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Individual risk assessment in the diagnosis of immediate type drug hypersensitivity reactions to betalactam and non-betalactam antibiotics using basophil activation test: a single center experience.

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ABSTRACT

Background: Drug hypersensitivity reactions of immediate type pose a challenging problem, especially, if standard diagnostic procedures do not lead to conclusive results. The aim of this investigation is to identify, whether basophil activation test (BAT) is able to provide additional benefit in the diagnostic evaluation of immediate type drug hypersensitivity reactions to antibiotics in comparison to the routine allergological diagnostic methods.

Materials and methods: We investigated patients, which presented to the Department of Dermatology and Allergology of the University Hospital of RWTH Aachen in Germany for diagnostic workup of type I allergic reactions to antibiotics during the period from 2009 to 2012. The analysis was performed retrospectively based on patient records. The inclusion criteria were performed standard allergological *in vivo* diagnostic and a basophil activation test (BAT) as a part of diagnostic workup.

Results: 82 diagnostic investigations were performed in 52 patients. BAT was positive in 9 of 12 cases with a positive clinical history but negative skin test results. Furthermore, all patients who reported severe drug hypersensitivity reactions (anaphylactic reaction grade 2 and above) showed positive BAT (5/5), while only 3 of these 5 cases demonstrated a positive skin testing that led to the conclusion of possible immediate type drug hypersensitivity.

Conclusions: Although skin tests remain the most important part of the primary diagnostic investigation, BAT is an additional valuable and sensitive *in vitro* test in the diagnostic procedure of immediate type allergic reactions to antibiotics. However, further standardized investigations are needed in order to calculate exact sensitivity and specificity of this diagnostic tool in both, adult and pediatric populations.

INTRODUCTION

Anaphylactic reactions are potentially life-threatening. Drugs are the second most common cause for anaphylactic reactions in adults according to the anaphylaxis register of the German speaking countries [1]. Antibiotics, including betalactam antibiotics, are considered to be one of the most frequently involved drugs in type-I hypersensitivity reactions [2-4].

Allergy diagnosis in case of an immediate reaction is usually based on detailed medical history, skin testing, drug provocation tests as well as *in vitro* diagnostic tests, such as measurement of specific IgE-antibodies, if available. However, in some cases these diagnostic tools may not lead to conclusive results. This diagnostic gap can possibly be filled by the basophil activation test (BAT), which is based on flow cytometric quantification of allergen-induced CD63 or CD203 expression. In addition, this test is an *in vitro* investigation, which does not constitute a risk (e.g. for a new reaction) for the patient. Specific IgE-diagnostic is validated just for a few antibiotics, whereas in BAT several other drugs can be used.

In the past two decades, a number of research articles on the use of BAT in the diagnosis of immediate type drug hypersensitivity reactions has been published [5-10]. However, it has not yet become part of standard diagnostic investigations. The advantage of BAT is its sensitivity (up to 50%) and specificity (up to 97%) as well as its non-invasiveness [5-10]. Therefore, BAT may be used especially in cases when *in vivo* diagnostic procedures like skin testing are not of diagnostic value (e.g. because of urticaria factitia) and drug provocation testing is considered to be too risky due to the severity of previous reaction or other patient specific conditions. Although the BAT provides fast results, it requires special conditions in the preparation as well as in the storage of the blood samples to ensure viability of the blood cells. Such conditions require a high level of effort and are generally given only in well established, specialized laboratories. Nevertheless, despite its complexity, BAT seems to be a useful diagnostic tool in IgE-mediated hypersensitivity reactions.

The aim of this investigation is to identify, whether BAT is able to provide additional benefit in the diagnosis of immediate type drug hypersensitivity reactions to betalactam and non-betalactam antibiotics in comparison to the standard allergy diagnostic methods, especially in assessment of the individual risk of the patient to experience another hypersensitivity reaction on re-exposure to the drug.

METHODS

Study design:

A retrospective cohort study was performed at the University Hospital of RWTH Aachen in Aachen, Germany, from 2009 to 2012 with the agreement of the local ethics committee (Ethics committee of the University Hospital of RWTH Aachen in Aachen, Germany, intern file number: EK 041/17).

Patients:

We investigated the patients, which presented to our department (Department of Dermatology and Allergology of the University Hospital of RWTH Aachen in Germany) for diagnostic workup of the type-I allergic reaction to betalactam and non-betalactam antibiotics during the period from 2009 to 2012. The inclusion criteria were suspicion of immediate type allergic sensitization to antibiotics based on clinical history, documented skin tests (e.g. skin prick test, intradermal test) and documented basophil activation tests (BAT) as a part of diagnostic workup.

We included patients with following symptoms after the intake of antibiotics: urticaria, angioedema, urticaria and angioedema, anaphylactic reaction, circulatory dysregulation or vomiting. Anaphylactic reaction was defined as described by European Academy of Allergy and Clinical Immunology (EAACI) Taskforce on Anaphylaxis [11].

Diagnostic workup:

After the accurate history taking, available skin tests were performed according to the International Consensus on Drug Allergy [12]. Skin prick test represented the first step in the diagnostic procedure, followed by intradermal test, if skin prick test was negative. As a next step, a patch test with suspected antibiotics was performed, especially if inaccuracies in the patients' history regarding the type of sensitization occurred. In children and adolescents younger than 17 years of age only skin prick test was regularly performed, but not intradermal and patch tests. After performing these *in vivo* tests, *in vitro* investigations were carried out in order to gain more conclusive information: Specific IgE for the culprit drugs, if available, was measured and also BAT was added to the diagnostic procedure (Figure 1A and 1B).

Skin prick test and intradermal test:

The skin prick and intradermal tests were performed at the volar side of the forearms. The procedure was carried out following the recommendations of the ENDA/EAACI Drug Allergy Interest Group position paper [13, 14]. The drug dilutions for the skin testing were prepared on the same day the tests were performed. Histamine dihydrochloride (10 mg/ml) served as a positive control and normal saline as a negative control. The skin reactions were read after 15-20 minutes. A wheal of at least 3 mm with a negative response to the control saline in the skin prick test was considered as positive. An increase in diameter of at least 5 mm in the intradermal test was considered as positive. The concentrations of

each tested drug are presented in the table 1. In case of a positive skin prick test an intradermal test with the drug was not performed. In children and adolescents younger than 17 years of age only skin prick test was regularly performed, but not intradermal test.

Patch test:

In addition, a patch test with suspected antibiotics was performed in order to increase the sensitivity and specificity of the investigation, especially if inaccuracies in the patients' history regarding the type of sensitization occurred or if SPT or IDT didn't lead to conclusive results e.g. due to urticaria factitia. Patch tests were done as the next diagnostic step after performed SPT and IDT. They were performed on the upper back of the patients on unaffected, untreated and uncleaned skin using Finn Chambers which were fixed with a hypoallergenic tape for 48h. The results were read after 48, 72 and 168 hours. The patients were investigated for erythema, infiltration, papules and vesicles on the exposed skin. The investigated results were scored according to the national guideline [14] and the recommendations of the European Environmental and Contact Dermatitis Research Group [15, 16]. The used drug concentrations are shown in the table 1. In children and adolescents younger than 17 years of age patch test was not regularly performed.

Specific IgE:

The investigation for specific IgE (sIgE) from patients' sera was performed for penicilloyl G and V, ampicilloyl, amoxicilloyl and cefaclor using ImmunoCAP™ 250 technology (Phadia). Also, a total IgE count was measured. The result was expressed using ImmunoCAP classes 0 to 6 and kU/l (< 0.35 kU/l for class 0; 0.35 – 0.70 kU/l for class 1; 0.70 – 3.5 kU/l for class 2; 3.5 – 17.5 kU/ for class 3; 17.5 – 50.0 kU/ for class 4; 50.0 – 100.0 kU/l for class 5 and > 100.0 kU/l for class 6) and the investigation was considered to be positive from class 1 (> 0.35 kU/l).

Basophil activation test:

50 µl of allergen (betalactam or non-betalactam antibiotics, all from BÜHLMANN LABORATORIES AG, Schönenbuch, Switzerland) was used in different concentrations as reflected in the table 2. Concentrations higher than those described below are cytotoxic and lower concentrations decrease sensitivity. To each tube 100 µl of stimulation buffer and 50 µl of K-EDTA (Potassium ethylenediaminetetraacetic acid) diluted whole blood were added. After mixing gently every tube was supplied with 20 µl of staining reagent (a mixture of monoclonal antibodies to human CD63 labeled with fluorescein isothiocyanate (anti-CD63-FITC) and to human chemokine receptor CCR3 labeled with phycoerythrin (Anti-CCR3-PE) all from BÜHLMANN LABORATORIES AG, Schönenbuch, Switzerland). The samples were incubated for 15 minutes at 37°C in a water bath. After this 2 ml of prewarmed lysing solution was added and incubated for 10 minutes at room temperature in order to remove the erythrocytes by a lysing reaction. After centrifugation for 5 minutes at 500 x g the cells were resuspended in 300 µl of wash buffer and then analyzed in a BD FACScan™ flow cytometer

using Flow CAST® by acquiring at least 500 to 600 basophilic cells per sample. For the negative control (the patient's individual background) 50 µl of stimulation buffer was used, furthermore two positive controls were performed with highly specific monoclonal antibody to the high affinity IgE binding receptor (Anti-FcεRI mAb) or the unspecific cell activator N-formyl-L-methionyl-L-leucyl-phenylalanine (fMLP) 50 µl (BÜHLMANN LABORATORIES AG, Schönenbuch, Switzerland) each.

Results were expressed as the percentage of CD63 and as the stimulation index (SI), calculated as the ratio between the percentage of degranulated basophils (CD63⁺ cells) with different allergens and the negative control. The BAT result was considered to be positive if the SI was ≥ 3 .

Drug provocation test:

We performed the drug provocation test (DPT) as a single blind placebo-controlled testing with the culprit drug, if there was no evidence of allergic sensitization in performed skin tests and *in vitro* tests or with an alternative drug, if performed skin tests or *in vitro* tests lead to conclusion of a possible allergic sensitization to the culprit drug. As example, a DPT with a cephalosporin was performed, if allergic type I sensitization to penicillin was confirmed by skin tests or *in vitro* diagnostic tools, in order to exclude cases of cross-reactivity. All DPTs were carried out orally over the two days with subsequent monitoring of the patients over the following nights. During one of the days the patients were exposed to placebo three times at intervals of 60 minutes each and during the drug provocation itself to the culprit drug in the increasing doses of 1:100, 1:10 and 1:1 of the therapeutic dose in the same time setting. Each following dose was administrated if there was no positive reaction to the previous one. In case of a positive reaction to one of the doses testing was stopped and the symptoms were treated if necessary. The therapeutic dose for each substance is presented in the table 3.

The prerequisite for the DPT was the informed consent of the patient (or patient's both parents) to undergo this diagnostic investigation and a signed agreement form. A part of the patients refused DPT, although it was recommended by a physician.

RESULTS

Demographic data:

In 52 patients that underwent medical examination for immediate type drug hypersensitivity reactions to betalactam and non-betalactam antibiotics using basophil activation test during the period from 2009 to 2012. 82 diagnostic investigations that met including criteria were performed. In 21 persons more than one antibiotic was tested based on medical history.

The study population included 19 male and 33 female individuals with a median age of 18years (ranging from 2 to 70 years). 24 patients were children and adolescents younger than 17 years of age, in this age group 31 diagnostic investigations were performed.

Clinical history:

The most common clinical symptom that led to the allergological diagnostic procedure was urticaria (55% of cases) or urticaria and angioedema (22%). Severe anaphylactic reactions (grade 2 and above) were rare, they occurred only in 5 cases (6%). An overview of the clinical presentation is presented in the figure 2.

Skin testing:

In altogether 82 performed diagnostic workups 77 skin prick tests were performed. In the remaining 5 cases the evaluation of the skin prick test result was not possible due to urticaria factitia. From these performed SPTs 10 (including 4 children and adolescents younger than 17 years of age) were positive, therefore no intradermal test was performed in these cases. From 67 cases with negative SPT results 38 intradermal tests were performed. In the remaining 25 children and adolescents younger than 17 years of age no IDT was performed on regular basis. Also in 4 more patients no IDT with substances cotrimoxazol (patient Nr. 4), azithromycin (patient Nr. 27) and roxithromycin (patients Nr. 15 and 28) was performed, as no recommendation for appropriate intradermal testing concentration was found in the literature. In 2 children IDT was performed in order to increase sensitivity of the skin testing, as there was a strong suspicion of a possible sensitization to the culprit drugs. In these cases, however, we were not able to gain additional information using IDT. IDT showed positive results in 9/38 (23.7%) cases. In 49 cases also a path test was performed, which was positive in 2 cases (patients Nr. 20 and 32) suggesting a possible delayed type drug hypersensitivity reaction, especially in absence of positive SPT and IDT results (Tables 4 and 5).

In vitro testing.

From the 82 diagnostic workups 62 were performed with betalactam antibiotics (15 with penicillin, 20 with aminopenicillins and 27 with cephalosporins) and 20 with non-betalactam antibiotics (7 with fluoroquinolones, 6 with macrolides and 7 others). From all 62 diagnostic investigations performed with betalactam antibiotics specific IgE was measured in 51 cases and revealed positive results in 4 cases, from which in 3 investigations this was the only positive test result (patients Nr. 14, 25 and 26).

From the overall of 82 performed investigations 48 (58.5%) were negative using both skin tests as well as *in vitro* tests (specific IgE, if available, and BAT). From these cases placebo controlled drug provocation tests were performed in 10 (21%) to the culprit drug and in 8 (17%) with an alternative drug from the same group (Table 4) and were well tolerated. Only in 1/48 (2%) case a placebo controlled drug provocation test was performed with an alternative drug from the same group and led to the objectified allergic symptoms (Figure 3, Table 5).

From the remaining 34 (41.5%) investigations 17 (50%) showed positive BAT-results. From these 17 BAT-positive cases 8 (47%) were also positive in one skin tests. Interestingly, BAT was positive in 9 (75%) of 12 cases with a positive clinical history but negative skin test results (Figure 3, Table 5).

From all 17 positive BAT results 12 (70.6%) were performed with betalactam antibiotics and also 12 (70.6%) were performed not longer than 12 months after the reported allergic reaction. The 5 non-betalactam antibiotics with positive BAT-results were cotrimoxazol (patient Nr. 4), ciprofloxacin and roxithromycin (patient Nr. 15), moxifloxacin (patient Nr. 20) and erythromycin (patient Nr. 46). Both fluoroquinolones showed also positive skin test results, but both macrolide antibiotics were positive only in BAT (Table 5).

13/34 (38%) cases showed positive results in at least one of the skin investigations, but negative BAT-results and 3 (9%) cases positive specific IgE antibodies, but no other positive test results. In one case (3%) skin test as well as specific IgE antibodies were positive, but BAT was unremarkable (Figure 3, Table 5).

In all 5 cases of severe anaphylactic reactions (grade 2 and above) BAT was positive, from which in 3 (60%) cases the workup was also positive in one of the skin tests.

However, there was also a false positive BAT result in our data. Patient Nr. 20 was investigated for possible drug hypersensitivity to penicillin and had negative SPT and IDT results and also negative result of sIgE investigation. In the performed patch test a positive reaction was seen. In addition BAT with penicillin was positive. Because of conflicting results (positive patch test a clue for delayed type reaction, but also positive BAT result) we performed BAT for alternative substance cefuroxim, which also was positive, however SPT, IDT and patch test for cefuroxim were negative and we had no clinical history for allergic sensitization to cefuroxim. Thus, DPT with cefuroxim was performed and

was well tolerated. In this case we considered the risk for severe allergic reaction on exposure to cefuroxim as low. The previous drug hypersensitivity reaction after intake of penicillin was limited by cutaneous symptoms. We assume in this case a false positive BAT result.

Interval from the drug hypersensitivity reaction to the diagnostic procedure:

We compared the results of positive diagnostic investigations regarding to the period of time from the drug hypersensitivity reaction to the diagnostic workup. Three big groups were observed. The group with positive BAT result alone (9 patients), the group with the positive skin test as the only positive investigation (13 patients) and the group with positive results of both skin test and BAT (8 patients). The remaining five patients with positive results of diagnostic workup (3 patients with positive sIgE results, 1 patient with positive DPT and 1 patient with positive results of both sIgE and skin test) were not compared to the other groups due to the small sample size. If the time interval from the drug hypersensitivity reaction till the diagnostic investigation couldn't be reported by the patient the result was excluded from the evaluation. 4 results were excluded (1 in the group with positive both BAT and skin test and 3 in the group with positive skin test only).

9 patients showed positive results in the BAT alone and were negative in further testing (SPT, IDT, etc.). In these cases 6/9 (66.7%) tests were performed with betalactam antibiotics. In 2/9 (22.2%) cases SPT and IDT results couldn't be evaluated due to urticaria factitia, in 6/9 (66.7%) patients only SPT was performed as 4/6 were children and IDT was not regularly performed in children in our setting and 2/6 patients were tested for roxithromycin or cotrimoxazol and we didn't performed IDT with these substances because of lacking strong recommendations for the appropriate concentration in IDT. 1/9 (11.1%) patient underwent SPT, IDT and patch test with negative results of these investigations. The median time from the reported drug hypersensitivity reaction to the diagnostic workup was 6 months (ranging from 2 to 41 months).

8 patients were investigated to be positive in both BAT and skin test. From these cases 6/8 (75%) were tested with for betalactam antibiotics. 5/8 (62.5%) of these investigations were positive in SPT, 2/8 (25%) showed positive IDT and 1/8 (12.5%) was positive in a patch test. The median time from the reported drug hypersensitivity reaction to the diagnostic workup was also 6 months (ranging from 4 to 72 months).

13 patients showed positive skin test during the diagnostic workup, but no positive *in vitro* results. 9/13 (69.2%) were evaluated for betalactam antibiotics. 5/13 (38.5%) of these investigations were positive in SPT, 7/13 (53.8%) showed positive IDT and 1/13 (7.7%) was positive in a patch test. The median time from the reported drug hypersensitivity reaction to the diagnostic workup was 12.5 months (ranging from 5 to >360 months).

DISCUSSION

BAT is a non-invasive diagnostic tool with good sensitivity and high specificity [5-10]. It may be used especially in cases when drug provocation testing is considered to be too risky due to the severity of previous reaction and *in vivo* diagnostic procedures like skin testing are not of diagnostic value (e.g. because of urticaria factitia). BAT is well established for diagnosis of type I hypersensitivity to betalactam antibiotics [5-9]. Furthermore, a number of investigations report about a usefulness of BAT in patients with perioperative anaphylaxis, especially, if neuromuscular blocking agents as culprit drugs are suspected [17, 18]. Thus, BAT might be of diagnostic advantage also in cases where skin tests yield negative results.

In about 75% (62/82) of the cases in our evaluation the included patients were investigated for immediate type drug hypersensitivity reactions to betalactam antibiotics, as this group of drugs is considered to be one of the most frequently involved in allergic reactions [3, 4]. Aminopenicillins (32% or 20/62) and cephalosporins (44% or 27/62) prevail in this group and reflect the shift in betalactam prescriptions, which has occurred in many European countries and particularly in Germany in the last decades [19-22].

Of considerable interest are 41.5% (34/82) of investigations with at least one positive test result, that again in 73.5% of cases (25/34) presented with a history of immediate type drug hypersensitivity reactions to betalactam antibiotics. In this collective the investigation for specific IgE antibodies was performed in 84% (21/25) of cases. From these patients with a positive clinical history but negative skin tests, specific IgE antibodies were positive in 25% (3/12), which is low, but still higher compared to the results of other investigators: A multicenter study on diagnosis of immediate type betalactam allergy *in vitro* conducted by ENDA (European Network for Drug Allergy) showed positive specific IgE in only 11.4% (4/35) of the cases in patients with positive clinical history but negative skin tests [20]. An important factor that should be considered while assessing results of these specific IgE values is the time between reported allergic reaction and the test, as a decline of sensitivity of sIgE may occur over the time [22]. In our case, however, all positive results of specific IgE antibodies were evaluated at least 12 months after the reported reaction. The reactions that lead to allergological workup in these cases were urticaria or/and angioedema.

Interestingly, BAT was positive in 9 of 12 cases (75%) with a positive clinical history but negative skin test results, which, again, was higher comparing to 37% in the above mentioned study [20]. Of these 9 skin test-negative and BAT-positive patients 6 were investigated for betalactams, but none had a positive specific IgE test results. This supports the advantage of BAT over specific IgE antibodies in this subgroup of patients, even if for both test methods negativization over the time was reported [22].

However, in one case with positive BAT result (cefuroxim) the DPT with the same substance was unremarkable (patient Nr. 20). Thus, false positive BAT results may occur, even if they are rare and therefore the specificity of BAT was estimated at up to 97% in further studies [5-10]. Furthermore, in

two cases with positive BAT result (to penicillin and cefaclor) the DPT with other substance from the same group (cefuroxim as alternative drug) was well tolerated. These cases however may be explained by lack of cross-reactivity of these betalactam antibiotics [24-27].

If evaluate the time from the reported drug hypersensitivity reaction to the diagnostic investigation in our study, the median interval to the diagnostic workup in patients with positive BAT results was 6 months, which was half as short comparing to the patients with positive skin test results (12.5 months). Thus, the possible negativization of the BAT with the time might be considered [22].

Although only immediate type reactions were studied, a patch test was performed because sometimes clinical history may be misleading. We observed 2 positive reactions in performed patch tests. In the first case penicillin was a culprit drug, there was no positive reaction in the skin prick and intradermal tests and no presence of specific IgE antibodies in serum of the patient, but positive reaction in BAT to the culprit drug and to cefuroxim. DPT with cefuroxim was performed and well tolerated (patient Nr. 20). Thus we interpret this case as an example for false positive results, as already mentioned above. In the other case clindamycin was a culprit drug, the BAT and skin prick test were negative, suggesting that in this case a delayed type reaction possibly occurred. A DPT, however, was not performed.

The limitation of the present investigation was the small number of performed DPT thus the percentage of false positive BAT results couldn't be evaluated. Also no control samples (of healthy, tolerant persons) were tested in parallel, especially for positive BATs performed with non-betalactam antibiotics, in order to exclude non-specific positive BATs. Due to the retrospective design of the study there is also some discrepancy in the performed diagnostic evaluations, e.g. in children below 17 years of age intradermal and patch tests were not performed on regular basis. Also skin prick tests were performed with fluoroquinolones, which are known for the uncertainties associated with skin testing, and are therefore difficult to interpret [28]. Recently it has been shown that fluoroquinones are able to bind to MrgprB2-receptors, which are present on mast cells, and the activation of this mast cell specific receptor may be a reason for pseudoallergic reactions to fluoroquinones [29]. Therefore, the history of an anaphylactic reaction to fluoroquinones together with a negative BAT and a positive skin reaction may support the diagnosis of a pseudoallergic reaction, in particular because the expression of CD 63 is less likely than the CD 203c upregulation [30]. Nevertheless, the advantages of BAT in diagnosis of immediate type drug hypersensitivity reactions to betalactam and non-betalactam antibiotics were clearly shown in our study. Without performing BAT we would have missed 11% (9/82) patients with the risk to experience an additional hypersensitivity reaction upon re-exposure to the drug. Furthermore, we observed that in all patients, who reported severe drug hypersensitivity reactions (anaphylactic reaction grade 2 and above) in the past (5/5) BAT was positive, but only in 60% of cases (3/5) skin tests led to the conclusion of possible immediate type drug hypersensitivity. *In vivo* tests in form of skin prick test and intradermal test remain the basis of the allergological

investigation, but BAT seems to be a useful diagnostic tool in assessment of the individual patient risk to experience an additional hypersensitivity reaction upon re-exposure to the drug, especially in patients with severe anaphylactic reactions in clinical history.

Taken together although skin tests are still the primary diagnostic investigation, BAT is – similar to the situation after immediate type reactions to atracurium and rocuronium [17, 18] induced anaphylaxis – an additional valuable *in vitro* test in the diagnostic procedure of immediate type allergic reactions to antibiotics, particularly if there are conflicting results in the skin test and the sIgE measurement. However, further studies using *in-vivo* and *in-vitro* diagnostic tools including BAT combined with double blind and placebo-controlled provocation tests are needed to evaluate the potential of BAT in the diagnosis of allergy to betalactam and non-betalactam antibiotics, especially in different age groups.

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REFERENCES

1. Worm M, Eckermann O, Dölle S, Abeler W, Beyer K, Hawranek T, Hompes S, Koehli A, Mahler V, Nemat K, Niggemann B, Pfohler C, Rabe U, Reissig A, Rietschel E, Scherer K, Treidler R, Ruëff F. Triggers and treatment of anaphylaxis: an analysis of 4,000 cases from Germany, Austria and Switzerland. *Dtsch Arztebl Int* 2014; 111(21): 367-75.
2. Sachs B, Fischer-Barth W, Merk HF. Reporting rates for severe hypersensitivity reactions associated with prescription-only drugs in outpatient treatment in Germany. *Pharmacoepidemiol Drug Saf* 2015; 24(10): 1076-84.
3. Viola M, Quarantino D, Gaeta F, Valluzzi RL, Caruso C, Rumi G, Romano A. Allergic reactions to antibiotics, mainly betalactams: facts and controversies. *Eur Ann Allergy Clin Immunol* 2005; 37(6): 223-9.
4. Antúnez C, Martín E, Cornejo-García JA, Blanca-Lopez N, R-Pena R et al. Immediate hypersensitivity reactions to penicillins and other betalactams. *Curr Pharm Des* 2006; 12(26): 3327-33.
5. Sanz ML, Gamboa PM, Antépara I, Uasuf C, Vila L et al. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002; 32(2): 277-86.
6. Torres MJ, Padial A, Mayorga C, Fernández T, Sanchez-Sabate E et al. The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004; 34(11): 1768-75.
7. De Week AL, Sanz ML, Gamboa PM, Aberer W, Sturm G et al. Diagnosis of immediate-type beta-lactam allergy in vitro by flow-cytometric basophil activation test and sulfidoleukotriene production: a multicenter study. *J Invest Allergol Clin Immunol* 2009; 19(2): 91-109.
8. Sanz ML, Gamboa PM, Mayorga C. Basophil activation tests in the evaluation of immediate drug hypersensitivity. *Curr Opin Allergy Clin Immunol* 2009; 9(4): 298-304.
9. Eberlein B, León Suárez I, Darsow U, Ruëff F, Behrendt H, Ring J. A new basophil activation test using CD63 and CCR3 in allergy to antibiotics. *Clin Exp Allergy* 2010; 40(3): 411-8.
10. Hoffmann HJ, Santos AF, Mayorga C, Nopp A, Eberlein B et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy* 2015; 70(11): 1393-405.
11. Muraro A, Roberts G, Worm M, Bilò MB, Brockow K, Fernández Rivas M, Santos AF, Zolkipli ZQ, Bellou A, Beyer K, Bindslev-Jensen C, Cardona V, Clark AT, Demoly

- P, Dubois AE, Dunn Galvin A, Eigenmann P, Halken S, Harada L, Lack G, Jutel M, Niggemann B, Ruëff F, Timmermans F, Vlieg-Boerstra BJ, Werfel T, Dhami S, Panesar S, Akdis CA, Sheikh A. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy* 2014; 69(8): 1026-45.
12. Demoly P, Adkinson NF, Brockow K, Castells M, Chiriac AM, Greenberger PA, Khan DA, Lang DM, Park HS, Pichler W, Sanchez-Borges M, Shiohara T, Thong BY. International Consensus on drug allergy. *Allergy* 2014; 69(4): 420-37.
13. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB, Bircher A, Blanca M, Bonadonna B, Campi P, Castro E, Cernadas JR, Chiriac AM, Demoly P, Grosber M, Gooi J, Lombardo C, Mertes PM, Mosbech H, Nasser S, Pagani M, Ring J, Romano A, Scherer K, Schnyder B, Testi S, Torres M, Trautmann A, Terreehorst I. Skin test concentrations for systemically administered drugs - an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy* 2013; 68(6): 702-12.
14. Schnuch A, Aberer W, Agathos M, Becker D, Brasch J, Elsner P, Frosch PJ, Fuchs T, Hillen U, Löffler H, Mahler V, Richter G, Szliska C; Deutsche Kontaktallergie-Gruppe. Performing patch testing with contact allergens. *J Dtsch Dermatol Ges* 2008; 6(9): 770-5.
15. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002; 57(1): 45-51.
16. Fischer T, Maibach HI. Patch testing in allergic contact dermatitis: an update. *Sem Dermatol* 1986; 5: 214–24.
17. Uyttebroek AP, Sabato V, Leysen J, Bridts C H, De Clerck LS, Ebo DG. Flowcytometric diagnosis of atracurium-induced anaphylaxis. *Allergy* 2014; 69: 1324–32.
18. Leysen J, Bridts CH, De Clerck LS, Vercauteren M, Lambert J, Weyler JJ, Stevens WJ, Ebo DG. Allergy to rocuronium: from clinical suspicion to correct diagnosis. *Allergy* 2011; 66: 1014–19.
19. Blanca M. Allergic reactions to penicillins. A changing world? *Allergy* 1995; 50: 777-82.
20. De Week AL, Sanz ML, Gamboa PM, Aberer W, Sturm G, Bilo MB, Montroni M, Blanca M, Torres MJ, Mayorga L, Campi P, Manfredi M, Drouet M, Sainte-Laudy J, Romano A, Merk H, Weber JM, Jermann TM; ENDA (European Network for Drug Allergy). Diagnosis of immediate-type beta-lactam allergy in vitro by flow-cytometric

- basophil activation test and sulfidoleukotriene production: a multicenter study. *J Investig Allergol Clin Immunol* 2009; 19(2): 91-109.
21. Kern WV, Fellhauer M, Hug M, Hoppe-Tichy T, Först G, Steib-Bauert M, de With K. Recent antibiotic use in German acute care hospitals - from benchmarking to improved prescribing and quality care. *Dtsch Med Wochenschr* 2015; 140(23): e237-46.
22. Bätzing-Feigenbaum J, Schulz M, Schulz M, Hering R, Kern WV. Outpatient Antibiotic Prescription. *Dtsch Arztebl Int* 2016; 113 (26): 454-9.
23. Fernández TD, Torres MJ, Blanca-López N, Rodríguez-Bada JL, Gomez E, Canto G, Mayorga C, Blanca M. Negativization rates of IgE radioimmunoassay and basophil activation test in immediate reactions to penicillins. *Allergy* 2009; 64(2): 242-8.
24. Martinez Tadeo JA, Perez Rodriguez E, Almeida Sanchez Z, Callero Viera A, Garcia Robaina JC. No cross-reactivity with cephalosporins in patients with penicillin allergy. *J Investig Allergol Clin Immunol* 2015; 25(3): 216-7.
25. Romano A, Gaeta F, ArribasPoves MF, Valluzzi RL. Cross-Reactivity among Beta-Lactams. *Curr Allergy Asthma Rep* 2016; 16(3): 24.
26. Romano A, Gaeta F, Valluzzi RL, Maggioletti M, Zaffiro A, Caruso C, Quarantino D. IgE-mediated hypersensitivity to cephalosporins: Cross-reactivity and tolerability of alternative cephalosporins. *J Allergy Clin Immunol* 2015; 136(3): 685-691.
27. Mayorga C, Sanz ML, Gamboa PM, García BE, Caballero MT, García JM, Labrador M, Lahoz C, Longo Areso N, López Hoyos M, Martínez Quesada J, Monteseirín FJ; Immunology Committee of the Spanish Society of Allergology and Clinical Immunology of the SEAIC. In vitro diagnosis of immediate allergic reactions to drugs: an update. *J Investig Allergol Clin Immunol* 2010; 20(2): 103-9.
28. Uyttebroek AP, Sabato V, Bridts CH, De Clerck LS, Ebo DG. Moxifloxacin hypersensitivity: uselessness of skin testing. *J Allergy Clin Immunol Pract* 2015; 3(3): 443-5.
29. McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, Dong X. Identification of a mast cell specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2015; 519: 237-241.
30. Decuyper II, Mangodt EA, van Gasse AL, Claesen K, Uyttebroek A, Faber M, Sabato V, Bridts CH, Mertens C, Hagendorens MM, de Clerck LS, Ebo DG: In vitro diagnosis of immediate drug hypersensitivity anno 2017: Potentials and limitations. *Drugs R D* 2017; 17(2): 265-78.

TABLES

Table 1: Concentrations of each substance used for skin prick test, intradermal test and patch test.

(If the concentration not given, the test was not performed (N/P) for example due to lack of literature / experience)

Substance used for skin prick test	Concentration used for skin prick test	Concentration used for intradermal test	Concentration used for patch test
Penicilloyl-poly-L-lysine	5×10^{-5} mM	5×10^{-5} mM	N/P
MDM (Minor determinant mixture)	2×10^{-2} mM	2×10^{-2} mM	N/P
Benzympenicillin	10.000 Units	10.000 Units	5%
Amoxicillin	20 mg/ml	20 mg/ml	5%
Ampicillin	20 mg/ml	20 mg/ml	5%
Cephalosporins (cefazolin, cefotaxim, ceftazidin, cefixim, cefuroxim, cefaclor, ceftibuten, cefadroxil)	2 mg/ml	2 mg/ml	5%
Ciprofloxacin	$2/3 \times 10^{-2}$ mg/ml	2×10^{-3} mg/ml	5%
Moxifloxacin	1,6 mg/ml (400 mg /250 ml)	0,05 mg/ml	5%
Levofloxacin	5 mg/ml	0,05 mg/ml	5%
Cotrimoxazol (Trimethoprim / Sulfamethoxazol)	80 mg/ml / 16 mg/ml	N/P	5%
Azithromycin	0,01 mg/ml	N/P	1%, 5%
Erythromycin	50 mg/ml	0,05 mg/ml	1%, 5%
Roxithromycin	30 mg/ml	N/A	1%, 5%
Clarithromycin	0,02 mg/ml	6×10^{-4} mg/ml	1%, 5%
Doxycycline	20 mg/ml	0,02 mg/ml	5%
Clindamycin	150 mg/ml	1,5 mg/ml; 15 mg/ml	5%

Table 2: Concentrations of each substance used for basophil activation test (BAT).

Substance used for BAT	Concentration used for BAT	Dilutions used for BAT
Penicillin G	4 mg/ml	1:1; 1:10; 1:100; 1:1000
Penicillin V	4 mg/ml	1:1; 1:10; 1:100; 1:1000
Ampicillin	2,5 mg/ml	1:1; 1:10; 1:100; 1:1000; 1:10000
Amoxicillin	2,5 mg/ml	1:1; 1:10; 1:100; 1:1000; 1:10000
Cefuroxim	2,5 mg/ml	1:1; 1:10; 1:100; 1:1000; 1:10000
Cefaclor	50 mg/ml	1:10; 1:100; 1:1000; 1:10000
Cefazolin	2,5 mg/ml	1:1; 1:10; 1:100; 1:1000
Cefotaxim	100 mg/ml; 200 mg/ml	1:100; 1:1000; 1:10000; 1:100000
Cefadroxil	5 mg/ml	1:1; 1:10; 1:100
Ceftazidin	50 mg/ml	1:100; 1:1000; 1:10000; 1:100000
Cefixim	20 mg/ml	1:10; 1:100; 1:1000; 1:10000; 1:100000
Ciprofloxacin	100 µg/ml	1:1; 1:10; 1:100
Moxifloxacin	1,6 mg/ml (400 mg /250 ml)	1:10; 1:100; 1:1000; 1:10000
Levofloxacin	5 mg/ml	1:10; 1:100; 1:1000; 1:10000
Trimethoprim	16mg/ml (80 mg/5 ml)	1:1; 1:10; 1:100; 1:1000
Sulfamethoxazol	80mg/ml (400 mg/5 ml)	1:1; 1:10; 1:100; 1:1000
Azithromycin	50 mg/ml	1:10; 1:100; 1:1000; 1:10000
Erythromycin	50 mg/ml	1:10; 1:100; 1:1000; 1:10000
Roxithromycin	30 mg/ml	1:10; 1:100; 1:1000; 1:10000
Clarithromycin	50 mg/ml	1:10; 1:100; 1:1000; 1:10000
Doxycycline	1 mg/ml	1:10; 1:100; 1:1000; 1:10000
Vancomycin	5 mg/ml	1:100; 1:1000; 1:10000; 1:100000
Clindamycin	150 mg/ml	1:10; 1:100; 1:1000; 1:10000

Table 3: The therapeutic dose for each substance used for drug provocation test (DPT).

Substance used for DPT	Standard therapeutic dose used for DPT	Therapeutic dose used for DPT in pediatric patients
Amoxicillin	1000 mg for persons with > 40 kg body weight	30 mg / kg body weight
Ampicillin	1000 mg for persons $\geq$ 6 years old	30 mg / kg body weight
Cefuroximaxetil	500 mg for persons > 12 years old	15 mg / kg body weight
Ceftibuten	200 mg for persons > 12 years old	9 mg / kg body weight
Cefaclor	250 mg for persons > 6 years old	25 mg / kg body weight
Doxycycline	100 mg for persons $\geq$ 8 years old	not suitable for persons < 8 years old
Erythromycin	500 mg mg for persons $\geq$ 8 years old (or $\geq$ 30 kg)	15 mg / kg body weight
Penicillin V	1 million units for persons > 12 years old	50.000 units

Table 4: Results of the skin testing.

	Skin prick test positive (from all performed)	Intradermal test positive (from all performed)	Patch test positive (from all performed)	Total (from all performed)
Skin prick test negative		9/38	2/49	67/77
Intradermal test negative	not performed if skin prick test positive		2/49	29/38
Patch test negative	2/77	9/38		47/49

Table 5: Results of the diagnostic workup on immediate type drug hypersensitivity reactions to systemic antibiotics in the Department of Dermatology and Allergology, University Hospital of RWTHAachen, Aachen, Germany, during the period from 2009 to 2012.

Legend: Nr.: Number of the patient, SPT: Skin prick test, IDT: Intradermal test, PT: Patch test, DPT: Drug provocation test, N/E: Evaluation of the test result was not possible due to urticaria factitia, N/P: The test was not performed, N/P* The test was not performed with culprit drug, but with an alternative drug. U: Urticaria, A: Angioedema, U/A: Urticaria and angioedema, AR: Anaphylactic reaction > grade 2, CD: Circulatory dysregulation, OA: Obstruction of upper airways, V: Vomiting, neg.: Negative, pos.: Positive, N/A: information was not available, the patient was not able to report the information despite careful history taking.

Nr.	Gender	Age (y)	culprit drug	type of reaction	time from reaction to the investigation (month)	SPT	IDT	PT	specific IgE	BAT	DPT
1	M	42	amoxicillin	U	2	N/E	N/E	neg.	neg.	neg. Penicillin G pos.	N/P* cefuroxim neg.
2	W	47	cefuroxim	U/A	2	N/E	N/E	neg.	neg.	neg.	N/P
2	W	47	ciprofloxacin	U/A	2	N/E	N/E	neg.	-	neg.	N/P
3	W	70	moxifloxacin	U	11	N/E	N/E	neg.	-	neg.	N/P
4	W	70	cotrimoxazole	AR	4	neg.	N/P	neg.	-	pos.	N/P
4	W	70	penicillin	U	>360	neg.	pos.	neg.	neg.	neg.	N/P* cefuroxim neg.
5	W	7	cefactor	U	48	neg.	N/P	N/P	N/P	neg.	N/P
5	W	7	amoxicillin	U	24	neg.	neg.	neg.	neg.	neg.	N/P
6	W	18	penicillin	U	36	neg.	neg.	neg.	neg.	neg.	neg.
6	W	18	cefuroxim	U	16	neg.	neg.	neg.	neg.	neg.	neg.
7	W	51	penicillin	U/A	>360	neg.	pos.	neg.	neg.	neg.	N/P* cefuroxim neg.
8	W	45	amoxicillin	U/A	5	neg.	pos.	neg.	neg.	neg.	N/P
8	W	45	cefuroxim	U/A	36	neg.	pos.	neg.	neg.	neg.	N/P
8	W	45	doxycycline	U	5	neg.	neg.	neg.	-	neg.	N/P
8	W	45	moxifloxacin	U	5	neg.	neg.	neg.	-	neg.	N/P
9	M	63	cefuroxim	U/A	10	neg.	neg.	neg.	neg.	neg.	N/P
10	W	42	cefazolin	U	13	neg. piperacilin pos.	neg.	neg.	neg.	neg.	N/P* cefuroxim neg.
11	W	45	cefuroxim	AR	5	pos.	N/P	N/P	neg.	pos.	N/P
12	W	41	penicillin	U	>360	neg.	neg.	neg.	neg.	neg.	N/P
13	W	18	levofloxacin	U/A	9	neg.	neg.	neg.	-	neg.	N/P
1	W	18	cefuroxim	U/A	7	neg.	neg.	neg.	neg.	neg.	neg.

3							g.	g.			
14	W	18	amoxicillin	U/A	14	neg.	ne g.	N/P	neg. cefaclor or pos.	neg.	N/P
15	M	50	amoxicillin	AR	10	N/E	N/P	ne g.	neg.	pos.	N/P
15	M	50	ciprofloxacin	AR	10	neg.	pos.	ne g.	-	pos.	N/P
15	M	50	roxithromycin	A	12	neg.	N/P	ne g.	-	pos.	N/P
16	W	18	penicillin	U	16	neg.	ne g.	ne g.	N/P	neg.	neg.
16	W	18	cefuroxim	U	16	neg.	ne g.	ne g.	N/P	neg.	neg.
17	M	4	cefaclor	U/A	20	neg.	N/P	ne g.	neg.	neg.	N/P* amoxicillin neg.
18	M	2	cefuroxim	U	7	neg.	N/P	N/P	neg.	neg.	N/P
19	W	37	cefazolin	CD	60	neg.	ne g.	ne g.	neg.	neg.	N/P
20	W	69	ampicillin	U	6	neg.	ne g.	ne g.	neg.	pos.	N/P
20	W	69	moxifloxacin	AR	6	pos.	N/P	ne g.	-	pos.	N/P
20	W	69	penicillin	U	N/A	neg.	ne g.	pos.	neg.	pos. cefuroxim pos.	N/P* cefuroxim neg.
21	M	35	doxycycline	U/A	N/A	neg.	ne g.	ne g.	-	neg.	neg.
21	M	35	penicillin	U/A	N/A	neg.	ne g.	ne g.	neg.	neg.	neg.
21	M	35	ceftibuten	U/A	N/A	neg.	ne g.	ne g.	-	N/P* cefuroxim neg.	neg.
22	W	49	penicillin	U	>360	neg.	ne g.	ne g.	neg.	neg.	N/P
23	M	42	penicillin	U	12	neg.	ne g.	ne g.	neg.	neg.	N/P
23	M	42	clindamycin	U	12	neg.	pos.	ne g.	-	neg.	N/P
23	M	42	cefuroxim	V	48	neg.	ne g.	ne g.	neg.	neg.	neg.
24	W	24	ampicillin	U	N/A	pos.	N/P	N/P	N/P	neg.	N/P
24	W	24	amoxicillin	U	N/A	pos.	N/P	N/P	N/P	neg.	N/P
24	W	24	penicillin	U	N/A	pos.	N/P	N/P	N/P	neg.	N/P* cefuroxim neg.
25	M	10	amoxicillin	U	96	neg.	ne g.	ne g.	pos.	neg.	N/P* cefuroxim neg.
26	W	57	penicillin	A	>300	neg.	ne g.	ne g.	pos.	neg.	N/P
27	W	21	amoxicillin	U	42	neg.	pos.	ne g.	neg.	pos.	N/P
27	W	21	azithromycin	V	17	neg.	N/P	ne g.	-	neg.	N/P* erythromycin neg.
28	W	29	roxithromycin	CD	4	neg.	N/P	ne g.	-	neg.	N/P
28	W	29	penicillin	CD	28	neg.	ne g.	ne g.	neg.	neg.	N/P
28	W	29	amoxicillin	U	180	neg.	ne g.	ne g.	neg.	neg.	N/P

29	W	53	clindamycin	U	6	pos.	N/P	neg.	-	neg.	N/P
30	W	42	penicillin	CD and OA	36	neg.	pos.	neg.	neg.	neg.	N/P
31	W	31	ampicillin	CD	180	neg.	neg.	neg.	neg.	neg.	N/P
32	M	31	clindamycin	U	7	neg.	neg.	pos.	-	neg.	N/P
32	M	31	ciprofloxacin	U	7	neg.	pos.	neg.	-	neg.	N/P
32	M	31	penicillin	U	>300	neg.	neg.	neg.	neg.	neg.	N/P
33	W	4	cefuroxim	U	41	neg.	N/P	N/P	neg.	pos.	N/P
34	W	5	amoxicillin	U	N/A	neg.	N/P	N/P	neg.	neg.	N/P
34	W	5	cefuroxim	U	N/A	neg.	N/P	N/P	neg.	neg.	N/P
35	M	3	amoxicillin	U/A	4	pos.	N/P	N/P	neg.	pos.	N/P
35	M	3	cefuroxim	U/A	4	pos.	N/P	N/P	neg.	pos.	N/P
36	W	13	penicillin	U	84	neg.	N/P	N/P	neg.	neg.	N/P
37	W	12	cefaclor	U	N/A	neg.	N/P	N/P	N/P	neg.	N/P* cefuroxim neg.
37	W	12	cotrimoxazole	U	N/A	neg.	N/P	N/P	-	neg.	N/P
38	M	4	amoxicillin	OA	15	neg.	N/P	N/P	N/P	neg.	N/P
39	M	7	erythromycin	U and OA	N/A	neg.	N/P	N/P	-	neg.	N/P
40	W	2	amoxicillin	U/A	3	neg.	N/P	N/P	neg.	neg.	N/P* cefuroxim neg.
41	W	2	cefaclor	U	1	neg.	N/P	N/P	neg.	neg.	N/P
42	M	4	ampicillin	U	N/A	neg.	N/P	N/P	neg.	neg.	N/P
42	M	4	erythromycin	U	N/A	neg.	N/P	N/P	-	neg.	N/P
43	W	4	cefaclor	U	9	neg.	N/P	N/P	neg.	neg.	neg.
44	M	15	cefadroxil	U	72	pos.	N/P	N/P	N/P	pos.	N/P
45	W	4	cefaclor	U	17	neg.	N/P	N/P	neg.	pos.	N/P
46	W	8	erythromycin	U	2	neg.	N/P	N/P	-	pos.	N/P
47	M	8	cefaclor	U	3	neg.	N/P	N/P	neg.	pos.	N/P* cefuroxim neg.
47	M	8	amoxicillin	U	> 60	neg.	N/P	N/P	neg.	neg.	N/P* cefuroxim neg.
48	M	7	cefaclor	U/A	<12	neg.	N/P	N/P	neg.	neg.	N/P* cefuroxim neg.
48	M	7	amoxicillin	U	<12	neg.	N/P	N/P	neg.	neg.	N/P* cefuroxim neg.
49	W	6	Cefaclor	U/A	22	neg.	N/P	N/P	neg.	neg.	N/P* amoxicillin pos.
5	M	3	cefuroxim	U	<12	neg.	N/P	N/P	neg.	neg.	N/P

0							P	P			
5 1	M	10	amoxicillin	OA	<12	pos.	N/ P	N/ P	pos.	neg.	N/P
5 2	M	3	cefaclor	A	3	neg.	N/ P	N/ P	N/P	neg.	N/P* cefuroxima nd ampicillin neg.

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FIGURES

Figure 1A: Detailed presentation of the allergological workup in adolescents and children younger than 17 years.

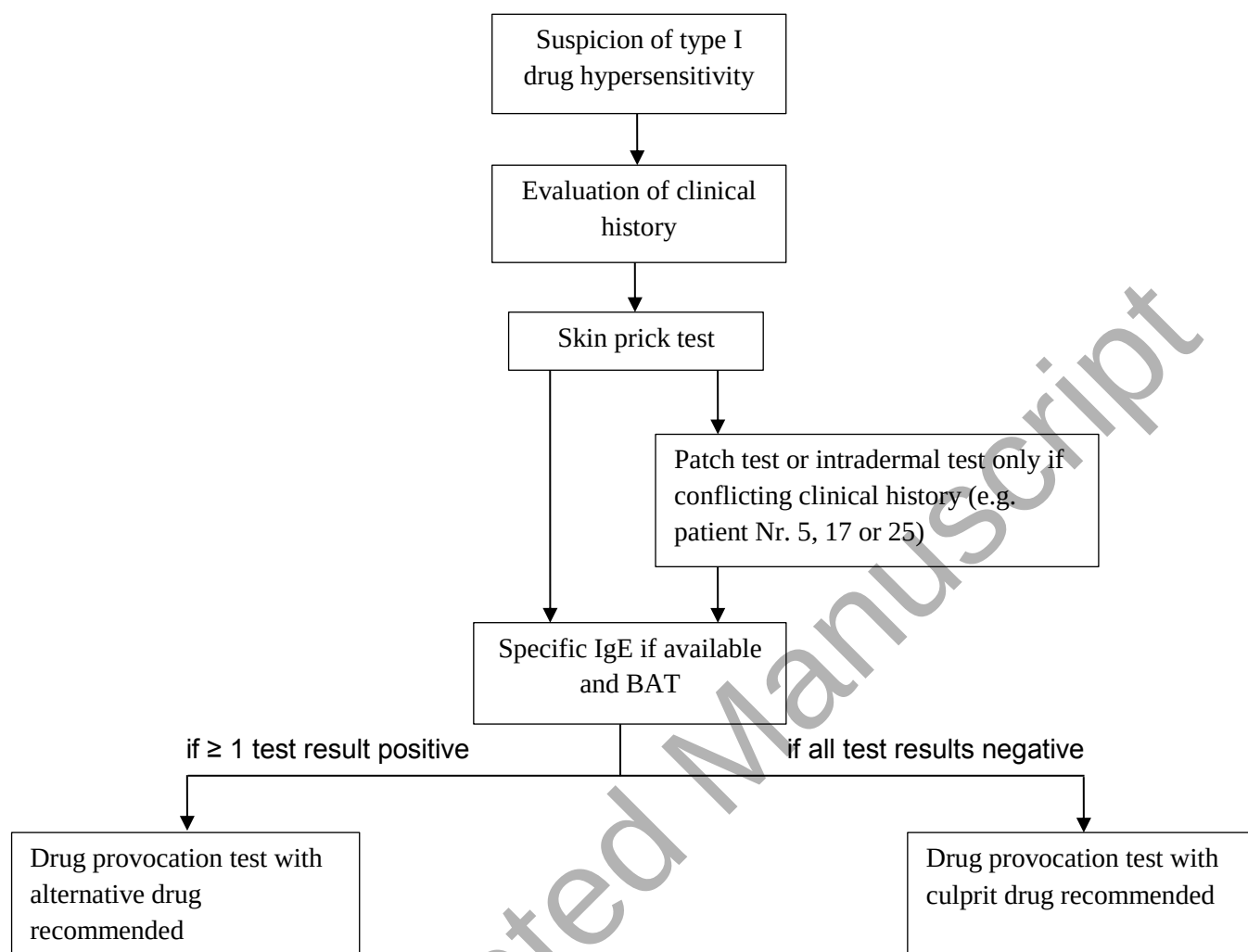
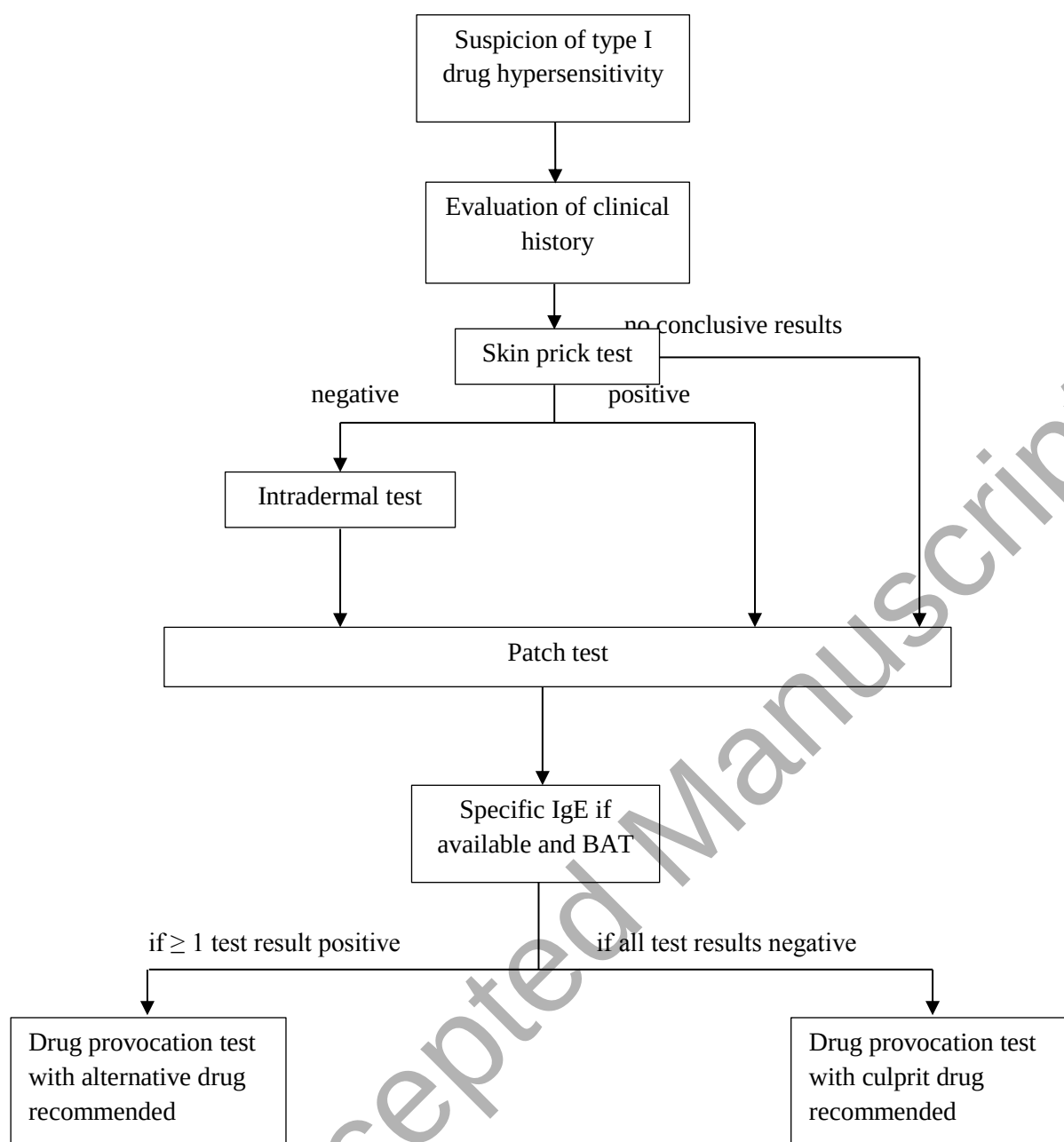


Figure 1B: Detailed presentation of the allergological workup in adults.



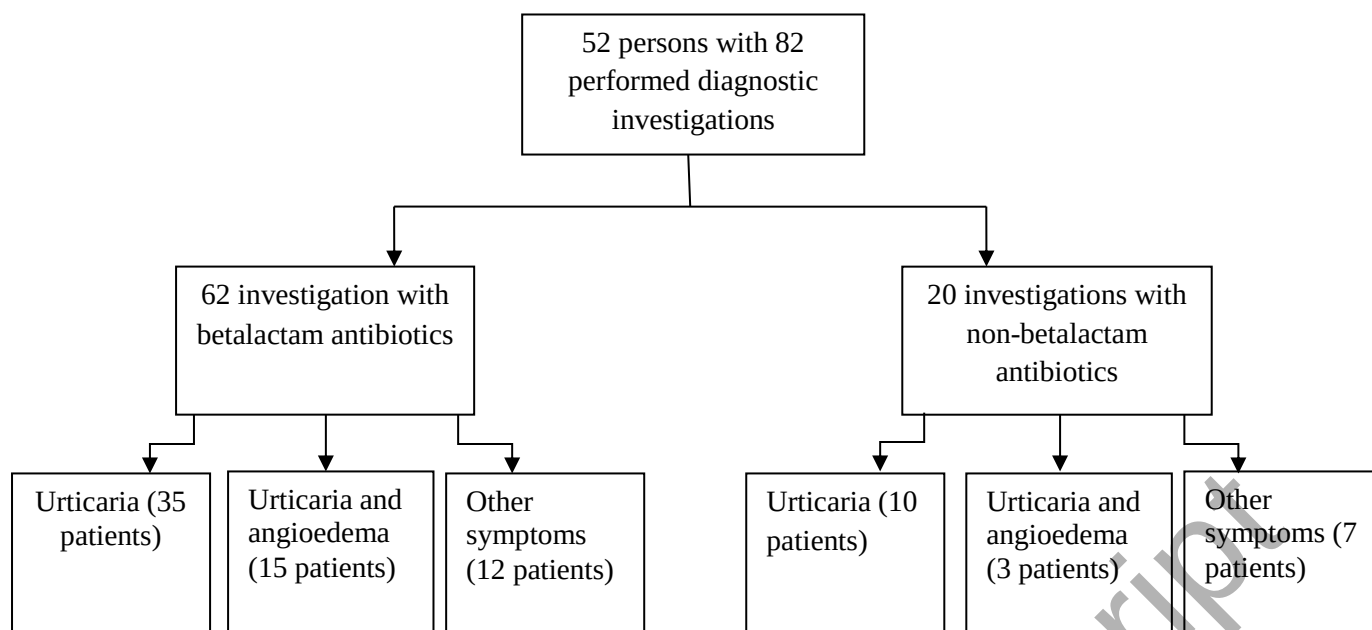


Figure 2: Epidemiological data of the patients that underwent allergological investigations for immediate type drug hypersensitivity reactions to betalactam and non-betalactam antibiotics using basophilic activation test during the period from 2009 to 2012 in the Department of Dermatology and Allergology, University Hospital of RWTH Aachen, Aachen, Germany.

Figure 3: Detailed results of the allergologic workup. All included patients received skin tests, sIgE (if available) and BAT.

