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Adding a second skin prick test reading and modifying the cut-off for beta-lactam–specific IgE enhances the sensitivity in the routine diagnostic workup for immediate beta-lactam hypersensitivity

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Abstract

Background: Beta-lactam (BL)–antibiotics are the most frequent reason for drug-induced hypersensitivity reactions. Because they are more efficient, less toxic, and less costly than other antibiotics, confirmation or exclusion of BL allergy is worthwhile. However, allergy tests for drug allergies are often false-negative.

Objectives: To evaluate the components of a stepwise diagnostic algorithm for immediate BL hypersensitivity with regard to sensitivity (SENS).

Methods: Consecutive patients with suspected allergy to BL antibiotics were retrospectively analyzed with regard to increasing sensitivity (plausible history of immediate BL hypersensitivity serving as external criterion) of (i) skin prick test (SPT) by adding a second reading (n = 746), (ii) BL-specific IgE-determination in vitro at two cut-offs (n = 539), and (iii) adding in vivo testing of minor and major BL determinants (n = 288).

Results: In the history-based population indicative of immediate BL hypersensitivity (n = 457), SPT with a sole 20-minute reading identified 99 (SENS: 0.21) and SPT with 20- and 40-minute-reading identified 133 cases (SENS: 0.29). in vitro specific IgE-examination identified 31 positives at a cut-off ≥0.35 kUA/L (5.8% of tested) and 99 at cut-off ≥0.11 kUA/L (18.4% of tested). In 203 SPT-negative individuals, immediate BL hypersensitivity was identified by additional tests: in 79 by specific IgE (cut-off ≥0.11 kUA/L) (thereof 53 identified solely by this test) and in 150 by in vivo testing of BL determinants in combination with Penicillin and Ampicillin intradermally (thereof 124 solely by this test); in 26 individuals both additional tests were positive. The combination of the three outpatient-based test modalities—(i) optimized SPT, (ii) specific IgE at optimized cut-off, and (iii) in vivo testing of BL determinants/Penicillin/Ampicillin—identified altogether 336/457 individuals with immediate BL-hypersensitivity (SENS: 0.73), whereas the combination of the two (i) + (ii) identified 212/457 (SENS: 0.46); (i) + (iii) 283/457 (SENS: 0.61).

Conclusions: To overcome the low sensitivity of allergological tests, optimized reading times of the SPT of BL, a lower cut-off for in vitro detection of BL-specific IgE, and intradermal testing of Penicillin, Ampicillin, and BL-determinants contribute to overall sensitivity under real life conditions to diagnose immediate BL-hypersensitivity.

KEYWORDS

allergy, beta-lactam antibiotics, sensitivity, skin prick test, reading times, specific IgE, cut-off

1 | INTRODUCTION

Beta-lactam (BL) antibiotics, mainly Penicillins, are often prescribed[1,2] because they are more efficient, less toxic, and less costly for the health care system than other antibiotics.[3-9] However, BLs are the most frequent reason for drug-induced hypersensitivity reactions due to their allergenicity and their common use.[10] Confirmation or exclusion of the diagnosis is important, both to prevent the prescription of BLs to allergic patients and to avoid unnecessary restrictions and prescription of less suitable alternatives in non-allergic individuals.[11] Because allergy tests for drug allergy are often false-negative, a stepwise approach for making the diagnosis is necessary.[12]

Hypersensitivity reactions to BLs are classified as immediate or non-immediate: The former usually appear within 1 hour of drug-intake and are mediated by specific immunoglobulin E (IgE) antibodies.[10] The European Network for Drug Allergy (ENDA) has devised diagnostic algorithms for the evaluation of immediate[13] and non-immediate reactions.[14] According to best practice algorithms,[10] in suspected cases the diagnosis of immediate BL hypersensitivity is based on one positive test out of four steps (skin prick test [SPT], intradermal test [IDT], in vitro test, and drug provocation test). However, differences exist in BL-allergy diagnostic pathways and pragmatic approaches concerning the sequence of test procedures as well as the selection of test substances.[15-17]

While the specificity of SPTs and IDTs of BLs in patients with an immediate-type reaction to Penicillins is good (97%–99%),[13,18] a comparatively low[13,18-24] sensitivity was found in a number of studies (Table S1), ranging from 0% to 70% for three or, respectively, four combined skin test substances[18,23] depending on the applied test (SPT vs IDT), and 21%–43% for single substances (Minor-Determinant-Mixture [MDM] 21%, Penicilloyl Polylysine [PPL] 22%, Ampicillin 33%, Amoxicillin 43%).[18]

The combination of PPL and MDM (resulting in at least one of the two positive) in patients with BL allergy yielded a sensitivity of 14.7%–61.5% (14.7%,[20] 26.5%,[19] 38%,[18] and 61.5%[22]) depending on several factors of study design/diagnostic approach (for details see Table S1), such as:

- patient selection (eg, only immediate allergic reactions versus inclusion of both immediate as well as non-immediate allergic reactions)
- definition/quality of external comparator to denominate the "true" entity of all diseased (eg, by use of several diagnostic aspects/methods combined vs a single comparator)
- time interval between allergic drug reaction and diagnostic procedure
- concentration of test substances (diluted or stock solution)
- number of test substances (alone or combined)
- test procedure (SPT and/or IDT; alone or combined)
- use of a late reading (between 8 and 72 hours)
- inclusion of a scheduled retest procedure in case of negative test results
- country (due to different prescription habits)
- year/decade of testing (due to changing prescription habits).

To improve sensitivity, harmonization of skin test procedures,[25] addition of test substances (such as minor determinants[10]), and standardization of test concentrations[26] have been suggested.

Based on investigations of ubiquitous environmental aeroallergens such as pollen and mite allergens, recommendations regarding SPTs were published,[27] which define 15 minutes as an optimal reading time. However, due to the different nature and exposure of allergens, as well as the amount and affinity of specific IgE (sIgE) antibodies, these standardized test conditions may not apply for BL antibiotics.

In the present analysis, we investigated the SPT sensitivity by adding an additional reading after 40 minutes to in vivo allergy testing of BL antibiotics in a cohort of patients with suspected BL hypersensitivity who were undergoing allergological work-up. Furthermore, BL-specific IgE determination in vitro at two cut-offs and/or adding in vivo testing of minor and major BL determinants combined with IDT of Penicillin G and Ampicillin was evaluated concerning their effect on sensitivity of ambulatory test procedures.

2 | METHODS

Consecutive patients (n = 769) presenting with a clinical history of suspected type I- or type IV-allergy to beta-lactam antibiotics between January 1, 2006 and September 11, 2012 in the Allergy clinics of the Department of Dermatology, University Hospital of Erlangen, were examined according to current medical practice

applying in-house standards. Data were retrieved and analyzed using the program Winalldat (Version 1.60, provided by the Information network of Departments of Dermatology, Göttingen, Germany).[28] The outpatient-based stepwise diagnostic algorithm to confirm or exclude immediate BL hypersensitivity comprised

- SPT of selected Penicillins/Cephalosporins (SPT substances see Table 1; results viable in n = 746 patients),
- specific IgE analysis in vitro (performed in n = 644),
- SPT and IDT of major and minor BL determinants (Penicilloyl Polylysine (PPL), MDM (Minor-Determinant-Mixture)) purchased from Diater, Madrid, Spain (DAP Penicillin) (results viable in n = 288 patients), thereof n = 254 in combination with IDT of Penicillin G (Infectocillin parenteral, InfectoPharm Arzneimittel und Consilium, Heppenheim, Germany) and Ampicillin (Ampicillin-ratiopharm 1.0 g, Merckle, Blaubeuren, Germany) (see Table 2).

As part of the skin test routine for suspected allergy to BL, patient ID, age, gender, and SPT results after 20 and 40 minutes were documented in Winalldat as structured data. In addition to a standard reading at 15–20 minutes,[29] an additional reading was routinely carried out after 40 minutes in all patients. SPT was regarded as positive based on a wheal diameter ≥ 3 mm, in the IDT ≥6 mm. Control test substances were histamine and NaCl 0.9% (Allergopharma, Reinbek, Germany) to verify the eligibility of patients for in vivo test procedures. Negative reactions to histamine or positive reactions to NaCl at the time of SPT of selected Penicillin/Cephalosporin (Table 1) (n = 16) and skin testing of DAP Penicillin combined with IDT of Penicillin G and Ampicillin (Table 2) (n = 15), respectively, documenting unsuitable test conditions, led to exclusion from the analysis.

Specific in vitro IgE results (test substances see Table 3) were retrieved from the LAURIS database (Swisslab, Version: 15.09.28, Berlin, Germany). Positive in vitro test results (Amoxicilloyl, Ampicilloyl, Penicilloyl G, Penicilloyl V and Cefaclor: Immuno-CAP (FEIA). ThermoFisher Scientific, Phadia, Uppsala, Sweden) were defined at a cut-off ≥0.35 kUA/L or ≥ 0.11 kUA/L, respectively. Data on the onset and morphology of previous skin reaction linked to BL as well as on atopic comorbidities (seasonal/perennial allergic rhinoconjunctivitis, allergic bronchial asthma, and/or atopic dermatitis) were retrieved from the hospital information system Soarian (Soarian Health Archive, Cerner Health Services Deutschland, Idstein, Germany).

TABLE 2 Results of combined test procedures: SPT and intradermal test (ie, intracutaneous application, i.c.) of (i) major and minor beta-lactam determinants, (ii) intradermal test of Penicillin G and Ampicillin

Tested substance	Test concentration	Tested patients n =	Patients with positive reaction n =
PPL prick	5×10^{-5} mM	284	3 (1.1%)
MDM 1:10 prick	2×10^{-2} mM	284	4 (1.4%)
MDM undiluted prick	2×10^{-2} mM	284	4 (1.4%)
PPL, i.c.	5×10^{-5} mM	272	36 (13.2%)
MDM 1:10, i.c.	2×10^{-2} mM	273	17 (6.2%)
MDM pure, i.c.	2×10^{-2} mM	272	38 (14.0%)
Penicillin G, i.c.	10.000 IU/mL	254	165 (65.0%)
Ampicillin, i.c.	20 mg/mL	254	117 (46.1%)

Abbreviations: i.c., intracutaneously; MDM, Minor-Determinant-Mixture; PPL, Penicilloyl Polylysine; SPT, skin prick test.
Altogether, n = 288 patients were tested with different frequency for different test substances (test substances freshly prepared according to[26] for non-irritant skin test concentrations).

TABLE 1 Skin prick test results to selected Penicillins and Cephalosporins freshly prepared according to[26] for non-irritant skin test concentrations

Drug	Test concentration	Tested patients n=	Patients with positive reaction in total n=	Patients with positive reaction after 20 and 40 min n=	Patients with positive reaction after 20 min n=	Patients with positive reaction after 40 min n=
Amoxicillin sodium	20 mg/mL	746	33 (4.4%)	16	10	7
Penicillin G(benzylpenicillin)	10000 IU/mL	746	33 (4.4%)	18	11	4
Phenoxy-methylpenicillin	20 mg/mL	746	31 (4.2%)	20	8	3
Ampicillin	20 mg/mL	746	21 (2.8%)	10	5	6
Amoxicillin/Clavulanic acid	20 mg/mL	746	23 (3.1%)	14	6	3
Cefotiam	2 mg/mL	746	37 (5.0%)	24	9	4
Ceftriaxone	2 mg/mL	746	20 (2.7%)	13	4	3
Cefuroxime axetil	2 mg/mL	746	25 (3.4%)	11	9	5
Cefotaxime	2 mg/mL	746	24 (3.2%)	9	12	3
Cephalexin	2 mg/mL	746	20 (2.7%)	9	5	6
Piperacillin	20 mg/mL	746	15 (2.0%)	8	6	1
Mezlocillin	20 mg/mL	720	16 (2.2%)	5	6	5
Flucloxacillin	20 mg/mL	746	25 (3.4%)	14	5	6

TABLE 3 Results of in vitro determination of beta-lactam-specific IgE at two different cut-offs

Tested substance	Tested patients n =	Patients with positive test result cut-off ≥0.35 kUA/L n =	Patients with positive test result cut-off ≥0.11 kUA/L n =
Amoxicilloyl	537	2(0.4%)	29(5.4%)
Ampicilloyl	537	12(2.2%)	73(13.6%)
Cefaclor	223	9(4.0%)	24(10.8%)
Penicilloyl G	538	13(2.4%)	33(6.1%)
Penicilloyl V	537	15(2.8%)	40(7.4%)

Examined in parallel in 539 patients during the period from February 2007 to September 2012: cut-off ≥0.35 kUA/L vs ≥0.11 kUA/L.

TABLE 4 Culprit beta-lactam drug and reaction type by history (n = 481)

Culprit drug by history	Immediate reaction	Combined reaction (immediate/non-immediate)	Non-immediate	Total
Amoxicillin sodium	84	91	33	208
Penicillin	88	33	13	134
Phenoxymethylpenicillin	8	3	0	11
Ampicillin	13	13	1	27
Amoxicillin/Clavulanic acid	7	18	4	29
Piperacillin	2	3	0	5
Mezlocillin	0	0	0	0
Flucloxacillin	2	3	1	6
Cefotiam	4	4	1	8
Ceftriaxone	7	1	1	9
Cefuroxime axetil	15	14	5	34
Cefotaxime	0	1	0	1
Cephalexin	5	2	1	8

The diagnostic approach to establish a plausible history classifying the hypersensitivity reaction linked to BL intake as immediate or non-immediate reaction succeeded in 481 cases (Table 4): In the examined cohort immediate hypersensitivity reaction prevailed (n = 235 cases, ≈49%), followed by non-immediate hypersensitivity in n = 186 (≈39%) and "mixed hypersensitivity" (with concomitant characteristics of both) in 60 cases (≈12%). In the other 288 cases, no robust history on symptom onset after drug intake or morphology of skin lesions could be established. Due to the post hoc character of our evaluations of the data collected according to clinical standards in medical practice, which are limited by the fact that not all patients were taking all diagnostic tests for confirmatory diagnosis, we chose in the present study as a conservative external comparator for the (hypothetical maximum) entity of diseased patients, the existence of a conclusive history for BL allergy (knowing that the "true" number of all BL allergic patients will be lower). Under this assumption, the history of immediate BL hypersensitivity served as external criterion for evaluation of sensitivity (proportion of actual positives, which are correctly identified amongst all individuals who have immediate BL hypersensitivity [sensitivity; SENS]). In this context, for the 288 cases that did not give a clear history on onset or morphology of their rash linked to BL intake, two different scenarios (Figure S1 and Figure S2) were adopted:

1 **Scenario 1 (proportions in cases with missing information are identical to cases with reported onset/morphology):** The pathophysiology of these 288 cases is distributed similarly to the cases with plausible history concerning immediate (≈49%), non-immediate (≈39%), or mixed (≈12%) onset. Under this assumption, an immediate IgE-mediated pathomechanism—being accessible to detection by SPT or specific IgE in vitro—can be assumed in 472/769 cases (≈61% of the total cohort) (Figure S1a) and, respectively, in 457/746 cases (≈61% of the SPT-cohort; n = 364 with solely immediate, n = 93 with mixed onset) (Figure S1b).
2 **Scenario 2 (proportions in cases with missing information are inverse to cases with reported onset/morphology):** Because usually non-immediate hypersensitivity reactions are more frequent,[10] in an alternative scenario the opposite assumption was adopted that the majority (61%) of 288 are of non-immediate pathomechanism (n = 176) and the remaining 112 cases (39%) are of immediate or mixed onset. In this scenario, in total 407/769 (≈53% of the total cohort [Figure S2a]) can be assumed to be of immediate hypersensitivity, which potentially could be detected by SPT or specific IgE and, respectively, 395/746 cases (≈53% of the SPT-cohort) (Figure S2b).

The evaluation of significant differences was made using the *t*-test for dependent and independent samples; a *P*-value < .05 was regarded as statistically significant.

3 | RESULTS

Of the total number of patients with immediate and non-immediate reactions (n = 769), the number of patients available for the evaluation of the different allergy tests varied according to a stepwise approach: SPT: n = 746, in vitro specific IgE test: n = 644, DAP Penicillin (n = 288), thereof n = 254 in combination with IDT of Penicillin G and Ampicillin.

3.1 | A second reading after 40 minutes increases sensitivity of SPT

Owing to the additional reading time at 40 minutes, in the entire SPT-examined cohort of patients with a clinical history of BL

hypersensitivity (n = 746), the share of patients with a positive SPT result increased from 13.3% (n = 99 with positive test result at the 20 minute-reading) to 17.8% (n = 133 with a positive test result at either reading) (two-sample *t*-test for dependent samples: $P < .00001$). Of these 133 patients, n = 30 (22.5%) showed a positive SPT result exclusively after 20 minutes (40-minute-reading negative), n = 34 patients (n = 34/133 = 25.6%) displayed a positive test reaction exclusively after 40 minutes (negative test result after 20 minutes), and 69 displayed a positive test result in both readings; however, n = 12 patients (n = 12/133 = 9.0%) showed a stronger reaction in the 40-minute reading compared to the 20-minute reading. Consequently, the addition of the 40-minute reading contributed additional value in more than one third (34.6%) of all n = 133 SPT-positive patients, either consisting in detection of the conversion of a negative into a positive test result or increased test reaction (Figure 1).

In the history-based population with assumed immediate BL hypersensitivity (n = 457) (scenario 1, FIGURE S1b), SPT with a sole 20-minute reading identified 99 cases (SENS: 0.21) and SPT with 20- and 40-minute reading 133 cases (SENS: 0.29). Under the less conservative assumption of scenario 2 (FIGURE S2) (immediate BL hypersensitivity present in only n = 407 patients of total cohort [FIGURE S2a]), and respectively in n = 395 of SPT-cohort (FIGURE S2b), SPT with a sole 20-minute reading identified 99 cases (SENS: 0.25) and SPT with 20- and 40-minute reading identified 133 cases (SENS: 0.33) of 395 individuals with immediate BL hypersensitivity (FIGURE S2b).

Among SPT-negative individuals, immediate BL hypersensitivity was identified by additional tests of the stepwise algorithm in n = 203 patients (see below).

3.2 | Specific IgE-detection of BL hypersensitivity in light of different cut-off-values

BL-specific IgE was examined in n = 644 patients. Using a sole cut-off value of ≥0.35 kUA/L (January 1, 2006 to September 11, 2012), in vitro examination of BL-specific IgE antibodies directed to at least one of the five beta-lactam components identified a sensitization to beta-lactams in n = 38 of n = 644 (5.9%) tested patients. From February 22, 2007 to September 11, 2012, two different cut-off values (≥0.35 kUA/L and ≥ 0.11 kUA/L) were examined in parallel in n = 539 patients with suspected BL hypersensitivity: With a cut-off ≥0.35 kUA/L, in n = 31 (5.8%) of n = 539 tested patients vs in n = 99 (18.4%) of n = 539 tested patients (at cut-off ≥0.11 kUA/L), positive test results confirmed the suspected diagnosis of beta-lactam allergy. The proportion of positive in vitro IgE results of individual BLs (ranging from 0.4% to 4% of tested patients at cut-off ≥0.35 kUA/L vs 5.4% to 13.6% at cut-off ≥0.11 kUA/L) is displayed in Table 3.

In 79/203 SPT-negative individuals, immediate BL hypersensitivity was identified by addition of BL-specific in vitro IgE-examination (n = 79 patients at cut-off ≥0.11 kUA/L vs n = 29 patients at cut-off ≥0.35 kUA/L), with thereof 53 identified solely by this test. At its best, the combination of SPT and BL-specific IgE examination in vitro identified 212/457 cases with immediate BL hypersensitivity (FIGURE S1b) (SENS: 0.46). Assuming scenario 2 (FIGURE S2b), 212/395 were identified (SENS: 0.53).

3.3 | In vivo testing of BL determinants combined with intradermal testing of Penciillin/Ampicillin increases the sensitivity of ambulatory test procedures

In vivo testing of BL determinants (DAP Penicillin) in combination with IDT of Penicillin and Ampicillin was performed within the stepwise diagnostic algorithm in n = 288 patients of whom not all were tested with all substances. Depending on the examined substance, between n = 254 and n = 284 patients were tested (Table 2). Of these, 199 patients (n = 199/288 = 69.1%) showed a positive test result to at least one of the test substances (n = 193 exclusively in the IDT, n = 2 exclusively in the SPT, and n = 4 in both test procedures); n = 89 patients (n = 89/288 = 30.9%) showed negative test results.

In our cohort, SPT und IDT of major and minor determinants alone (without IDT of Penicillin G and Ampicillin) resulted in positive test reactions in 61/284 (21.4%) tested patients and in the identification of additional 43/203 BL-sensitized individuals (21.1%), who had

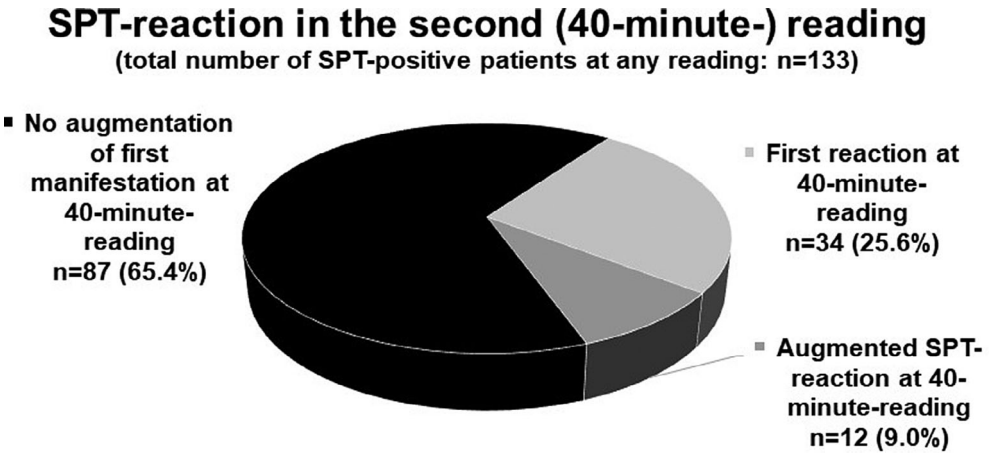

FIGURE 1 Benefit of a second SPT reading: In 133/746 patients immediate BL hypersensitivity was diagnosed by SPT: Thereof, 34/133 (25.6%)—negative at the 20-minute reading—were exclusively identified by a positive SPT in the second reading at 40 minutes. In addition, an augmented SPT reaction (compared to a positive reaction at the 20-minute reading) was present in 12/133 patients (9%)

been SPT negative to the SPT panel of selected Penicillins/cephalosporins (displayed in Table 1).

The addition of in vivo testing of BL determinants (DAP Penicillin) in combination with IDT of Penicillin G and Ampicillin (resulting in at least one positive skin reaction to one of four of the haptens) identified immediate BL hypersensitivity in 150/203 SPT-negative patients (thereof 124 only by adding this in vivo test combination, not by specific IgE). Among the above 150 patients, 28 had a positive skin test result to PPL, 34 to MDM, 130 to Penicillin G, and 86 to Ampicillin. Of these:

- 16 recognized at least one of the two BL determinants, PPL or MDM (without a concomitant reaction to Ampicillin or Penicillin G)
- 107 recognized at least one of the two, Ampicillin or Penicillin G (without a concomitant reaction to PPL or MDM)
- One recognized at least one of the two BL determinants, PPL or MDM, and additionally Ampicillin (not Penicillin G)
- 8 recognized at least one of the two BL determinants, PPL or MDM, and additionally Penicillin G (not Ampicillin)
- 18 recognized at least one of the two BL determinants, PPL or MDM, and additionally Penicillin G and Ampicillin.

Of the 124 individuals, who were SPT-negative to the test series from Table 1 and negative for specific IgE, 21 had a positive skin test result to PPL, 23 to MDM, 108 to Penicillin G, and 71 to Ampicillin. Of these:

- 13 recognized at least one of the two BL determinants, PPL or MDM (without a concomitant reaction to Ampicillin or Penicillin G)
- 92 recognized at least one of the two, Ampicillin or Penicillin G (without a concomitant reaction to PPL or MDM)
- One recognized least one of the two BL determinants, PPL or MDM, and additionally Ampicillin (not Penicillin G)
- 8 recognized least one of the two BL determinants, PPL or MDM, and additionally Penicillin G (not Ampicillin)
- 10 recognized least one of the two BL determinants, PPL or MDM, and additionally Penicillin G and Ampicillin.

The combination of SPT (test series from Table 1) with in vivo testing of BL determinants (SPT and IDT)/Penicillin (IDT)/Ampicillin (IDT) identified 283/457 cases (FIGURE S1b) with immediate BL-hypersensitivity (SENS: 0.61). Assuming scenario 2 (FIGURE S2b) 283/395 were identified (SENS: 0.71). SPT of the test series displayed in Table 1 together with BL determinants alone identified 176/457 (SEN: 0.38), assuming scenario 2 176/395 (SENS 0.44).

3.4 | The combination of tests in the stepwise algorithm increases the detection rate of immediate BL hypersensitivity

In n = 203 SPT-negative patients (at 20 and 40 minutes), further ambulatory test procedures revealed an existing immediate BL hypersensitivity: in n = 124 patients BL hypersensitivity was exclusively identified by in vivo testing of BL determinants (DAP Penicillin)/Penicillin (IDT)/Ampicillin (IDT), in n = 53 patients exclusively by in vitro specific IgE (cut-off ≥0.11 kUA/L), and in n = 26 patients by in vivo testing of BL determinants/Penicillin (IDT)/Ampicillin (IDT), and in vitro specific IgE (cut-off ≥0.11 kUA/L). With all of these diagnostic procedures taken together, in n = 336 of 746 patients with suspected beta-lactam allergy an immediate BL hypersensitivity was diagnosed. Using the history of immediate hypersensitivity as external criterion, 336/457 cases (Figure S1b) could be identified with the combination of all ambulatory test procedures (SENS: 0.73; 0.85 under the assumption of scenario 2 [336/395, Figure S2b])

3.5 | Cross- and co-reactivity to Penicillins and Cephalosporins affect one-third of diagnosed beta-lactam allergic individuals in the investigated cohort

Cross-reactivity between Penicillins and Cephalosporins is based on similarities of their side chains.[30] Therefore, it was investigated whether and how many patients with positive SPT to a particular Penicillin simultaneously react to a specific Cephalosporin in this cohort. Almost one-third (n = 39/133 = 29.3%) of all patients with a positive SPT to BL antibiotics reacted simultaneously to Penicillins *and* Cephalosporins, 45.1% (n = 60/133) had a positive SPT exclusively to Penicillins and n = 34/133 (25.6%) exclusively to Cephalosporins (Figure 2). The analysis of the test results of eight Penicillins (listed in Table 5) reveals that concomitant positive SPT reactions to the Cephalosporins Cefotiam (second generation) and Cephalexin (first generation) were most frequent, but concomitant reactions also occurred to third-generation Cephalosporins.

3.6 | BL hypersensitivity and concomitant atopic diseases

Recently, it has been reported that patients with high total IgE and IgE against prevalent allergens had a slower decrease of BL-specific IgE than nonatopic patients.[31] Therefore, the frequency of clinically manifest atopic diseases was assessed in our cohort. Among the n = 769 patients assessed for BL hypersensitivity, 274 patients (35.6%) reported rhinoconjunctival symptoms (n = 208 of seasonal preponderance, n = 66 perennially). Allergic bronchial asthma was reported by n = 110 patients (14.3%) and atopic dermatitis by n = 52 patients (6.8%).

Patients with positive SPT results to BL antibiotics showed a slightly higher proportion of patients with one or more concomitant atopic diseases (47.4%) compared to patients with negative SPT results (40.1%). Using two-sample *t*-test for independent samples with $P = .12$, no significant difference was found. A positive or negative correlation between atopy and BL skin test reactivity could not be identified in the investigated cohort.

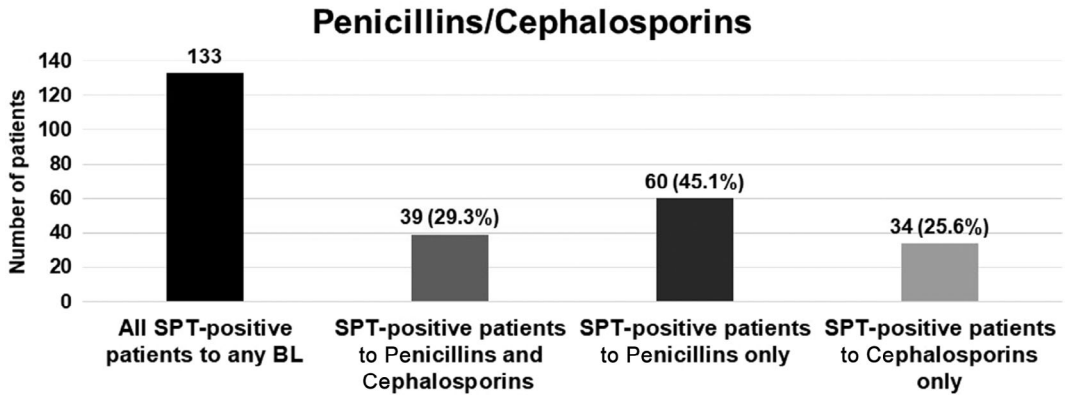

FIGURE 2 Skin prick test results to Penicillins and/or Cephalosporins

TABLE 5 Concomitant positive skin prick test results to Penicillins and to Cephalosporins

Positive reaction to	Patients with positive reaction n =	Most frequent positive reactions to (Cephalosporin)…	Patients with positive reaction n =
Amoxicillin sodium	33	Cefotiam (second generation) Cefuroxime axetil (second generation) Cephalexin (first generation)	7(21.2%) 7(21.2%) 7(21.2%)
Penicillin	33	Cefotiam (second generation) Cephalexin (first generation)	11(33.3%) 9(27.3%)
Phenoxymethylpenicillin	31	Cefotiam (second generation) Ceftriaxone (third generation)	12(38.7%) 10(32.3%)
Ampicillin	21	Cefotiam (second generation) Cephalexin (first generation) Ceftriaxone (third generation)	11(52.4%) 8(38.1%) 8(38.1%)
Amoxicillin/Clavulanic acid	23	Cefotiam (second generation) Cefotaxime (third generation) Ceftriaxone (third generation)	9(39.1%) 9(39.1%) 8(34.8%)
Piperacillin	15	Cefotiam (second generation) Ceftriaxone (third generation) Cefuroxime axetil (second generation)	10(66.7%) 8(53.3%) 8(53.3%)
Mezlocillin	16	Cefotiam (second generation) Cephalexin (first generation)	8(50.0%) 8(50.0%)
Flucloxacillin	25	Cephalexin (first generation) Cefotiam (second generation)	10(40.0%) 8(32.0%)

4 | DISCUSSION

Despite recent improvement of the sensitivity of skin tests in the diagnostic workup for beta-lactam allergies, for example, by SPT and IDT of Benzylpenicilloyl-octa-L-lysine (BP-OL) and Benzylpenilloate, increasing sensitivity in the investigated population of a prospective multicenter clinical trial (with previously confirmed immediate type BL allergy) to up to 61.36%,[32] sensitivity of skin tests is still low compared to inhalation allergens with reported sensitivity of 80%–97%.[33] The pan-European GA^{2}LEN (Global Allergy and Asthma European Network) skin test study found that SPT of 8 to 10 aeroallergens read after 15 minutes is sufficient to identify the majority of sensitized patients; depending on the country, between 4 and 13 of n = 18 allergens was sufficient to determine all sensitized patients.[34] In clinical practice, based on these findings identified for aeroallergens, frequently a reading time after 15–20 minutes is extrapolated also for in vivo allergy testing with medicinal products (drugs).

However, due to the differences concerning the nature of allergen sources (aeroallergens vs drugs), exposure routes, and frequency of exposure, the test conditions validated and standardized for aeroallergens may not be applicable to drug allergy testing. Therefore, the relevance of a second SPT reading (40-minute reading) in addition to the standard reading after 15–20 minutes in the diagnostic workup of BL antibiotics was investigated.

Based on this noninvasive and inexpensive measure, in n = 34/133 additional SPT-positive individuals (25.6%), immediate BL hypersensitivity could be identified by a positive SPT, which had been negative at the 20-minute-reading, allowing to shorten further diagnostics

in these patients. In another n = 12 patients (9.0%), a stronger, unequivocal SPT reaction was seen in the 40-minute reading compared to the 20-minute reading. However, because n = 30 patients showed a positive SPT result exclusively in the 20-minute reading (40-minute reading negative), the 20-minute reading cannot be replaced, but appears to be feasibly supplemented by a second reading after 40 minutes.

Using a cut-off value of ≥0.11 kUA/L, in n = 99 (18.4%) of n = 539 tested patients with suspected BL hypersensitivity, BL-specific IgE was identified in vitro compared to n = 31 (5.8%) with a cut-off ≥0.35 kUA/L, indicating a benefit of the lower cut-off value. This is in concordance with the reported finding that the diagnostic performance of an ImmunoCAP specific IgE test for β-lactams (cut-off positivity >0.10 kUA/L for at least one hapten) is maximized for patients with low to moderate total IgE levels (<200 kU/L)[35,36]; however, the application of specific/total IgE ratio >0.002 was reported in the same reports to be significantly better than conventional positivity.

Our results of a low detection rate of specific IgE in individuals with a positive history of BL sensitivity are in concordance with published evidence, however, depending on the investigated cohort:

- sensitivity of 0%–25% depending on initial clinical manifestation (technique: CAP-FEIA (cut-off >0.35 kUA/L; BL in vitro tested: Penicilloyl G, Amoxicillin, Ampicillin, Cefaclor; investigated cohort: n = 45 patients who previously underwent BL skin test and/or drug provocation; clinical manifestation: urticaria or anaphylaxis),[37]
- sensitivity of 25% (technique: CAP-FEIA >0.35 kUA/L; tested beta-lactam: Benzylpenicilloyl; cohort: n = 88 patients with immediate hypersensitivity reactions to BL confirmed within 1 year prior to the published prospective multicenter clinical trial),[32]
- reactivity of 62.2% (for the combination of three haptens; single haptens: 14 cases [32.14%] were positive for Benzylpenicillin, n = 16 [39.28%] for Ampicillin, and n = 17 (42.85%) for Amoxicillin (technique: CAP-FEIA >0.35 kUA/L; BL in vitro tested: Benzylpenicillin, Amoxicillin, Ampicillin; subgroup investigated by CAP-FEIA: n = 45 patients with immediate reaction to BL within 1 year prior to testing).[20]

A reason for the reported low sensitivity might be that sIgE levels decrease dramatically over time after the exposure to the drug, which will affect the test results if the interval between hypersensitivity reaction and allergological workup is rather long.[20,37] Despite the low sensitivity of these in vitro tests, which certainly require improvement,[10,37] analysis of BL-specific IgE may contribute to the identification of BL-sensitized patients (especially in circumstances that make in vivo tests impracticable). Furthermore, patients with clinically relevant sensitization who could not be diagnosed by skin testing but only by BL-specific serum IgE have been described: Torres et al reported 40 of 290 patients (13.8%) who were skin test negative but showed a positive sIgE against BLs and a clinically relevant sensitization.[38] This is in accordance with the finding in our cohort with BL hypersensitivity in which even 26.1% (53/203) of SPT-negative patients were exclusively identified as BL reactive by BL-specific serum IgE.

The use of minor and major determinants in the diagnostic workup for BL hypersensitivities is discussed controversially. The reasons for this are the high costs of commercial test substances, problems with the availability of test substances that have not been licensed in most European member states, the required time for stepwise testing and regionally changing prescription habits of BL-antibiotics, and the associated change in allergologically relevant allergenic structures. In our cohort, SPT und IDT of major and minor determinants alone (without IDT of Penicillin G and Ampicillin) identified additional 43/203 BL-sensitized individuals (21.1%), who had been SPT negative to the SPT panel of selected Penicillins/Cephalosporins (displayed in Table 1) and thereof n = 13/203 (6.9%), respectively, which were not identified by any other test (for comparison n = 92/203 [45.3%] were exclusively identified by IDT of Penicillin and Ampicillin). Our results are broadly in concordance with a study in 195 patients (74 with a history of immediate reactions to BL, 74 with non-immediate reactions, and 47 undergoing prophylactic tests) which, besides skin testing a panel of BL-antibiotics, compared PPL and MDM from Allergopharma (Allergopen) with those from Diater (DAP Penicillin) by parallel testing[39]: Minor determinant mixture reagents produced identical results in all 195 patients. Results of skin testing with PPL reagents were concordant in 190 (97.4%). One hundred two patients (52.3%) in this study had a positive skin test to any BL antibiotic; 29/195 tested patients (14.9%) were positive to PPL and/or MDM, and MDM was positive in 22 patients. The rate in which a benefit of testing BL determinant can be deduced varies dependent on the patient selection, which in our case was a "real world" cohort presenting to a university hospital for allergological workup.

The limitation of our investigation is that the true prevalence of BL hypersensitivity is not known. According to guidelines, patients are diagnosed with Penicillin allergy if skin test result or specific IgE to Penicillin is positive.[40] However, the true sensitivity and specificity of these tests are presently not known.[40]

To estimate the contribution of each test within the stepwise algorithm for the identification of sensitized individuals, an external criterion is required for which we chose a plausible history of immediate BL hypersensitivity (sensitivity calculation included two scenarios based on different assumptions). Another external criterion could have been drug provocation test; however, our patients with positive test results were not routinely admitted for re-exposure to unequivocally ascertain clinical relevance of positive in vitro or in vivo skin tests. This is in concordance with published algorithms,[10,15] which recommend drug provocation test only when other tests failed to detect specific IgE. This also refers to the positive predictive value (PPV) as a parameter for assessing the performance of medical test procedures, which is of utmost interest. It indicates how many people who have been diagnosed with a certain disease by a test procedure are actually ill (PPV = number of correct positive/(number of correct positive + number of false-positive). This question could be addressed within a clinical study in which all BL-sensitive individuals with a positive skin test (or respectively sIgE) are confirmed by an oral provocation test. The presented data analysis of our cohort of patients with assumed BL hypersensitivity was confined to outpatient diagnostics according

to medical practice and does not allow for the determination of the PPV.

Few case series exist that performed provocation in test-positive individuals: for example, Macy et al. described in a case series four persons with positive specific IgE and negative drug provocation (DPT), as well as three patients with positive DPT and negative specific IgE.[41] In another sample of 25 patients with positive Penicillin skin test results, specific IgE results, or both, patients were challenged with their culprit Penicillin[40]: Only 9 (36%) of 25 were challenge-positive. There was an increased probability of being Penicillin allergic if both skin test result and specific IgE were positive at T_0.

However, also a negative drug provocation test is not entirely reliable due to rarefication of BL-specific IgE antibodies over time. Some published recommendations therefore suggest a second drug provocation test 2 to 4 weeks after the first negative one,[10] whereas others rely their diagnosis on a single provocation test.[17] In clinical practice it is difficult to manage a second provocation test because inpatient hospital resources are required as well as compliance of patients who are generally rather reluctant toward inpatient procedures. A recent attempt to design predictive models for BL allergy using a drug allergy and hypersensitivity database based on history alone failed.[42] Two different independent methods using clinical history predictors could not accurately predict BL allergy and replace a conventional allergy evaluation for suspected BL allergy. However, patients who report an anaphylactic history have a 2- to 4-fold increased risk of true allergy.[42] Anaphylactic history additionally confers an increased risk of anaphylaxis during allergy testing, and cross-reactivity with other beta-lactams.[16]

R_1 and R_2 chemical side chains of the cephalosporins may cause IgE-mediated cross-reactivity with Penicillin and other Cephalosporins. Skin tests predict IgE-mediated reactions and showed cross-reactivity between Penicillins and early generation Cephalosporins that shared side chains, but confirmatory challenge data are lacking.[43] Aminopenicillins are known to be cross-reactive with aminocephalosporins such as Cefaclor, Cefadroxil, and Cefalexin in some patients. In the literature, the group of penicillin-allergic individuals also includes reports on sensitizations against other cephalosporins such as Cefoperazone,[44] Ceftriaxone,[44] Cefuroxim[45,46] Cefpodoxim, and Cefixim[47] as well as against Cephalothin and Cefamandol.[4] In our cohort, concomitant SPT reactions to penicillin and cephalosporins concerned one-third of SPT-positive individuals, including third-generation cephalosporins, which have been generally regarded as safe alternatives in penicillin-allergic individuals. However, it is of utmost importance to verify the dissimilarity of side chains prior to administering third-generation cephalosporins.[16,43] In our cohort, frequently a coexisting sensitization was found against (in SPT-positive individuals to Phenoxymethylpenicillin, Ampicillin, Amoxicillin/Clavulanic acid or Piperacillin and Cefotaxime (in SPT-positive individuals to amoxicillin/clavulanic acid). Ceftriaxone and Cefotaxime have an identical side chain in R1-position (methoxyimino group); however, this is not identical to R1-side chains of Penicillin.[43] In addition to genuine cross-sensitivities, there are also co-sensitizations and false-positive findings to be discussed.

In our cohort assessed for beta-lactam allergy (n = 769), clinical manifestations of atopic diseases were rather frequent comorbidities: 274 patients (35.6%) reported rhinoconjunctival symptoms (n = 208 of seasonal preponderance, n = 66 perennially). Allergic bronchial asthma was reported by n = 110 patients (14.3%) and atopic dermatitis by n = 52 patients (6.8%). These frequencies of reported atopic comorbidities are above the reported prevalence in the general population in Germany: In the latest German Health Survey (DEGS1) the following lifetime prevalence for atopic diseases was identified in adults: allergic rhinoconjunctivitis: 14.8%, allergic bronchial asthma: 8.6%, and atopic dermatitis: 3.5%.[49] In the past, frequently an association of BL hypersensitivity and atopy has been discussed.

In the published evidence (for details see Table S2), controversial findings and opinions exist concerning atopy as a comorbidity or even as a risk factor for BL allergy, which in part is due to different definitions/criteria used to define atopy, as well as selection of investigated population.[11,20,31,49-62] Of note, most studies exclusively examined immediate BL reactions, whereas three studies included immediate and non-immediate reactions (for detailed information see the Online Supplement). In summary, there is no consistent opinion regarding the association between atopic diathesis/atopic comorbidities and BL allergy. Although in our cohort atopic diseases were more frequent than in the general population, the fact that patients with positive SPT against BL antibiotics did not significantly differ from SPT-negative patients concerning atopic diseases argues against this association in our cohort.

BL is the most common cause of adverse drug reactions according to published literature. Antibiotic use has increased by 65% between 2000 and 2015, fueled by increased use in low- to middle-income countries, whereas high-income countries, which have processes implemented aimed at curbing antibiotic resistance (antibiotic stewardship), experienced slower growth.[13,63] Confirmation or exclusion of the diagnosis of BL allergy is important with regard to the individual patient as well as public health.[11,64]

In summary, to overcome the low sensitivity of allergological tests, optimized reading times of the SPT of BL, a lower cut-off for in vitro detection of BL-specific IgE, and intradermal testing of Penicillin, Ampicillin, and BL determinants contribute to overall sensitivity under real-life conditions to diagnose immediate BL hypersensitivity. The diagnostic gap that had been imposed to the diagnostic workup of BL allergy in Germany by the withdrawal of the marketing authorization of one the BL-determinant test kits (Allergopharma, Merck, Darmstadt, Germany) (Table S1) in February 2007 has recently been closed by a novel marketing authorization (for DAP penicillin kit by Diater, Madrid, Spain; containing nowadays Benzylpenicilloyl-octa-L-lysine (BP-OL) as major determinant Benzylpenilloate as minor determinant) granted in September 2019 by the Paul-Ehrlich-Institut, Langen, Germany.

CONFLICT OF INTEREST

The authors declare that they have no competing interests. V. Mahler indicates that the retrospective study was designed and carried out at the University Hospital Erlangen Department of Dermatology (prior to the current employment at the Paul-Ehrlich-Institut).

The paper is a scientific contribution by the author as expert in the field of allergology and may not be understood or quoted as being made on behalf of or reflecting the position of the respective national competent authority, the European Medicines Agency, or one of its committees or working parties.

AUTHOR CONTRIBUTIONS

Vera Mahler: Conceptualization; data curation; formal analysis; investigation; methodology; resources; supervision; validation; visualization; writing-original draft; writing-review and editing. **Bernadette Rosti:** Data curation; formal analysis; investigation; methodology; resources; validation; visualization; writing-original draft; writing-review and editing.

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REFERENCES

1. Cars O, Mölstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet*. 2001;357(9271):1851-1853.
2. Bätzing-Feigenbaum J, Schulz M, Hering R, Kern WV. Outpatient antibiotic prescription. *Dtsch Arztebl Int*. 2016;113(26):454-459.
3. Chandra RK, Joglekar SA, Tomas E. Penicillin allergy: anti-penicillin IgE antibodies and immediate hypersensitivity skin reactions employing major and minor determinants of penicillin. *Arch Dis Child*. 1980;55(11):857-860.
4. Forrest DM, Schellenberg RR, Thien VV, et al. Introduction of a practice guideline for penicillin skin testing improves the appropriateness of antibiotic therapy. *Clin Infect Dis*. 2001;32(12):1685-1690.
5. Fox S, Park MA. Penicillin skin testing in the evaluation and management of penicillin allergy. *Ann Allergy Asthma Immunol*. 2011;106(1):1-7.
6. Hamzaoui A. Allergie aux anti-infectieux. *Rev Mal Respir*. 2006;23(4 Pt 2):10S70-10S72.
7. Sade K, Holtzer I, Levo Y, Kivity S. The economic burden of antibiotic treatment of penicillin-allergic patients in internal medicine wards of a general tertiary care hospital. *Clin Exp Allergy*. 2003;33(4):501-506.
8. Silva R, Cruz L, Botelho C, et al. Work up of patients with history of beta-lactam hypersensitivity. *Allergol Immunopathol (Madr)*. 2009;37(4):193-197.
9. Trcka J, Schäd SG, Pfeuffer P, et al. Penicillintherapie trotz Penicillinallergie? Plädoyer für eine allergologische diagnostik bei verdacht auf penicillinallergie. *Dtsch Arztebl*. 2004;101(43):A2888-A2892.
10. Blanca M, Romano A, Torres MJ, et al. Update on the evaluation of hypersensitivity reactions to betalactams. *Allergy*. 2009;64(2):183-193.
11. Chang C, Mahmood M, Teuber S, Gershwin ME. Overview of penicillin allergy. *Clinic Rev Allerg Immunol*. 2012;43(1-2):84-97.
12. Brockow K, Przybilla B, Aberer W, et al. Guideline for the diagnosis of drug hypersensitivity reactions. S2K-guideline of the German Society for Allergology and Clinical Immunology (DGAKI) and the German Dermatological Society (DDG) in collaboration with the Association of German Aller gologists (AeDA), the German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Contact Dermatitis Research Group, the Swiss Society for Allergy and Immunology (SGAI), the Austrian Society for Allergology and Immunology (ÖGAI), the German Academy of Allergology and Environmental Medicine (DAAU), the German Center for Documentation of Severe Skin Reactions and the German Federal Institute for Drugs and Medical Products (BfArM). *Allergo J Int*. 2015;24(3):94-105.
13. Torres MJ, Blanca M, Fernandez J, et al. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy*. 2003;58(10):961-972.
14. Romano A, Blanca M, Torres MJ, et al. Diagnosis of nonimmediate reactions to beta-lactam antibiotics. *Allergy*. 2004;59(11):1153-1160.
15. Torres MJ, Romano A, Celik G, et al. Approach to the diagnosis of drug hypersensitivity reactions: similarities and differences between Europe and North America. *Clin Transl Allergy*. 2017;7(1):7.
16. Chiriac AM, Banerji A, Gruchalla RS, et al. Controversies in drug allergy: drug allergy pathways. *J Allergy Clin Immunol Pract*. 2018;S2213-2198(18):30513.
17. Wurpts G, Aberer W, Dickel H, et al. Guideline on diagnostic procedures for suspected hypersensitivity to beta-lactam antibiotics. Guideline of the German Society for Allergology and Clinical Immunology (DGAKI) in collaboration with the German Society of Allergology (AeDA), German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Contact Dermatitis Research Group (DKG), the Austrian Society for Allergology and Immunology (ÖGAI), and the Paul-Ehrlich Society for Chemotherapy (PEG). *Allergo J Int*. 2019;28(5):121-151.
18. Torres MJ, Romano A, Mayorga C, et al. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy*. 2001;56(9):850-856.
19. Bousquet PJ, Pipet A, Bousquet-Rouanet L, Demoly P. Oral challenges are needed in the diagnosis of β-lactam hypersensitivity. *Clin Exp Allergy*. 2007;38(1):185-190.
20. García Núñez I, Barasona Villarejo MJ, Algaba Mármol MA, Moreno Aguilar C, Guerra Pasadas F. Diagnosis of patients with immediate hypersensitivity to ß-lactams using retest. *J Investig Allergol Clin Immunol*. 2012;22(1):41-47.
21. McLean-Tooke A, Aldridge C, Stroud C, Spickett GP. Practical management of antibiotic allergy in adults. *J Clin Pathol*. 2011;64(3):192-199; Correction in: *J Clin Pathol* 2019;72(12):840. https://doi.org/10.1136/jcp.2010.077289corr1
22. Richter AG, Wong G, Goddard S, et al. Retrospective case series analysis of penicillin allergy testing in a UKspecialist regional allergy clinic. *J Clin Pathol*. 2011;64(11):1014-1018.
23. Sagar PS, Katelaris CH. Utility of penicillin allergy testing in patients presenting with a history of penicillin allergy. *Asia Pac Allergy*. 2013;3(2):115-119.
24. Jost BC, Wedner HJ, Bloomberg GR. Elective penicillin skin testing in a pediatric outpatient setting. *Ann Allergy Asthma Immunol*. 2006;87(6):807-812.
25. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy*. 2002;57(1):45-51.
26. Brockow K, Garvey LH, Aberer W, et al. On behalf of the ENDA/EAACI Drug Allergy Interest Group. Skin test concentrations for systemically administered drugs – an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy*. 2013;68(6):702-712.
27. Bousquet J, Heinzerling L, Bachert C, et al. Practical guide to skin prick tests in allergy to aeroallergens. *Allergy*. 2012;67(1):18-24.
28. Schnuch A, Uter W, Lessmann H, Geier J. Klinische epidemiologie und prävention der kontaktallergien. Der Beitrag des Informationsverbundes Dermatologischer Kliniken (IVDK). *Bundesgesundheitsbl*. 2012;55(3):329-337.
29. Ruëff F, Bergmann KC, Brockow K, et al. Hauttests zur Diagnostik von allergischen SofforttypreaktionenLeitlinie der Deutschen Gesellschaft für Allergologie und klinische Immunologie (DGAKI) in Abstimmung mit dem Ärzteverband Deutscher Allergologen (ÄDA), dem Berufsverband Deutscher Dermatologen (BVDD), der Deutschen Dermatologischen Gesellschaft (DDG), der Deutschen Gesellschaft für Hals-Nasen-Ohren-Heilkunde und Kopf- und Hals-Chirurgie (DGHNOKHC), der Deutschen Gesellschaft für Pneumologie und Beatmungsmedizin (DGP) und der Gesellschaft für Pädiatrische Allergologie und Umweltmedizin (GPA). *Allergo J*. 2010;19(6):402-415.

30. Dickson SD, Salazar KC. Diagnosis and management of immediate hypersensitivity reactions to cephalosporins. *Clinic Rev Allerg Immunol*. 2013;45(1):131-142.
31. Cornejo-García JA, Guéant-Rodriguez R-M, Torres MJ, et al. Biological and genetic determinants of atopy are predictors of immediate-type allergy to betalactams, in Spain. *Allergy*. 2012;67(9):1181-1185.
32. Fernández J, Torres MJ, Campos J, Arribas-Poves F, Blanca M, on behalf of the DAP-Diater Group. Prospective, multicenter clinical trial to validate new products for skin tests in the diagnosis of allergy to penicillin. *J Investig Allergol Clin Immunol* 2013;23(6):398–408.
33. Chiriac AM, Bousquet J, Demoly P. In vivo methods for the study and diagnosis of allergy. *Middleton's Allergy - Principles and Practice*. Vol 1128. Philadelphia, PA: Elsevier Saunders; 2013.
34. Bousquet PJ, Burbach G, Heinzerling LM, et al. GA^{2}LEN skin test study III: minimum battery of test inhalent allergens needed in epidemiological studies in patients. *Allergy*. 2009;64(11):1656-1662.
35. Vultaggio A, Matucci A, Virgili G, et al. Influence of total serum IgE levels on the in vitro detection of beta-lactams-specific IgE antibodies. *Clin Exp Allergy*. 2009;39(6):838-844.
36. Vultaggio A, Virgili G, Gaeta F, Romano A, Maggi E, Matucci A. High serum β-lactams specific/total IgE ratio is associated with immediate reactions to β-lactams antibiotics. *PLoS One*. 2015;10(4):e0121857. https://doi.org/10.1371/journal.pone.0121857.
37. Fontaine C, Mayorga C, Bousquet PJ, et al. Relevance of the determination of serum-specific IgE antibodies in the diagnosis of immediate β-lactam allergy. *Allergy*. 2007;62(1):47-52.
38. Torres MJ, Majorga C, Cornejo-García JA, Romano A, Blanca M. IgE antibodies to penicillin skin test negative patients. *Allergy*. 2002;57(10):965.
39. Romano A, Viola M, Bousquet PJ, et al. A comparison of the performance of two penicillin reagent kits in the diagnosis of beta-lactam hypersensitivity. *Allergy*. 2007;62(1):53-58.
40. Tannert LK, Mortz CG, Skov PS, Bindslev-Jensen C. Positive skin test or specific IgE to penicillin does not reliably predict penicillin allergy. *J Allergy Clin Immunol Pract*. 2017;5(3):676-683.
41. Macy E, Goldberg B, Poon KYT. Use of commercial anti-penicillin IgE fluorometric enzyme immunoassays to diagnose penicillin allergy. *Ann Allergy Asthma Immunol*. 2010;105(2):136-141.
42. Chiriac AM, Wang Y, Schrijvers R, et al. Designing predictive models for beta-lactam allergy using the drug allergy and hypersensitivity database. *J Allergy Clin Immunol Pract*. 2018;6(1):139-148.e2.
43. Zagursky RJ, Pichichero ME. Cross-reactivity in β-lactam allergy. *J Allergy Clin Immunol Pract*. 2018;6(1):72-81.e1.
44. Liu XD, Gao N, Quiao HL. Cephalosporin and penicillin crossreactivity in patients allergic to penicillins. *Int J Clin Pharmacol Ther*. 2011;49(3):206-216.
45. Buonomo A, Nucera E, Pecora V, et al. Cross-reactivity and tolerbility of cephalosporins in patients with cell-mediated allergy to penicillins. *J Investig Allergol Clin Immunol*. 2014;24(5):331-337.
46. Caimmi S, Galéra C, Bousquet-Rouanet L, Arnoux B, Demoly P, Bousquet PJ. Safety of cefuroxime as an alternative in patients with a proven hypersensitivity to penicillins: a DAHD cohort survey. *Int Arch Allergy Immunol*. 2010;153(1):53-60.
47. Trcka J, Seitz CS, Bröcker EV, Gross GE, Trautmann A. Aminopenicillin-induced exanthema allows treatment with certain cephalosporins or phenoxymethyl penicillin. *J Antimicrob Chemother*. 2007;60(1):107-111.
48. Romano A, Guéant-Rodriguez RM, Viola M, Pettinato R, Guéant JO. Cross-reactivity and tolerability of cephalosporins in patients with immediate hypersensitivity to penicillins. *Ann Intern Med*. 2004;141(1):850-856.
49. Langen U, Schmitz R, Steppuhn H. Prevalence of allergic diseases in Germany. Results of the German health interview and examination survey for adults (DEGS1). *Bundesgesundheitsb*. 2013;56(5-6):698-706.
50. Choi IS, Han E, Lim S, et al. Beta-lactam antibiotic sensitization and its relationship to allergic diseases in tertiary hospital nurses. *Allergy Asthma Immunol Res*. 2010;2(2):114-122.
51. Kurt E, Demir AU, Cadirci O. Immediate type drug hypersensitivity reactions and associated risk factors in an adult Turkish men population. *Iran J Allergy Asthma Immunol*. 2010;9(4):245-250.
52. Yang J, Qiao H, Zhang Y, et al. HLA-DRB geno-type and specific IgE responses in patients with allergies to penicillins. *Chin Med J*. 2006;119(6):458-466.
53. Aron Y, Desmazes-Dufeu N, Matran R, et al. Evidence of a strong, positive association between atopy and the HLA class II alleles DR4 and DR7. *Clin Exp Allergy*. 1996;26(7):821-828.
54. Apter AJ, Schelleman H, Walker A, Addya K. Clinical and genetic risk factors of self-reported penicillin allergy. *J Allergy Clin Immunol*. 2008;122(1):152-158.
55. Mirakian R, Ewan PW, Durham SR, et al. BSACI guidelines for the management of drug allergy. *Clin Exp Allergy*. 2009;39(1):43-61.
56. Co Minh HB, Bousquet PJ, Fontaine C, Kvedariene V, Demoly P. Systemic reactions during skin tests with beta-lactams: a risk factor analysis. *J Allergy Clin Immunol*. 2006;117(2):466-468.
57. Demoly P, Kropf R, Bircher A, Pichler WJ. Drug hypersensitivity: questionnaire. *Allergy*. 1999;54(9):999-1003.
58. Fazlollahi MR, Bidad K, Shokouhi R, et al. Frequency and risk factors of penicillin and amoxicillin allergy in suspected patients with drug allergy. *Arch Iran Med*. 2017;20(1):34-37.
59. Haddi E, Charpin D, Tafforeau M, et al. Atopy and systemic reactions to drugs. *Allergy*. 1990;45(3):236-239.
60. Arikoglu T, Aslan G, Batmaz SB, Eskandari G, Helvaci I, Kuyucu S. Diagnostic evaluation and risk factors for drug allergies in children: from clinical history to skin and challenge tests. *Int J Clin Pharm*. 2015;37(4):583-591.
61. Ponvert C, Perrin Y, Bados-Albiero A, et al. Allergy to betalactam antibiotics in children: results of a 20-year study based on clinical history, skin and challenge tests. *Pediatr Allergy Immunol*. 2011;22(4):411-418.
62. Adkinson NF. Risk factors for drug allergy. *J Allergy Clin Immunol*. 1984;74(4 Pt 2):567-572.
63. Friedrich MJ. Antibiotic consumption increasing globally. *JAMA*. 2018;319(19):1973.
64. https://www.pei.de/SharedDocs/Downloads/vigilanz/bulletin-zur-arzneimittelsicherheit/2018/1-2018.pdf?__blob=publicationFile&v=5. Accessed May 18, 2018.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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