Original Article

The Importance of Prolonged Provocation in Drug Allergy — Results From a Danish Allergy Clinic

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What is already known about this topic? Currently, drug provocation tests are recommended only in skin test negative patients and performed as a single challenge. Prolonged provocation to rule out nonimmediate reactions is scarcely discussed in literature and not yet addressed in guidelines.

What does this article add to our knowledge? In Denmark, drug provocation, including prolonged provocation, with culprit drug is a safe and useful method to diagnose drug allergy. Most provocations are with narrow-spectrum penicillins, reflecting the local pattern of antibiotic use.

How does this study impact current management guidelines? Prolonged provocation increases the number of patients diagnosed with nonimmediate reactions and should always be considered when drug provocation is included in allergy investigation.

BACKGROUND: Drug provocation is the "Gold Standard" in drug allergy investigation. Recent studies suggest that a negative drug provocation on first dose should be followed by a prolonged provocation over several days.
OBJECTIVE: To evaluate drug allergy investigations on the basis of drug provocation, including prolonged provocation.
METHODS: Data from adult patients investigated for drug allergy in a Danish Allergy Clinic during the period 2010 to 2014 were entered into a database. Data included clinical details and results of provocations with suspected culprit drug (for penicillins performed only in specific IgE-negative patients). If provocation was negative on first dose, treatment was continued for 3 to 10 days.
RESULTS: A total of 1,913 provocations were done in 1,659 patients, median age 46 years, of whom 1,237 (74.6%) were females. Drugs investigated were antibiotics, 1,776 (92.8%), of which 1,590 (89.5%) were penicillins; analgesics, 59 (3.1%); local anesthetics, 33 (1.7%); and other drugs, 45 (2.4%). In total, 211 of 1,913 (11.0%) provocations were positive. Causes were antibiotics, 198 (93.8%), of which 167 (84.3%) were penicillins; analgesics, 7 (3.3%); local anesthetics, 0; and other drugs, 6 (2.8%). Only 43 (20.4%) provocations were positive on first dose, whereas 95 (45.0%) turned positive more than 3 days later.
CONCLUSIONS: Only 11.0% of the provocations were positive. Importantly, only 1 of 5 patients tested positive on the first dose, indicating that prolonged exposure should always be considered when drug provocation is included in allergy investigations. Most provocations were with penicillins, reflecting the pattern of antibiotic use in Denmark, which differs from that in other countries, especially outside Northern Europe. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;■:■-■)

Key words: *Prolonged provocation; Drug challenge; Narrow-spectrum penicillin; Drug provocation test; Drug allergy; Betalactam antibiotics*

Suspected drug allergy is a widespread problem with great impact on daily medical practice. Many nonallergic adverse drug effects are mislabeled allergy and both health personnel and patients fear that minor reactions may be the precursor for life-threatening anaphylaxis. Increased understanding about the relative rarity of true allergic reactions and the underlying mechanisms would be useful in putting these fears into perspective.[1] Patients with suspected drug allergy are often prescribed more expensive drugs,[2] have longer hospitalizations,[3] and are prescribed broad-spectrum antibiotics contributing to antimicrobial resistance.[4] Investigations to confirm or rule out allergy are therefore important.

Large epidemiological studies of drug allergy are scarce. The prevalence of alleged penicillin allergy in 2006 in a Danish hospital population was 2.6%.[5] The true prevalence is difficult to determine because of overdiagnosing of patients who are not investigated, as well as underestimation of cases due to underreporting.[6-9]

The fact that only a small proportion of suspected allergies are confirmed on investigation is well described in literature.

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Abbreviations used
ASA- Acetylsalicylic acid
IR- Immediate reaction
NIR- Nonimmediate reaction
sIgE- Specific IgE
IDT- Intradermal test
ENDA- European Network for Drug Allergy
NSAID- Nonsteroidal anti-inflammatory drug

In populations with suspected drug allergy, the confirmed penicillin allergy rate differs between 28.7%[10] in Denmark, using the European Network for Drug Allergy (ENDA) guidelines, and 13.5%[11] in Slovenia, using local guidelines. In the United States, the reported rate is lower, with less than 10% being diagnosed with penicillin allergy after a suspected reaction.[12,13] The reported differences between countries and centers seem related to differences in investigation protocols,[10,11,14-19] populations,[20,21] and patterns of antibiotic use.[22,23]

In many countries, betalactam antibiotics is the most common drug group suspected of causing allergy, followed by other antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs).[3,7,17,24] Narrow-spectrum penicillins are the most frequently prescribed drugs in Denmark,[25] due to low price, favorable side effect profile, and low risk of inducing resistance. Broad-spectrum antibiotics, especially aminopenicillins, are more commonly used in countries outside Northern Europe because of higher incidence of resistance to narrow-spectrum penicillins.[22]

As opposed to American guidelines,[13] European guidelines[26-28] suggest different investigation protocols for immediate reactions (IRs) (<1 hour of drug intake) and nonimmediate reactions (NIRs) (>1 hour after drug intake) on the basis of history of the suspected reaction. However, a recent study questioned patient recall and showed that clinical history cannot be used to predict whether reactions will be IRs or NIRs.[10] Investigation protocols in most allergy centers use skin tests: Skin prick tests and intradermal tests (IDTs) for IRs and patch tests/late reading of IDTs for NIRs. Drug provocation, or drug challenge, is usually performed only if skin test results are negative. Drug provocation is the "Gold Standard" with good diagnostic value.[29] Recently, prolonged provocation, that is, a repeated dose provocation over several days, has been introduced in another Danish center for adult patients. Results suggest that a negative drug provocation on first dose does not rule out an NIR, which can be diagnosed during prolonged provocation.[10,15] This has also been suggested in children from other parts of Europe.[30,31] However, the issue of prolonged provocation is not yet addressed in guidelines.

In our clinic, data on allergy investigation using drug provocation tests, including prolonged provocation with culprit drugs, have been collected since 2010. The aim of this study was to evaluate the results from drug allergy investigations in our clinic during the period 2010 to 2014. This is the largest study of prolonged provocation in drug allergy investigation reported so far.

METHODS

Data from adult patients undergoing drug provocation as part of investigations for drug allergy (excluding perioperative reactions) in the Allergy Clinic, Gentofte Hospital, Denmark, during the period 2010 to 2014 were collected prospectively in a database. Data were collected in case record forms filled in by the attending physician, inspired by the ENDA guidelines.[32] Contraindications for provocation followed recommendations in the ENDA position paper on drug provocation.[28] Patients with suspected allergy to penicillins underwent drug provocation only if specific IgE (sIgE) for penicillins were negative.

Drug allergy investigation at the clinic includes clinical history, analysis of sIgE (for penicillins), and drug provocation with culprit drug if known (Figure 1). Skin testing is not presently included in the standard investigation protocol in the region. sIgE is measured using the ImmunoCAP method (ThermoFisher Scientific, Uppsala, Sweden) with a cutoff value of more than 0.35 kUA/L. sIgE analysis is carried out for penicillin V, penicillin G, ampicillin, and amoxicillin (all commercially available) and in addition for penicillin degradation products (minor determinants), a noncommercially available test that our clinic has special access to on a research basis. If sIgE to 1 or more penicillins is detected, further testing is usually abandoned because of a suspected high probability of a clinical allergy and the patient is issued with a warning card against penicillins. Data from these patients are not entered into the provocation database. An exception was made in 8 cases in which sIgE was slightly elevated to only 1 penicillin, and the patient had a strong clinical indication for needing penicillins. If no sIgE is found, the patient undergoes drug provocation with suspected culprit drug. If culprit type of penicillin is unknown, provocation with penicillin V is performed.

Drug provocation is performed under observation including access to emergency room facilities. Intravenous access is obtained when provocation is considered high risk either because of history of a severe IR or because of comorbidities. The provocation is either titrated or nontitrated, depending on the estimated risk of inducing a reaction on the basis of severity of the reaction and the patient's comorbidities. The route of provocation is usually the original route of administration, that is, mainly oral, or in a few cases intravenous and subcutaneous.

Titrated provocation is usually performed in three 10-fold steps 30 to 45 minutes apart depending on the route of administration, starting with 1:100 dilution of therapeutic dose and ending with full therapeutic dose.

Nontitrated provocation is performed with a single full therapeutic dose. Both types of provocation are followed by 2-hour observation in the clinic. If provocation is negative, an IR is ruled out and in most cases provocation is continued at home for 3 to 10 days, to match timing of exposure and symptom onset of the initial reaction. For the purpose of this study, *immediate reactions* are defined as developing within the observation time at the clinic, that is, less than 2 hours after the first full dose. *Nonimmediate reactions* are defined as developing after 2 hours. A provocation is considered positive, for both IRs and NIRs, on the development of objective symptoms. For IRs, this would be rash/urticaria with or without pruritus, and very rarely, respiratory and/or circulatory symptoms and for NIRs typically maculopapular exanthema. Subjective symptoms only are not considered sufficient to diagnose a clinical allergy. NIRs are generally confined to the skin and patients are issued with a written treatment plan for out-of-hours treatment plus antihistamines and in many cases steroids on leaving the clinic. If patients develop symptoms they are instructed to call the clinic, where they will speak to a doctor who assesses their reported symptoms and gives advice about treatment.

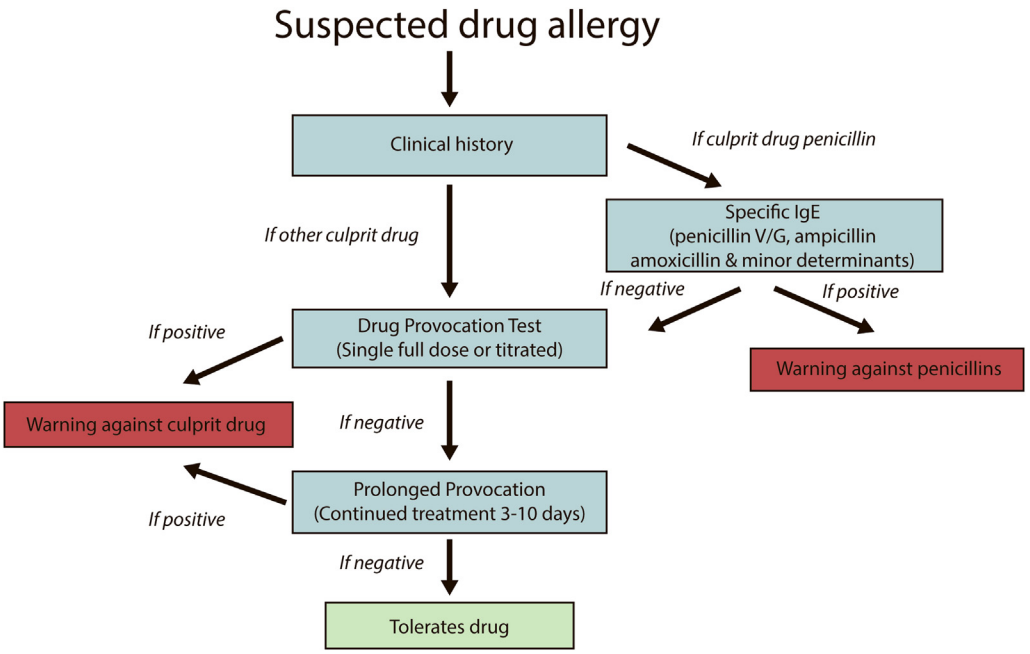

FIGURE 1. The investigation algorithm for suspected drug allergic reactions at Gentofte Hospital.

Statistical analyses

Statistical analyses were performed using the chi-square test (for symptoms and treatment) in Excel 2010 (Microsoft Corporation, Redmond, WA) and the Mann-Whitney *U* test (for time interval between initial reaction and provocation) in SPSS version 22 (IBM Corp., Armonk, NY). *P* values of less than .05 were considered statistically significant.

RESULTS

A total of 1,913 provocations were carried out in 1,659 patients, median age 46 years (range, 18-90 years), of whom 1,237 (74.6%) were females. Titrated provocation was performed in 443 (23.2%) cases. A total of 14% of provocations were a single-dose challenge, many with local anesthetics or vaccines, where continued treatment was not relevant, or in patients who had reported first-dose reactions.

The most common drug types investigated was antibiotics, with 1,776 (92.8%) provocations performed of which 21 (1.1%) were cephalosporins and 1,590 (89.5%) penicillins (Figure 2). In 1,582 cases sIgE were negative and in 8 cases sIgE to 1 of the penicillins was slightly elevated, with values ranging between 0.35 and 0.53 kUA/L. In 7 of these cases, provocation was negative and in 1 case provocation was positive for dicloxacillin (culprit drug), but negative for penicillin V. Of the 21 cephalosporin provocations, 19 were with cefuroxime and 2 with cefalexin. The 165 (8.6%) other antibiotics included macrolides (62), quinolones (24), and metronidazole (39). Analgesics were the suspected culprit drug in 59 (3.1%) provocations, of which 45 (76.3%) were NSAIDs/acetylsalicylic acid (ASA), 23 were with ibuprofen, 10 ASA, 7 ASA in combination with codeine, 2 diclofenac, 1 etodolac, 1 celecoxib, and 1 tiaprofenic acid. The remaining 45 (2.5%) provocations were with drugs such as vaccines (8), proton pump inhibitors (6), biologicals (5), antifungals (3), contrast media (2), and others (21).

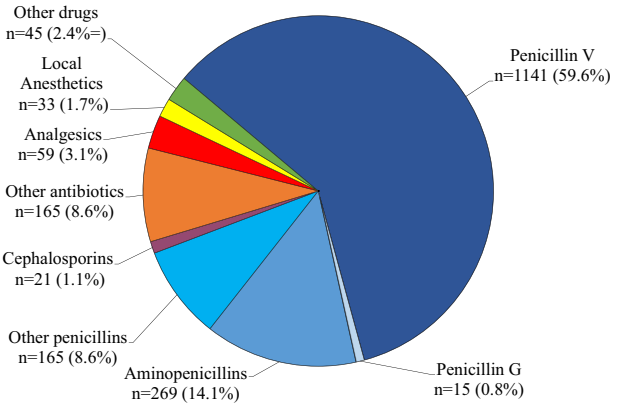

FIGURE 2. Total drug provocation tests (N = 1913).

In total, 211 of 1,913 (11.0%) provocations were positive. Positive provocations were caused by antibiotics in 198 (93.8%) cases, of which 167 (84.3%) were penicillins. Seven (3.3%) provocations with analgesics proved positive, 6 (85.7%) NSAIDs/ASA (3 ASA, 2 ibuprofen, and 1 celecoxib) and 1 paracetamol (Figure 3). No local anesthetics tested positive.

Of the 211 positive provocations, only 43 (20.4%) were IRs (occurring within the 2-hour observation period), 73 (34.6%) turned positive later than 2 hours after the first dose, but no later than 3 days. The remaining 95 (45.0%) turned positive after 3 or more days of prolonged provocation (Figure 4). Overall, aminopenicillins tested positive most frequently, with 29.4% (79 of 269) positive provocations (Figure 3); of these, 29 (36.7%) turned positive between 2 hours and 3 days and 37 (46.8%) after 3 days (Figure 4). Only 13 (16.5%) were IRs (Figure 4). Penicillin V provocations were positive in 6.0% (68 of 1,141) (Figure 3) of cases, with 19 (27.9%) turning positive between

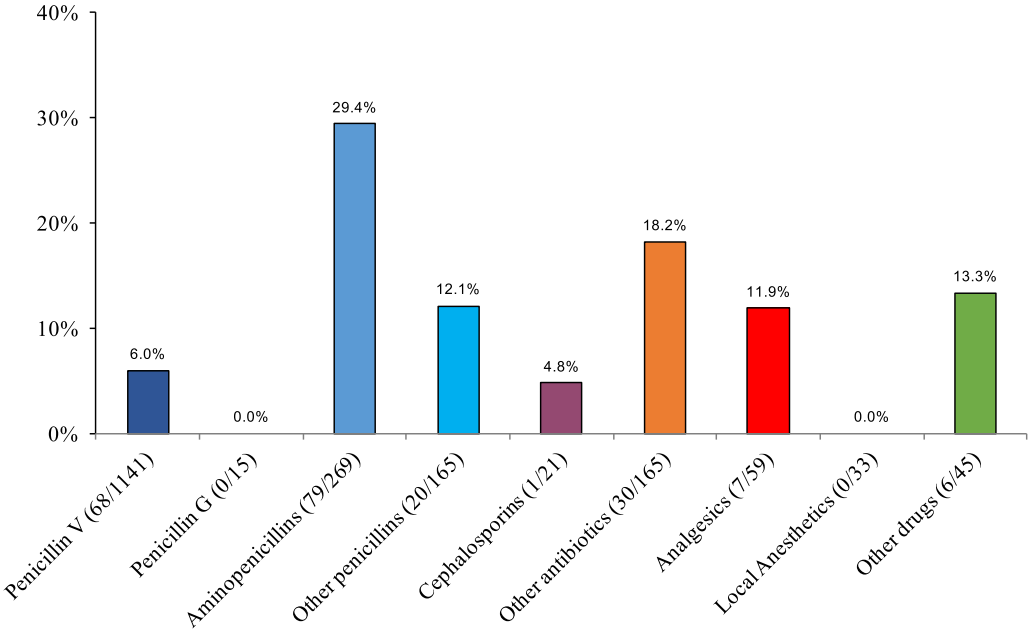

FIGURE 3. Drugs in positive provocation tests (n = 211).

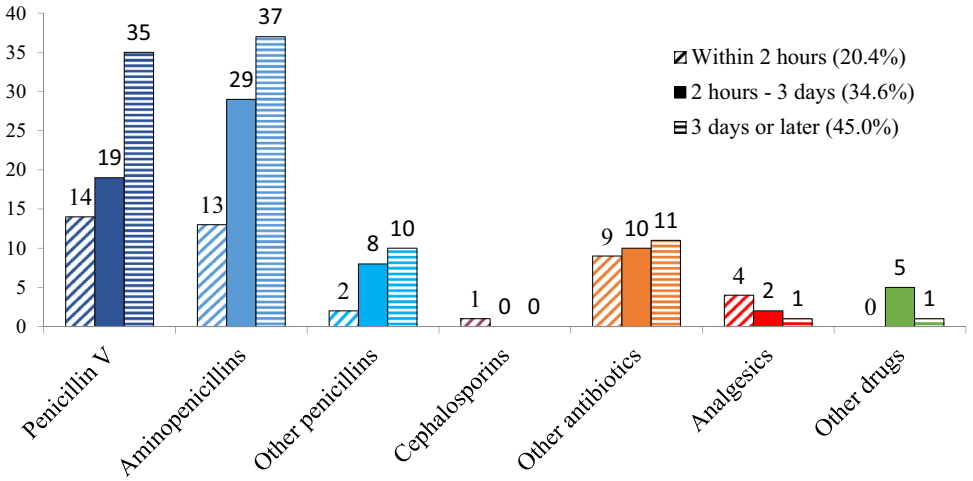

FIGURE 4. Timing of positive tests.

2 hours and 3 days and 35 (51.5%) after 3 days (Figure 4). Only 14 (20.6%) were IRs (Figure 4).

NIRs were also seen for other penicillins: dicloxacillin (16), mecillinam (1), and flucloxacillin (1). Other antibiotics with NIRs were metronidazole (7), macrolides (4), nitrofurantoin (3), trimethoprim (3), clindamycin (2), quinolone (1), and sulphonamide (1). Among other drugs, 6 (2.8%) provocations were positive. All reactions were NIRs, where 2 positive drug provocation test results were with antivirals and 1 each of contrast media, cyclosporine, letrozol, and nystatin.

For all patients, the median time interval between initial reaction and investigation was 6 years (range, 0-67 years). For patients with positive provocations, median time interval was 2 years (range, 0-48 years) versus 9 years (range, 0-67 years) ($P < .0001$) for patients with negative provocation. Repeated reactions with the same drug were reported for 127 patients and only 22 (17.3%) had a positive drug provocation. For symptoms elicited during provocations, see Table I. A total of 98.1% had objective skin symptoms and provocation was considered positive. For IRs, only 1 patient developed circulatory symptoms and was the only patient fulfilling the criteria for anaphylaxis, with severe respiratory and circulatory symptoms about 30 minutes after full dose provocation requiring treatment with adrenaline (Table I). In total, 6 (14.0%) patients had immediate airway symptoms, in addition to skin symptoms. For NIRs, 4 (2.4%) reported minor airway symptoms occurring at home. Three reported minor swelling of throat, tongue, or face, all responding to 1 dose of antihistamine and/or steroids or resolving spontaneously. One patient experienced shortness of breath, which responded to an antihistamine tablet.

TABLE I. Symptoms and treatment reported at initial reaction and at the time of positive provocation

	Negative DPT		Positive DPT			Symptoms at DPT (n = 211)			
	Reported symptoms at initial reaction (n = 1702)		Reported symptoms at initial reaction (n = 211)			IRs (n = 43)		NIRs (n = 168)	
Symptoms and treatment	n	%	n	%	*P* value	n	%	n	%
Symptoms									
Skin	1472	86.5	207	98.1	<.0001	42	97.7	167	99.4
Airway	158	9.3	19	9.0	.895	6	14.0	4	2.4
CVS	62	3.6	3	1.4	.093	1	2.3	0	0.0
CNS	104	6.1	7	3.3	.102	6	14.0	1	0.6
Other	142	8.3	13	6.2	.273	8	18.6	17	10.1
Treatment									
Yes	562	33.0	135	64.0	<.0001	40	93.0	153	91.1
No	631	37.1	45	21.3	<.0001	2	4.7	15	8.9
Unknown	509	29.9	31	14.7	<.0001	1	2.3	0	0.0
Antihistamines only	326	58.0	65	48.1	.038	8	20.0	79	51.6
Steroids only	12	2.1	3	2.2	.950	0	0.0	0	0.0
Antihistamines and steroids combined	188	33.5	59	43.7	.025	28	70.0	70	45.8
Adrenaline	11	2.0	2	1.5	.714	1	2.5	0	0.0
Others*	25	4.4	6	4.4	.998	3	7.5	4	2.6

CVS, Cardiovascular system; CNS, central nervous system; DPT, drug provocation test.
*Others include 1 of the following: bronchodilators, paracetamol, metoclopramide, or topical steroids.

TABLE II. Comparison of the reported delay between drug intake and onset of initial reaction, with delay between drug intake and onset of reaction during drug provocation test (DPT)

Initial reaction	DPT positive IR	DPT positive NIR 2-24 h	DPT positive NIR 2-3 d	DPT positive NIR >3 d	DPT positive NIR after treatment	Total
First dose	**9**	2	3	0	1	15
2-24 h	5	**5**	3	0	1	14
2-3 d	8	10	**9**	9	9	45
>3 d	10	9	17	**29**	10	75
After treatment	5	2	9	5	**23**	44
Unknown	6	2	1	4	5	18
Total	43	30	42	47	49	211
Concordance	20.9%	16.7%	21.4%	61.7%	46.9%	

Bold numbers indicate concordance, which was generally low.

Seven (3.3%) patients experienced symptoms from the central nervous system, mostly dizziness. For the NIRs, 153 of 168 (91.1%) received treatment, with 70 (45.8%) receiving a combination of antihistamines and steroids. No NIRs were associated with significant morbidity.

Data on reported symptoms at the initial reaction in provocation-positive and provocation-negative patients, respectively, are presented in Table I. Skin symptoms were reported by most patients in both groups, but significantly more often in provocation-positive patients (98.1%) versus the negative group (86.5%; $P < .0001$). There were no significant differences in rates of reported airway or circulatory symptoms and rates were low in both groups.

Table I also presents treatment administered at the initial reaction. Significantly more provocation-positive patients required treatment (64.0%) than patients who did not react on the provocation (33.0%; $P < .0001$). Treatment with adrenaline for the initial reaction was reported rarely for both groups, with 1.5% and 2.0%, respectively (nonsignificant).

Generally, information about initial reactions was limited. For 536 (28.0%), the time interval from the first dose to reaction was unavailable. In 218 (11.3%) cases, symptoms were reported to occur on first dose (IRs) and in 1159 (60.6%) as occurring more than 2 hours after the first dose or after subsequent doses (NIRs). Table II compares the reported delay between drug intake and onset of initial reaction with delay between drug intake and onset of reaction during provocation. The bold numbers indicate concordance, which was generally low. Of 43 patients with IRs on provocation, only 9 (20.9%) had reported a first-dose initial reaction. A total of 168 patients had a reaction more than 2 hours after the first dose, of which 6 (3.5%) had reported a first-dose initial reaction. The highest concordance was found for delayed reactions, such as more than 3 days into treatment (61.7%) or after ending treatment (46.9%).

DISCUSSION

This study of 1,913 provocations is the largest study of drug provocations, including prolonged provocations with culprit drugs, reported to date. The most common drug type investigated was antibiotics, with penicillins representing more than 80% of the total number of provocations (Figure 2). Penicillin V was the suspected drug in 60% of the investigations, a high proportion in comparison with studies from other countries,[7,16,20] but similar to findings by another Danish group.[10] The high percentage of narrow-spectrum penicillin is explained by differences in antibiotic use,[22,23,33] where narrow-spectrum preparations are still widely used in Northern Europe, but not in Southern Europe. Other differences are a lower proportion of suspected reactions to macrolides, quinolones, cephalosporins, and NSAIDs in Denmark compared with Southern Europe. These results emphasize the importance of acknowledging differences in drug sensitization patterns in different countries.

Only 11% patients had a drug allergy confirmed, highlighting the importance of drug allergy investigation to ensure that relevant treatment is not avoided unnecessarily. In theory false-negative challenge test results may have occurred because data on renewed allergic reactions after a negative provocation were not collected systematically. However, the re-referral rate for patients with drug allergy in our clinic is very low. Recommendations for drug allergy investigation vary and most guidelines include skin testing.[6,13,34,35] Sensitivity and specificity of skin testing is dependent on variables such as drug type, concentrations used, timing of reaction (IR or NIR), and selection of patients for testing. Positive skin test results contraindicate drug provocation in current guidelines and thus the positive predictive value for skin testing is rarely measured against the "Gold Standard" of drug provocation. One study considered the positive predictive value for skin testing in penicillin allergy to be low, after performing drug provocation test on skin test positive patients.[18] Another Danish study of adults, and an American study of children, has questioned the value of skin testing in the investigation of immediate type penicillin allergy.[10,36] This also has been discussed in delayed-type allergy investigation in a study by Romano et al[20] who found false-negative results in delayed-type allergy investigation. In our center, therefore, drug allergy investigations do not include skin testing, but we screen patients using sIgE for penicillins and carry out provocation only when sIgE cannot be detected. This approach yielded 11% confirmed allergies, which is lower than in studies in which skin testing is included in the investigation protocol and provocation is either not performed or performed only in skin test negative patients.[10,15-17,37,38] However, 2 other studies including skin testing in their investigation protocol find a similar rate of positives to our study.[18,19] Both these studies were conducted outside Northern Europe and differences in antibiotic use, investigation protocols, or genetic factors may have influenced the patterns of drug allergy sensitization.[39] Direct comparison of rates of sensitization between different centers and countries is therefore problematic as long as investigation protocols are not standardized.

In our center we have chosen not to follow commonly used guidelines, which may be seen as controversial and a limitation to our study. In our population, most patients presented with mild initial reactions with skin symptoms only, and less than 10% had cardiovascular and/or respiratory symptoms. Provocation was planned as either titrated or nontitrated depending on the estimated risk of a reaction during provocation and the patient's comorbidity. In the course of 1,913 drug provocations using a 2-hour observation period, only 1 patient had a reaction fulfilling the criteria for anaphylaxis and responded quickly to standard treatment with adrenaline and fluids. Because most drugs in this study were penicillins, the duration of the observation period has not been sufficiently studied for other drug groups such as proton pump inhibitors and NSAIDs and a longer observation period may be needed for these drug groups. No reactions were reported with significant morbidity, but some, especially maculopapular rashes, were described as unpleasant. Four patients reported nonimmediate airway symptoms but all resolved either spontaneously or after a single dose of oral antihistamine and/or prednisolone. About 46% were treated with steroids after a positive prolonged provocation (NIR) usually to treat maculopapular rashes. Steroid use could be interpreted to be a sign of a more severe reaction. However, patients were sent home with antihistamines and steroids after provocation and therefore because of the easy access to treatment, one may assume a lower threshold for starting treatment. This study shows that serious reactions are rare and manageable in a specialized setting and drug provocation with culprit drug in our population of sIgE−negative patients is a valuable and safe method, even without a previous skin test. We hypothesize that not including patients with positive sIgE to penicillin probably reduced the number of potentially serious IgE-mediated IRs. To precisely assess the contribution of sIgE in correctly and safely labeling or de-labeling patients with penicillin allergy, studies comparing results of sIgE, skin tests, and provocation should be performed. Such studies of all 3 test modalities are currently not available.

We included 8 patients with borderline positive sIgE (0.35-0.53 kUA/L) and 7 of 8 were negative on provocation. This questions the specificity of sIgE to penicillins. Patients with higher value sIgEs did not undergo provocation, but were diagnosed with a drug allergy solely on the basis of sIgE results. It cannot be ruled out that some of these patients had false-positive testing on IgE and were therefore mislabeled with an allergy. Sensitivity and specificity of sIgE for penicillins is unknown, but it has been shown for other allergens, for example, chlorhexidine, that borderline values may be false positive.[40,41] sIgE values decline over time and it is possible that some patients with negative sIgE had elevated levels previously and might be at risk of future reactions.[41,42]

Penicillin V was the most commonly investigated drug but only 6.0% (68 of 1,141) of the provocations were positive, primarily during prolonged provocation. It is well known that rashes during childhood might be the result of viral exanthemas[43] and because many patients reported rashes during childhood, it may explain the high proportion of negative provocations with penicillin V. It could be argued that this subpopulation of usually healthy patients with a vague history of a reaction in childhood has a low risk of reacting and could perhaps undergo provocation without previous testing for the presence of sIgE.[14] For the 269 provocations performed with aminopenicillins, a higher proportion of positives (29.4%) was seen, primarily NIRs, and this parallels findings in Southern European studies.[16,20] In our study, about 45% developed skin symptoms (often maculopapular rashes) at least 3 days into

prolonged provocation with aminopenicillins, which confirms findings in the literature.[7,27,44-46]

Most positive provocations (79.6%) in fact occurred after the initial provocation in the clinic. Only 20.4% were IRs occurring on first dose, while 34.6% reactions occurred in the interval more than 2 hours to less than 3 days into the provocation and could be either delayed reactions to the initial provocation dose, or a result of subsequent doses of the prolonged provocation. Even though comparison with pediatric studies might be problematic, in a recent study with single dosing, 29% of the reactions in children appeared after 24 hours and the median time to symptom onset was 12 hours (interquartile range, 5-36 hours).[36] This supports the notion that only very few patients develop delayed reactions to a single dose after 36 hours.[36] In our study, the remaining 45.0% reactions occurred more than 3 days into the prolonged provocation, not likely related to the first dose, and these reactions would have been missed if prolonged provocation had not been performed. Although de novo sensitization to the culprit drug during a prolonged challenge cannot be ruled out, we consider the risk to be small and not very different from the risk of single-dose challenge.

The literature regarding prolonged provocation is currently quite limited.[30,31] Another Danish study by Hjortlund et al[10] found an additional 11.4% positive reactions by including 7-day prolonged provocation with penicillins. On the contrary, Solensky[47] argues that the value of prolonged provocation is limited because of the low rate of diagnosed delayed reactions. In Hjortlund et al's study, drug provocation was preceded by skin testing (both skin prick test and IDT with immediate and delayed reading). It is likely that some NIRs were diagnosed on delayed reading of IDT, explaining the lower rate of NIRs during prolonged provocation in Hjortlund et al's study. The findings in our study imply that at least 45% of patients would not have been diagnosed with a drug allergy if only single-day provocation had been performed. Although most NIRs are short-lived maculopapular rashes, a few patients develop more long-lasting (weeks) reactions requiring treatment with corticosteroids. Identifying these more severe reactions is important but not possible on the history alone. By doing a prolonged provocation these reactions are picked up and the patients can be warned against future exposure. If these patients had only had a single-dose challenge, they might not have reacted during provocation, but symptoms may instead recur while they are receiving a full treatment course during a subsequent illness, which is inconvenient and unpleasant for the patients. We thus recommend that prolonged provocation should be considered an essential part of drug allergy investigation, not exclusively for penicillins but also for other drugs. It is possible that the relatively high rate of NIRs on provocations could be reduced by introducing IDT with delayed reading for penicillins[48] in the investigation protocol and this should be investigated further in our population.

The time interval between initial reaction and drug provocation was significantly shorter in the provocation-positive group (2 vs 9 years, respectively). It could be speculated that time itself increases the chance of a negative drug provocation; that is, clinical reactivity is lost with time. Alternatively, because nonallergic reactions might be less severe, patients with these reactions may delay contact to a specialist for investigation. A short interval between the first reaction and investigation has been mentioned as an important factor for identifying positive sIgE results[14,15,38] but the relevance of IgE levels for the clinical reaction is still uncertain.[41]

A significantly larger proportion of provocation-positive patients required treatment at the initial reaction (64.0%) than among the provocation-negative patients (33.0%). There was a very low rate of reported treatment with adrenaline for the initial reaction in both groups. Interestingly, 11 patients reported treatment with adrenaline in the provocation-negative group, suggesting that the reaction was not anaphylactic, that clinical reactivity was lost, or that investigation was performed with the wrong drug.

Only 20.9% patients with first-dose reactions during drug provocation reported a first-dose reaction at the initial reaction, confirming the mismatch between reported clinical history and drug provocation results (Table II) found in other studies.[10,20] Concordance between reported history and findings on provocation was generally low, but improved for the delayed reactions occurring more than 3 days into prolonged provocation. These mismatches suggest that separate investigation protocols for IRs and NIRs are less useful and that a standardized combined protocol for both reaction types may be a better strategy for drug allergy investigation.

In conclusion, in this large study of 1,913 drug provocations including prolonged provocation with culprit drug, only 11.0% tested positive. The vast majority of provocations were with antibiotics, primarily narrow-spectrum penicillins, reflecting the pattern of antibiotic use in Denmark. Importantly, only 1 of 5 patients tested positive on first dose, indicating that prolonged exposure should always be considered when drug provocation is included in allergy investigations. Drug provocation should always be performed under supervision by specialists and with access to emergency treatment, but this study shows that serious reactions are very rare and thus provocation is a safe and valuable tool for diagnosing drug allergy.

REFERENCES

1. Blumenthal KG, Park MA, Macy EM. Redesigning the allergy module of the electronic health record. Ann Allergy Asthma Immunol 2016;117:126-31.
2. Li M, Krishna MT, Razaq S, Pillay D. A real-time prospective evaluation of clinical pharmaco-economic impact of diagnostic label of "penicillin allergy" in a UK teaching hospital. J Clin Pathol 2014;67:1088-92.
3. Chalabianloo F, Berstad A, Schjøtt J, Riedel B, Irgens Å, Florvaag E. Clinical characteristics of patients with drug hypersensitivity in Norway: a single-centre study. Pharmacoepidemiol Drug Saf 2011;20:506-13.
4. Macy E, Contreras R. Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: a cohort study. J Allergy Clin Immunol 2014;133:790-6.
5. Borch JE, Andersen KE, Borch JE, Al ET. The prevalence of suspected and challenge-verified penicillin allergy in a university hospital population. Basic Clin Pharmacol Toxicol 2006;98:357-62.
6. Demoly P, Adkinson NF, Brockow K, Castells M, Chiriac AM, Greenberger PA, et al. International consensus on drug allergy. Allergy 2014; 69:420-37.
7. Gomes ER, Demoly P. Epidemiology of hypersensitivity drug reactions. Curr Opin Allergy Clin Immunol 2005;5:309-16.
8. Gomes E, Cardoso MF, Praça F, Gomes L, Mariño E, Demoly P. Self-reported drug allergy in a general adult Portuguese population. Clin Exp Allergy 2004; 34:1597-601.
9. Albin S, Agarwal S. Prevalence and characteristics of reported penicillin allergy in an urban outpatient adult population. Allergy Asthma Proc 2014;35:489-94.
10. 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;68:1057-64.
11. Kopač P, Zidarn M, Košnik M. Epidemiology of hypersensitivity reactions to penicillin in Slovenia. Acta Dermatovenerol Alp Pannonica Adriat 2012;21: 65-7.

12. Macy E, Ngor EW. Safely diagnosing clinically significant penicillin allergy using only penicilloyl-poly-lysine, penicillin, and oral amoxicillin. J Allergy Clin Immunol Pract 2013;1:258-63.
13. Weiss ME, Bernstein DI, Blessing-Moore J, Cox L, Lang DM, Nicklas RA, et al. Drug allergy: an updated practice parameter. Ann Allergy Asthma Immunol 2010;105:259-273.e78.
14. Holm A, Mosbech H. Challenge test results in patients with suspected penicillin allergy, but no specific IgE. Allergy Asthma Immunol Res 2011;3:118-22.
15. 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;92:307-12.
16. Bousquet PJ, Pipet A, Bousquet-Rouanet L, Demoly P. Oral challenges are needed in the diagnosis of beta-lactam hypersensitivity. Clin Exp Allergy 2008; 38:185-90.
17. Çelik GE, Karakaya G, Öztürk AB, Gelincik A, Abadoğlu O, Sin A, et al. Drug allergy in tertiary care in Turkey: results of a national survey. The ADAPT study: adult drug allergy perception in Turkey. Allergol Immunopathol (Madr) 2013;42:573-9.
18. 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.
19. Bourke J, Pavlos R, James I, Phillips E. Improving the effectiveness of penicillin allergy de-labeling. J Allergy Clin Immunol Pract 2015;3:365-74.
20. Romano A, Viola M, Mondino C, Pettinato R, Di Fonso M, Papa G, et al. Diagnosing nonimmediate reactions to penicillins by in vivo tests. Int Arch Allergy Immunol 2002;129:169-74.
21. Messaad D, Sahlam H, Benahmed S, Godard P, Bousquet J, Demoly P. Drug provocation tests in patients with a history suggesting an immediate drug hypersensitivity reaction. Ann Intern Med 2004;140:1001-6.
22. Coenen S, Ferech M, Malhotra-Kumar S, Hendrickx E, Suetens C, Goossens H. European Surveillance of Antimicrobial Consumption (ESAC): outpatient penicillin use in Europe. J Antimicrob Chemother 2006;58:418-22.
23. Cars O, Mölstad S, Melander A. Variation in antibiotic use in the European Union. Lancet 2001;357:1851-3.
24. Stevenson D. An epidemic of over diagnosing drug allergies. Allergy Asthma Proc 2014;35:92-4.
25. Magnussen MD, Gudnason T, Jensen US, Frimodt-Møller N, Kristinsson KG. Antibacterial use in the Faroe Islands, Iceland, and Denmark 1999-2011. Scand J Infect Dis 2014;46:502-7.
26. 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;58:961-72.
27. Romano A, Blanca M, Torres MJ, Bircher A, Aberer W, Brockow K, et al. Diagnosis of nonimmediate reactions to beta-lactam antibiotics. Allergy 2004; 59:1153-60.
28. 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;58:854-63.
29. Demoly P, Romano A, Botelho C, Gaeta F, Silva R, Rumi G, et al. Determining the negative predictive value of provocation tests with beta-lactams. Allergy 2010;65:327-32.
30. Ponvert C, Perrin Y, Bados-Albiero A, Le Bourgeois M, 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;22:411-8.
31. Mori F, Cianferoni A, Barni S, Pucci N. Amoxicillin allergy in children: five-day drug provocation test in the diagnosis of nonimmediate reactions. J Allergy Clin Immunol Pract 2015;3:375-380.e1.
32. Demoly P, Kropf R, Pichler WJ, Bircher A. Drug hypersensitivity: questionnaire. Allergy 1999;54:999-1003.
33. Solensky R, Khan DA. Evaluation of antibiotic allergy: the role of skin tests and drug challenges. Curr Allergy Asthma Rep 2014;14:459.
34. Mirakian R, Leech SC, Krishna MT, Richter AG, Huber PA, Farooque S, et al. Management of allergy to penicillins and other beta-lactams. Clin Exp Allergy 2015;45:300-27.
35. Blanca M, Romano A, Torres MJ, Férnandez J, Mayorga C, Rodriguez J, et al. Update on the evaluation of hypersensitivity reactions to betalactams. Allergy 2009;64:183-93.
36. Mill C, Primeau MN, Medoff E, Lejtenyi C, O'Keefe A, Netchiporouk E, et al. Assessing the diagnostic properties of a graded oral provocation challenge for the diagnosis of immediate and nonimmediate reactions to amoxicillin in children. JAMA Pediatr 2016;170:e160033.
37. García Núñez I, Barasona Villarejo MJ, Algaba Mármol MA, Moreno Aguilar C, Guerra Pasadas F. Diagnosis of patients with immediate hypersensitivity to beta-lactams using retest. J Investig Allergol Clin Immunol 2012;22: 41-7.
38. Celik GE, Aydin Ö, Dogu F, Çipe F, Boyvat A, Ikinciogullari A, et al. Diagnosis of immediate hypersensitivity to β-lactam antibiotics can be made safely with current approaches. Int Arch Allergy Immunol 2012;157:311-7.
39. Guéant JL, Romano A, Cornejo-Garcia JA, Oussalah A, Chery C, Blanca-López N, et al. HLA-DRA variants predict penicillin allergy in genome-wide fine-mapping genotyping. J Allergy Clin Immunol 2015;135:253-259.e10.
40. Opstrup MS, Malling HJ, Krøigaard M, Mosbech H, Skov PS, Poulsen LK, et al. Standardized testing with chlorhexidine in perioperative allergy—a large single-centre evaluation. Allergy 2014;69:1390-6.
41. Hjortlund J, Mortz C, Stage T, Skov P, Dahl R, Bindslev-Jensen C. Positive serum specific IgE has a short half-life in patients with penicillin allergy and reversal does not always indicate tolerance. Clin Transl Allergy 2014;4:34.
42. Garvey LH, Krøigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L, et al. IgE-mediated allergy to chlorhexidine. J Allergy Clin Immunol 2007;120: 409-15.
43. Hocqueloux L, Guinard J, Buret J, Causse X, Guigon A. Do penicillins really increase the frequency of a rash when given during Epstein-Barr Virus primary infection? Clin Infect Dis 2013;57:1661-2.
44. Trautmann A, Seitz CS, Stoevesandt J, Kerstan A. Aminopenicillin-associated exanthem: lymphocyte transformation testing revisited. Clin Exp Allergy 2014; 44:1531-8.
45. Torres MJ, Blanca M. The complex clinical picture of β-lactam hypersensitivity: penicillins, cephalosporins, monobactams, carbapenems, and clavams. Med Clin North Am 2010;94:805-20.
46. Bircher AJ, Scherer K. Delayed cutaneous manifestations of drug hypersensitivity. Med Clin North Am 2010;94:711-25.
47. Solensky R. Penicillin allergy as a public health measure. J Allergy Clin Immunol 2014;133:797-8.
48. Barbaud A. Skin testing and patch testing in non-IgE-mediated drug allergy. Curr Allergy Asthma Rep 2014;14:442.