

ORIGINAL ARTICLE

SKIN AND EYE DISEASES

Diagnosis of penicillin allergy revisited: the value of case history, skin testing, specific IgE and prolonged challenge

J. Hjortlund, C. G. Mortz, P. S. Skov & C. Bindslev-Jensen

Department of Dermatology and Allergy Centre, Odense University Hospital, Odense C, Denmark

To cite this article: Hjortlund J, Mortz CG, Skov PS, Bindslev-Jensen C. Diagnosis of penicillin allergy revisited: the value of case history, skin testing, specific IgE and prolonged challenge. *Allergy* 2013; DOI: 10.1111/all.12195.

Keywords

ENDA guidelines; immediate vs nonimmediate reactions; penicillin allergy; prolonged oral drug challenge; skin testing reproducibility.

Correspondence

Carsten Bindslev-Jensen, Department of Dermatology and Allergy Centre, Odense University Hospital, Sdr. Boulevard 29, DK-5000 Odense C, Denmark.
Tel.: +45 65413622
Fax: +45 66123819
E-mail: carsten.bindslev-jensen@rsyd.dk

Accepted for publication 30 April 2013

DOI:10.1111/all.12195

Edited by: Thomas Bieber

Abstract

Background: Skin testing in duplicate, correlation between case history of immediate and nonimmediate reactions and challenge outcome and prolonged oral treatment with penicillin in the diagnostic evaluation of allergic reactions to β -lactam antibiotics, mimicking real-life situations, have only been addressed in few studies.

Methods: A total of 342 patients suspected of having β -lactam allergy were investigated according to the European Network for Drug Allergy (ENDA) guidelines and patients found to be negative in the ENDA program were supplemented with a 7-day oral treatment with penicillin. Skin testing with penicillins was performed in duplicate.

Patients with case histories of reactions to other β -lactams were also subsequently challenged with the culprit drug.

Results: Nineteen patients were IgE-sensitized to penicillin. Then, intracutaneous tests (ICTs) were performed, in which 35 patients tested positive for allergy, 21 with delayed and 14 with immediate reactions. Only three patients tested positive for the major (PPL) and/or minor (MDM) penicillin determinants, all being positive for penicillin G in ICT. The remaining 291 patients were challenged with penicillin: 10 tested positive in single-dose challenge and 23 tested positive in the 7-day challenge. A total of 17 of 78 patients with a negative penicillin challenge tested positive during challenges with other β -lactams.

We found no correlation between case histories of immediate and nonimmediate reactions and reaction time during challenge.

Conclusion: The data suggest that case history is often insufficient to discriminate between immediate reactors and nonimmediate reactors. A 7-day challenge with the culprit β -lactam may yield more positive reactions than the accepted one- or 2-day challenge. Interpretation of skin testing should be made with caution.

The diagnosis of allergic reactions to β -lactam antibiotics is based on the European Network for Drug Allergy (ENDA) guidelines, including skin tests, *in vitro* measurements, and drug challenge (1–3). Very little data are available on reproducibility of skin tests and relevance of the proposed subdivision of reactions into immediate (IR) and nonimmediate (NIR) reactions, based on case history. Further, the challenge regimen proposed by ENDA does not reflect the conventional 4–7-day therapy with penicillin.

The aim of this study was to substantiate our previous findings (4) with additional inclusion of skin testing in duplicate with more allergens and also with inclusion of patch testing, to compare the immediate and nonimmediate

reactions during challenge and to assess selective reactions to specific β -lactams.

Materials and methods

Adult patients referred to our department with a clinical history suspected of having a β -lactam allergy during the period September 2010–September 2011 were included in this prospective study. All patients were investigated according to ENDA guidelines (1–3) with the following modifications:

- 1 The definitions of immediate reactions (IR) and nonimmediate reactions (NIR) in the ENDA guidelines are based on patients' case history; we extended this

Table 1 Demographic data of the 452 patients investigated with ENDA Questionnaire

	Total	Any test result		Only Questionnaire
		Positive	Negative	
Number	342	98 (28.7%)	244 (71.3%)	110
Sex (female)	240 (70.2%)	65 (66.3%)	175 (71.7%)	88 (80.0%)
Age				
<40 years	113 (33.0%)	34 (34.7%)	79 (32.4%)	44 (40.0%)
40–60 years	149 (43.6%)	49 (50.0%)	100 (41.0%)	37 (33.6%)
>60 years	80 (23.4%)	15 (15.3%)	65 (26.6%)	29 (26.4%)
Atopy	16 (4.7%)	2 (2.0%)	14 (5.7%)	7 (6.4%)
Asthma	28 (8.2%)	6 (6.1%)	22 (9.0%)	12 (10.9%)
Time since primary reaction				
<1 year	150 (43.9%)	57 (58.2%)	93 (38.1%)	41 (37.3%)
1–5 years	36 (10.5%)	16 (16.3%)	20 (8.2%)	12 (10.9%)
>5–10 years	23 (6.7%)	7 (7.1%)	16 (6.6%)	9 (8.2%)
>10 years	132 (38.6%)	18 (18.4%)	114 (46.7%)	48 (43.6%)
Unknown (time)	1 (0.3%)	0 (0%)	1 (0.4%)	0 (0%)
Symptoms at primary reaction				
Anaphylaxis	11 (3.2%)	4 (4.1%)	7 (2.9%)	0 (0%)
Angioedema and/or Urticaria	135 (39.5%)	54 (55.1%)	80 (32.8%)	51 (46.4%)
Unclassified Cutaneous rash	180 (52.6%)	36 (36.8%)	145 (59.4%)	52 (47.3%)
Other cutaneous symptoms	3 (0.9%)	2 (2.0%)	1 (0.4%)	1 (0.9%)
None cutaneous symptoms	13 (3.8%)	2 (2.0%)	11 (4.5%)	6 (5.4%)
Distribution of cutaneous symptoms (primary)				
Localized	105 (30.7%)	30 (30.6%)	75 (30.7%)	42 (38.2%)
Generalized	192 (56.1%)	65 (66.3%)	127 (52.1%)	54 (49.1%)
Unknown	45 (13.2%)	3 (3.1%)	42 (17.2%)	14 (12.7%)
Respiratory symptoms	41 (12.0%)	13 (13.3%)	28 (11.5%)	20 (18.2%)
Other manifestations	40 (11.7%)	12 (12.2%)	28 (11.5%)	15 (13.6%)
Type of culprit drug				
Penicillin V or G	188 (55.0%)	46 (46.9%)	142 (58.2%)	67 (60.9%)
Amoxicillin	33 (9.6%)	20 (20.4%)	13 (5.3%)	7 (6.4%)
Ampicillin	24 (7.0%)	7 (7.1%)	17 (7.0%)	12 (10.9%)
Dicloxacillin	39 (11.4%)	15 (15.3%)	24 (9.8%)	10 (9.1%)
Other	22 (6.4%)	4 (4.1%)	18 (7.4%)	5 (4.5%)
Unrecalled (culprit drug)	63 (18.4%)	8 (8.2%)	55 (22.5%)	16 (14.5%)
Time interval between dosage and reaction				
<1 h	36 (10.5%)	19 (19.4%)	16 (6.6%)	12 (10.9%)
1–2 h	13 (3.8%)	6 (6.1%)	7 (2.9%)	3 (2.7%)
<24 h	35 (10.2%)	7 (7.1%)	28 (11.5%)	16 (14.5%)
>24 h, but during treatment (days)	163 (47.7%)	39 (39.8%)	125 (51.2%)	52 (47.3%)
After treatment (days)	50 (14.6%)	19 (19.4%)	31 (12.7%)	13 (11.8%)
Unknown (time interval)	72 (21.1%)	10 (10.2%)	62 (25.4%)	21 (19.1%)
Treatment				
No treatment of the reaction	121 (35.4%)	17 (17.4%)	104 (42.6%)	36 (32.7%)
Treatment of the reaction	154 (45.0%)	66 (67.3%)	88 (36.1%)	51 (46.4%)
Unknown (treatment)	67 (19.6%)	15 (15.3%)	52 (21.3%)	23 (20.9%)

No significant differences between the included and excluded patients regarding patient characteristics were found ($P > 0.05$).

- definition to include symptoms and signs elicited during challenge. Specifically, we define IR as a reaction commencing within 1 h after last drug intake and NIR as a reaction commencing 1 h after last drug intake.
- All patients, regardless of time between drug intake and reaction, were diagnosed using the same protocol, including measurement of specific IgE and patch testing.
- Skin testings were carried out in duplicate, and all patients were tested with all drugs described below.
- The challenge procedures differed by number of doses and steps and by route of administration.
- If the above program proved negative, investigations continued with a prolonged oral (7 days) treatment (p.o.7) with penicillin.
- All patients were initially challenged with penicillin. If this challenge proved negative and if case history pointed at a different β -lactam, the patient was subsequently challenged also with their culprit drug.

On the first evaluation day, case history was analyzed, ENDA questionnaire for drug allergy was filled out by participants (5), clinical examination was performed, and specific IgE levels against phenoxymethylpenicillin (penV), benzylpenicillin (penG), ampicillin (AMP), and amoxicillin (AX) were measured using ImmunoCap (ThermoFisher Scientific). On the second evaluation day, skin prick test (SPT), intracutaneous test (ICT), and patch test (PT) were performed. In patients with negative results, penicillin challenge was initiated 1 week later.

Skin prick test was performed with penV (25 mg/ml), AX (1.25 mg/ml and 25 mg/ml), and the major (PPL, benzylpenicilloyl-poly-L-lysine) and minor determinant mix (MDM) from Diater Laboratory, Spain. ICT was performed with penG and AMP (1.25 mg/ml and 25 mg/ml), dicloxacillin (DX) 1 mg/ml, mecillinam (ME) 4 mg/ml, and PPL and MDM (in patients with anaphylaxis, PPL and MDM were performed initially with 1 : 10 dilutions as recommended by the manufacturer). ICT was not performed with AX due to unavailability of a formulation for intravenous use on the Danish market.

Histamine 10 mg/ml (ALK-Abello, Denmark) and isotonic NaCl were used as controls. Freshly prepared penicillins diluted with saline were used for SPT and ICT. SPT and ICT were performed on the volar forearm and read after 20 min. ICT (0.05 ml of each hapten) and SPT were considered positive if the increase in the largest diameter (in duplicate) was ≥ 3 mm and surrounded by flare (6–9). SPT and ICT were also read after 72 h and 7 days.

Patch test (PT) was performed according to the International Contact Dermatitis Research Group (ICDRG) recommendations (10, 11) with commercialized forms of penG and penV, AMP, AX, DX, and ME (10% in petrolatum). All vehicles were manufactured by Chemotechnique (Sweden) (6–10). Reactions from + to +++ were regarded as positive (2).

Penicillin challenge

On the third day, challenge was initiated under anaphylaxis surveillance by intravenous injection of penG from 100 IE to 1,000,000 IE in 10-fold increments with 20-min interval, followed by administration of 400 mg of oral penV 20 min later at the center and continued with a 7-day course (800 mg t.i.d) at home. Challenge was stopped when a clinical reaction occurred. Anaphylaxis was defined according to Simons (12).

Patients who tested negative in the above program and those with case histories of reactions to other β -lactam antibiotics were subsequently challenged with the 500 mg culprit drug, 2 (AMP, AX) or 3 (DX) doses per day for 7 days (p.o.7). Antihistamines or other drugs affecting the result were discontinued. The study was approved by the regional ethical committee (project ID. S-20100091).

Statistical analysis

Data were collected in duplicate in the EpiData database. Statistical analyses were performed with STATA 11, StataCorp, College Station, TX, USA, using Fisher's exact test. Statistical significance was defined as $P < 0.05$.

Results

A total of 452 adult patients, referred to the allergy center for evaluation of history of allergic reactions to penicillin, were included in this prospective study; 110 patients refrained from further testing, as shown in Table 1.

The most frequently reported symptoms were unclassified cutaneous rash (180 cases, 52.6%), urticaria and/or angioedema (135 cases, 39.5%), and anaphylaxis (11 cases, 3.2%). Respiratory or gastrointestinal symptoms without cutaneous reactions were reported by 13 (3.8%).

The β -lactam eliciting the initial reaction (the culprit drug) was penV and penG in 188 cases (55%), DX in 11.4%, AX in 9.6%, AMP in 7%, and unknown in 18.4%.

According to case history, reactions commencing within 1 h after last drug intake were subclassified as immediate reactions (IR, 36 patients) and those commencing 1 h after last drug intake as nonimmediate reactions (NIR, 235 patients), as described in the Materials and Methods section. Seventy-one patients could not be subclassified. Only patients reporting anaphylaxis were over-represented in the IR group ($P < 0.05$), as shown in Table 2.

Diagnostic tests

Sensitization is defined hereafter as a positive outcome of any of the tests applied. IgE sensitization was demonstrated in 19 patients: 18 to penV, 1 to penG, 9 to AMP, and 6 to AX. Monosensitization was only seen to penV (8 cases) and penG (1 case). There was no correlation between positive

Table 2 Type of reaction compared with symptoms at the primary reaction

Symptomatology (Case history)							
Type of reaction (<i>n</i> = 342)	Anaphylaxis (<i>n</i> = 11)	Urticaria/angioedema (<i>n</i> = 135)	Unclassified cutaneous Rash (<i>n</i> = 180)	Vasculitis (<i>n</i> = 1)	AGEP (<i>n</i> = 1)	Mb. Sweet (<i>n</i> = 1)	None (<i>n</i> = 13)
IR (<i>n</i> = 36)*	11	12	11	0	0	0	2
NIR (<i>n</i> = 235)*	0	106	121	1	1	0	6
Unknown (<i>n</i> = 71)**	0	17	48	0	0	1	5

*In all cases, except anaphylaxis, no significant difference between the IR and NIR groups was found ($P > 0.05$).

**Seventy one patients did not recall the exact timing.

Table 3 Skin test results

Reagents		Skin test result							NT
		pos/pos			pos/neg			neg/neg	
		Total	I	D	Total	I	D		
SPT									
Amoxicillin 1.25 mg/ml	0	0	0	0	0	0	339	0	
Amoxicillin 25 mg/ml	1	0	1	0	0	0	338	0	
PPL 1 : 1	0	0	0	0	0	0	339	0	
MDM 1 : 1	0	0	0	0	0	0	339	0	
ICT									
Penicillin G 1.25 mg/ml	7	3	4	1	1	0	331		
Penicillin G 25 mg/ml	12	7	5	1	1	0	325	1	
Ampicillin 1.25 mg/ml	14	2	12	2	0	2	323		
Ampicillin 25 mg/ml	26	9	17	5	2	3	308		
Dicloxacillin 1 mg/ml	6	4	2	1	1	0	332		
Mecillinam 4 mg/ml	5	4	1	1	1	0	333		
PPL 1 : 10*	0	0	0	0	0	0	19	320	
PPL 1 : 1	1	0	1	2	2	0	335	1	
MDM 1 : 10*	0	0	0	0	0	0	19	320	
MDM 1 : 1	3	1	2	0	0	0	335	1	
Patch test									
Penicillin G 10% pet.	2	0	2	1	0	1	332	4	
Penicillin V 10% pet.	2	0	2	0	0	0	290	47	
Ampicillin 10% pet.	7	0	7	0	0	0	304	28	
Amoxicillin 10% pet.	9	0	9	1	0	1	325	4	
Dicloxacillin 10% pet.	4	0	4	0	0	0	330	5	
Mecillinam 10% pet.	0	0	0	0	0	0	260	79	

I = Immediate reaction in skin test (at 20 min).

D = Delayed reaction.

The patients were not tested with AX in ICT because of unavailability during the study.

*Since As we only used the recommended 1 : 10 dilution (PPL and MDM) in patients with a case history of a severe reaction, most patients (320) were only tested with the 1 : 1 concentration, whereas the many cases of 'not tested, NT' in PT was caused by supply unavailability during a part of the study period.

Three patients were not skin-tested (one with vasculitis, one AGEF, one Mb. Sweet).

outcome in IgE testing and culprit drug reported (data not shown).

The results of SPT, ICT, and PT are presented in Table 3. A total of 35 of 339 patients (99 positive results, 24 patients

Table 4 Correlation between positive test results (IgE, ICT and PT)

	IgE + (n = 19)	IgE – (n = 323)	PT + (n = 9)	PT – (n = 333)
Immediate				
ICT + (n = 14)	6	8	0	14
ICT – (n = 328)	13	315	9	319
Delayed				
ICT + (n = 21)	0	21	9	12
ICT – (n = 321)	19	302	0	321

All 9 patients positive in PT also had a positive delayed reaction in ICT.

The 6 ICT positive results of the 19 patients with positive IgE were all immediate reactions.

Bold values denote how many were positive in the different types of tests.

positive to more than one) tested positive. Discrepancy between duplicate test results was seen in seven patients (11 tests, three with a borderline and 8 with a clearly unilateral positive reaction). Reproducibility was thus approximately 90% when calculating number of tests but only 83% when calculating the number of patients. As expected, the recommended concentration of penicillin for ICT (25 mg/ml) yielded more positive reactions in the same patients than 1.25 mg/ml.

The majority of positive reactions in ICT were elicited by AMP 25 mg/ml (26 cases, 74.3%; 14 also positive to AMP 1.25 mg/ml). An identical pattern was seen with penG (12 resp. seven patients). No patient was positive only to 1.25 mg/ml. As a formulation of AX for parenteral use was not available in Denmark, we did not perform ICT with this drug.

The majority of ICT reactions were NIR (60%). No dual responses (IR and NIR in the same patient) were elicited. The only patient positive in SPT was also positive in ICT to AMP. SPT and ICT with PPL and MDM resulted in very few positive reactions (one and three, respectively, concomitantly positive in ICT to penG 25 mg/ml).

Nine patients were positive in PT (+ or ++ reactions on day 3 or 7), all to AX; seven were also positive to AMP (two not tested with AMP).

The only consistent correlation between positive test results was found between a positive PT and positive delayed reaction in ICT, as shown in Table 4; especially, the lack of correlation between IgE-positive patients and ICT-positive patients is noteworthy. In contrast to what normally is seen with classical type I allergens, only six of the 19 IgE-positive patients were also positive in ICT.

Outcome of challenge procedures

A total of 291 patients tested negative in all tests were challenged with penicillin, as shown in Table 5. We found no correlation between a case history of IR and NIR, and the probability of a positive outcome of any test in parts A, B, or C ($P > 0.05$). A case history of anaphylaxis, urticaria, and/or angioedema showed a significantly higher probability

Table 5 Distribution of type of reaction according to case history (IR or NIR) and diagnostic evaluation

Challenge	PART A (n = 342)					PART B (n = 291)				PART C (n = 78)					
	Total* (n = 342)	IgE pos. (n = 19)	SPT pos. (n = 1)	ICT immediate pos. (n = 14)	ICT Late reading pos. (n = 21)	Patch test		i.v. pos. (n = 4)	p.o. pos. (n = 6)	p.o. 7 pos. (n = 23)	p.o. pos. (n = 1)	p.o. 7 pos. (n = 16)	Any positive test (n = 114)	Any positive patient (n = 98)	Negative patient (n = 244)
Type of reaction	n														
Immediate reaction	36	4	0	5	0	0	3	5	1	0	2	20	17	19	
Non-immediate reaction	235	12	1	6	18	8	1	1	20	1	13	81	69	166	
Unknown	71	3	0	3	3	1	0	0	2	0	1	13	12	59	

Part A: (Diagnostic tests: IgE analysis, skin testing (SPT, ICT and PT).

Part B: (Penicillin challenge: i.v. titrated part, single oral dose (p.o.), prolonged oral challenge (p.o.7).

Part C: (Oral challenge with other culprit drug (dicloxacillin, ampicillin, amoxicillin).

*Three patients only participated in measurement of specific IgE (one vasculitis, one AGEP, and one Mb. Sweet).

than unspecific rash of being positive in any test in parts A, B, or C (40% vs 20.7%, $P < 0.05$).

During i.v. challenge, four patients reacted with mild cutaneous maculopapular rash (one to 10.000 IE, 3 to 1.000.000 IE). Six of the remaining 287 patients reacted within 2 h in the single-dose oral challenge with penV. Of the 281 patients continuing with p.o.7 with penV, 23 reacted during (14 patients, day 2 and days 6–7) or after treatment (nine patients, mostly days 2–3). The main sign elicited during challenge was urticaria/angioedema (18 patients), followed by maculopapular rash in five patients. No severe reactions were seen.

Of the remaining 258 patients, 78 presented with a case history of reaction to other penicillins. They were subsequently challenged with their culprit drug. Seventeen reacted to their culprit drug, 7 to DX, 7 to AX, and 3 to AMP (10 urticaria and/or angioedema and 7 maculopapular rash).

No reactions were elicited within 1 h in the group reporting IR, but seen in six patients reporting NIR, as shown in Table 6. A significant correlation between patients reporting IR and patients reacting within 2 h was found (8 of 11 patients, $P < 0.05$). Seven of 36 patients reporting NIR reacted during the first 2 h of challenge.

In all, this modification of the ENDA guidelines resulted in 98 (28.7%) positive outcomes, confirmed by either specific IgE (19 patients, 5.6%), skin tests (35 patients, 10.2%), i.v. challenge (four patients, 1.2%), single-dose challenge (seven patients, 2.0%), or prolonged oral challenge (39 patients, 11.4%).

Discussion

This prospective study is a continuation of our previous retrospective study in 2011(4). In the present study, we included PT with penicillins, PPL, and MDM in SPT and ICT, and all testings were performed in duplicate. All patients were investigated with a modification of ENDA guidelines and, if negative, a prolonged oral treatment (p.o.7) was added.

Any food or drug challenge, regardless of eliciting agent, should ideally aim to replicate the circumstances present at the initial reaction. Although in case of drug challenge with penicillins, the immunologic events taking place during the infection normally cannot be included, it is important to mimic the initial circumstances. We therefore included p.o.7 because single-dose penicillin treatment is normally confined to perioperative treatment.

This extended procedure yielded more positive outcomes: A total of 98 patients (28.7%) yielded positive outcome in this study, whereas only 59 patients (7.3%) yielded positive outcome after excluding p.o.7.

Prolonged oral treatment with penicillin has been attempted only in few studies. Blanca-Lopez (13) studied 39 children given a prolonged oral treatment with amoxicillin, where 19 reacted with cutaneous symptoms. Ponvert (14) described 130 positive challenges in 111 children, in which 88 were positive during a prolonged oral treatment with the culprit β -lactam. Two additional studies (15, 16) have described similar, although less frequent, results.

Table 6 Correlation between case history of IR and NIR and outcome of challenge including time course

Type of challenge		Penicillin			Ampicillin/Amoxicillin/ Dicloxacillin	
Type of reaction (Case history)	Time of reaction (Challenge)	i.v. pos. (n = 4)	p.o. pos. (n = 6)	p.o. 7 pos. (n = 23)	p.o. pos. (n = 1)	p.o. 7 pos. (n = 16)
Immediate reaction	<1 h					
	1–2 h	3	5			
	>24 h, but during treatment			1		
	After treatment (days)					2
Non-immediate reaction	<1 h	1		4		1
	1–2 h		1			
	<24 h				1	
	>24 h, but during treatment			9		7
	After treatment (days)			7		5
Unknown	>24 h, but during treatment			1		1
	After treatment (days)			1		

Concerning the practical approach for patient evaluation, different algorithms for diagnosing IR and NIR have been suggested (1–3), but data on reliability of the case history have never been compared with the outcome of challenge. We were unable to reproduce the case history-based classification of IR and NIR in 87% of our patients (41 of 47 who reported IR or NIR in the questionnaire) using the challenge outcome as true outcome. Among these patients, we saw six patients categorized as NIR on the basis of their case history of reacting immediately during challenge, whereas no reactions were elicited within 1 h in the group reporting IR, as shown in Table 6. According to ENDA guidelines, measurement of specific IgE is not included in NIR patients. This could pose a risk to the patients' safety. In this study, we found 12 of the 19 patients with IgE sensitization belonging to the group of NIR according to case history and only four patients in the group reporting IR, so according to guidelines, these patients would have been challenged with penicillin, because they tested negative in skin testing. Finally, many patients in this study (21.1%) are unable to give a detailed case history regarding time interval between dosage and reaction (17, 18).

Other studies (19, 20) have also previously stated that clinical history is not a reliable predictor of subsequent penicillin skin testing.

In the literature, the two main signs of IR are urticaria with or without angioedema and anaphylaxis. The main signs of NIR are maculopapular rashes, but also delayed-appearing urticaria/angioedema (1–3). In this study, the most frequent reaction during challenge was urticaria and/or angioedema, followed by maculopapular rashes. Only 22.2% of the patients reacting with urticaria and/or angioedema showed reaction within 2 h on day 1, and the remaining (77.8%) reacted during or even after treatment. Whether a patient reacting during continuous treatment on, for example, day 4 would have experienced a similar reaction after

challenge with a single dose of penicillin (i.e., a true NIR) cannot be deducted from the present study.

Predicting positive predictive value (PPV) and negative predictive value (NPV) of specific IgE and skin tests is difficult when omitting a gold standard (i.e., knows for certain whether a patient is clinically reactive or not). Romano et al. (21) challenged five patients with positive delayed responses to ICT, in which only two tested positive. In another study (22), 270 challenges were performed regardless of skin test outcome. A total of 137 patients positive in ICT were challenged, with nine positive outcomes, while 5 of 135 patients negative in ICT tested positive. PPV is then as low as 0.07, and NPV 0.95. Three other studies (23–25) also challenged skin test-positive patients with penicillin and obtained similar results.

One study has even tested the diagnostic value of specific IgE by challenging patients with IgE sensitization to penicillin (26): two of seven patients with positive IgE (0.4–2.9) challenged with penicillin tested positive, both with an immediate reaction (urticaria), and 2 of 15 patients with negative IgE tested positive in challenge, one with IR and one with NIR.

We found a lower number of positive skin tests to β -lactams (10.5%) than reported in studies from the southern part of Europe (12–70%) even when utilizing identical concentrations of allergen (1, 2, 27, 28). This can only partly be explained by the nonavailability of AX for ICT, because, although we do not know the number of positive ICTs with AX in our population, only seven patients tested positive to AX. Also, in contrast to what we had expected from the literature, where the frequency of positive reactions to PPL and MDM varies from 5.5 to 22% (27, 29, 30), SPT and ICT with PPL and MDM resulted in only 4 positive reactions in three patients (0.9%), all also being positive in ICT to penG 25 mg/ml. Besides regional geographic differences in drug consumption, we have no apparent explanation for these findings.

We found consistent correlation between ICT (delayed) and PT. All nine patients positive in PT were also with positive NIR in ICT. The majority of patients positive in ICT were PT negative, confirming previous findings that ICT has a higher sensitivity than PT (21, 31, 32).

Discrepancies in the results of repeated skin testing in patients have led to the concept of (re-)sensitization induced by testing (15, 33, 34). The reports describing a positive skin test result in a previously skin test-negative patient have used single testing and found that a few percentage of the patients became positive when repeated. Lack of reproducibility in a small proportion of patients thus provides a more plausible explanation than procedural sensitization. Further, a positive outcome of a second challenge in a previously negative patient has also been interpreted as (re-)sensitization (1, 3, 33, 35, 36). In these cases, however, the initial negative challenge was followed by a regular treatment course with a β -lactam probably mimicking our results obtained by the prolonged challenge. As sensitization by skin testing with classical, complete allergens has so far not been proven, this is also unlikely to happen with haptens such as β -lactams. SPT and ICT were performed simultaneously with all allergens despite warnings in the literature of risk of elicitation of systemic reactions. Although we found no such reactions,

such a potential risk should always be considered especially in populations with high rates of true IR.

Our study points toward that testing for allergy to β -lactams conceptually does not differ from other testing procedures in allergology, except perhaps in the fact that a higher proportion of the patients are not demonstrably IgE-sensitized and also that a higher proportion follow another time course by reacting with symptoms and signs after hours to days of exposure. Non-IgE mechanisms seem plausible, but further studies are needed. Until such studies have shed light on possible differences between IR and NIR, we suggest for practical and for safety reasons to evaluate both groups using the same diagnostic workup.

Author contributions

Janni Hjortlund has performed the data analysis and drafted the manuscript. All coauthors have contributed to conception and design of the study and interpretation and discussion of the results and approved the final version of the manuscript.

Conflict of interest

There are no conflicts of interest for each named author.

Reference

- Blanca M, Romano A, Torres MJ, Fernandez J, Mayorga C, Rodriguez J et al. Update on the evaluation of hypersensitivity reactions to betalactams. *Allergy* 2009;**64**:183–193.
- Romano A, Blanca M, Torres MJ, Bircher A, Aberer W, Brockow K et al. Diagnosis of nonimmediate reactions to beta-lactam antibiotics. *Allergy* 2004;**59**:1153–1160.
- Torres MJ, Blanca M, Fernandez J, Romano A, Weck A, Aberer W et al. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy* 2003;**58**:961–972.
- Hjortlund J, Mortz CG, Skov PS, Eller E, Poulsen JM, Borch JE et al. One-week oral challenge with penicillin in diagnosis of penicillin allergy. *Acta Derm Venereol* 2012;**92**:307–312.
- Demoly P, Kropf R, Bircher A, Pichler WJ. Drug hypersensitivity: questionnaire. EAACI interest group on drug hypersensitivity. *Allergy* 1999;**54**:999–1003.
- Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002;**57**:45–51.
- Dreborg S. Skin test used in type I allergy testing. *Allergy* 1989;**44**:14–59.
- Dreborg S. Allergen standardization and skin tests. *Allergy* 1993;**48**:49–82.
- Romano A, Bousquet-Rouanet L, Viola M, Gaeta F, Demoly P, Bousquet PJ. Benzylpenicillin skin testing is still important in diagnosing immediate hypersensitivity reactions to penicillins. *Allergy* 2009;**64**:249–253.
- Barbaud A, Goncalo M, Bruynzeel D, Bircher A. Guidelines for performing skin tests with drugs in the investigation of cutaneous adverse drug reactions. *Contact Dermatitis* 2001;**45**:321–328.
- Frosch PJ, Menné T, Lepoittevin J-P. *Contact Dermatitis*. Berlin: Springer-Verlag, 2006.
- Simons FE. Anaphylaxis. *J Allergy Clin Immunol* 2010;**125**:161–181.
- Blanca-Lopez N, Zapatero L, Alonso E, Torres MJ, Fuentes V, Martinez-Molero MI et al. Skin testing and drug provocation in the diagnosis of nonimmediate reactions to aminopenicillins in children. *Allergy* 2009;**64**:229–233.
- Ponvert C, Perrin Y, Bados-Albiero A, Le BM, Karila C, Delacourt C 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**:411–418.
- Mendelson LM, Ressler C, Rosen JP, Selcow JE. Routine elective penicillin allergy skin testing in children and adolescents: study of sensitization. *J Allergy Clin Immunol* 1984;**73**:76–81.
- Pichichero ME, Pichichero DM. Diagnosis of penicillin, amoxicillin, and cephalosporin allergy: reliability of examination assessed by skin testing and oral challenge. *J Pediatr* 1998;**132**:137–143.
- Demoly P, Bousquet J. Drug allergy diagnosis work up. *Allergy* 2002;**57**:37–40.
- Schnyder B, Pichler WJ. Nonimmediate drug allergy: diagnostic benefit of skin testing and practical approach. *J Allergy Clin Immunol* 2012;**129**:1170–1171.
- Macy E. How predictive is a history of penicillin allergy? *JAMA* 2001;**286**:1174.
- Wong BB, Keith PK, Wasserman S. Clinical history as a predictor of penicillin skin test outcome. *Ann Allergy Asthma Immunol* 2006;**97**:169–174.
- Romano A, Viola M, Mondino C, Pettinato R, Di FM, Papa G et al. Diagnosing nonimmediate reactions to penicillins by in vivo tests. *Int Arch Allergy Immunol* 2002;**129**:169–174.
- Goldberg A, Confino-Cohen R. Skin testing and oral penicillin challenge in patients with a history of remote penicillin allergy. *Ann Allergy Asthma Immunol* 2008;**100**:37–43.
- Green GR, Rosenblum AH, Sweet LC. Evaluation of penicillin hypersensitivity: value of clinical history and skin testing with penicilloyl-polylysine and penicillin G. A cooperative prospective study of the penicillin study group of the American Academy of Allergy. *J Allergy Clin Immunol* 1977;**60**:339–345.
- Sogn DD, Evans R III, Shepherd GM, Casale TB, Condemi J, Greenberger PA et al. Results of the National Institute of Allergy and Infectious Diseases Collabora-

- tive Clinical Trial to test the predictive value of skin testing with major and minor penicillin derivatives in hospitalized adults. *Arch Intern Med* 1992;**152**:1025–1032.
25. Solley GO, Gleich GJ, Van Dellen RG. Penicillin allergy: clinical experience with a battery of skin-test reagents. *J Allergy Clin Immunol* 1982;**69**:238–244.
26. Silva R, Cruz L, Botelho C, Castro E, Cadinha S, Castel-Branco MG et al. Immediate hypersensitivity to penicillins with negative skin tests—the value of specific IgE. *Eur Ann Allergy Clin Immunol* 2009;**41**:117–119.
27. Torres MJ, Romano A, Mayorga C, Moya MC, Guzman AE, Reche M et al. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy* 2001;**56**:850–856.
28. Torres MJ, Blanca M. The complex clinical picture of beta-lactam hypersensitivity: penicillins, cephalosporins, monobactams, carbapenems, and clavams. *Med Clin North Am* 2010;**94**:805–820.
29. Romano A, Viola M, Bousquet PJ, Gaeta F, Valluzzi R, Caruso C et al. A comparison of the performance of two penicillin reagent kits in the diagnosis of beta-lactam hypersensitivity. *Allergy* 2007;**62**:53–58.
30. Romano A, Gaeta F, Valluzzi RL, Caruso C, Rumi G, Bousquet PJ. The very limited usefulness of skin testing with penicilloyl-polylysine and the minor determinant mixture in evaluating nonimmediate reactions to penicillins. *Allergy* 2010;**65**:1104–1107.
31. Romano A, Quarantino D, Di FM, Papa G, Venuti A, Gasbarrini G. A diagnostic protocol for evaluating nonimmediate reactions to aminopenicillins. *J Allergy Clin Immunol* 1999;**103**:1186–1190.
32. Torres MJ, Sanchez-Sabate E, Alvarez J, Mayorga C, Fernandez J, Padial A et al. Skin test evaluation in nonimmediate allergic reactions to penicillins. *Allergy* 2004;**59**:219–224.
33. Lopez-Serrano MC, Caballero MT, Barranco P, Martinez-Alzamora F. Booster responses in the study of allergic reactions to beta-lactam antibiotics. *J Investig Allergol Clin Immunol* 1996;**6**:30–35.
34. Matheu V, Perez-Rodriguez E, Sanchez-Machin I, Garcia-Robaina JC, de la Torre MF. Importance of repeat testing in the diagnosis of penicillin allergy. *Br J Dermatol* 2006;**154**:198.
35. Aberer W, Bircher A, Romano A, Blanca M, Campi P, Fernandez J et al. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. *Allergy* 2003;**58**:854–863.
36. Solensky R, Earl HS, Gruchalla RS. Lack of penicillin resensitization in patients with a history of penicillin allergy after receiving repeated penicillin courses. *Arch Intern Med* 2002;**162**:822–826.