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**Quantification of specific IgE antibodies in immediate drug hypersensitivity: more shortcomings than potentials?**

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Running title: IgE in immediate drug hypersensitivity

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## **Abstract**

**Background:** For many physicians, quantification of serum drug-specific IgE (sIgE) antibodies constitutes the first measure in the diagnostic approach of immediate drug hypersensitivity reactions (IDHR).

**Aim:** To review the accuracy and limitations of the main drug-sIgE tests, especially those that are commercially available.

**Methods:** A literature search was conducted, using the key-words allergy, diagnosis, drugs, hypersensitivity, specific IgE antibodies; this was complemented by the authors' own experience.

**Results:** The drugs that have mostly been studied appeared to be  $\beta$ -lactam antibiotics, neuromuscular blocking agents (NMBA) and morphine, the latter as a biomarker for sensitisation to substituted ammonium structures that constitute the major epitope of NMBA. For  $\beta$ -lactams sensitivity and specificity varied between 0-85% and 52-100%, respectively. For NMBA, sensitivity and specificity varied between 38.5-92% and 92-100%, respectively. With respect to sIgE to morphine it appears this drug to be a sensitive biomarker for sensitisation to rocuronium and suxamethonium but not for atracurium. However, sIgE morphine should not be applied in isolation to diagnose IDHR to NMBA nor opiates.

**Conclusions:** Although drug-sIgE assay can provide valuable information they should not be performed in isolation to establish correct diagnosis, as their predictive value is not per se absolute. Larger comprehensive studies are urgently required to determine the accuracy of drug-sIgE assays.

## Introduction

Although controlled drug provocation tests (DPT) are considered as the absolute gold standard for correct diagnosis of immediate drug hypersensitivity reactions (IDHR), DPT entail a risk of severe, life-threatening complications and can even be contraindicated (*i.e.*, patients having suffered from extreme reactions, patients taking  $\beta$ -blockers or angiotensin-converting enzyme inhibitors) or not be possible for obvious reasons (*i.e.*, hypersensitivity to curarizing NMBA). Besides, DPT do not show absolute predictive values and yield false negative results (1). Consequently, hitherto, diagnostic DPT have mainly been applied for research purposes and have still not entered mainstream clinical practice. Therefore, diagnosis of IDHR generally starts with a thorough history complemented with skin tests or *in vitro* quantification of (commercially available) sIgE antibodies when an IgE-mediated mechanism with activation of mast cells and basophils is suspected. Unfortunately, only a few drug-sIgE assays are available, and most of them have not been thoroughly clinically validated. Starting from our clinical priorities and experience, the objective of this article is to review the literature on the value of commercially available drug-sIgE assays and serum tryptase in the diagnostic approach of IDHR. Emphasis is put on some misconceptions, shortcomings, and unmet needs. As with any subject still beset by many questions, alternative interpretations, hypotheses, or explanations expressed here may not find universal acceptance.

## Quantification of serum tryptase

Although quantification of acute and baseline serum tryptase cannot be considered as confirmatory testing, serum tryptase has proven to be relevant in the diagnostic management of IDHR, mainly to confirm mast cell activation and to rule out or confirm (clonal) mast cell disorders (2) and mast cell activation syndromes (3). Today, one assay that quantifies total serum tryptase as the sum of continuously secreted baseline tryptase and  $\beta$ -tryptase only released from degranulating mast cells is commercially available (ImmunoCAP Thermofisher, Uppsala, Sweden). Although its recommended threshold is 11.4  $\mu\text{g/L}$ , it has been suggested to abandon this absolute decision cut-off, mainly as relevant increases have been observed well below this cut-off (4, 5). Moreover, recently a new algorithm for interpretation of serum tryptase has been proposed in which the acute level (30-240 minutes after the event) is considered significant when it exceeds  $1.2 \times \text{baseline} + 2$  (baseline sample is drawn 24h after the acute event) (6). With this approach anaphylaxis can be confirmed for values lower than 11.4  $\mu\text{g/L}$ . Importantly as recently suggested, the sensitivity of this approach is higher if basal (post-reaction) levels is obtained within 2 months from the acute event (7). Alternatively, by comparing the two measurements anaphylaxis could be ruled out even for acute tryptase values  $> 11.4 \mu\text{g/L}$ , as underlying diseases such as chronic renal failure, might induce hypertryptasemia (6).

The importance of baseline tryptase extends beyond ensuring a return to baseline after a mast cell-mediated IDHR as elevated baseline levels might be indicative for underlying (clonal) mast cell disorders (2). Note that in patients suffering from severe IDHR with hypotension without urticaria and angioedema a mast cell disorder has to be ruled out, particularly in men (8). Levels of mature tryptase > 1 µg/L indicate mast cell degranulation. However this test is not commercially available.

### **Quantification of drug sIgE antibodies**

IgE was discovered in 1967 as the “reagine” responsible for so-called type I hypersensitivity reactions (9, 10). Approximately 5 years later the first *in vitro* assay for serum sIgE called the radioallergosorbent test (RAST) was developed and commercialised. The original RAST was configured as a cyanogen-bromide activated paper disc on which native allergen extracts were covalently coupled. In this assay sIgE antibodies that complex with the allergen are quantified with radio-iodinated polyclonal antihuman IgE antibodies using a  $\gamma$ -counter (11). Today, traditional RAST has been almost completely abandoned and quantification of drug-sIgE antibodies generally relies upon quantification of a drug-(hapten)-carrier antibody complex in which the secondary antihuman IgE is not conjugated to a radioisotope anymore but coupled to an enzyme with colorimetric reading in the enzyme linked immunosorbent test (ELISA) or with a fluorescence reading in the fluorescent enzyme immunoassay (FEIA) (12). However, unlike protein allergens, for routine application of immunoassays to drug sIgE antibodies, the current situation is characterized by a limited availability of well-validated drug-specific tests. The only drug-sIgE assays that are commercially available from Thermofisher are penicilloyl G (c1), penicilloyl V (c2), ampicilloyl (c5) and amoxicilloyl (c6) determinants, cephaclor (c7), chlorhexidine (c8, antiseptic), chymopapain (c209), (bovine) gelatin (c74), human (c73), bovine (c71) and porcine (c70) insulin, morphine (c260, marker for sensitization to tertiary and quaternary substituted ammonium determinants), pholcodine (c261) and suxamethonium (c202). In addition, and for research purposes only, adrenocorticotrophic hormone, atracurium, bacitracin, carboplatin, cefamandole, ceftioxin, cefotaxime, cefuroxime, cisplatin, mepivacaine, methylprednisolone-21-succinate, nafamostat (4-guanidinobenzoic acid), oxaliplatin, penicillin minor determinants (*e.g.*, penicillanyl), pholcodine, propylphenazone, protamine, rocuronium, and tetanus toxoid are offered via the Thermofisher Scientific special allergen service. However, hitherto, most of these assays have not been sufficiently clinically validated, mainly as a result of the unavailability of sufficient numbers of accurately phenotyped patients and exposed control individuals.

*$\beta$ -lactam antibiotics*

Probably the one of the most studied sIgE tests are those for  $\beta$ -lactam antibiotics, especially amoxicillin and benzyl penicilloyl. The most used antibiotics of this class in clinical practice consist of monocyclic molecules, such as monobactams, and bicyclic molecules, such as penicillins and clavams, cephalosporins, and carbapenems. Although, as shown in table 1, sIgE assays for  $\beta$ -lactams usually show a low sensitivity, with decreasing sensitivity over time (13), several cases of positive sIgE results in IDHR with negative skin tests have been described (14-18). Besides these disappointing sensitivity data, there is also increasing and significant evidence upon the low specificity of the tests (15, 17, 19-22). In some studies false positivity could have resulted from nonspecific binding in the solid phase assay as a result of elevated total IgE titers (20-23). However, Johansson *et al* (23) found that 26% of the patients with a positive sIgE for penicillin have clinically irrelevant sIgE antibodies to phenylethylamine (PEA) and that these anti-PEA antibodies test negative in a basophil activation assay. In summary, sIgE antibodies to  $\beta$ -lactams seem of limited value and should not be used in isolation to diagnose IDHR to these antibiotics and should, in order to avoid misdiagnosis, be supplemented with basophil activation tests (BAT), skin testing and, where appropriate a DPT (24, 25). For a recent review on the potential and limitations of BAT in IDHR the reviewer is referred elsewhere (26).

#### *Neuromuscular blocking agents*

Curarizing NMBA constitute a major cause of perioperative anaphylaxis (27-31). DPT with NMBA not being an option for obvious reasons, skin tests are the primary diagnostic method to document IDHR to NMBA (32). However, their predictive value is not absolute thus leaving room for additional *in vitro* test. As displayed in table 2, in the absence of readily available assays, for about 2 decades, several groups have tried to determine the accuracy of various home-made NMBA-sIgE assays (33-36). Today, IDHR to NMBA is generally serologically assessed indirectly through various methods that measure IgE reactivity to tertiary and quaternary ammonium structures that have been demonstrated the major epitopes of NMBA (37, 38). Most frequently, applied methods are a choline chloride (33, 34, 39-44), a p-aminophenyl phosphoryl choline (PAPPC) (33, 39, 40, 45) and/or morphine-based assays (33-35, 45-50). With respect to the ImmunoCAP FEIA suxamethonium, rocuronium, atracurium and morphine, all available from Phadia Thermofisher it seems that sensitivity and specificity for the individual NMBA-specific varies between 38.5-92% and 85.7-100%, respectively. Alternatively, it appears that a morphine-based immuno assay constitutes a valuable test for the detection of suxamethonium and rocuronium-reactive antibodies but not to depict atracurium-reactive antibodies (46, 48). Note, however, that IgE reactivity to tertiary and quaternary substituted ammonium structures is prevalent in the general population (47, 48, 50) and can therefore not be used as a screening technique to identify patients at risk or in isolation to document

NMBA hypersensitivity (51, 52). Most important explanations for these false-positive sIgE results are an elevated total IgE (48) and intake of the opiate antitussive pholcodine (53).

### *Opiates*

Despite their frequent and ubiquitous use, genuine IgE-mediated allergy to opiates (morphine, codein, pholcodine and heroin) remains exceedingly rare. Also, correct diagnosis is not straightforward, mainly because of uncertainties associated with measurement of drug-specific IgE antibodies and skin testing (54). Recently, it has been suggested that the two commercially available sIgE assays for a *Papaver somniferum* (poppy seed) extract and morphine would be reliable in the diagnosis of IgE-mediated opiate allergy (55, 56). However, using DPT we were unable to confirm these data (57), mainly because the high prevalence of sIgE antibodies to these compounds in an allergic population. This observation is highly relevant when facing patients for whom correct identification of the causative drug is impeded because of simultaneous intake or administration of different compounds, *e.g.*, during general anaesthesia. Erroneous opiate allergy diagnosis might not only entail unnecessary avoidance measures but also, most importantly, ultimately put patients at risk by overlooking alternative diagnoses such as an allergy to rocuronium or suxamethonium. For the time being the sole *in vitro* method to document opiate allergy are basophil activation tests (BAT), as these cells, unlike cutaneous mast cells, are unresponsive to opiates (58). Moreover, negative BAT, along with negative skin testing for different NMBA and negative provocation tests for the structurally almost similar opiates suggest these drugs probably to be safe in pholcodine hypersensitivity (58).

### *Chlorhexidine*

Chlorhexidine, a cationic bisguanide antiseptic and disinfectant, is used as the (di)acetate or (di)glucuronide salt. These chlorhexidine salts can trigger irritant dermatitis, allergic contact dermatitis (59), IDHR (including life-threatening anaphylaxis) (60-63) and even a combination of both, contact dermatitis and IDHR (64). For a traditional arbitrarily chosen decision threshold of 0.35 kUA/L, the sensitivity of sIgE chlorhexidine varied between 84.2-91.6% and specificity between 93.7-100%. For a ROC-generated threshold of 0.20 kUA/L sensitivity was 94.1% and specificity 90.7% (62, 63). Like for  $\beta$ -lactam (21-23) and NMBA (48), raised total IgE levels were shown to have an impact on chlorhexidine sIgE measurement at levels higher than 500 kU/L and more particularly at levels higher than 2,000 kU/L (63). Recently, it was demonstrated the optimal sampling time for sIgE chlorhexidine to be between 1 and 4 months (65), but sIgE might persist for years (29).

### *Miscellaneous*

Many (severe) IDHR occur in hospital settings, especially in the perioperative period. Besides NMBA,  $\beta$ -lactam antibiotics and chlorhexidine these reactions can be related to other drugs and related compounds such as plasma substitutes and *Hevea* latex for which sIgE assays are also readily available. Discussing the accuracy of the different sIgE assays for *Hevea* latex extends beyond the scope of this review. Briefly, it is clear that correct diagnosis of IgE-mediated allergy to natural latex is not always straightforward, mainly because of the false-positive sIgE results (66, 67), especially in patients suffering from grass and weed pollen allergy who are sensitized to cross-reactive carbohydrate determinants and/or profilin (68-72). Therefore, in a significant number of patients additional tests such as skin tests, component resolved diagnosis (69-72) and eventually BAT (67, 73-76) might be required to establish correct diagnosis. For a review on component resolved diagnosis the reader is referred elsewhere (77).

Another compound that merits our attention is bovine gelatin, as this compound constitutes the active component in certain plasma substitutes, haemostatic sponges and can be present in various other drugs such as vaccines. Since the first descriptions of the allergenicity of gelatin (78), IgE-mediated IDHR to this compound, including fatal anaphylaxis, have been increasingly reported. Today, 2 distinct types of IgE-mediated bovine gelatin allergy are recognized. First, genuine gelatine allergy that results from sensitization to the protein part of the molecule. Second, gelatin allergy resulting from a sensitization to a glycan moiety of the molecule, *i.e.* galactose- $\alpha$ (1,3)-galactose ( $\alpha$ -gal) (79-81), as first described by Chung *et al* (82) and Commins *et al* (83). To our knowledge, there are no studies that have determined the diagnostic accuracy of sIgE gelatin. However, it is of note that patients with life-threatening anaphylaxis to gelatin as a result of  $\alpha$ -gal sensitization are generally

overlooked by traditional gelatin-sIgE assay and need additional testing including quantification of  $\alpha$ -gal specific IgE antibodies and gelatin skin testing (79-81).

### **Summary**

From this review it appears that drug-sIgE antibody testing can provide useful information but can rarely be applied in isolation to exclude or document IDHR, as they lack absolute predictive values. For  $\beta$ -lactam determinants the main issue is low sensitivity, sensitivity which could not be increased without significant loss of specificity (21). For NMBA, accuracy of the tests seem divergent. However, in general NMBA and chlorhexidine-specific assays attain acceptable sensitivity and specificity, especially when drug-specific cut-offs are applied (48, 63). Although quantification of sIgE to morphine appears as a reliable biomarker of sensitization to tertiary and quaternary ammonium structures, IgE reactivity to this compound in general and allergic population is as high as 5-10%. Therefore, the test should not be applied in isolation to diagnose IDHR to NMBA or opiates.

With respect to the low sensitivity of some tests it has been argued this observation to relate upon the time-interval elapsed between the acute reaction and testing. Although we agree that late testing can result in lower sensitivity we do not adhere to the recommendation of the ENDA/EAACI Drug Allergy Interest Group. Based upon a single publication about negatization of sIgE to  $\beta$ -lactam antibiotics (13), in their Position Paper (84) further use of drug-sIgE is dissuaded when the time-interval exceeds 3 years. However, this is not our experience (51) and drug-sIgE may persist as long as 5-30 years (85, 86). With respect to the low specificity of some tests it is reemphasized that correct interpretation of sIgE results requires taking into account total IgE values (21, 48, 63). Whether the introduction of sIgE/total IgE ratio's increases specificity (21) remains to be confirmed.

Table 1: $\beta$ -lactams (updated from Ebo D <i>et al.</i> (87))						
Compound	Reference test	Assay	Sensitivity	Specificity	N	Reference
Various $\beta$ -lactams	H + ST	CAP-FEIA	BPO + AXO + peni G + AMP: 31.8%	BPO + AXO + peni G + AMP: 88.6%	58	(88)
Various $\beta$ -lactams	H $\pm$ ST $\pm$ DPT	CAP-FEIA	BPO: 32% AXO: 43% BPO+AXO: 50%	BPO: 98% AXO: 98% BPO+AXO: 96%	129	(89)
Various $\beta$ -lactams	H $\pm$ ST $\pm$ DPT	CAP-FEIA	BPO: 10-68% AXO: 41-53%	BPO: 98% AXO: 95%	410	(18)
Various $\beta$ -lactams	H	CAP-FEIA	37.9%	86.7%	58	(90)
Various $\beta$ -lactams <sup>1</sup>	H $\pm$ ST $\pm$ DPT	CAP-FEIA RAST <sup>2</sup>	0-25% <sup>2</sup> 42.9-75% <sup>2</sup>	83.3-100% <sup>2</sup> 66.7-83.3% <sup>2</sup>	45	(14)
Various $\beta$ -lactams	H $\pm$ ST	CAP-FEIA CAP-FEIA	85% <sup>3</sup> 44% <sup>4</sup>	54% <sup>3</sup> 80% <sup>4</sup>	176	(21)
Various $\beta$ -lactams	H $\pm$ ST	CAP-FEIA	66%	52%	293	(22)

<sup>1</sup> home-made assay, <sup>2</sup> sensitivity and specificity vary according to clinical manifestations, <sup>3</sup> for threshold 0.10 kUA/L, <sup>4</sup> for threshold 0.35 kUA/L.  
H: history, ST: skin test, DPT: drug provocation test, N: number  
CAP-FEIA: fluorescence enzyme immunoassay available from Phadia Thermofisher. RAST: radio allerge sorbent test.  
Peni G: penicillin G, AMP: ampicillin, BPO: benzyl penicilloyl, AXO: amoxicillin

Table 2: Curarizing neuromuscular blocking agents (NMBA) (updated from Ebo D <i>et al.</i> (87))						
Compound	Reference test	Assay	Sensitivity	Specificity	N	Reference
Various NMBA	H ± ST	RIA RIA RIA	PAPPC: 97% MOR: 83% QAS: 86%	PAPPC: 97% NA NA	75	(33)
Various NMBA	H + ST	RIA RAST RAST	QAS: 87.9% SUC: 66.7% Alcuronium: 40.7%	NA	83	(34)
Various NMBA	H + ST	RIA RIA	MOR: 85% NMBA-specific: 52%	98%	118	(46)
Various NMBA	H + ST	CAP-FEIA CAP-FEIA	SUXA: 38.5% MOR: 67.7%	SUX: 96.3-99.6% MOR: 90-95%	866	(47)
Rocuronium <sup>1</sup>	H + ST	CAP-FEIA	SUXA: 72% <sup>2</sup> SUXA: 60% <sup>3</sup> ROCU: 92% <sup>2</sup> ROCU: 68% <sup>3</sup> MOR: 88% PHOL: 86%	SUXA: 100% <sup>2</sup> SUXA: 100% <sup>3</sup> ROCU: 93% <sup>2</sup> ROCU: 93% <sup>3</sup> MOR: 100% PHOL: 100%	82	(48)
Various NMBA <sup>1</sup>	H + ST	CAP-FEIA	QAM <sup>4</sup> : 87.7%	QAM <sup>4</sup> : 90.7%	168	(50)
Atracurium <sup>1</sup>	H + ST	CAP-FEIA	SUXA: 28.6% ATRA: 57.1% MOR: 14.2%	SUXA: 85.7% ATRA: 100% MOR: 85.7%	78	(49)

<sup>1</sup> applying ROC-generated drug-specific thresholds, <sup>2</sup> for a ROC-generated threshold of 0.11 kUA/L for suxamethonium and 0.13 kUA/L for rocuronium, <sup>3</sup> for a traditional threshold of 0.35 kUA/L.  
<sup>4</sup>“optimized” morphine-based assay  
H: history, ST: skin tests, RIA: radio immunoassay, RAST: radio allergosorbent test, CAP-FEIA: fluorescence enzyme immunoassay available from Phadia Thermofisher, PAPPC: p-aminophenyl phosphoryl choline, MOR: morphine, QAS: quaternary ammonium structure, SUC: succinyl choline, SUX: suxamethonium, ROCU: rocuronium, QAM: quaternary ammonium morphine, ATRA: atracurium. N: number. NA: not available.

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