to report or show any reactions appearing within this time interval, as well as to take pictures of the latter.

(ii) Some groups consider the tolerance of a MSUD or DTD enough to exclude a delayed hypersensitivity.[18,29,73,74,80,87,89,92,93,97,98,103,106-108] In the related studies,[18,29,73,74,80,87,89,92,93,97,98,103,106-108] the rate of positive responses to DPT with suspected BL ranged from 0%[29,89] to 7.7%.[106]

Other groups believe that a too short exposure to BL (in terms of days) can be responsible for false negative DPT, this being the rationale for performing DPT with a duration of 2 to 5 days[69-71,75-79,82,83,85,86,95,99,104,105,109,111,112] or even of 7 to 10 days[70,81,88,90,95,104,111] to confirm or rule out a delayed hypersensitivity.

It should be noted that false negative results may also occur because of a too low dosage, missing cofactors like infections, and/or potential tolerance induction by the challenge procedure.[67]

In studies that performed prolonged DPT,[69-71,75-79,81-83,85,86,88,90,95,99,104,105,109,111,112] a rate of positive results ranging from 2%[78] to 17.2%[90] was found. Because such rate was greater than 10% in several studies,[69,70,88,90,95,105,111] it has been suggested that prolonged DPT increase the allergy

workup sensitivity in nonimmediate reactors to BL. However, there is evidence that one-day DPT can elicit reactions, as far as 48 hours after the last administered dose and, in some single cases, even later,[80,106,111] making it clear that at least a proportion of the positive results from prolonged DPT that did not respect a wash-out period (i.e., a time interval between the achievement of the therapeutic dose and subsequent administrations of this dose, which should be equal to the time elapsed between the administration of the first therapeutic dose and the appearance of the symptoms of the index reaction[111]) could have been achieved by one-day DPT. This emphasizes the importance of considering a wash-out period after the initial provocation, but only two studies[71,111] were conceived to respect a wash-out period of 24 hours and up to 10 days, respectively.

Few studies organized a follow-up, assessing the negative predictive value (NPV) of DPT in real-life conditions.[72,80,83,84,92,118-121] In two studies that carried out one-day DPT in subjects with nonimmediate reactions to BL,[80,118] the NPV of such DPT was 89.1% and 94.9%, respectively, whereas in four studies concerning pediatric populations, which performed prolonged DPT,[72,83,84,119] the NPV was 92.5%, 95.5%, 96.7%, and 93.3%, respectively.

Nonimmediate reactions following a negative one-day or prolonged DPT are described as mild/moderate. Nevertheless, in the studies that carried out one-day DPT,[80,92,118,120] the rate of subjects who have taken again the BL concerned in real-life conditions ranged from 22%[80] to 39%,[118] whereas in the studies that performed prolonged DPT with BL[72,83,84,109] such rate ranged from 52%[72] to 71%.[84] Moreover, Ratzon et al[122] found that 100% (n =18/18) of the patients who had undergone prolonged DPT and needed a BL used it, compared with only 76% (n = 16/21) of patients who had undergone one-day DPT.

Overall, in subjects who are otherwise healthy, potential drawbacks of prolonged DPT with antibiotics, such as microbial resistance and new sensitizations, should be balanced by potential benefits, such as picking up more nonimmediate reactions and convincing patients, or their parents and/or physicians, that treatments with the BL concerned in real life conditions will be tolerated.[72,83,84,109] According to most of the authors of this paper, one-day DPT should be preferred to prolonged DPT because it is sufficient to establish a firm diagnosis. Considering the literature data,[72,80,83,84,92,109,118,120] however, prolonged DPT might be done if it is necessary to convince patients, or their parents and/or physicians, that treatments with the BL concerned will be tolerated in real life conditions. In patients who undergo prolonged DPT, the wash-out period and duration of these DPT could be chosen according to the chronology of the index reaction.

In conclusion, there are still important areas regarding DPT protocols where consensus is lacking, even among the authors of this article. Geographical differences highlight the fact that a “one size fits all” approach is unlikely to be successful and therefore no firm recommendations on DPT

protocols are made in this paper and instead current data from recent studies have been presented. The introduction of a risk stratification to distinguish between high- and low-risk patients will enable the investigation strategies to be tailored to the individual patient ensuring a safe but more effective approach. Once risk stratification is more widely implemented and more studies have been published, it will be possible to reach consensus and make firm recommendations for DPT protocols.

The case for re-evaluation of patient sensitivity

Some studies demonstrated that up to 30% of patients with an IgE-mediated hypersensitivity to penicillins and/or cephalosporins may lose sensitivity and become ST negative within 1 year and more than 60% within 5 years.[123,124] In other studies performed on samples of at least 50 subjects with a suspected hypersensitivity to BL and negative allergy tests, including DPT, who underwent a re-evaluation about 1 month later,[94,100,125-127] a resensitization (i.e., a conversion to ST positivity) has been observed with a frequency ranging from 2%[127] to 15.9%.[100] Therefore, in strong suspicion of a hypersensitivity, although there is no clear consensus among experts,[128] according to the large majority of the authors of this paper, it is advisable to retest (2-4 weeks later) patients who suffered severe immediate reactions to BL more than 6 months before and display negative results in the allergy evaluation, including DPT (high/strong).

Cases of natural antibiotic tolerance acquisition have recently been reported in children with initial mild delayed exanthema proven by DPT,[84] suggesting that some forms of hypersensitivity to BL may not be a permanent condition.

CROSS-REACTIVITY AMONG BL AND TOLERABILITY OF ALTERNATIVE BL

Administration of alternative penicillins to patients with penicillin allergy

The literature data indicate a high degree of cross-reactivity among semi-synthetic penicillins, especially aminopenicillins (i.e., amoxicillin, ampicillin, bacampicillin, and pivampicillin) which share an amino group in their side chain, as well as between semi-synthetic penicillins and benzylpenicillin.[15,16] Nevertheless, there are studies in which subjects with either an IgE-mediated[91] or T-cell-mediated hypersensitivity to aminopenicillins[129] underwent graded challenges with penicillin G and penicillin V found negative in allergy tests and tolerated them.

Administration of cephalosporins to patients with penicillin allergy

In studies performed since 1990 on samples of at least 30 subjects with a documented IgE-mediated hypersensitivity to penicillins,[130-134] the rate of positive responses to ST with cephalosporins ranged from 0%[131,133] to 33.3%.[134] The highest rate was found in a study,[134] in which ST were performed with a panel of 9 cephalosporins, including cefamandole and aminocephalosporins that share similar or identical side-chains with penicillins. In these studies,[130-134] penicillin-allergic participants displaying negative results to cephalosporin ST

underwent challenges with cephalosporins like cefazolin, cefuroxime, ceftazidime, and ceftriaxone that do not share similar or identical side chains with penicillins. Of a total of 912 challenges, only 2 (0.2%) were positive.[133]

Of the 3 studies[38,129,135] that assessed cross-reactivity with cephalosporins in adults with a T-cell-mediated hypersensitivity – by performing ST and/or PT and, in case of negative results, challenges with the entire panel of tested cephalosporins – [238,135] found a rate of cross-reactivity with aminocephalosporins of 19.1% and 31.2%, respectively. In these studies,[38,129,135] of a total of 1,083 cephalosporin challenges, only 3 (0.3%) were positive.[38,129]

Administration of aztreonam and carbapenems to patients with penicillin allergy

Prospective studies on subjects with an IgE-mediated penicillin allergy have demonstrated a rate of cross-reactivity between penicillins and carbapenems and/or aztreonam lower than 1%.[136-140] Two studies regarding subjects with a T-cell-mediated hypersensitivity to penicillins[38,141] have documented an absence of cross-reactivity with either aztreonam or carbapenems. In the aforesaid studies concerning hundreds of subjects with either IgE-mediated[136-140] or T-cell-mediated hypersensitivity to penicillins,[38,141] all participants displaying negative results to ST with aztreonam and carbapenems tolerated challenges.

Administration of penicillins, aztreonam, carbapenems, and alternative cephalosporins, to patients with cephalosporin allergy

In a study regarding 98 subjects with an IgE-mediated hypersensitivity to cephalosporins,[142] about 25% of the subjects had positive allergy tests to penicillins, 3% to aztreonam, 2% to imipenem/cilastatin, and 1% to meropenem. In this study,[142] all subjects displaying negative ST and SsIgE with these alternative BL tolerated them, except for one subject who reacted to imipenem/cilastatin.

Another study[143] on 102 adults with such hypersensitivity identified 3 groups of cephalosporins: group A, which included those with a methoxyimino group in their R1 side chains (i.e., cefuroxime, ceftriaxone, cefotaxime, cefodizime, and cefepime) plus ceftazidime, group B, which was composed of aminocephalosporins, and group C, which included cephalosporins other than those belonging to groups A and B. This study[143] demonstrated the usefulness of considering side-chain groups when selecting alternative cephalosporin in cephalosporin-allergic subjects. In effect, all 323 challenges with alternative cephalosporins found negative in skin testing were tolerated.

Overall, the literature data indicate that cross-reactivity related to the common BL ring, which entails positive allergy tests to all BL, is very rare in subjects with an IgE-mediated hypersensitivity and is absent in those with a T-cell-mediated hypersensitivity.[15] More frequently,

cross-reactivity among BL is connected with structural similarities or identities among their side-chain structures.[15,16] In this regard, the similarity or identity of the branch chain moiety of cephalosporin R1 structure is more frequently related to cross-reactivity among cephalosporins than the similarity or identity of the ring of the R1 structure is.[15,16,144] However, some studies concerning cross-reactivity among BL have found patterns of allergy-test positivity which cannot be explained by either the common BL ring or by similar or identical side chains[129,132-134,142,143] thus indicating the possibility of coexisting sensitivities to different BL because of prior exposures to them. For this reason, prophylactic ST with the BL concerned are advisable before their administration via graded challenges to BL-allergic patients who need alternative BL (high/strong). In graded challenges, usually, an initial dose of one tenth of the MSUD is administered. In patients with negative results, a full MSUD is administered 1 hour later.

If it is not possible to carry out a complete allergy workup, patients with histories of immediate reactions to penicillins and a compelling need of a cephalosporin or another alternative BL can undergo ST with cephalosporins (or carbapenems, or aztreonam) that have side chains different from those of the responsible penicillins, and, in case of negative results, graded challenges with the alternative BL concerned (high/strong). A similar approach can be chosen in patients with histories of cephalosporin allergy who need an alternative BL, including another cephalosporin.

In low-risk patients with mild nonimmediate reactions to BL who require alternative BL, if there's no time to wait for the delayed reading of pre-treatment ST, giving under close surveillance a full dose of a structurally non-related BL (Figure 1) can be considered.

Conclusion

This document provides an update of previous guidelines of the EAACI DAIG for the diagnosis of BL hypersensitivity,[31] which concerns availability and concentrations of ST reagents, ST and DPT protocols, as well as diagnostic algorithms and administration of alternative BL in subjects with BL hypersensitivity. About DPT, considering the literature data of the last decade and the results of a recent EAACI DAIG survey,[5] which documented deviations from DAIG recommendations and stressed the need to standardize them, authors tried to meet this need. However, further studies are necessary to provide data supporting the standardization of DPT protocols, especially of those regarding nonimmediate reactions, for which there is currently no consensus.

Unlike the previous guidelines,[31] this paper provides a risk stratification in BL hypersensitivity according to index reaction(s), as well as an algorithmic approach, based on cross-reactivity studies, in patients with a suspicion of BL hypersensitivity and an immediate need for an antibiotic therapy, when referral to an allergist is not feasible.

Regarding the allergy workup, the main novelty of this document is the fact that in some low-risk nonimmediate reactions ST are not mandatory, especially in children. Overall, a positive ST or in

vitro test result allows the allergist to diagnose a BL hypersensitivity (even with a small risk of false positivity), whereas a negative test result has more limited value in determining the risk for future reactions. In this case, it is advisable to complete the allergy workup by performing the DPT if it is not contraindicated.

Acknowledgements

We thank Professor Massimo Castagnola (Istituto di Biochimica e Biochimica Clinica, Università Cattolica del Sacro Cuore, Rome) for his valuable help regarding structural similarities between beta-lactams.

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FIGURE 1 Comparison of R1 and R2 structural similarities between beta-lactams.
Drugs that have identical R1 or R2 structures are listed as R1 (orange cell) or R2 (yellow cell). If only the ring or branch chain moiety of the R1 structure is identical, it is listed as R1' or R1'', respectively. Drugs that have similar R1 or R2 structures are listed as r1 or r2. If only the ring or branch chain moiety of the R1 structure is similar, it is listed as r1' or r1'', respectively. Blank cells imply no R1 or R2 structural similarities.
Bold type for penicillins indicates that the related use represented more than 1% of total penicillin use in Europe in 2009. Bold type for cephalosporins indicates that the related use represented more than 1% of total outpatient cephalosporin use in Europe in 2009.

FIGURE 2 Algorithm for the diagnosis of immediate hypersensitivity reactions to beta-lactams.

FIGURE 3 Algorithm for the diagnosis of nonimmediate hypersensitivity reactions to beta-lactams.

TABLE 1 Danger signs in beta-lactam allergy

Immediate reactions	Nonimmediate reactions
• Palmo-plantar, genital, ear, and/or head itching • Conjunctival redness • Blood pressure drop symptoms (e.g., dizziness, fainting, need to lie down etc) • Cough • Sneezing • Wheezing • Dyspnea • Dysphonia • Dysphagia	• Intense facial involvement • Atypical target lesions • Bullous lesions • Widespread dark-red erythema • Extensive pustulosis • Painful skin • Mucosal involvement • Generalized lymphadenopathy • Elevated liver enzymes • Impaired renal function tests • Fever > 38.5°C • Alterations in blood cell counts (i.e., anemia, granulocytopenia, thrombocytopenia, neutrophilia, eosinophilia) • Hypocomplementemia • Hepatitis, nephritis, pneumonitis

TABLE 2 Risk stratification in beta-lactam allergy according to index reaction(s)

High-risk patients*	*Immediate reactions*	○ Anaphylaxis ○ Hypotension ○ Laryngeal edema ○ Bronchospasm ○ Urticaria and/or angioedema ○ Generalized erythema
	Nonimmediate reactions	○ Stevens-Johnson syndrome ○ Toxic epidermal necrolysis ○ Drug reaction (or rash) with eosinophilia and systemic symptoms ○ Generalized bullous fixed drug eruption ○ Acute generalized exanthematous pustulosis ○ Linear IgA bullous dermatosis ○ Severe maculopapular exanthema (MPE)❶ ○ Systemic vasculitis/Serum-sickness–like reaction ○ Specific organ manifestations❷ ○ Drug-induced autoimmune diseases❸
Low-risk patients¶	*Immediate reactions*	○ Isolated generalized pruritus that did not require treatment ○ Isolated gastrointestinal symptoms (e.g., nausea, diarrhea, vomiting)

		○ Local urticaria to parenteral administration
	Nonimmediate reactions	○ Contact dermatitis ○ Systemic contact dermatitis ○ Local infiltrated reaction to intramuscular administration ○ Palmar exfoliative exanthema ○ Fixed drug eruption ○ Delayed-appearing urticaria ○ Mild❹/Moderate❺MPE (especially in children) ○ Symmetric drug-related intertriginous flexural exanthema

*Pregnant women and subjects with severe cardiovascular, renal, and/or respiratory compromise, as well as those with systemic mastocytosis or treated with beta-blockers should be considered at high risk, independently of their index reactions.

¶Patients who experienced reactions with unspecified morphology (characteristics) and/or chronology can be considered at low risk if danger signs can be excluded with confidence.

❶Widespread rash that may become confluent and develop into erythroderma; >1-week duration, with systemic involvement (e.g., fever, eosinophilia); rarely, with minimal vesicles or pustules.

❷Blood cytopenia (i.e., anemia, granulocytopenia, thrombocytopeni), hepatitis, nephritis, pneumonitis.

❸Systemic (or cutaneous) lupus erythematous, pemphigus vulgaris, bullous pemphigoid.

❹ More or less widespread rash; <1-week duration, without systemic involvement.

❺More or less widespread rash; >1-week duration, without systemic involvement.

TABLE 3 Algorithmic approach to patients with histories of hypersensitivity reactions to specified penicillins and/or cephalosporins and an immediate need for antibiotic therapy, when referral to an allergist is not feasible

Nonimmediate reactions to penicillins and/or cephalosporins High-risk subjects	**Immediate reactions to penicillins and/or cephalosporins** High-risk subjects	**Nonimmediate or immediate reactions to penicillins and/or cephalosporins** Low-risk subjects
↓	↓	↓
Avoid using penicillins and cephalosporins; use non-BL antibiotics by microbial coverage OR Use carbapenems or aztreonam* by graded challenge¶, after careful risk benefit analysis in SJS/TEN OR Use by graded challenge 3rd/4th/5th generation cephalosporins in subjects who reacted to penicillins¶ or penicillins with side chains different from those of the responsible cephalosporins in subjects who reacted to cephalosporins¶, very carefully in SJS/TEN	Avoid using the entire class of the responsible BL; OR Use by graded challenge 3rd/4th/5th generation cephalosporins in subjects who reacted to penicillins§ or penicillins with side chains different from those of the responsible cephalosporins in subjects who reacted to cephalosporins§ OR Use carbapenems or aztreonam* by graded challenge§ OR Use non-BL antibiotics by microbial coverage	Use full dose 3rd/4th/5th generation cephalosporins in subjects who reacted to penicillins or full dose penicillins with side chains different from those of the responsible cephalosporins in subjects who reacted to cephalosporins OR Use full dose carbapenems or aztreonam* OR Use non-BL antibiotics by microbial coverage

SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

*Except in subjects who experienced reactions to ceftazidime.

¶In the case of non-BL-therapy clinical failure.

§Except in subjects who experienced severe anaphylaxis.

TABLE 4 Highest nonirritating concentrations recommended for both prick and intradermal testing with beta-lactams

Hapten	Concentration
Benzylpenicilloyl-poly-L-lysine	6.0×10^{-5} mol/L
Benzylpenicilloyl-octa-L-lysine	8.64×10^{-5} mol/L
Sodium benzylpenilloate	1.5×10^{-3} mol/L
Benzylpenicillin	10,000 IU/mL
Amoxicillin and other semi-synthetic penicillins	20 mg/mL*
Cefepime	2 mg/mL
Other cephalosporins	20 mg/mL
Clavulanic acid	20 mg/mL
Aztreonam	2 mg/mL
Imipenem-cilastatin	0.5 mg/ml-0.5 mg/mL
Meropenem and ertapenem	1 mg/mL

*Concentrations of 25 mg/mL have also been used and accepted for semi-synthetic penicillins because they are nonirritanting.[36]

Table 5. Areas of agreement on the general principles of drug provocation test (DPT) with beta-lactams (BL)

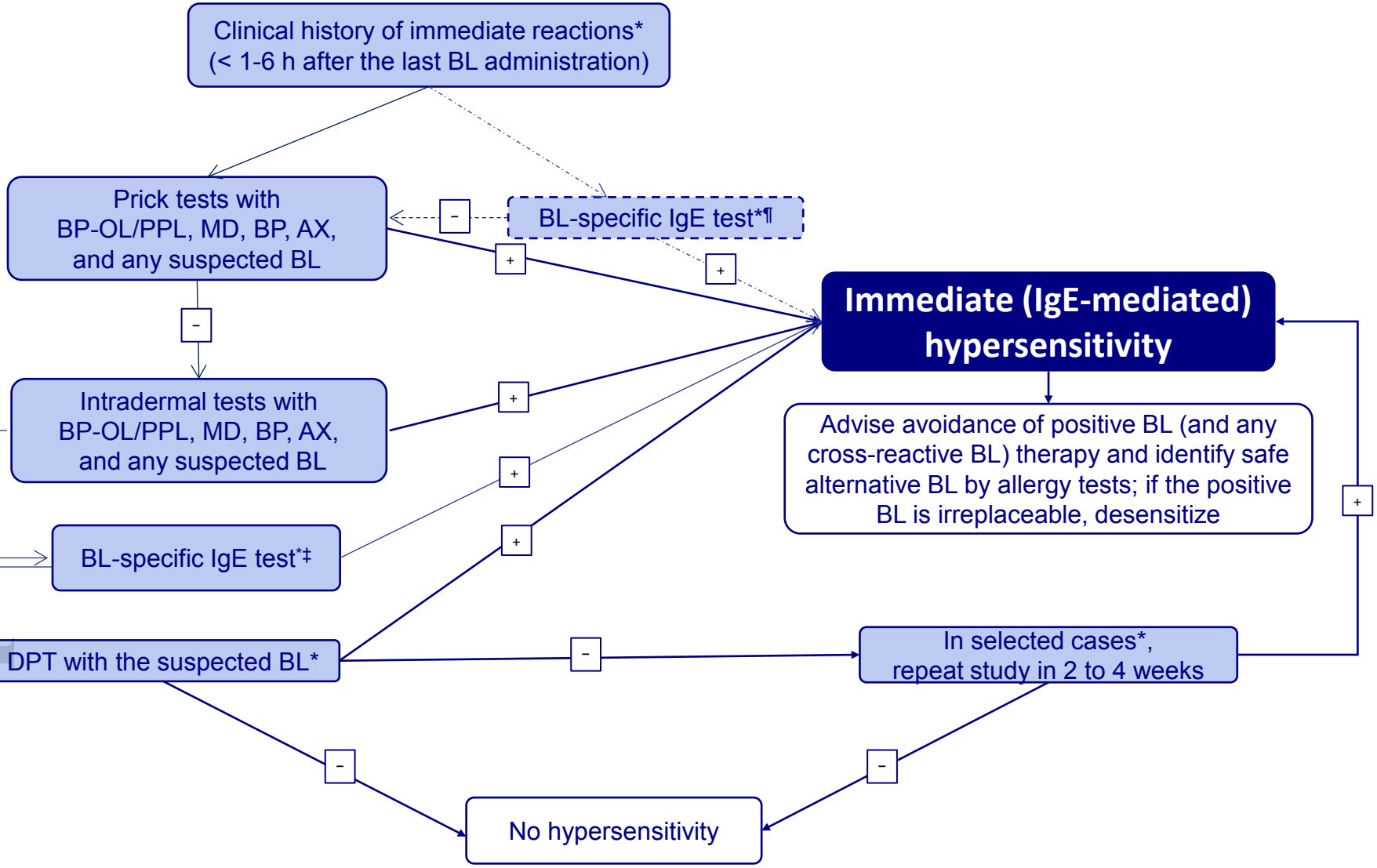
DPT remains the gold standard to diagnose both immediate and nonimmediate hypersensitivity reactions to BL.
DPT should be performed in an appropriate setting by a specialized team trained to recognize and treat allergic symptoms.
Antihistamines, systemic corticosteroids, and other medications potentially interfering with symptoms during provocation (e.g., beta-blockers, ACE-inhibitors, immunosuppressive drugs etc) should be stopped before performing DPT according to the EAACI[67] and local guidelines.
An open, single, or double-blinded procedure can be chosen based on the clinical profile of the patient.
An observation period of at least 1-2 hours is indicated at the end of the DPT.*
DPT with the suspected BL is indicated in low-risk subjects with histories of nonimmediate reactions (Table 2).
DPT with the suspected BL is indicated in patients with histories of immediate reactions presenting negative skin tests and *in vitro* tests.
There is a relative contraindication to performing DPT in patients with uncontrolled asthma, active urticaria, or an underlying disease limiting the use of rescue medications such as adrenaline.
DPT with the suspected BL is contraindicated in high-risk patients with histories of severe nonimmediate reactions (Table 2) or near fatal anaphylaxis, and caution is recommended in patients with systemic mastocytosis, who are at increased risk of severe reactions because of an increased number of effector cells.

*The recommended observation period is 1 to 2 hours after the last administered dose because severe reactions to BL, such as anaphylaxis, generally appear within this time interval.[106]

TABLE 6 Doses recommended for drug provocation tests in subjects with immediate reactions

Low-risk subjects	High-risk subjects
10% → 40% → 50% of the maximum single unit dose Interval between doses: 30-60 minutes (depending on that of the index reaction)	1% → 10% → 40% → 49% of the maximum single unit dose *[or 1% → 5% → 15% → 30% → 49% of the maximum single unit dose]* Interval between doses: 30-60 minutes (depending on that of the index reaction)

		Penicillins (Peni)								Cephalosporins (Ceph): 1st generation						2nd									3rd												4th		5th				Mono
		Flucloxacillin	Dicloxacillin	Penicillin G	**Penicillin V**	Piperacillin	Ampicillin	**Amoxicillin**	**Pivmecillinam**	**Cefadroxil**	Cefatrizine	**Cephalexin**	**Cefazolin**	Cephalothin	Cefradine	Cefoxitin	**Cefuroxime**	Cefotiam	**Cefprozil**	**Cefaclor**	Cefonicid	Cefamandole	Ceforamide	Loracarbef	Cefoperazone	**Ceftibuten**	**Cefixime**	**Ceftriaxone**	Cefditoren	Cefodizime	Cefotaxime	**Cefpodoxime**	Ceftizoxime	Cefetamet	Ceftazidime	Cefepime	Cefpirome	Cefataroline fosamil	Ceftolozane	Cefiderocol	Aztreonam
Peni	Flucloxacillin		r1																																						
	Dicloxacillin	r1																																							
	Penicillin G				r1'	r1'	r1'	r1'		r1	r1	r1							r1	r1	r1	r1	r1	r1	r1'																
	Penicillin V			r1'		r1'	r1'	r1'		r1	r1	r1							r1	r1	r1	r1	r1	r1	r1'																
	Piperacillin			r1'	r1'		R1'	r1'		r1'	r1'	R1'							r1'	R1'	r1'	r1'	r1	R1'	R1''																
	Ampicillin			r1'	r1'	R1'		r1'		r1	r1	R1			r1				r1	R1	r1	r1	r1	R1	r1'																
	Amoxicillin			r1'	r1'	r1'	r1'			R1	R1	r1			r1				R1	r1	r1	r1	r1	r1	R1'																
	Pivmecillinam																																								
Ceph 1st	**Cefadroxil**			r1	r1	r1'	r1	R1			R1	r1			r1				R1	r1	r1	r1	r1	r1	R1'																
	Cefatrizine			r1	r1	r1'	r1	R1		R1		r1			r1			r2	R1	r1	r1	r1	r1r2	r1	R1'																
	Cephalexin			r1	r1	R1'	R1	r1		r1	r1				r1				r1	R1	r1	r1	r1	R1	r1'																
	Cefazolin																																								
	Cephalothin															R1r2	r1'r2														R2										
	Cefradine						r1	r1		r1	r1	r1							r1	r1				r1																		
2nd	Cefoxitin													R1r2			r1'R2														r2										
	Cefuroxime													r1'r2		r1'R2											r1''	R1''	R1''	R1''r2	R1''	R1''	R1''	r1''	R1''	R1''	R1''	r1''	r1''	r1''
	Cefotiam										r2								r2	r2	r2		r2	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	r1'	r1'	R1'	R1'
	Cefprozil			r1	r1	r1'	r1	R1		R1	R1	r1			r1					r1	r1	r1	r1	r1	R1'																
	Cefaclor			r1	r1	R1'	R1	r1		r1	r1	R1			r1				r1		r1	r1	r1	R1	r1'																
	Cefonicid			r1	r1	r1'	r1	r1		r1	r1	r1						r2	r1	r1		R1r2	r1r2	r1	r1'r2																
	Cefamandole			r1	r1	r1'	r1	r1		r1	r1	r1						r2	r1	r1	R1r2		r1r2	r1	r1'R2																
	Ceforamide			r1	r1	r1	r1	r1		r1	r1r2	r1						r2	r1	r1	r1r2	r1r2		r1	r1r2																
	Loracarbef			r1	r1	R1'	R1	r1		r1	r1	R1			r1				r1	R1	r1	r1	r1		r1'																
3rd	Cefoperazone			r1'	r1'	R1''	r1'	R1'		R1'	R1'	r1'						r2	R1'	r1'	r1'r2	r1'R2	r1r2	r1'																		
	Ceftibuten																	R1'									R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	r1	r1	R1'	R1'
	Cefixime																r1''	R1'								R1'		R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	r1	r1	R1'	R1'
	Ceftriaxone																R1''	R1'								R1'	R1'		R1	R1	R1	R1	R1	R1	R1	R1	R1''	r1	R1'	R1'
	Cefditoren																R1''	R1'								R1'	R1'	R1		R1	R1	R1	R1	R1	R1	R1	R1''	r1	R1'	R1'
	Cefodizime																R1''	R1'								R1'	R1'	R1	R1		R1	R1	R1	R1	R1	R1	R1	R1''	r1	R1'	R1'
	Cefotaxime													R2		r2	R1''r2	R1'								R1'	R1'	R1	R1	R1		R1	R1	R1	R1	R1	R1''	r1	R1'	R1'
	Cefpodoxime																R1''	R1'								R1'	R1'	R1	R1	R1	R1		R1	R1	R1	R1	R1	R1''	r1	R1'	R1'
	Ceftizoxime																R1''	R1'								R1'	R1'	R1	R1	R1	R1	R1		R1	R1	R1	R1	R1''	r1	R1'	R1'
	Cefetamet																R1''	R1'								R1'	R1'	R1	R1	R1	R1	R1	R1		R1	R1	R1	R1''	r1	R1'	R1'
	Ceftazidime																r1''	R1'								R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'		R1'	R1'r2	r1	R1''	R1	R1
4th	Cefepime																R1''	R1'								R1'	R1'	R1	R1	R1	R1	R1	R1	R1	R1'		R1	R1''	r1	R1'r2	R1'
	Cefpirome																R1''	R1'								R1'	R1'	R1	R1	R1	R1	R1	R1	R1	R1'r2	R1		R1''	r1	R1'	R1'
5th	Cefataroline fosamil																R1''	r1'								r1	r1	R1''	R1''	R1''	R1''	R1''	R1''	r1	R1''	R1''		R1'	r1	r1
	Ceftolozane																r1''	r1'								r1	r1	r1	r1	r1	r1	r1	r1	R1''	r1	r1	R1'		R1''	R1''
	Cefiderocol																r1''	R1'								R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1	R1'r2	R1'	r1	R1''		R1
Mono	Aztreonam																r1''	R1'								R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1'	R1	R1'	R1'	r1	R1''	R1	



AX = amoxicillin
BL = beta-lactam
BP = benzylpenicillin
BP-OL = benzylpenicilloyl-octa-L-lysine
DPT = drug provocation test
MD = minor determinant
PPL = benzylpenicilloyl-poly-L-lysine
* = see text

¶The dotted lines refer to the first part of the diagnostic algorithm for patients who experienced severe anaphylaxis and undergo BL-specific IgE test before skin tests, whereas continuous lines refer to the algorithm for other patients with immediate reactions.

‡Do not retest patients who experienced severe anaphylaxis.

Low-risk subjects with histories of nonimmediate reactions*
(> 1 h after the initial BL administration)

Prick and intradermal tests with
PPL/BP-OL*, MD*, BP, AX, and the suspected BL

20 min

\+

IgE-mediated
hypersensitivity

\–

Late skin test reading
(48 h to 1 week)

\+

T-cell-mediated
hypersensitivity

\–

Perform DPT with the suspected BL*

\+

Undetermined
pathogenic mechanism

\–

Suspected BL therapy may be advised

Advise avoidance of positive BL (and any cross-reactive BL) therapy and identify safe alternative BL by allergy tests

--- = These tests are not mandatory in children with mild maculopapular exanthema and in subjects with palmar exfoliative exanthema*
AX = amoxicillin
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* = see text