Skin and in vitro tests reduce the need for drug provocation tests in drug hypersensitivity to betalactams

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Abstract

BACKGROUND: Many patients report questionable drug hypersensitivity reactions (DHR) to betalactams. Allergological evaluation is required for objectivation. Recently, some researchers advocated direct drug provocation tests (DPTs) omitting a prior allergy-workup. However, DPTs bare the risk of severe side effects and are a scarce resource in overloaded healthcare-systems. We investigated the value of an approach using only the broadly available methods drug-specific history, specific IgE, and skin tests without DPT. METHODS: We conducted a chart review in a retrospective cohort of 932 patients in an allergy outpatient centre from 2016-2017. Patients had been submitted to drug-specific history and specific IgE-, skin prick-, intradermal- and patch-tests with early and late readings with a series of penicillins and cephalosporins but DPTs were no option. RESULTS: Overall, positive in vitro and/or skin tests were found in 96/932 (10.3%) patients. Drug-specific IgE was detected in 40/932 (4.3%) patients, 61/787 (7.8%) patients had positive skin tests. In vitro tests to Pencillin V showed the highest rate of positivity 24/479 (5.0%) and early readings of ampicillin the highest amongst the skin tests 3/49 (6.1%). Immediate skin tests were more often positive than delayed ones (75:45). The combination of all parameters including drug-specific history solved 346/932 (37.1%) cases while 586/932 (62.9%) remained unresolved. Females and younger children carried a lower risk for positive tests ($p<0.05$, X^2-test). CONCLUSIONS: Testing with betalactams applying simple, cheap, and safe skin and blood tests can solve a third of DHR-cases on a high throughput scale.

Introduction

About 10% of the population report an adverse reaction to "penicillin" (1). While the term "penicillin" in common language is often misused as a representant for the large group of β-lactam antibiotics including cephalosporins (common structure see supplementary Figure 1) or more general as a synonym for "antibiotic", the word "allergy" in this context commonly represents various sorts of drug hypersensitivity reactions (DHR). "Drug allergy" entries appear as red flags in electronic health records of up to 35% of patients upon which "penicillin" is the most frequently mentioned suspected drug (2). However, only 4% of these patients will show positive reactions when tested with penicillin either by skin, blood or provocation tests (3). There are several reasons to explain this high rate of obviously false histories:

1. Although a DHR may have been caused by a drug, the pathophysiological mechanism may not be an immunological one. Most adverse reactions are caused by the specific pharmacological mechanism of the antibiotic and are classified as non-allergic *type A* reactions. Antibiotics not only kill the pathogenic but also – as an obligatory side effect – the essential symbiotic bacteria leading to e.g. gastrointestinal malfunction. Type A reactions comprise more than 80% of all DHR and are no contraindication for a future reintroduction of the suspected drug even without testing (4, 5).

2. A true allergic reaction (also referred as *B-type* DHR(4)) is usually regarded as a contraindication for future use. It may be of the immediate, anaphylactic type I (IgE-mediated), of the delayed type IV allergy (T-cell mediated), and rarely of the type II (IgG mediated) or type III (IgG/IgM mediated). Still, also immunological memory may diminish and even completely disappear over the years (6).
3. Differential diagnoses such as urticaria/angioedema driven by infection for immediate type reactions (7) and viral exanthema for delayed reactions are much more frequent than DHRs (8). Infections can frequently result in a prescription of an antibiotic, which in turn may easily be misinterpreted as being the cause of the DHR.

This situation bears considerable risks not only to the patient, who may be prescribed unnecessary alternative, sometimes less effective antibiotics, but also to society because the overuse of alternative antibiotics can propagate antibiotic resistance (9, 10). Hence, current guidelines on the management on DHRs demand an allergy workup for a "de-labelling" of false histories of DHR in the case of important drugs and β-lactam antibiotics are generally regarded as belonging to such a kind (4, 11).

About how to reach this goal, there seems to be some disagreement on both sides of the Atlantic (12). While Europeans and US experts agree on the importance of skin testing, there are different views on the usefulness of *in vitro* tests (13). Skin tests offer the advantage of giving an immediate result and are cheap for the healthcare system (14). While their sensitivity is limited their specificity is high (15).*In vitro* tests are the safest test for patients. Unfortunately, there are not many marketed, standardized tests except for drug-specific IgE (sIgE) to β-lactam antibiotics including the cephalosporin cefaclor. Measurement of sIgE has a low sensitivity but at a high specificity (16).

In 2018, a basic DHR test without drug provocation tests (DPT) was calculated causing costs of US$ 220 for the US healthcare system. Including DPTs increased the price to at least US$ 359 (17). Nevertheless, in recent times some US experts have gone even one step further and started propagating DPTs for mild reactions even without a prior skin or *in vitro* tests (10, 18-20).

With this study we wanted to take a step back and ask, how many cases of suspected DHRs to β-lactams could be solved by applying simple, broadly available methods causing only limited costs without the resource of performing DPTs. We report the results of a retrospective chart review of a cohort of 932 patients with a history of DHRs to β-lactam from the years 2016 to 2017 from a single centre outpatient clinic. The patients underwent the following algorithm: 1) DHR-specific history, 2) drug-specific IgE test (depending upon availability), 3) a series of skin prick, intradermal and patch tests.

Methods & Patients

Patients

From January 1st, 2016 to December 31st, 2017 a cohort of 48.629 routine patients or sera of routine patients were referred to the Floridsdorf Allergy Center with 91.438 diagnoses (many had more than one referral diagnosis). Of these, 3.875 (8.0%) carried a diagnosis compatible with a history of a DHR. In 1.532 individuals (1085 female / 447 male; 40.6 years ± 22.1) the suspected drug belonged to penicillin and/or cephalosporine antibiotics and a serological test was made. As the laboratory of the allergy clinic also serves as a tertiary referral centre for external serological tests, clinical data was not available for 76 patients that were eliminated leaving 1456 patients for the intention-to-treat analysis (Figure 1). A significant proportion of 523 patients had to be excluded from the per-protocol population because they did not show up for their scheduled skin tests and another single patient stepped down from skin testing on the day of the test (low compliance). This resulted in 932 individuals (669 female / 263 male; 42.5 years old ± 22.1) available for the per-protocol analysis (Table 1 and Figure 1).

Supplementary Figure 2 depicts the standard algorithm, which was a modified approach according to the guideline of the German speaking countries (21) adapted for the needs of our allergy outpatient clinic without

a possibility for performing DPTs. The attending physician could deviate from the algorithm according to individual patient-specific factors. The primary outcome (DHR 'confirmed', or 'possible', or 'unresolved') depended on the interpretation of the summary of all available tests by the attending physician.

Materials & Methods

Specific IgE, total IgE and serum tryptase were measured on an ImmunoCAP 250 laboratory robot with commercially available tests from ThermoFisher (Uppsala, Sweden): Penicilloyl G (c1) & V (c2), Amoxicilloyl (c6), Ampicillin (c5), Minor determinate mixture (MDM) (U233), Cefaclor (c7). Due to production limits of the manufacturer and the high demand at our centre, not all test reagents were available during the whole study period (especially c5, c6, c7 and U233).

Skin prick (SPT), intradermal (IDT) and patch tests (PT) were performed with nationally licensed drugs for intravenous use in nationally recommended concentrations and read accordingly (21, 22): Penicillin G, Amoxicillin/Clavulanic Acid, Cefazolin and Ceftriaxon: "Penicillin G-Natrium Sandoz", "Curam®", "Cefazolin Sandoz", "Ceftriaxon Sandoz", all from Sandoz, Kundl, Austria; Ampicillin/Sulbactam: "Unasyn®", Pfizer, Borgo San Michele, Italy; Cefuroxim: "Cefuroxim MIP", Cephasaar, Sankt Ingbert, Germany). The commonly used penicillin derivatives MDM & PPL for skin tests marketed by Diater, Madrid, Spain are not licensed in Austria and cannot be used in routine settings outside academic hospitals. PTs were performed using Curatest® (Lohman und Rauscher, Vienna, Austria) and read after 24 hours together with the late reading of the IDT.

Generally, skin tests were performed in the following order:

1st) all SPTs at once, when negative after 20 min followed by

2nd) all IDTs at once, when negative after 20 min followed by

3rd) all PTs at once followed by

4th) late readings of all tests after 24 hours.

Ethics and Statistics

The study was approved by the ethics committee of the Medical University of Vienna, Austria, during Christian Ostermayer's medical diploma thesis according to the Helsinki Declaration of Human Rights (ECS 1103/2018). X^2-tests were calculated using MedCalc Statistical Software version 19.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020).

Results

Patients

In 796/932 cases (85.4%) the primary suspected drug was a penicillin, in 136 (14.6%) a cephalosporin. As observed in many allergy studies before, the sex ratio was balanced in children of up to 10 years, while the rate of female patients steadily increased among the teenagers to finally increase to a ratio of 3 females per 1 male in the adults through all older age groups (Table 1).

A DHR could already be excluded by history in 135/932 patients (14.5%) before even entering the allergy workup. In most cases, the patients had already unintentionally but safely taken a β-lactam again e.g. in the form of a generic drug under a different brand name.

Two patients had a history of severe cutaneous adverse reactions (SJS/TEN) of whom one was patch tested negatively; patients with a clear history for an immediate DHR (< 1h after drug intake): 243/932 (26.1%); for a delayed DHR (> 24h after drug intake) 365/932 (39.2%).

Specific IgE

Drug-specific IgE was determined in all study patients and 40/932 (4.3%) had at least one positive test above the standard cut off threshold level [?]0.35kU/l (Table 2). In more detail, there were 83/2227 (3.7%) positive sIgE tests. Surprisingly, Pencillin V and not Amoxicillin was the most often positive drug (24/479=5.0%; Table 2 and Figure 2a). Measuring sIgE to minor determinants mixture MDM or Cefaclor added little value in the diagnosis of type I allergy to β-lactams.

It is known that the specificity of sIgE to penicillin decreases with higher total IgE levels while sensitivity decreases with very low total IgE levels (23, 24). The calculation of the ratio drug-specific IgE/total IgE had been introduced to overcome the problem with high total IgE. Vultaggio et al. published a threshold of > 0.0022 with a sensitivity of 43%, a specificity of 95%, and a positive predictive value of 93% for the β-lactam ImmunoCAP® assays (25). We found elevated total IgE of [?]100kU/l in 244/917 (26.6%) sera. Hence, we also calculated sIgE/total IgE ratios. Applying the hypothetical 0.0022 cut off would have largely increased the positivity rates to Amoxicilloyl (from 2.8% to 16.3%), Penicilloyl V (from 5.0% to 11.7%), Ampicillin (from 4.0% to 7.5%) and Cefaclor (1.0% to 6.3%) but not to Penicilloyl G and MDM (grey columns in Supplementary Figure 3).

Another published recommendation is lowering the cut off from [?]0.35kU/l to [?]0.1kU/l (16) to expand the sensitivity of sIgE especially in patients with low total IgE levels. This lower threshold level would double the overall positivity rates from 83/2227 (3.7%) to 171/2227 (7.8%), double all penicillins and septuple cefaclor but leave MDM sensitivity unchanged (Table 2 and purple columns in Supplementary Figure 3). For the further per-protocol-analysis we decided to exclude both possibilities to maintain comparability with previous studies.

Interestingly, 7/365 (1.9%) of the patients with a history suggestive for a delayed type-response had drug-specific IgE suggesting rather an immediate than a delayed mechanism. Considering, that there were only 40 patients with a positive sIgE test, the history concerning the timing and type of DHR was not reliable in 17.5% (7/40), which can have consequences when assigning safety precautions during DPTs.

Skin tests

Skin tests were positive in 61/787 (7.8%) patients. IDTs were the most sensitive tests for both immediate (= blue) and delayed reactions (red) (Table 2 and Figure 2c). Contrary to the *in vitro* tests, Aminopenicillins were more often positive than Penicillin G in early and late readings. This was true for SPT and IDT (Figure 2b and 2c). The relatively highest rates of positive SPTs were observed with cephalosporins (Figure 2b). In our algorithm, a positive SPT in the immediate reading abrogated a continuation with IDTs (see Methods). That is why cephalosporins had lower rates of positive IDTs than aminopenicillins (Figure 2c).

Overall, Ampicillin had the highest percentage of positive IDT reactions (Figure 2c). This reflects the recent trend to turn away from prescribing Amoxicillin/Clavulanic acid back to the stronger allergen Ampicillin/Sulbactam due to the high liver-toxicity (26).

PT showed low additive value to late readings of IDTs. There were only two cases, each with a history of maculopapular rash, where the PT rendered positive with an at the same time negative late reading of the IDT.

Safety

Safety is always a matter of concern when doing skin tests in patients with a history of DHR. In 5050 skin test (1718 SPT, 1697 IDT, 1635 PT) we did not observe a single systemic reaction in any patient.

Cross-reactivity of type I responses

Cross-reactivity within β-lactam antibiotics is caused by structural similarities (Supplementary Figure 1) (22). That is why many patients had more than one positive test. The 83 positive *in vitro* tests represented only 40 patients and the 120 positive skin tests only 61 patients.

There were only 2 patients with concomitant positive reactions to penicillins and cephalosporins and that is why we could not investigate them thoroughly (sIgE to Amoxicillin & immediate IDT to Cefuroxim; positive immediate IDT to Amoxillin & Cefuroxim).

The amount of solved cases

The primary endpoint of this study consisted in the number of solved cases without performing a DPT: Of the 932 patients in the per-protocol-analysis, 135 (14.5%) had not been submitted to tests because the DHR had already been excluded by history (Figure 3). In another 115 patients (12.3%), a DHR could be ruled out because a very improbable history was further underlined by negative *in vitro*and *in vivo* tests. Drug allergy was confirmed by positive*in vitro* and/or *in vivo* test in 96 (10.3%) of the patients and the patients received an allergy passport, which is the usual way of handling this situation in central European countries (27). Summing up, 346 (37.1%) cases were solved while 586 (62.9%) remained unresolved. Histories of DHR to cephalosporin were more often solved than the ones to penicillin (54% vs 34% Figure 3).

A successful diagnostic procedure depended a lot on the patients' compliance. Although we tried to make the workup as convenient as possible, DHR cases could usually not be resolved at the first visit by obtaining the drug-specific history only. The intention-to-treat population also included the patients not showing up for their scheduled skin tests (Figure 1). Including these patients markedly increased the numbers of unsolved cases from 62.9% to 76.4% (Penicillin 65.7% to 78.5% and Cephalosporin from 46.3% to 62.2%).

Unsolved cases were referred to 4 different hospital-based dermatological and 2 paediatric departments in Vienna that perform DPTs. However, due to their limited capacities (see Discussion), only some patients ended up in a DPT (personal communication with the aforementioned departments). Very strict national data protection laws inhibited a structured follow up of these routine patients after leaving our allergy centre. Only a single 52-year-old woman returned to us reporting about her negative DPT, why we also included her into the 'solved negative' category.

Risk Factors for positive reactions

Finally, we looked for risk factors for a confirmation or disapproval of the suspected DHR (Figure 4). For this analysis, we only considered patients where the algorithm resulted in a clear yes/no situation (according to Figure 3). Of the several possible risk factors, we identified two that were significant:

1. Male (40%) vs. female sex (29.5%) ($p = 0.0012$, $\mathbf{X^2}$ test). This was remarkable as the number of female patients with a referral history for DHR was 2.54 times higher than the one of males (Table 1).

2. Age >10: Younger children carried a lower risk for a confirmation of the DHR, than the older study population <10 years ($p = 0.0411$, $\mathbf{X^2}$-test).

All other risk factors shown in Figure 4 such as a parental history for a DHR in childhood, an underlying atopy (defined as a positive allergy test to inhalative or nutritive allergens, a history of atopic dermatitis, allergic rhinoconjunctivitis or bronchial asthma), underlying chronic urticaria, a history suggestive of an

immediate or a delayed reaction pattern of the DHR did not differ significantly between confirmed and disapproved cases.

In a previous study, we had reported elevated serum tryptase levels as a risk factor for severe DHR (28). In contrast, in the present study elevated serum tryptase of [?]11.4 ng/ml was only detectable in 17/447 (3.8%) and only one of these patients had a confirmed reaction.

Discussion

Recently, the voices in the allergy community have been growing louder favouring a turn away from the classical allergological approach including *in vitro* plus skin tests putting DPT at the end as the final method and instead heading to a direct DPT without prior testing. This has been propagated especially in the United States (13, 18, 19). With our study, we would like to stress the point, that aiming for such an extreme standpoint puts patients at unnecessary risks while roughly a third of DHR cases can be solved with a safer and cheaper approach.

It may be argued that looking at sIgE and skin tests separately results in only low positivity rates and that these tests may be regarded as dispensable, therefore. Rightly, the low rate of 4.3% drug-specific IgE to β-lactams on the ImmunoCAP® system nearly replicated the 3.4% that we had already described 14 years ago (29). However, these tests are cheap and can be applied on a large patient group (14). Tweaking read out parameters such as lowering the threshold to 0.1 kU/l could have doubled or calculating sIgE/total IgE ratios could have even quadrupled the positivity rates in our patients, but this would have come at the price of a lowered specificity (25). Basophil activation tests are reported as having superior sensitivity and specificity (30, 31). However, they are more expensive, require especially trained personal, expensive laboratory equipment and consume a lot of laboratory time. This makes them a difficult system for use on a broader routine dimension.

Positive skin tests in our study occurred nearly in twice as many patients (7.8%) than drug-sIgE (4.3%). Generally, skin tests tend to be more sensitive than blood tests at an also high specificity with good negative predictive value (32). Our study underlines the role of skin testing and we performed them successfully even in 98 children [?]10 years. However, also their specificity had been challenged (33) while later studies confirmed the high specificity of IDTs especially to cephalosporins (34). IDTs are the most useful skin tests with early and late readings. They cause a little bit of pain, which is usually tolerated by all patients, and they are safe as we experienced no systemic reaction in 1697 IDTs. When a late reading of the IDT is available, PTs added only little additional value and might be omitted in the routine setting. Still, PTs to β-lactams may have a role in the history of severe cutaneous adverse reactions or in other situations, where IDTs are impossible due to patient-specific factors.

Interestingly, the higher rate of confirmed reactions to cephalosporins was just based on the immediate reaction pattern. This had already been described by Romano et al. who confirmed a lot of immediate but hardly any delayed type reactions by skin testing (35).

DPTs are the golden standard to rule out or confirm a DHR (36, 37). This is especially true for the non-immunologically mediated reactions to non-steroidal anti-inflammatory drugs (38). While representing the gold-standard, they are not perfect tests, as numerous studies described false-positive reactions even upon placebo tests (29, 39) and false-negative tests (40). DPTs put patients at risks, are an expensive and resource-consuming measure and are a limited resource, even in well-developed health care systems. In Austria, one of the top-ten countries concerning access to healthcare (41), there is currently no reimbursement scheme for DPTs neither by public nor private healthcare insurances. Because of this lacking financial incentive for hospitals and outpatient facilities, Austria is faced with scarce resources for performing DPTs. The metropolitan area of the Austrian capital city Vienna counts around 3 million inhabitants. Four dermatological and two paediatric wards are the only institutions offering DPTs with a long waiting list. In a personal communication with these institutions, the yearly capacity turns out to be at an astonishingly

low 465 DPTs / year. The majority of these (300) are offered by the department of dermatology at the Medical University of Vienna. In an own reference study at this institution (29), only 130/291 (44.7%) DHR evaluations were reserved for antibiotics, the rest for other drugs e.g. NSAIDs. Assuming, that this ratio has not changed much over time, the yearly capacity for performing DPTs with antibiotics in greater Vienna can be estimated at around 207/year. Of the 932 patients in our 2-year study, 37.1% of the cases were solved by the application of the diagnostic algorithm with a clear yes/no outcome. While these 346 individuals already received an allergy passport (27) or the instruction that they can safely re-introduce β-lactams in their antibiotic regimen, the majority of 62.9% cases remained unsolved with an ongoing need for DPTs. These 586 patients/2 years (=269/year) of our single allergy outpatient clinic alone, would have greatly overwhelmed all DPT capacities of Eastern Austria. To up the ante, there are 4 additional Viennese allergy outpatient clinics and many more specialists for dermatology and paediatrics who see additional cases. Hence, the full conventional allergy workup of an allergy outpatient clinic can help to reduce the pressure on underpowered DPT capacities.

Conclusions

We believe that the combination of easy and cheap methods, each of limited sensitivity in a conventional DHR workup as recommended by many international and national guidelines (4, 22) is still required for an efficient management of unevaluated DHRs to β-lactam antibiotics. Relying on DPTs alone would simply overstrain most healthcare systems.

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Figures & Tables

Table 1

Study patients as defined by the per-protocol analysis in Figure 1. Age was defined as the age at the 1st presentation in the allergy clinic.

Table 2

Overview of all test results in absolute and relative numbers: sIgE = specific IgE (positive/negative). Results of specific IgE ([?]0.35 kU/l) is displayed in the first column. Prick and intradermal tests: 20 min / 24 hours / all tests. Patch tests 24 hours / all. The highest value of each category is in underline format. (blue for immediate allergy, red for delayed allergy)

Figure 1

Flow diagram and definition of the patient populations.

Figure 2

Percentage of positive tests in relation to all tests: Positive tests for specific IgE (2a) and early readings in skin prick tests (2b) and intradermal tests (2c) were suggestive for immediate type I allergy (blue). Late readings (red) in skin prick tests (2b), intradermal tests (2c) and patch tests (2d) after 24 hours were suggestive for delayed type IV reactions.

Figure 3

As the primary endpoint of this study, it was possible to solve 37.1% of the cases (confirmed in green and disapproved in red) by exploiting all possibilities of the algorithm depicted in Figure 1. Penicillin and Cephalosporin differed as only 34% of the Penicillin but nearly half of the Cephalosporin cases could be solved.

Figure 4

Risk factor analysis for a confirmation (red) of the drug hypersensitivity reaction (DHR) versus its' disapproval (green). Men and younger children had a significantly higher risk for a confirmed DHR than women and teenagers/adults. This analysis included only the 346 solved cases according to Figure 1 & 3. Significance was calculated using a X^2-test.

Supplementary Figure 1

Cross-reactivity in penicillins and cephalosporins is caused by similarities in the chemical structure of the drugs, which share their common β-lactam ring structure (light pink). That is why they are known as "β-lactam" antibiotics. The thiazolidine ring of penicillins (light green) differentiates them from the dihydrothiazin ring of cephalosporins (light grey). Cefaclor and ampicillin share their acyl side chain, and the one of amoxicillin is also rather similar (light blue). Pictures were drawn according to Blumenthal et al. (42) and Zagursky and Pichichero (43).

Supplementary Figure 2

Algorithm for the workup of the study patients. Each individual physician could deviate from this procedure according to the patient's individual needs. Drug provocation tests were not part of this study.

Supplementary Figure 3

The hypothetical lowering of the standard threshold for drug-specific IgE from [?]0.35 kU/l (blue) to [?]0.1 kU/l (purple) is recommended by some guidelines to increase the weak sensitivity in patients with low total IgE (16). Another hypothetical indirect measure in patients with a high total IgE is to determine the sIgE / total IgE ratio. Vultaggio et al. published a cut off of > 0.0022 for β-lactam antibiotics (grey) (25).

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Figure 1