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Detection of drug-specific immunoglobulin E (IgE) and acute mediator release for the diagnosis of immediate drug hypersensitivity reactions

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ABBREVIATIONS

BAT: Basophil activation test

BL: Betalactams

DAO: Diamine oxidase

DHR: Drug hypersensitivity reactions

DPT: Drug provocation tests

ECP: Eosinophil cationic protein

ELISA: Enzyme immunoassay

FEIA: Fluoroimmunoassay

LTT: Lymphocyte transformation test

NSAIDs: Non-steroidal anti-inflammatory drugs

RAST: Home-made radioallergosorbent test

RIA: Radioimmunoassay

slgE: Specific IgE

ST: Skin testing

α -gal: galactose- α -1,3-galactose

ABSTRACT

The diagnosis of a drug hypersensitivity reaction (DHR) is complex. The first step after taking the clinical history is to look for a sensitization to confirm or exclude the diagnosis and to identify the culprit drug. Skin tests are the primary means of detecting sensitization in DHR, but are associated with a risk for a severe reaction and may be contraindicated. In vitro tests offer the potential to support or confirm a diagnosis of DHR and influence medical decision making. For immediate-type DHR, a few validated assays for measurement of specific IgE (sIgE) are commercially available to a limited number of drugs. In addition, several home-made sIgE radioimmunoassays have been used in other studies. The sensitivity of the sIgE assay is drug-dependant and generally low (0-85%) for betalactams and reported heterogeneous for other drugs ranging from 20% for chlorhexidine and 44% for suxamethonium to 92% for chlorhexidine. However, as all these studies included patients, in whom DHR was confirmed only by skin tests and not by provocation, the results have to be interpreted carefully and may be unreliable. Determination of mediators during an acute phase of a reaction may indirectly support the diagnosis of a DHR by demonstrating mast cell and basophil mediator release. Negative in vitro tests do not exclude a DHR or imputability of a drug, but a positive result may support causality and eliminate the necessity for a drug provocation test. Unfortunately, evidence is limited with a lack of well-controlled studies in larger numbers of well-phenotyped patients, which results in susceptibility for bias and a need for future multi-center studies.

1. INTRODUCTION

Drug hypersensitivity reactions (DHR) are a matter of concern in both outpatient care and hospital setting for doctors and patients alike. Many patients report DHRs in the past, but only about 10-20% of these reported reactions can be confirmed by a proper allergy workup.^{1,}

² The high number of reported reactions in patient histories hampers the selection of a suitable therapy. Presumed drug allergy (immune-mediated hypersensitivity) may also disappear after a certain period of time as memory cells will not prevail forever.^{1, 3, 4} Possible consequences of incorrectly classified DHRs in the patient history include the increased use of alternative medications, which are often less effective, associated with a longer morbidity, more adverse events and higher costs.^{5, 6} Thus, it is important to clarify a presumed drug hypersensitivity. Allergy diagnosis is based on a detailed clinical history, in vivo procedures, such as skin testing (ST), in vitro methods and drug provocation tests (DPT).⁷ Tests are selected according to the assumed mechanism of the DHR. Many in vitro tests can help in the diagnosis and in the identification of the culprit drug (Fig. 1).⁸ The most often applied in vitro tests to identify the culprit drug having elicited a DHR in the past are determination of specific immunoglobulin E (sIgE) and cellular tests. Cellular tests, such as basophil activation test, have demonstrated usefulness for some drugs and are not within the scope of this review. On the other hand, as will be discussed, only few immunoassays are sufficiently validated.^{8, 9} Even in those, the correlation to skin tests results is often questionable to poor. Determination of mediators and markers of mediator release during an acute phase of a reaction may support the diagnosis of an immediate DHR.¹⁰

Allergic DHRs comprise about 10% of adverse drug reactions and mostly belong to type I (immediate) IgE-mediated drug allergy or type IV (non-immediate) T cell-mediated drug allergy.^{1, 11} Immediate allergic reactions occurring within one (up to six) hours after contact with the drug are induced by specific IgE (sIgE) antibodies, produced against a hapten-carrier conjugate during a usually asymptomatic sensitization phase.¹² Subsequent attachment to high-affinity IgE receptors on mast cells or basophils and renewed contact and

cross-linking of sIgE lead to cell activation and release of several mediators (histamine, tryptase, leukotrienes, and others), which cause the symptoms and can be measured in the blood. Specific IgE to certain drugs and mediators or marker substances can be measured by immunoassays. The aim of the present review is to summarize the current applications of sIgE and mediator detection currently available for the diagnosis of DHR and to give recommendations for their use.

2. IN VITRO DIAGNOSTIC TEST METHODS

In general, *in vitro* diagnostic tests may either be used to identify or confirm the culprit drug having caused a reaction in the past or for the evaluation of mediators and markers released during the acute phase of the reaction in order to confirm a DHR in contrast to e.g. toxic, vasovagal or somatoform reactions.⁸ All methods for the diagnosis of a DHR depend on the mechanism involved and chronology of the reaction.¹¹ Specific IgE to drugs and mediators or markers during the acute phase of the reaction are only helpful for the evaluation of immediate reactions, such as anaphylaxis and urticaria occurring within the first 1-6 hours after drug intake. Whereas presence of sIgE together with a compatible history of DHR to this drug will be interpreted as a demonstration of an allergic mechanism, increased levels of mediators and markers during an acute reaction can also be detected in non-allergic DHRs, such as to non-steroidal anti-inflammatory drugs (NSAIDs).

3. SPECIFIC IGE DETERMINATION

These methods are performed after the resolution of the reaction and depend on the mechanism involved.

3.1. Principle

IgE antibodies as the molecules responsible for immediate type I hypersensitivity reactions were discovered by Ishizaka in 1966. Specific IgE (sIgE) describes the fraction of total IgE antibodies in serum with a specificity towards certain allergens, which can be determined using *in vitro* testing. The first *in vitro* assay for sIgE, the so-called radio allergosorbent test

(RAST), was a cyanogen-bromide activated paper disc with covalently coupled native allergen extracts, which can bind the sIgE antibodies.¹³ Those were quantified with radioactively marked polyclonal antihuman IgE antibodies. Whereas the same principle of coupling specific allergen extracts to either a solid phase or to liquid carriers are used, nowadays radioactive labelling has been largely replaced by nonradioactive immunoassays.¹⁴ The most available commercial method, is the fluoroimmunoassay (FEIA), where the drug is covalently bound to poly-L-lysine. However, there are also homemade radioimmunoassays or enzyme immunoassay (ELISA) using different carriers, such as human serum albumin, poly-L-lysine, amino-aliphatic spacers and dendrimers structures as well as different solid phases (cellulose, sepharose, zeolites, or silica particles).^{8, 15} Quantification of drug-sIgE is more complicated than for sIgE to protein allergen extracts, because drugs and their metabolites are low-molecular haptens, which have to be bound to a carrier to become immunologically active and quantification predominantly relies upon measurement of a drug-(hapten)-carrier antibody complexes.¹⁴ Advantages of the sIgE assay in comparison to other in-vitro methods, such as cellular assays (which need processing within 24 hours) are that serum samples can be stored for a long time, can more easily be transported to other laboratories and that the assays can be automated.⁸

3.2. Specific IgE antibodies to drugs

Skin tests are the primary means for detecting sensitization for every single drug.^{16 17} However, in vitro tests, such as sIgE determination and basophil activation test may complement the diagnosis, particularly in patients with very severe prior DHR or those in whom skin tests and drug provocation tests are contraindicated. Only a limited number of drug-specific sIgE immunoassays are available and these assays are much less validated than those directed against allergenic proteins.⁹ In addition, many immediate DHRs, such as against the vast majority of NSAIDs and radiocontrast media, are not IgE-mediated. In these pseudoallergic reactions, specific IgE cannot be measured. Thermo Fisher, a commercial provider of sIgE assays (ImmunoCAP Thermo-Fisher, Uppsala, Sweden), markets sIgE tests

for the drugs penicilloyl G, penicilloyl V, ampicilloyl and amoxicilloyl determinants, cephaclor, chlorhexidine, chymopapain, bovine gelatin, human insulin, morphine, pholcodine, and suxamethonium only.¹⁴ For research purposes, tests only to very few additional drugs are available. Drug-specific sIgE immunoassays do cover only a small fraction of all drugs available.¹⁸ Importantly, in most studies on sIgE results in patients with suspected DHR, DHR was confirmed only by skin tests and not by provocation, which is not a proper validation (Tab. 1-3). Furthermore, the correlation between sIgE and skin test results to drugs has been found to be generally poor.^{19,20} Although this has been interpreted by authors that these methods would be complementary to each other, it may alternatively rather indicate a lack of validity of one or both methods.^{19,20} In one study in the USA, none of four patients with a history of penicillin DHR and sIgE to penicilloyl G $\pm$ penicilloyl V $\pm$ amoxicilloyl determinants reacted in the provocation test or had a concordant positive skin test.¹⁹ This status quo reflects the situation, that many suspected DHRs cannot be confirmed and that safe drug alternatives cannot be reliably identified by skin or in vitro allergy testing alone, an IgE-mediated mechanism is only demonstrated to a minority of drugs (e.g. betalactam drugs) and that insufficient numbers of accurately phenotyped patients are available to develop such assays.⁹

According to published studies, the accuracy of the sIgE assay by ImmunoCAP depends on the drug involved, but generally sensitivity is rather low (0-85%) for BL allergy (Tab. 1),^{8,18} heterogeneous for NMBA ranging from 83-92% for rocuronium, 78-84% for morphine and 44% for suxamethonium in NMBA allergy (Tab. 2)^{21 22 23 8, 18} In contrast to the situation in Europe, experts from the United States consider the current commercial immunoassay to determine sIgE levels to penicillin not useful because of its suboptimal sensitivity and low concordance with STs and DPTs.⁹

The low sensitivity of sIgE to drugs could be a result of the drug-binding to a solid phase, the dependence on a carrier as part of the antigenic determinant, an inadequate density of haptens in the conjugate, or that metabolites are responsible, which are not always included

in the assays.⁸ In addition, sensitivity of sIgE assays decrease over time.⁴ Thus, patients who present for diagnosis might have already lost their circulating IgE antibodies, whereas in individual others they may also retain for decades.²⁴ Although data is insufficient, it has generally been recommended to do specific allergy workup, if possible, four to six weeks after complete resolution of all clinical symptoms and signs, as after a time interval of more than 6–12 months, some drug tests may already have already turned negative.¹

3.2.1 Betalactams

Commercially available IgE tests exist for several penicillins (penicillin G, penicillin V, amoxicillin and ampicillin) and for the cephalosporin cefaclor but not for other betalactams (Tab. 1), which is an unmet need.⁸ For betalactams, commercially available ImmunoCAP sensitivity is heterogeneous in different studies (0% - 85%), but generally low, whereas the specificity is mostly high (54% - 100%).^{3, 8, 25-34} Penicilloyl determinants are well-established, and non-commercial inhouse assays have been developed.^{8, 27, 35, 36} RAST sensitivity ranges from 42.9% - 75% and its specificity from 67.7% - 83.3%.⁸ The sensitivity depends on the severity of the clinical symptoms and is higher in anaphylaxis as compared to urticaria.²⁹ Decreasing of the threshold to 0.1 kU/L in ImmunoCAP increases the sensitivity, but reduces the specificity, particularly in cases of total IgE>200 kU/L.³⁰ False-positive sIgE results to penicillins do occur in some patients due to IgE to a cross-reactive epitope, phenylethylamine, an allegenic structure related to penicillin but different from the major and minor allergens.^{9, 37} It has been reported that using the sIgE/total IgE ratio increase the specificity; thus the total IgE level should be considered when taking the results into account.³⁰ However, using a ratio is more complicated and in this study also decreased the sensitivity from 66% for sIgE against a betalactam to only 43%. Also homemade assays for cephalosporins, such as Sepharose-RIA and cellulose-RIA have reported a good sensitivity of up to 74,3%.^{27, 36}

3.2.2 Neuromuscular blocking agents (NMBA)

Commercial ImmunoCAP is only available for suxamethonium-specific IgE.^{38, 39} However, further data is available for inhouse and non-commercially licensed NMBA.³⁹⁻⁴⁶ Cross-reactivity exists between different NMBA as tertiary and quaternary substituted ammonium structures that have been shown to be the major epitopes.⁴⁴ As also morphine and pholcodine provide these structures. Specific IgE to these substances may be an evidence for a sensitization against NMBA and in individual studies displays even better sensitivity than the suxamethonium ImmunoCAP.²² A morphine-based immunoassay has been discussed to add to the diagnosis of anaphylaxis from suxamethonium and rocuronium anaphylaxis antibodies, but appears not to be cross-reactive with atracurium-reactive antibodies.^{14, 47} However, in the general population, IgE reactivity to tertiary and quaternary ammonium structures are common, e.g. after taking the antitussive pholcodine or in patients with high total IgE, resulting in nonspecific binding of IgE to morphine, which considerably impacts with the specificity of a positive result.^{11, 12}

3.2.3 Other drugs

Sensitivity and specificity of sIgE to other drugs is given in Tab. 3.⁸ In chlorhexidine allergy, ImmunoCAP had an excellent sensitivity (91.6%) in patients with a positive ST to this drug and 100% specificity.⁴⁸

IgE against galactose- α -1,3-galactose residues (α -gal) can be measured in Cetuximab-allergic patients, as it is present in the Fab portion of this antibody. The ImmunoCAP sensitivity ranges from 68-92% with a specificity from 90-92%.⁴⁹ Patients with Cetuximab-allergy often have pre-existing α -gal-specific IgE related to previous tick bites and detecting this antibody by ELISA may predict severe reactions to cetuximab at first infusion with a sensitivity 71.4% and specificity of 82.1%.⁵⁰

For further drugs, no controlled studies with commercially available assays have been reported. Quinolones are unspecific histamine-releasers more often than that an IgE-

mediated mechanism has been demonstrated and skin tests are often irritative. Nevertheless, two homemade Sepharose-RIA to quantify sIgE to quinolones have reported an excellent specificity (100%) and low sensitivity (31.6% - 54.5%); however, data is limited.^{51, 52} Most DHR to nonsteroidal anti-inflammatory drugs (NSAID) are due to a dysbalance of the prostaglandine/lipotriene pathway and no evidence for an IgE-mediated mechanism has been found in ELISA using diclofenac conjugates. Only pyrazolones such as metamizole often elicit an IgE-mediated reaction and sIgE antibodies in patients' sera have been demonstrated in ELISA against propyphenazonone conjugates.⁵³ Anti-infliximab IgE in ImmunoCAP has been reported with a sensitivity of 26% and a specificity of 90%.^{54, 55} Platins have a reported sensitivity for carboplatin and oxaliplatin of 58% and 75% with a specificity of 100% (5/5) and 75%, respectively, in a small scale study employing the ImmunoCAP system.⁵⁶

4.- MEDIATORS AND BIOMARKERS FOR THE ACUTE PHASE OF AN IMMEDIATE DRUG HYPERSENSITIVITY REACTION

Several mediators and biomarkers have been studied for their potential to indicate a diagnosis of an anaphylaxis, some being caused by a DHR. As mast cells and basophils are believed to be the cells primarily involved in anaphylaxis, several mast cell or basophil mediators in serum have been studied as markers for anaphylaxis elicited by different triggers including drugs, insect venoms and food. Histamine may be most important causing clinical reactions; however, the analysis of histamine levels is difficult and has practical problems. Mast cell tryptase has evolved clinically as the most practical biomarker of anaphylaxis, because of a commercially available assay. It remains unproven, if allergic and pseudoallergic DHRs differ in the type of mediators they predominantly release. Mast cell mediators such as tryptase and histamine, may be detectable both, after IgE-mediated allergic as well as after pseudoallergic reactions. However, an allergic DHR typically yields higher mediator levels and increased reaction severity.⁵⁷

There has been an interest in the detection of newer biomarkers in anaphylaxis; however, only preliminary experimental data is available for these and further studies are needed to confirm their validity.⁵⁸

4.1.- Tryptase

Tryptases, largely mast cell-specific serine-proteases, constitute a major part of pre-stored pro-inflammatory mediators of mast cells. They are either in mature monomer isoforms or mature heterotetramers.⁵⁹ Immature forms (pro- α tryptases) are constitutively secreted and reflect the concentration of “baseline tryptase in serum” (BST). Mature forms (pro- β tryptases) are stored in secretory mast cell granules and are released as a consequence of mast cell activation during the process of degranulation.⁶⁰ Mature beta-tryptase had previously been measured by an assay, which is not commercially available any longer (Pharmacia Tryptase RIACT) and had been increased only in a minority of patients with immediate DHRs.^{61, 62} To date, total tryptase (i.e. mature plus immature forms), can be measured by a widely commercially available assay (Phadia ThermoFisher, Uppsala, Sweden) in serum or other tissue fluids. Levels are a reflection of both, total mast cell number in the body plus mast cell activation levels.¹⁰ The assay is robust and the mediator in serum is stable at room temperature. The manufacturer gives a geometric mean of 3.8 $\mu\text{g/L}$ and an upper 95th percentile of 11.4 $\mu\text{g/L}$ for baseline serum tryptase levels (BST) in healthy individuals. This upper limit has been recently questioned, as levels above 8 $\mu\text{g/L}$ have been associated with persons with hereditary alpha-tryptasemia.⁶³ Furthermore, BST levels have been reported to be influenced by obesity, older age and decreased kidney function and are strongly elevated in mastocytosis and a few other less common diseases.⁶⁴⁻⁶⁸

4.1.1- Tryptase as marker for mast cell activation

Elevations of concentrations of tryptase are not a primary marker of an immediate DHR, but of a mast cell activation and degranulation, which can in part support this diagnosis. The half-

life of tryptase is 90 - 120 minutes.⁵⁸ Thus, it should be evaluated between 30 minutes and two hours after the onset of symptoms, ideally after both, 30 and 120 minutes. In earlier studies, total BST levels of >11.4 ng/ml, >12 ng/ml or >13.5 ng/ml have been set as cut-off values for a positive result (Tab.4).⁸ In more recent studies, either the changes of BST in serial measurements after the reaction have defined optimal cut-off points or values have been compared with BST concentrations. BST levels are stable in a given individual. In those patients with elevated BST, mastocytosis and other diseases leading to increased values should be ruled out.⁶⁷ To determine BST for comparison, either a recent value or a sample obtained at least 24 hours after the resolution of the symptoms are taken. The minimal elevation of the acute total tryptase level that is considered to be clinically significant is unknown, however, an increase of $\geq 20\%$ above baseline level plus 2 $\mu\text{g/l}$ during or within 4 hours after a symptomatic period has been suggested to be relevant in a consensus conference of experts.⁶⁸ This international consensus formula (elevated, if $\geq 1.2 \times [\text{basal tryptase value}] + 2 \mu\text{g/L}$) has been considered to be the best equation for detecting immediate DHR in terms of sensitivity and specificity.⁶⁹⁻⁷² In individual studies, some of them selecting their optimal cut-off point, the sensitivity of tryptase in DHR varies from 30-94.1% (Tab.4).^{8, 70, 73-83} It is strongly dependent on the cut-off value, which reciprocally influences specificity. The time interval between reaction and collection, the reaction severity, and possibly the mechanism (IgE-mediated versus non-allergic) influence the sensitivity (Tab. 4). The reported specificity is influenced by the cut-off value (52%-100%), but is mostly high. Serum tryptase increases mainly in more severe immediate-type reactions, such as anaphylaxis, whereas it was less helpful to differentiate mild allergic or non-allergic reactions from "hysterical reactions".⁸⁴ Furthermore, in perioperative anaphylactic shock, tryptase levels were even more elevated >13.5 $\mu\text{g/l}$ in 12/18 patients.⁷⁶ Serum tryptase measurements have been particularly helpful to confirm DHR in the controlled setting of perioperative drug reactions (Tab. 4). In patients with sequential serum measurements, levels were highest directly after the reaction, but remained increased after 3 hours and after 6 hours in 5/5 patients.⁷⁶

4.2.- Histamine and histamine metabolites

Histamine is probably biologically the most important mediator for immediate-type allergic reactions and anaphylaxis. It is derived from enzymatic processing of histidine by L-histidine decarboxylase. Large amounts of this compound are stored in granules of basophils and mast cells.⁶⁰ It fulfils its physiological functions in the organism through four different receptors. Histamine elicits the symptoms of an immediate allergic reaction through action via H1-receptors on end organs, such as vessels and bronchia.

Histamine levels in blood increase during anaphylaxis and still may be used as an experimental tool for diagnosis of a mast cell activation, e.g. indicating potential DHR. The sensitivity may even be somewhat higher than the sensitivity for tryptase and has been reported between 61 and 92% with a specificity from 51-91%.^{75, 78, 79, 85} The disadvantages of this method are that histamine levels show considerable variability among healthy individuals, show a pronounced circadian variation, have a very short half-life of about 10-30 minutes and that standardization of the assays is difficult, making this test primarily used for experimental studies.⁸⁶ Thus, blood should be collected within the first hour after the reaction and has to be permanently cooled and immediately processed. Increased histamine level must be compared with each patient baseline level and increases have to be interpreted taking into account a limited specificity due to a high inter-individual and intra-individual variability.⁸⁷ Elevated levels of histamine can be measured in the urine, however, local synthesis of histamine by bacteria in the urinary tract has been reported to influence the results. Thus, measurements of histamine metabolites, N-methylhistamine and N-methylimidazoleacetic acid, in urine in the hours after a reaction have become the preferred indirect methods, used only by a few centers worldwide.^{69, 88}

4.2.- Other potential biomarkers

Other biomarkers reported to increase concentrations after anaphylaxis, such as mast cell carboxypeptidase A3, chymase, mast cell cytokines, platelet-activating factor, CCL-2, prostaglandins or sulfidoleukotrienes in urine have reported increased concentrations in anaphylaxis as compared to controls in individual studies, but all still need further validation for DHR.^{58, 78, 89, 90 91-93} Biomarkers and mediators from other sources, such as blood eosinophilia or proteins of the eosinophil granule, e.g. eosinophil cationic protein (ECP) have been associated with aspects of allergic disease, such as aspirin-exacerbated respiratory disease.⁹⁴ However, for DHR, they are more likely to be a secondary bystander and data is scarce.⁹⁵ Fernandez found no clear increases for ECP at 2 or 24 hours after an immediate allergic reaction to drugs.⁶² Likewise, the histamine-degrading enzyme diamine oxidase (DAO) has been described to be elevated during severe anaphylaxis together with tryptase levels in two mastocytosis patients, but has not been associated with immediate DHR and results have to be confirmed in larger cohorts and by other researchers.⁹⁶

CONCLUSIONS

In vitro methods, such as measurement of sIgE and determination of mediators during an acute phase of an immediate DHR have the potential to support the diagnosis of a DHR, to confirm a culprit drug and to avoid DPT. They are free of risk for a patient. Serum can be frozen and stored for later use. Automated test systems do exist. However, in vitro test results should not be used in isolation to confirm or exclude the diagnosis, whereas they may give helpful information for decision making, which are interpreted together the clinical history and with available in vivo test results. There are several unmet needs in this area. Unfortunately, only few sIgE assays for drugs are available in routine diagnostics. Additionally, they generally have a low sensitivity depending on the drug involved that cannot be increased without reducing specificity. Good sensitivity has only been described for chlorhexidine, alpha-gal, latex and some non-commercially available NMBAs. Concerning biomarkers in the clinical routine to support the diagnosis of an acute event of anaphylaxis to

elicitors such as a drug, automated commercially available assays are primarily limited to tryptase measurement. The sensitivity of tryptase elevation for immediate DHRs is moderate and related to the cut-off level and to severity. The evidence for validity of all available in vitro tests is generally insufficient. Most data are old with questionable methodology and there is a lack of recent well-designed studies. Further studies are needed requiring multicenter studies with large numbers of well-phenotyped provocation-tested patients and controls to confirm the sensitivity, specificity, negative and positive predictive value of the described in vitro tests. For optimal validation, patients with a history for immediate DHR and positive provocation may be compared to those with negative provocation.

Figure legend

Figure 1. Application of in vitro tests in the diagnostic algorithm of drug hypersensitivity reactions (DHR). They may either support a diagnosis of an immediate DHR or supplement skin tests in detecting a sensitization to drugs. For high risk patients, those with severe reactions or if drug skin tests are not available or contraindicated, in vitro tests are performed before skin tests. Positive results have to be interpreted together with the history and all other available information.

REFERENCES

1. Demoly P, Adkinson NF, Brockow K, Castells M, Chiriac AM, Greenberger PA, et al. International Consensus on drug allergy. *Allergy* 2014; 69:420-37.
2. Romano A, Atanaskovic-Markovic M, Barbaud A, Bircher AJ, Brockow K, Caubet JC, et al. Towards a more precise diagnosis of hypersensitivity to beta-lactams - an EAACI position paper. *Allergy* 2019.
3. Torres MJ, Romano A, Mayorga C, Moya MC, Guzman AE, Reche M, et al. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy* 2001; 56:850-6.
4. Fernandez TD, Torres MJ, Blanca-Lopez N, Rodriguez-Bada JL, Gomez E, Canto G, et al. Negativization rates of IgE radioimmunoassay and basophil activation test in immediate reactions to penicillins. *Allergy* 2009; 64:242-8.
5. Brockow K. Triage Strategies for Clarifying Reported Beta-lactam Allergy. *J Allergy Clin Immunol Pract* 2019; 7:1066-7.
6. Querbach C, Biedermann T, Busch D, Eisenhardt-Kocher R, Feihl S, Filser C, et al. Suspected penicillin allergy: risk assessment using an algorithm as an antibiotic stewardship project. *Allergo J Int* 2020:174–80.
7. Brockow K, Przybilla B, Aberer W, Bircher AJ, Dreher R, Dickel H, et al. Guideline for the diagnosis of drug hypersensitivity reactions: S2K-Guideline of the German Society for Allergology and Clinical Immunology (DGAKI) and the German Dermatological Society (DDG) in collaboration with the Association of German Allergologists (AeDA), the German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Contact Dermatitis Research Group (DKG), the Swiss Society for Allergy and Immunology (SGAI), the Austrian Society for Allergology and Immunology (OGAI), the German Academy of Allergology and Environmental Medicine (DAAU), the German Center for Documentation of Severe Skin Reactions and the German Federal Institute for Drugs and Medical Products (BfArM). *Allergo J Int* 2015; 24:94-105.
8. Mayorga C, Celik G, Rouzaire P, Whitaker P, Bonadonna P, Rodrigues-Cernadas J, et al. In vitro tests for drug hypersensitivity reactions: an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy* 2016; 71:1103-34.
9. Mayorga C, Ebo DG, Lang DM, Pichler WJ, Sabato V, Park MA, et al. Controversies in drug allergy: In vitro testing. *J Allergy Clin Immunol* 2019; 143:56-65.
10. Brockow K, Behrendt H, Ring J. Diagnostischer Wert von Mastzellmediatoren bei der Anaphylaxie. *Allergologie* 2005; 28:125-9.
11. Brockow K. Drug Allergy: Definitions and Phenotypes. In: Khan DA, Banerji A, editors. *Drug Allergy Testing*. St. Louis, USA: Elsevier; 2018. p. 19-26.
12. Schnyder B, Brockow K. Pathogenesis of drug allergy--current concepts and recent insights. *Clin Exp Allergy* 2015; 45:1376-83.
13. Johansson SG. In vitro diagnosis of reagin-mediated allergic diseases. *Allergy* 1978; 33:292-8.
14. Decuyper, II, Mangodt EA, Van Gasse AL, Claesen K, Uyttebroek A, Faber M, et al. In Vitro Diagnosis of Immediate Drug Hypersensitivity Anno 2017: Potentials and Limitations. *Drugs R D* 2017; 17:265-78.
15. Garcia JJ, Blanca M, Moreno F, Vega JM, Mayorga C, Fernandez J, et al. Determination of IgE antibodies to the benzylpenicilloyl determinant: a comparison of the sensitivity and specificity of three radio allergo sorbent test methods. *J Clin Lab Anal* 1997; 11:251-7.

16. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002; 57:45-51.
17. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB, et al. Skin test concentrations for systemically administered drugs -- an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy* 2013; 68:702-12.
18. van der Poorten MM, Van Gasse AL, Hagendorens MM, Faber MA, De Puyseleir L, Elst J, et al. Serum specific IgE antibodies in immediate drug hypersensitivity. *Clin Chim Acta* 2020; 504:119-24.
19. Macy E, Goldberg B, Poon KY. Use of commercial anti-penicillin IgE fluorometric enzyme immunoassays to diagnose penicillin allergy. *Ann Allergy Asthma Immunol* 2010; 105:136-41.
20. 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.
21. Rouzair P, Proton G, Bienvenu F, Guilloux L, Benoit Y, Jiriau V, et al. IgE antibody detection in the diagnosis of hypersensitivity to neuromuscular blocking agents. *Acta Anaesthesiol Scand* 2012; 56:263-4.
22. Laroche D, Chollet-Martin S, Leturgie P, Malzac L, Vergnaud MC, Neukirch C, et al. Evaluation of a new routine diagnostic test for immunoglobulin E sensitization to neuromuscular blocking agents. *Anesthesiology* 2011; 114:91-7.
23. Ebo DG, Bridts CH, Hagendorens MM, Meertens CH, De Clerck LS, Stevens WJ. Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. *Allergy* 2006; 61:935-9.
24. Romano A, Gaeta F, Valluzzi RL, Zaffiro A, Caruso C, Quarantino D. Natural evolution of skin-test sensitivity in patients with IgE-mediated hypersensitivity to cephalosporins. *Allergy* 2014; 69:806-9.
25. Blanca M, Mayorga C, Torres MJ, Reche M, Moya MC, Rodriguez JL, et al. Clinical evaluation of Pharmacia CAP System RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. *Allergy* 2001; 56:862-70.
26. Sanz ML, Gamboa PM, Antepara I, Uasuf C, Vila L, Garcia-Aviles C, 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; 32:277-86.
27. Romano A, Gueant-Rodriguez RM, Viola M, Amoghly F, Gaeta F, Nicolas JP, et al. Diagnosing immediate reactions to cephalosporins. *Clin Exp Allergy* 2005; 35:1234-42.
28. Garcia-Aviles C, Sanz ML, Gamboa PM, Urrutia I, Antepara I, Jauregui I, et al. Antigen specific quantification of sulfidoleukotrienes in patients allergic to Betalactam antibiotics. *J Investig Allergol Clin Immunol* 2005; 15:37-45.
29. Fontaine C, Mayorga C, Bousquet PJ, Arnoux B, Torres MJ, Blanca M, et al. Relevance of the determination of serum-specific IgE antibodies in the diagnosis of immediate beta-lactam allergy. *Allergy* 2007; 62:47-52.
30. Vultaggio A, Matucci A, Virgili G, Rossi O, Fili L, Parronchi P, et al. Influence of total serum IgE levels on the in vitro detection of beta-lactams-specific IgE antibodies. *Clin Exp Allergy* 2009; 39:838-44.
31. De Week AL, Sanz ML, Gamboa PM, Aberer W, Sturm G, Bilo MB, et al. 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; 19:91-109.

32. Vultaggio A, Virgili G, Gaeta F, Romano A, Maggi E, Matucci A. High serum beta-lactams specific/total IgE ratio is associated with immediate reactions to beta-lactams antibiotics. *PLoS One* 2015; 10:e0121857.
33. Salas M, Fernandez-Santamaria R, Mayorga C, Barrionuevo E, Ariza A, Posadas T, et al. Use of the Basophil Activation Test May Reduce the Need for Drug Provocation in Amoxicillin-Clavulanic Allergy. *J Allergy Clin Immunol Pract* 2018; 6:1010-8 e2.
34. Van Gasse AL, Sabato V, Degerbeck F, DeWitt AM, Oulkadi R, Faber MA, et al. Specific IgE to cefazolin: Does it benefit diagnosis? *J Allergy Clin Immunol Pract* 2019; 7:2932-4.
35. Romano A, Mayorga C, Torres MJ, Artesani MC, Suau R, Sanchez F, et al. Immediate allergic reactions to cephalosporins: cross-reactivity and selective responses. *J Allergy Clin Immunol* 2000; 106:1177-83.
36. Antunez C, Blanca-Lopez N, Torres MJ, Mayorga C, Perez-Inestrosa E, Montanez MI, et al. Immediate allergic reactions to cephalosporins: evaluation of cross-reactivity with a panel of penicillins and cephalosporins. *J Allergy Clin Immunol* 2006; 117:404-10.
37. Johansson SG, Adedoyin J, van Hage M, Gronneberg P, Nopp A. False-positive penicillin immunoassay: an unnoticed common problem. *J Allergy Clin Immunol* 2013; 132:235-7.
38. Florvaag E, Johansson SG, Oman H, Venemalm L, Degerbeck F, Dybendal T, et al. Prevalence of IgE antibodies to morphine. Relation to the high and low incidences of NMBA anaphylaxis in Norway and Sweden, respectively. *Acta Anaesthesiol Scand* 2005; 49:437-44.
39. Rouzaire P, Nosbaum A, Denis L, Bienvenu F, Berard F, Cozon G, et al. Negativity of the basophil activation test in quinolone hypersensitivity: a breakthrough for provocation test decision-making. *Int Arch Allergy Immunol* 2012; 157:299-302.
40. Mata E, Gueant JL, Moneret-Vautrin DA, Bermejo N, Gerard P, Nicolas JP, et al. Clinical evaluation of in vitro leukocyte histamine release in allergy to muscle relaxant drugs. *Allergy* 1992; 47:471-6.
41. Guilloux L, Ricard-Blum S, Ville G, Motin J. A new radioimmunoassay using a commercially available solid support for the detection of IgE antibodies against muscle relaxants. *J Allergy Clin Immunol* 1992; 90:153-9.
42. Gueant JL, Mata E, Masson C, Gerard P, Moneret-Vautrin DA, Mouton-Faivre C, et al. Non-specific cross-reactivity of hydrophobic serum IgE to hydrophobic drugs. *Mol Immunol* 1995; 32:259-66.
43. Monneret G, Bencat Y, Debard AL, Gutowski MC, Topenot I, Bienvenu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002; 102:192-9.
44. Ebo DG, Venemalm L, Bridts CH, Degerbeck F, Hagberg H, De Clerck LS, et al. Immunoglobulin E antibodies to rocuronium: a new diagnostic tool. *Anesthesiology* 2007; 107:253-9.
45. Leysen J, Bridts CH, De Clerck LS, Vercauteren M, Lambert J, Weyler JJ, et al. Allergy to rocuronium: from clinical suspicion to correct diagnosis. *Allergy* 2011; 66:1014-9.
46. Uytendaele AP, Sabato V, Bridts CH, De Clerck LS, Ebo DG. Immunoglobulin E antibodies to atracurium: a new diagnostic tool? *Clin Exp Allergy* 2015; 45:485-7.
47. Fisher MM, Baldo BA. Immunoassays in the diagnosis of anaphylaxis to neuromuscular blocking drugs: the value of morphine for the detection of IgE antibodies in allergic subjects. *Anaesth Intensive Care* 2000; 28:167-70.
48. Garvey LH, Kroigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L, et al. IgE-mediated allergy to chlorhexidine. *J Allergy Clin Immunol* 2007; 120:409-15.

49. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose- α -1,3-galactose. *N Engl J Med* 2008; 358:1109-17.
50. Mariotte D, Dupont B, Gervais R, Galais MP, Laroche D, Tranchant A, et al. Anti-cetuximab IgE ELISA for identification of patients at a high risk of cetuximab-induced anaphylaxis. *MAbs* 2011; 3:396-401.
51. Manfredi M, Severino M, Testi S, Macchia D, Ermini G, Pichler WJ, et al. Detection of specific IgE to quinolones. *J Allergy Clin Immunol* 2004; 113:155-60.
52. Aranda A, Mayorga C, Ariza A, Dona I, Rosado A, Blanca-Lopez N, et al. In vitro evaluation of IgE-mediated hypersensitivity reactions to quinolones. *Allergy* 2011; 66:247-54.
53. Himly M, Jahn-Schmid B, Pittertschatscher K, Bohle B, Grubmayr K, Ferreira F, et al. IgE-mediated immediate-type hypersensitivity to the pyrazolone drug propyphenazone. *J Allergy Clin Immunol* 2003; 111:882-8.
54. Vultaggio A, Matucci A, Nencini F, Pratesi S, Parronchi P, Possi O, et al. Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions. *Allergy* 2010; 65:657-61.
55. Matucci A, Pratesi S, Petroni G, Nencini F, Virgili C, Milla M, et al. Allergological in vitro and in vivo evaluation of patients with hypersensitivity reactions to infliximab. *Clin Exp Allergy* 2013; 43:659-64.
56. Caiado J, Venemalm L, Pereira-Santos MC, Costa L, Barbosa MP, Castells M. Carboplatin-, oxaliplatin-, and cisplatin-specific IgE: cross-reactivity and value in the diagnosis of carboplatin and oxaliplatin allergy. *J Allergy Clin Immunol Pract* 2013; 1:494-500.
57. Clement O, et al. Immediate Hypersensitivity to contrast agents: the french Cirtaci study. *eClinical Medicine* 2018.
58. Beck SC, Wilding T, Buka RJ, Pareto RL, Huissoon AP, Krishna MT. Biomarkers in Human Anaphylaxis: A Critical Appraisal of Current Evidence and Perspectives. *Front Immunol* 2019; 10:494.
59. Vitte J. Human mast cell tryptase in biology and medicine. *Mol Immunol* 2015; 63:18-24.
60. Metcalfe DD, Pawankar R, Ackerman SJ, Akin C, Clayton F, Falcone FH, et al. Biomarkers of the involvement of mast cells, basophils and eosinophils in asthma and allergic diseases. *World Allergy Organ J* 2016; 9:7.
61. Ordoqui E, Zubeldia JM, Aranzabal A, Rubio M, Herrero T, Tornero P, et al. Serum tryptase levels in adverse drug reactions. *Allergy* 1997; 52:1102-5.
62. Fernandez J, Blanca M, Moreno F, Garcia J, Segurado E, del Cano A, et al. Role of tryptase, eosinophil cationic protein and histamine in immediate allergic reactions to drugs. *Int Arch Allergy Immunol* 1995; 107:160-2.
63. Lyons JJ. Hereditary Alpha Trypsinemia: Genotyping and Associated Clinical Features. *Immunol Allergy Clin North Am* 2018; 38:483-95.
64. Gonzalez-Quintela A, Vizcaino L, Gude F, Rey J, Meijide L, Fernandez-Merino C, et al. Factors influencing serum total tryptase concentrations in a general adult population. *Clin Chem Lab Med* 2010; 48:701-6.
65. Schliemann S, Seyfarth F, Hipler UC, Elsner P. Impact of age and heterophilic interference on the basal serum tryptase, a risk indication for anaphylaxis, in 1,092 dermatology patients. *Acta Derm Venereol* 2012; 92:484-9.
66. Sirvent AE, Gonzalez C, Enriquez R, Fernandez J, Millan I, Barber X, et al. Serum tryptase levels and markers of renal dysfunction in a population with chronic kidney disease. *J Nephrol* 2010; 23:282-90.

67. Valent P, Akin C, Escribano L, Fodinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. *Eur J Clin Invest* 2007; 37:435-53.
68. Valent P, Akin C, Arock M, Brockow K, Butterfield JH, Carter MC, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012; 157:215-25.
69. Egner W, Sargur R, Shrimpton A, York M, Green K. A 17-year experience in perioperative anaphylaxis 1998-2015: harmonizing optimal detection of mast cell mediator release. *Clin Exp Allergy* 2016; 46:1465-73.
70. Gastaminza G, Lafuente A, Goikoetxea MJ, D'Amelio CM, Bernad-Alonso A, Vega O, et al. Improvement of the Elevated Tryptase Criterion to Discriminate IgE- From Non-IgE-Mediated Allergic Reactions. *Anesth Analg* 2018; 127:414-9.
71. Vitte J, Amadei L, Gouitaa M, Mezouar S, Zieleskiewicz L, Albanese J, et al. Paired acute-baseline serum tryptase levels in perioperative anaphylaxis: An observational study. *Allergy* 2019; 74:1157-65.
72. Valent P, Bonadonna P, Hartmann K, Broesby-Olsen S, Brockow K, Butterfield JH, et al. Why the 20% + 2 Tryptase Formula Is a Diagnostic Gold Standard for Severe Systemic Mast Cell Activation and Mast Cell Activation Syndrome. *Int Arch Allergy Immunol* 2019; 180:44-51.
73. Passia E, Janduns P. Using Baseline and Peak Serum Tryptase Levels to Diagnose Anaphylaxis: a Review. *Clinical Reviews in Allergy & Immunology* 2020; 58:366-76.
74. Enrique E, Garcia-Ortega P, Sotorra O, Gieg P, Richart C. Usefulness of UniCAP-Tryptase fluoroimmunoassay in the diagnosis of anaphylaxis. *Allergy* 1999; 54:602-6.
75. Lin RY, Schwartz LB, Curry A, Tesola GR, Knight RJ, Lee HS, et al. Histamine and tryptase levels in patients with acute allergic reactions: An emergency department-based study. *J Allergy Clin Immunol* 2000; 106:65-71.
76. Dybendal T, Guttormsen AB, Elvay S, Askeland B, Harboe T, Florvaag E. Screening for mast cell tryptase and serum IgE antibodies in 18 patients with anaphylactic shock during general anaesthesia. *Acta Anaesthesiol Scand* 2003; 47:1211-8.
77. Malinovsky JM, Decagay S, Wessel F, Guilloux L, Mertes PM. Systematic follow-up increases incidence of anaphylaxis during adverse reactions in anesthetized patients. *Acta Anaesthesiol Scand* 2008; 52:175-81.
78. Stone SF, Coterelli C, Isbister GK, Holdgate A, Brown SG, Emergency Department Anaphylaxis I. Elevated serum cytokines during human anaphylaxis: Identification of potential mediators of acute allergic reactions. *J Allergy Clin Immunol* 2009; 124:786-92 e4.
79. Berroa F, Lafuente A, Javaloyes G, Ferrer M, Moncada R, Goikoetxea MJ, et al. The usefulness of plasma histamine and different tryptase cut-off points in the diagnosis of perianaesthetic hypersensitivity reactions. *Clin Exp Allergy* 2014; 44:270-7.
80. Krishna MT, York M, Chin T, Gnanakumaran G, Heslegrave J, Derbridge C, et al. Multi-centre retrospective analysis of anaphylaxis during general anaesthesia in the United Kingdom: aetiology and diagnostic performance of acute serum tryptase. *Clin Exp Immunol* 2014; 178:399-404.
81. Baretto RL, Beck S, Heslegrave J, Melchior C, Mohamed O, Ekbote A, et al. Validation of international consensus equation for acute serum total tryptase in mast cell activation: A perioperative perspective. *Allergy* 2017; 72:2031-4.
82. Egner W, Cook TM, Garcez T, Marinho S, Kemp H, Lucas DN, et al. Specialist perioperative allergy clinic services in the UK 2018: Results from the Royal College

- of Anaesthetists Sixth National Audit Project (NAP6) investigation of perioperative anaphylaxis. *Clin Exp Allergy* 2018; 48:846-61.
83. Sala-Cunill A, Cardona V, Labrador-Horrillo M, Luengo O, Estes O, Garriga T, et al. Usefulness and limitations of sequential serum tryptase for the diagnosis of anaphylaxis in 102 patients. *Int Arch Allergy Immunol* 2013; 160:192-9.
 84. Komericki P, Arbab E, Grims R, Kranke B, Aberer W. Tryptase as severity marker in drug provocation tests. *Int Arch Allergy Immunol* 2006; 140:164-9.
 85. Laroche D, Gomis P, Gallimidi E, Malinovsky JM, Mertes PM. Diagnostic value of histamine and tryptase concentrations in severe anaphylaxis with shock or cardiac arrest during anesthesia. *Anesthesiology* 2014; 121:272-9.
 86. Rehn D, Reimann HJ, von der Ohe M, Schmidt U, Schmel A, Hennings G. Biorhythmic changes of plasma histamine levels in healthy volunteers. *Agents Actions* 1987; 22:24-9.
 87. Hon YY, Jusko WJ, Zhou HH, Chen GL, Guo D, Zhou G, et al. Endogenous histamine and cortisol levels in subjects with different histamine N-methyltransferase C314T genotypes : a pilot study. *Mol Diagn Ther* 2008; 10:109-14.
 88. Keyzer JJ, de Monchy JG, van Doormaal JJ, van Vooijst Vader PC. Improved diagnosis of mastocytosis by measurement of urinary histamine metabolites. *N Engl J Med* 1983; 309:1603-5.
 89. Dass C, Eyck PT, Ballas Z, Lee S. Characterization of serum biomarkers during anaphylaxis in emergency department patients. *J Allergy Clin Immunol Pract* 2020; 8:3213-5 e1.
 90. Nassiri M, Eckermann O, Babina M, Edelhafer G, Worm M. Serum levels of 9alpha,11beta-PGF2 and cysteinyl leukotrienes are useful biomarkers of anaphylaxis. *J Allergy Clin Immunol* 2016; 137:312-4 e7.
 91. Baumann R, Untersmayr E, Zissler UM, Eyerich S, Adcock IM, Brockow K, et al. Non-invasive and minimally-invasive techniques for the diagnosis and management of allergic diseases. *Allergy* 2020.
 92. Simons FE. Anaphylaxis: Recent advances in assessment and treatment. *J Allergy Clin Immunol* 2009; 124:625-36; quiz 37-8.
 93. Vadas P, Perelman B, Less G. Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis. *J Allergy Clin Immunol* 2013; 131:144-9.
 94. Choi GS, Kim JH, Shin YS, Ye YM, Kim SH, Park HS. Eosinophil activation and novel mediators in the aspirin-induced nasal response in AERD. *Clin Exp Allergy* 2013; 43:730-40.
 95. Aihara Y, Ito S, Aihara M, Kobayashi Y, Yokota S. Different patterns of cytokines, ECP and immunoglobulin profiles at two adverse drug reactions in a patient. *Pediatr Int* 2005; 47:616-21.
 96. Boehm T, Reiter B, Ristl R, Petroczi K, Sperr W, Stimpfl T, et al. Massive release of the histamine-degrading enzyme diamine oxidase during severe anaphylaxis in mastocytosis patients. *Allergy* 2019; 74:583-93.

Table 1. Studies with specific IgE antibodies to betalactam antibiotics

Year	Drugs tested	Number (Pat./Contr.)	Method (CH/ST/DPT)	Assay	Time delay (days)	Sensitivity (%)	Specificity (%)	Reference
2000	CEPH, CEFAM, CEFTA, CEFT, CEFU, CEFO	30/0	CH/ST	RAST	371	CEPH: 30 PG: 10	NA	35
2001	PG, AX, CLV	74/55	CH/ST/DPT	CAP- FEIA	252	PG: 32; AX: 43; PG+AX: 50	BPO: 98; AXO: 98; BPO+AX O: 96	25
2001	PG, AX, AMP	290/120	CH/ST/DPT	CAP- FEIA	3670	PG: 10– 62; AX: 41–53	BPO: 95 AXO: 95	3
2002	PG, AX, Amp, CEFU, CEFZ	58/30	CH/ST	CAP- FEIA	NA	38	87	26
2005	CEFT, CEFO, CEFTA	76/0	CH/ST	CAP- FEIA, Seph RIA	725	74	NA	27
2005	AX, PG, CEFU, CEPHA	67/30	CH/ST/DPT	CAP- FEIA	NA	38	83	28
2006	CEFAC, CEFON, CEFO, CEFTA, CEFT, CEFU	51/NA	CH/ST/DPT	RAST	401.2	47	NA	36
2007	PG, AX, AMP, CEPH	30/15	CH/ST/DPT	CAP- FEIA RAST	999	CAP: 0–25 RAST: 43–75	CAP: 83– 100 RAST: 67–83	29
2009	PG, PV, AX, AMP	61/115	CH/ST	CAP- FEIA	96	85	54	30
2009	BP, AX, AMP, CEFs	178/81	CH/ST/DPT	CAP- FEIA	NA	28	87	31
2015	PG, PV, AX, AMP	171/122	CH/ST	CAP- FEIA	89.1	66	52	32
2018	Amoxicillin	57/28	CH/ST/DPT	CAP- FEIA	44%<1 82	19	NA	33
2019	Cefazolin	43/31	CH/ST/DPT	CAP- FEIA	NA	49	94	34

If not specified otherwise, sensitivity and specificity are calculated based on all drugs tested. Number (Pat./Contr.), number of patients and controls; CH, clinical history; ST, skin test; DPT, drug provocation test; RIA, radioimmunoassay; Seph-RIA, sepharose radioimmunoassay; CAP-FEIA, CAP fluoroimmunoassay; NA, not available; AX, amoxicillin; AMP: Ampicillin; CEFU: Cefuroxime; CEFZ: Cefazolin; CEFAC: Cefaclor; CEPH, Cephalothin; CEFAM, cefamandole; CEFTA, Ceftazidime; CEFT, Ceftriaxone; CEFO Cefotaxime; CEFZ, cefazolin; CEFs: Cephalosporins; CLV: Clavulanic acid; PG, penicillin G

Table 2. Studies with specific IgE antibodies to neuromuscular blocking agents

Year	Drugs tested	Number (Pat./Contr.)	Method (CH/ST/DPT)	Assay	Sensitivity (%)	Specificity (%)	Reference
1992	SUX, VEC, PAN, ALC, ATRA, GAL	40/44	CH, ST	Seph-RIA	83	100	40
1992	MOR, TMA, TEA, SUX, ALC	31/34	CH/ST	RAST (SUX, ALC) PAPPC RIA Seph-RIA	PAPPC RIA: 97 RIA: MOR 82 QAS 80, TMA 94, TEA 60 RAST SUX:70 RAST-ALC:39	PAPPC RIA: 97	41
1991	SUX, VEC, ALC, PAN, GAL	83/0	CH	Seph-RIA RAST	Seph-RIA: 88 RAST-SUX: 67 RAST-ALC: 41	NA	42
2002	ROC, SUX, ATRA	39/17	CH/ST	RIA	62	100	43
2005	MOR, SUX	65/801	CH	CAP-FEIA	Mor: 68 Sux: 39	Mor: 90-95 Sux: 96-100	38
2007	ROC	25/55	CH/ST	CAP-FEIA	ROC:92 SUX: 72 MOR: 88	ROC:93 SUX: 100 MOR: 100	44
2011	MOR	114/34	CH/ST	CAP-FEIA	84	91	22
2011	ROC	41/25	CH/ST	CAP-FEIA	83	72	45
2012	SUX, ROC, MOR	11/20	CH/ST	CAP-FEIA / RIA (PAPPC)	SUX:44; ROC:83; MOR: 78 PAP: 85	SUX: 100 ROC: 68 MOR: 85 PAP: 95	39
2015	ATRA	7/7	CH/ST	CAP-FEIA	57	100	46

If not specified otherwise, sensitivity and specificity are calculated based on all drugs tested. Time delays between reaction and test was not given in any of these studies. Number (Pat./Contr.), number of patients and controls; CH, clinical history; ST, skin test; DPT, drug provocation test; RIA, radioimmunoassay; Seph-RIA, sepharose radioimmunoassay; CAP-FEIA, CAP fluoroimmunoassay; NA, not available; ATRAC: Atracurium; ALC, Alcuronium; GAL: gallamine; MOR, Morphine; PAN: Pancuronium; PAPPC: p-aminophenyl phosphoryl choline; ROC: Rocuronium; SUX: Suxamethonium

Table 3. Studies with specific IgE antibodies to miscellaneous drugs

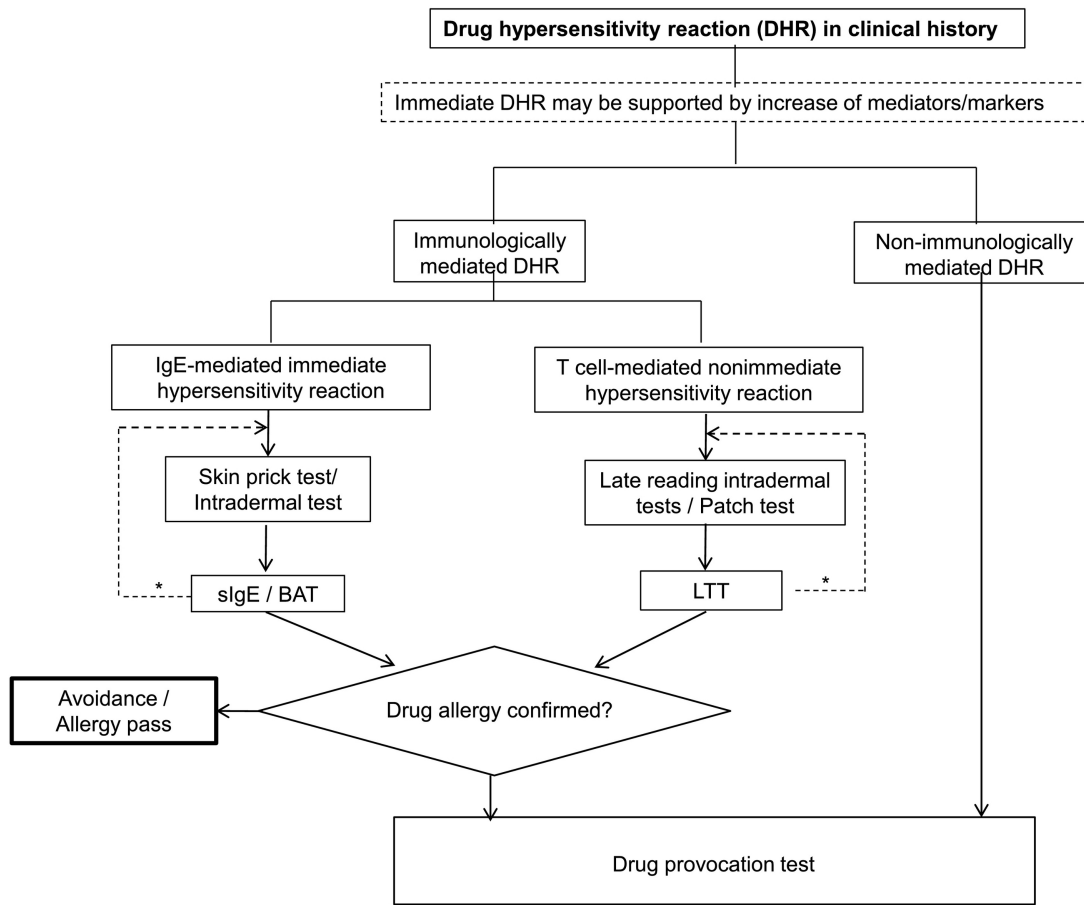
Year	Drugs tested	Number (Pat./Contr.)	Method (CH/ST/DPT)	Assay	Time delay (days)	Sensitivity (%)	Specificity (%)	Reference
2004	PIP, RUFL, PEFL, NALI, CINOX, LOME, CIPRO, NORF, OFLO	55/32	CH	Seph-RIA	371	55	100	51
2011	MOXI, CIPRO, LEVO	38/35	CH/DPT	Seph-RIA	120	32	100	52
2007	Chlorhexidine	12/10	CH/ST	CAP-FEIA	NA	92	100	48
2008	Propyphenazone	53/20	CH/ST	ELISA	652	58	NA	53
2010	Infliximab	11/20	CH	CAP-FEIA	NA	27	NA	54
2013	Infliximab	30/50	CH/ST	CAP-FEIA	NA	26	90	55
2008	Cetuximab	27/513	CH/DPT	CAP-FEIA	NA	68	98	49
2013	Cetuximab	14/195	CH	ELISA	1	71	82	50
2013	Carboplatin, Oxaliplatin	24/17	CH/ST	CAP-FEIA	NA	58/75	100/75	56

If not specified otherwise, sensitivity and specificity are calculated based on all drugs tested. Number (Pat./Contr.), number of patients and controls; CH, clinical history; ST, skin test; DPT, drug provocation test; RIA, radioimmunoassay; Seph-RIA, sepharose radioimmunoassay; CAP-FEIA, CAP fluoroimmunoassay; NA, not available; CINOX, cinoxacin; CIPRO: Ciprofloxacin; LEVO: Levofloxacin; LOME: Lomefloxacin; MOXI: Moxifloxacin; NALI, nalidixic acid; NORF: Norfloxacin; PEFL, Pefloxacin; OFLO, Ofloxacin; PIP, Pipemidic acid; RUFL, Rufloxacin

Table 4. Studies with total tryptase determination in the acute phase of an anaphylaxis including drug hypersensitivity reactions

Year	Number Patients	Setting / patients with drug as elicitors	Time blood sampling after reaction (hrs)	Cutoff Tryptase	Sensitivity (%)	Specificity (%)	Reference
1999	17	Emergency room Drugs in 7/16	<6	>8.23ng/ml	94	92	74
2000	66	Emergency room Drugs in 31/66	<12	>13.5ng/ml	30	NA	75
2003	18	Perioperative	3/6/24	>13.5ng/ml	67	100	76
2008	22	Perioperative	0.5-1	>12ng/ml >25ng/ml Tryptase/BST >3	64 41 62	100 100 83	77
2009	76	Emergency room Drugs in 26/76	<2	Increase >2ng/ml	75 severe, 57 moderate	NA	78
2013	102	Emergency room Drugs in 51/102	1-2/4-6/12-24	>11.4ng/ml	61.8	NA.	83
2014	37	Perioperative	<2	>11.4ng/ml	57 in skin test + 6 in skin test -	94	79
2014	161	Perioperative	Immediate/ 1-2	Peak tryptase and change from baseline	>15.7 ng/ml: 75 >25 ng/ml: 64 Increase > 24,6%: 94 Increase > 141%: 75	>15.7 ng/ml: 68 >25 ng/ml: 74 Increase > 24,6%: 53 Increase > 141%: 68	80
2017	82	Perioperative	Immediate/ 1-2	>1.2x BST+2 ng/ml	78	91	81
2018	266	Perioperative	Immediate / sequential < 6	Anaphylaxis with peak tryptase < 14 ng/ml 1.2xBST+2 ng/ml	< 14 ng/ml: 71 1.2xBST+2 ng/ml: 87 Tryptase dependent on drugs, severity, but not reaction speed and IgE mechanism	NA	82
2018	111	Emergency room, perioperative, during hospitalization Drugs in 61/111	Immediate +/- 2	1.2x BST +2 ng/ml	IgE-mediated: 69 Non-IgE-mediated: 20	NA	70
2019	85	Perioperative	0.5-2	>1.35xBST >1.2xsBST+2 ng/ml >sBT+3ng/ml >11.4 ng/ml	>1.35xBST: 81 >1.2xBST + 2 ng/ml: 75 >BST + 3 ng/ml: 72 >11.4 ng/ml: 53	>1.35xsBT: 52 >(1.2xsBT) + 2 ng/mL: 86 >sBT+3 ng/mL: 86 >11.4 ng/ml: 95	71

NA, not available; BST, baseline serum tryptase



*For high risk patients, those with severe reactions or if drug skin tests are not available or contraindicated, *in vitro* tests are performed before skin tests; slgE= specific IgE antibodies to drugs; BAT= basophil activation test; LTT= lymphocyte transformation test (mainly used for non-immediate reactions)

Figure 1