Drugs R D (2017) 17:265–278
DOI 10.1007/s40268-017-0176-x

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REVIEW ARTICLE

In Vitro Diagnosis of Immediate Drug Hypersensitivity Anno 2017: Potentials and Limitations

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Published online: 4 March 2017
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Abstract

Background For most physicians, quantification of drug-specific immunoglobulin E (drug-sIgE) antibodies constitutes the primary in vitro measure to document immediate drug hypersensitivity reactions (IDHR). Unfortunately, this is often insufficient to correctly identify patients with IgE-mediated IDHR and impossible for non-IgE-mediated IDHR that result from alternative routes of basophil and mast cell activation. In these difficult cases, diagnosis might benefit from cellular tests such as basophil activation tests (BAT).

Aim The aim was to review the potential and limitations of quantification of sIgE and BAT in diagnosing IDHR. The utility of quantification of serum tryptase is discussed.

Methods A literature search was conducted using the key words allergy, basophil activation, CD63, CD203c, diagnosis, drugs, hypersensitivity, flow cytometry, specific IgE antibodies; this was complemented by the authors' own experience.

Results The drugs that have been most studied with both techniques are β-lactam antibiotics and curarizing neuromuscular blocking agents (NMBA). For sIgE morphine, data are available on the value of this test as a biomarker for sensitization to substituted ammonium structures that constitute the major epitope of NMBA, especially rocuronium and suxamethonium. For the BAT, there are also data on non-steroidal anti-inflammatory drugs (NSAIDs) and iodinated radiocontrast media. For β-lactam antibiotics, sensitivity and specificity of sIgE varies between 0 and 85% and 52 and 100%, respectively. For NMBA, sensitivity and specificity varies between 38.5 and 92% and 85.7 and 100%, respectively. Specific IgE to morphine should not be used in isolation to diagnose IDHR to NMBA nor opiates. For the BAT, sensitivity generally varies between 50 and 60%, whereas specificity attains 80%, except for quinolones and NSAIDs.

Conclusions Although drug-sIgE assays and BAT can provide useful information in the diagnosis of IDHR, their predictive value is not absolute. Large-scale collaborative studies are mandatory to harmonize and optimize test protocols and to establish drug-specific decision thresholds.

Key Points

Although drug provocation tests are considered the gold standard for immediate drug hypersensitivity reactions, their entrance in mainstream application is severely hampered for obvious ethical reasons.

Although drug-specific immunoglobulin E antibody assays and basophil activation tests can add to the diagnosis of immediate drug hypersensitivity reactions, their predictive value for a future clinical outcome is not absolute.

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1 Introduction

The gold standard for correct diagnosis of immediate drug hypersensitivity reactions (IDHR) are controlled drug provocation tests (DPT) with the culprit compound(s). However, DPT entail a considerable risk of severe, life-threatening complications and can simply be contraindicated (i.e. in patients having already suffered from life-threatening reactions and patients taking β-blockers or angiotensin-converting enzyme inhibitors) or impossible for obvious reasons [i.e. hypersensitivity to curarizing neuromuscular blocking agents (NMBA)]. Moreover, DPT do not show absolute predictive values and might yield false negative results [1]. Consequently, diagnostic DPT are still mainly confined to research settings. As a result, a diagnostic workup for IDHR comprises a thorough history complemented with skin tests and/or in vitro quantification of (commercially available) specific immunoglobulin E (sIgE) antibodies when an IgE-mediated mechanism with activation of mast cells and basophils is suspected. Unfortunately, only a few drug-specific IgE (drug-sIgE) assays are available, and most of them have not been thoroughly validated. Furthermore, IDHR might not per se involve IgE/high-affinity IgE receptor (FcεRI)-cross-linking, but may also result from alternative pathways, such as a ligation of the Mas-related G-protein receptor MRGPRX2 [2, 3], that cannot be detected by an sIgE antibody assay. The development and validation of cellular tests such as basophil activation tests (BAT) might, somewhat, hold promise in such cases. Starting from our clinical priorities and expertise, the objective of this manuscript is to review the literature on the value of serum tryptase, commercially available drug-sIgE assays and BAT in the diagnosis of IDHR. Emphasis is put on some particular 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.

2 Principles of Quantification of Drug-Specific Immunoglobulin E Antibodies and Basophil Activation Tests

IgE antibodies were discovered in 1967 as the 'reagines' responsible for so-called type I hypersensitivity reactions [4, 5]. Five years later, the first in vitro assay for serum sIgE antibodies, the so-called radio allergosorbent test (RAST), was developed and commercialized. The original RAST was designed as a cyanogen-bromide activated paper disc, on which native allergen extracts were covalently coupled and sIgE antibodies that bind with the allergen were quantified with radio-iodinated polyclonal antihuman IgE antibodies using a γ-counter [6]. At present, quantification of drug-sIgE antibodies predominantly relies upon quantification of a drug-(hapten)-carrier antibody complex in which the secondary antihuman IgE is conjugated to an enzyme with colorimetric reading in the enzyme-linked immunosorbent test (ELISA) or with a fluorescence reading in the fluorescent enzyme immunoassay (FEIA) [7]. However, unlike protein allergens, only a limited number of drug-specific immunoassays are available. The only drug-sIgE assays that are currently commercially available from Thermo Fisher are penicilloyl G, penicilloyl V, ampicilloyl and amoxicilloyl determinants, cephaclor, the antiseptic chlorhexidine, chymopapain, bovine gelatin, human, bovine and porcine insulin, morphine (marker for sensitization to tertiary and quaternary substituted ammonium determinants), pholcodine and suxamethonium. For research purposes only, additional assays such as adrenocorticotropic hormone, atracurium, bacitracin, carboplatin, cefamandole, cefoxitin, cefotaxime, cefuroxime, cisplatinum, mepivacaine, methylprednisolone-21-succinate, nafamostat (4-guanidinobenzoic acid), oxaliplatin, penicillin minor determinants (e.g. penicillanyl), propyphenazone, protamine, rocuronium, and tetanus toxoid are offered via the Thermo Fisher Scientific special allergen service. However, most of these assays have not been thoroughly validated, mainly as a result of the unavailability of sufficient numbers of accurately phenotyped patients and exposed or challenged control individuals.

Basophils represent less than 1% of the peripheral blood leukocytes. Basophils develop from CD34+ pluripotent progenitor stem cells, exhibit a segmented nucleus and are identifiable by metachromatic staining with basic dyes (e.g. toluidine blue). Like tissue resident mast cells, basophils can be triggered by IgE-dependent and various IgE-independent ways. Cross-linking of the surface-bound FcεRI generally occurs through (glyco)proteins, chemical allergens or auto-antibodies directed against the FcεRI receptor or membrane-bound IgE antibodies. If not IgE-dependent, activation will mainly result from coupling of receptors with endogenous (e.g. cytokines, anaphylatoxins, chemokines, IgG, neuropeptides) or exogenous (e.g. pathogen-associated molecular patterns) elements. Recently, McNeil et al. [2] described the potential of MRGPRX2-related mast cell activation by various drugs containing a tetrahydroisoquinoline (THIQ) motif, such as some quinolones and NMBA. Alternatively, other largely unknown pathways (e.g. direct mast cell degranulation by opiates, iodinated contrast media and vancomycin) might also induce degranulation of basophils and mast cells. Upon activation, basophils and mast cells will release a myriad of mediators that are responsible for the early and

late phase manifestations of the immediate allergic reaction.

The foundations of current flow-assisted BAT were laid 25 years ago [8], and in the meantime, the technique has largely supplanted older mediator release assays that rely upon difficult quantification of mediators released in the supernatant. The technical principles and requirements of BAT have been detailed elsewhere [9]. Traditional BAT relies upon a flow cytometric analysis of various activation and degranulation markers on the surface membrane. These changes can be detected and quantified on a single-cell level using specific monoclonal antibodies conjugated with different LASER-excitable fluorochromes. For example, basophils are traditionally identified by markers such as CCR3 (CD193)/CD3, CD123/HLA-DR or IgE/CD203c. Of these markers, only CD203c, the ectonucleotide pyrophosphatase/phosphodiesterase family member 3 enzyme (E-NPP3), is lineage specific. After activation, the appearance or up-regulation of surface activation and/or degranulation markers, such as CD63 and/or CD203c, is quantified [9–14]. Although there is controversy about CD63 being the optimal readout for basophil activation [15], it is of note that for the time being, only the appearance of CD63 seems to reflect anaphylactic degranulation with significant release of histamine [16] (see also Fig. 1).

Alternative methods to measure basophil activation imply quantification of surface inhibitory receptor CD300a expression [17] and phosphorylation of signalling molecules such as p38 mitogen-activated protein kinase (MAPK) [18] and signal transducer and activator of transcription (STAT) 5 [19]. In addition, it was demonstrated that histamine release can also be quantified by flow cytometry. In this technique, designated as HistaFlow, the intracellular content of histamine and its release are analysed by an enzyme affinity method using the histaminase diamine oxidase [20]. Figures 1 and 2 show a representative HistaFlow dotplot of a cefazolin- and pholcodine-reactive patient, respectively. Note the specificity of the test, as basophils of the pholcodine allergic patient do not respond to structurally similar opiates, i.e. morphine and codeine, that are tolerated by the patient.

3 β-Lactam Antibiotics

The most studied sIgE assays are those for β-lactam antibiotics, especially amoxicillin and benzyl penicilloyl. Although, several cases of positive sIgE results in IDHR with negative skin tests have been described [21–25], sIgE assays for β-lactams generally show a low sensitivity that decreases over time [26], as is shown in Table 1. In contrast, specificity generally appears to be high, but in some studies, disappointing specificity data have been observed [22, 24, 27–30]. In some studies, false positivity could have resulted from nonspecific binding in the solid phase assay as a result of elevated total IgE titres [28–31]. However, Johansson et al. [31] 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 β-lactams seem of limited value and should not be used in isolation to diagnose IDHR to these antibiotics. In order to avoid misdiagnosis, these assays should be complemented with BAT, skin testing and, where appropriate, a DPT [32, 33]. Table 2 summarizes the data of BAT in IDHR to β-lactams. Hitherto, ten studies have investigated the BAT as a diagnostic in IDHR to β-lactam antibiotics, mainly to amoxicillin. Compared with the quantification of sIgE antibodies, BAT shows a comparable sensitivity and specificity. As for sIgE, sensitivity of BAT to β-lactams is rather low and decreases over time [26].

4 Quinolones

IDHR to quinolones constitute a difficult pathomechanistic conundrum and pose a significant diagnostic challenge, mainly because of the absence of readily available quinolone sIgE assays and serious uncertainties associated with skin testing [34–36]. For example, we observed that moxifloxacin skin testing yielded a positive predictive value of 36% and negative predictive value of 25% [36].

Studies on the BAT with quinolones (Table 3) show that CD63-based assays frequently yield negative results [37–39], except for the study of Aranda et al. [40]. This might suggest that fluoroquinolones can trigger basophil activation which is difficult to depict by traditional CD63-based assays. We speculate that the more consistent results with CD203c up-regulation [41, 42] might indicate mediator release in response to quinolones results from alternative degranulation pathways such as ligation of MRGPRX2 [2]. Moreover, such an IgE-independent activation mechanism might explain IDHR to quinolones upon first exposure in naïve patients and the frequent false-negative sIgE results [40, 43].

5 Neuromuscular Blocking Agents

In many countries, curarizing NMBA represent a significant cause of anaesthesia-related anaphylaxis [44–48]. Skin tests are the primary instrument to confirm IDHR to NMBA [49]. However, the predictive value of skin testing is not absolute thereby leaving room for additional in vitro

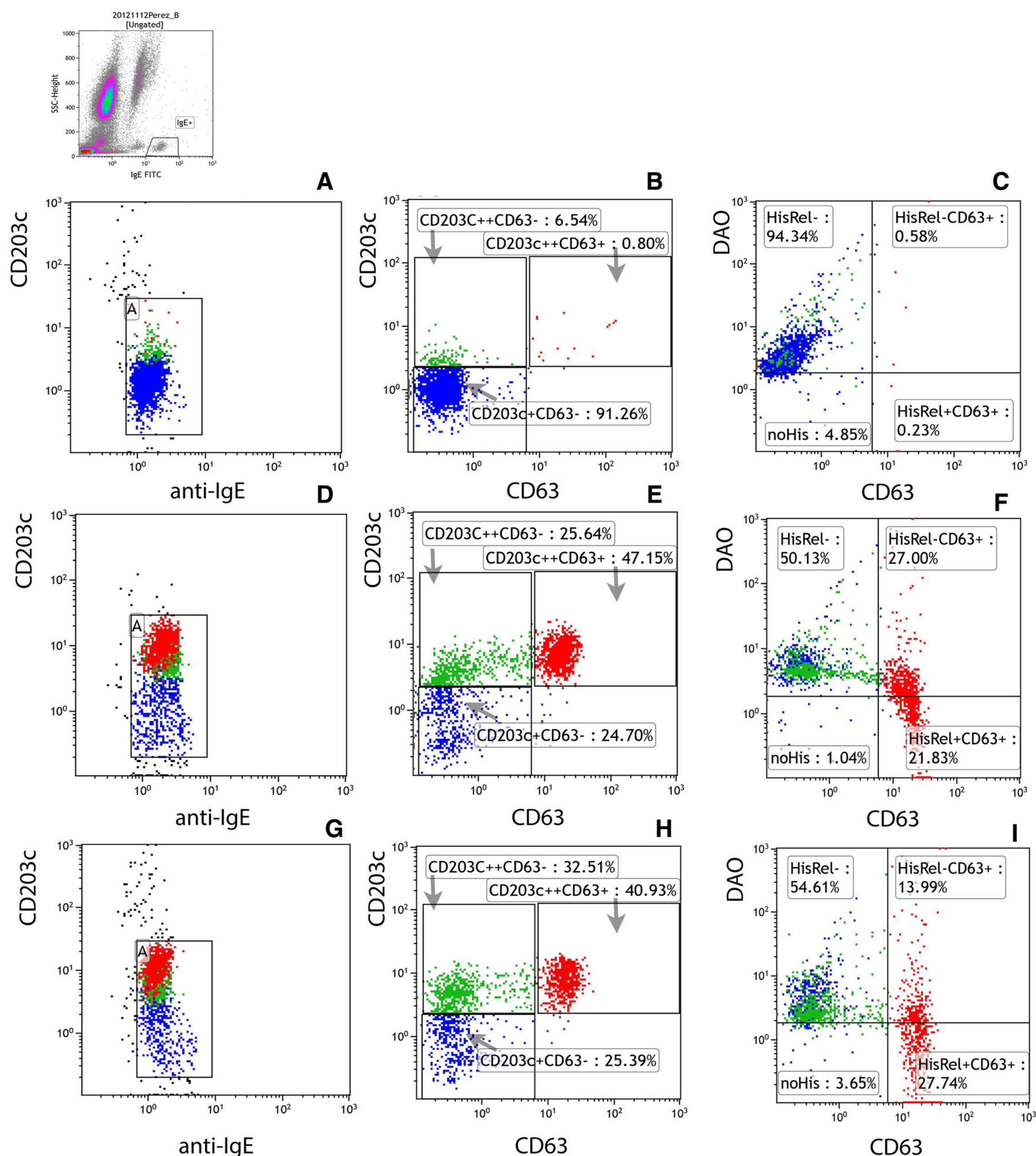

Fig. 1 HistaFlow plots in a cefazolin-reactive patient showing clear anaphylactic degranulation of basophils in response to cefazolin (100 µg/mL). **a**–**c** Resting cells stimulated with buffer; **d**–**f** display the responses to positive control stimulation with anti-IgE; and **g**–**i** THE response upon stimulation with the antibiotic. Note that only CD203c++/CD63+ cells release histamine (decrease of DAO, **c**, **f**, **i**). See [110]. *DAO* diamine oxidase, *HisRel* histamine release, *IgE* immunoglobulin E

tests. In the absence of readily available assays, for about two decades, several groups have tried to define the accuracy of various home-made NMBA-sIgE assays (Table 4) [50–53]. At present, IDHR to NMBA are serologically assessed indirectly through assays measuring IgE reactivity to tertiary and quaternary substituted ammonium structures that have been shown to be the major epitopes of NMBA [54, 55]. Most frequently applied methods are a choline chloride [50, 51, 56–61], a p-aminophenyl phosphoryl choline (PAPPC) [50, 56, 57, 62] and/or morphine-based assays [50–52, 62–67]. With respect to the ImmunoCAP FEIA for suxamethonium, rocuronium, atracurium and morphine, the sensitivity and specificity for the individual NMBA varies between 38.5 and 92% and 85.7 and 100%, respectively. Furthermore, it has been demonstrated that a morphine-based immunoassay is a valuable test to detect suxamethonium- and rocuronium-reactive antibodies, but not to depict atracurium-reactive antibodies

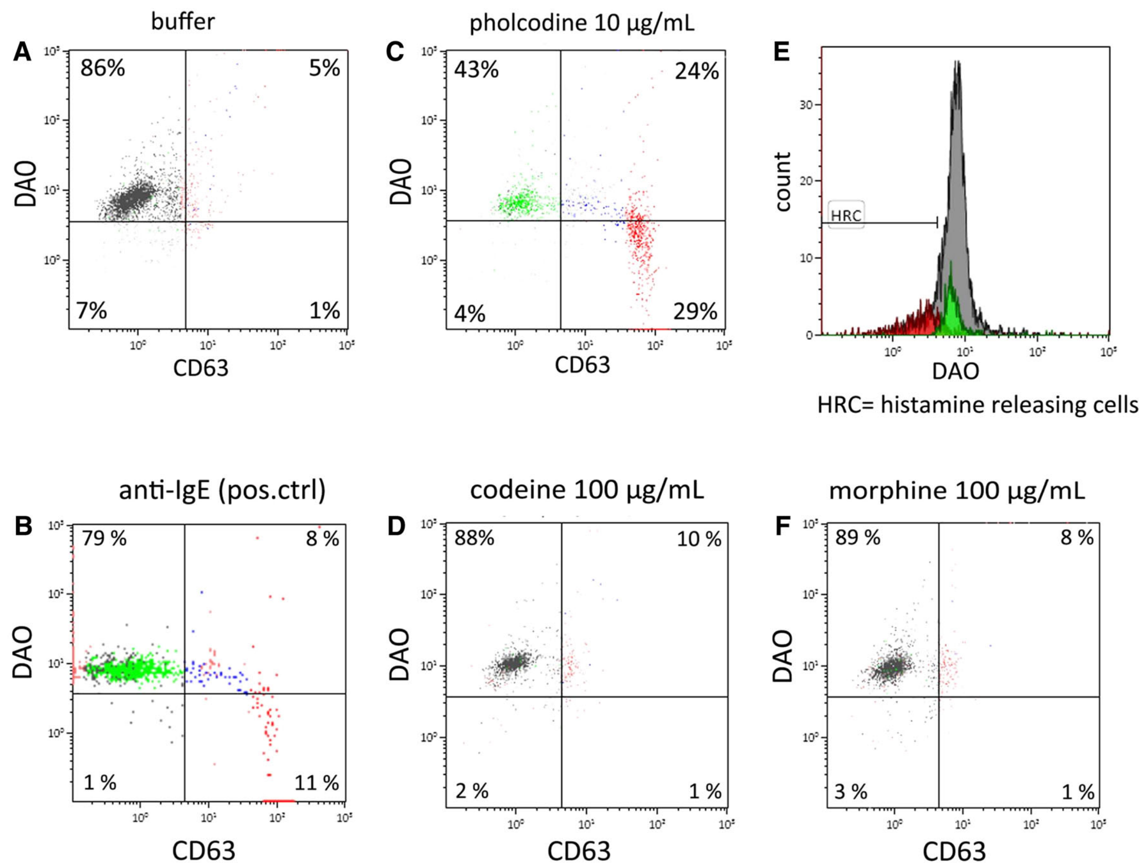

Fig. 2 Representative plot of CD63 appearance and histamine release in response to buffer, anti-IgE as a positive control, pholcodine 10 µg/mL, and the structurally almost similar opiates codeine (100 mg/mL) and morphine (100 µg/mL) in a patient with pholcodine allergy and a negative challenge for codeine and morphine [82]. *DAO* diamine oxidase, *IgE* immunoglobulin E, *pos.ctrl* positive control

[63, 65]. Quantifying IgE reactivity to tertiary and quaternary substituted ammonium structures to identify patients at risk or to document NMBA hypersensitivity [68, 69] might cause a large number of false-positive results as they are prevalent in the general population [64, 65, 67]; these assays can therefore not be used as a screening technique to identify patients at risk or to document NMBA hypersensitivity [68, 69]. The most important hypotheses for these false-positive sIgE results are an elevated total IgE [65] and intake of the opiate antitussive pholcodine [70]. Alternatively, as recently stressed by Spoerl et al. [3], IDHR to NMBA such as rocuronium might occur independently from IgE/FcεRI cross-linking and relate to MRGPRX2-mediated activation of mast cells [2] and, therefore, not be depicted by sIgE assays.

Table 5 displays the data about BAT in IDHR to NMBA. In general, sensitivity of the assay varies between 36 and 92%, whereas specificity easily reaches 95%. Importantly, BAT not only enables identification of the culprit drug, but also provides the opportunity to study cross-reactivity and tailor safe alternatives for future anaesthesia [71, 72].

6 Non-Steroidal Anti-inflammatory Drugs

IDHR to non-steroidal anti-inflammatory drugs (NSAIDs) are extremely common, and it is generally accepted that the large majority of these reactions are independent of IgE/FcεRI cross-linking, but correspond to a pharmacological mechanism caused by the inhibition of cyclo-oxygenase (COX)-1 isoenzyme, resulting in depletion of prostaglandin E2 with unstrained synthesis of cysteinyl leukotrienes and mediator release from basophils, mast cells and eosinophils. Consequently, as displayed in Table 6, the sensitivity of the BAT (about 20–40%) leaves a lot to be desired regarding diagnosis of IDHR to NSAID hypersensitivity. However, only a minority of IDHR to NSAIDs appear to be 'genuine' IgE-mediated reactions, and in that case, patients appear to react exclusively to a single NSAID family. So far, five publications have reported on BAT in selective hypersensitivity to pyrazolones [73–77]. In these cases, BAT yielded a sensitivity between 42 and 70% and a specificity of 86–100%. However, one study showed a significant lower sensitivity of the BAT, contradicting the other studies [76].

Table 1 Specific IgE to β-lactam antibiotics

Compound	Ref. test	Assay	Sensitivity	Specificity	*N*	Ref.
Various β-lactams	H + ST	CAP-FEIA	BPO + AXO + peni G + AMP: 31.8%	BPO + AXO + peni G + AMP: 88.6%	58	[111]
Various β-lactams	H + ST + DPT	CAP-FEIA	BPO: 32%	BPO: 98%	129	[112]
			AXO: 43%	AXO: 98%		
			BPO + AXO: 50%	BPO + AXO: 96%		
Various β-lactams	H + ST + DPT	CAP-FEIA	BPO: 10–68%	BPO: 98%	410	[25]
			AXO: 41–53%	AXO: 95%		
Various β-lactams	H	CAP-FEIA	37.9%	86.7%	58	[113]
Various β-lactams[a]	H + ST + DPT	CAP-FEIA	0–25%[b]	83.3–100%[b]	45	[21]
		RAST[b]	42.9–75%[b]	66.7–83.3%[b]		
Various β-lactams	H + ST	CAP-FEIA	85%[c]	54%[c]	176	[29]
		CAP-FEIA	44%[d]	80%[d]		
Various β-lactams	H + ST	CAP-FEIA	66%	52%	293	[30]

AMP ampicillin, *AXO* amoxicillin, *BPO* benzyl penicilloyl, *CAP-FEIA* fluorescence enzyme immunoassay (available from Phadia Thermo Fisher), *DPT* drug provocation test, *H* history, *IgE* immunoglobulin E, *N* number, *peni G* penicillin G, *RAST* radio allergo sorbent test, *Ref.* reference, *ST* skin test

[a] Home-made assay

[b] Sensitivity and specificity vary according to clinical manifestations

[c] For a threshold of 0.10 kUA/L

[d] For a threshold of 0.35 kUA/L

Table 2 BAT in immediate β-lactam hypersensitivity

Stimulus	Ref. test	Activation marker	Sensitivity (%)	Specificity (%)	Number of patients and controls	Ref.
β-Lactam	H	CD63	50	93	88	[113]
β-Lactam	H + DPT	CD63	39	93	53	[114]
β-Lactam	H + ST + IgE + DPT	CD63	49	91	110	[115]
Amoxicillin	H + ST	CD203c	52	100	41	[116]
		CD63	22	79		
β-Lactam	H	CD63	50	89–97	262	[117]
β-Lactam	H + ST + IgE	CD63-CCR3	55	100	39	[118]
		CD63-IgE	53			
Amoxicillin	H	CD63	29	–	14 patients, no controls	[119]
Amoxicillin	H + ST + DPT	CD63	50	–	61 patients, number of controls not mentioned	[120]
Amoxicillin	H + ST	CD63	50	–	30 patients	[121]
Cefazolin	H + ST	CD63	33	94	16 patients, 17 controls	[122]
		CD203c	67	94		

BAT basophil activation test, *DPT* drug provocation test, *H* history, *IgE* immunoglobulin E, *Ref.* reference, *ST* skin test

7 Opiates

Genuine IgE-mediated allergies to opiates (morphine, codeine, pholcodine) remain rare notwithstanding the frequent and universal use of these drugs. Additionally, correct diagnosis is not straightforward, mainly because of uncertainties associated with measurement of drug-sIgE antibodies and skin testing [78]. Recently, it has been suggested that the two commercially available sIgE assays for a *Papaver somniferum* (poppy seed) extract and morphine can add to the diagnosis of IgE-mediated opiate allergy [79, 80]. However, using DPT we were unable to confirm these data [81], mainly because of 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

Table 3 BAT in immediate quinolone hypersensitivity

Stimulus	Ref. test	Activation marker	Sensitivity (%)	Specificity (%)	Number of patients and controls	Ref.
Various quinolones	H + DPT	CD63	0	–	4	[37]
Various quinolones	H + ST + DPT	CD63	0	100	18	[38]
Various quinolones	H	CD203c	100	100	5	[41]
Various quinolones	H + DPT	CD63	71	–	73	[40]
Various quinolones	H + DPT	CD203c	NA	100	34	[42]
Moxifloxacin	H	CD63	9.1	77.8	11	[39]
		CD203c	36.4	94.4	11	
Ciprofloxacin		CD63	83.3	88.9	6	
		CD203c	0	94.4	6	
Moxifloxacin	H	CD63	13.3	100	24	Unpub
		CD203c	46.7	100	24	

BAT basophil activation test, *DPT* drug provocation test, *H* history, *NA* not available, *Ref.* reference, *ST* skin test, *Unpub* unpublished data

Table 4 Specific IgE to NMBA and substituted ammonium structures

Compound	Ref. test	Assay	Sensitivity	Specificity	*N*	Ref.
Various NMBA	H + ST	RIA	PAPPC: 97%	PAPPC: 97%	75	[50]
		RIA	MOR: 83%	NA		
		RIA	QAS: 86%	NA		
Various NMBA	H + ST	RIA	QAS: 87.9%	NA	83	[51]
		RAST	SUC: 66.7%			
		RAST	Alcuronium: 40.7%			
Various NMBA	H + ST	RIA	MOR: 85%	98%	118	[63]
		RIA	NMBA-specific: 52%			
Various NMBA	H + ST	CAP-FEIA	SUXA: 38.5%	SUXA: 96.3–99.6%	866	[64]
		CAP-FEIA	MOR: 67.7%	MOR: 90–95%		
ROCU[a]	H + ST	CAP-FEIA	SUXA: 72%[b]	SUXA: 100%[b]	82	[65]
			SUXA: 60%[c]	SUXA: 100[c]		
			ROCU: 92%[b]	ROCU: 93%[b]		
			ROCU: 68%[c]	ROCU: 93%[c]		
			MOR: 88%	MOR: 100%		
			PHOL: 86%	PHOL: 100%		
Various NMBA[a]	H + ST	CAP-FEIA	QAM[d]: 87.7%	QAM[d]: 90.7%	168	[67]
ATRA[a]	H + ST	CAP-FEIA	SUXA: 28.6%	SUXA: 85.7%	78	[66]
			ATRA: 57.1%	ATRA: 100%		
			MOR: 14.2%	MOR: 85.7%		

ATRA atracurium, *CAP-FEIA* fluorescence enzyme immunoassay (available from Phadia Thermo Fisher), *H* history, *IgE* immunoglobulin E, *N* number, *NA* not available, *NA* not available, *NMBA* neuromuscular blocking agent, *MOR* morphine, *PAPPC* p-aminophenyl phosphoryl choline, *PHOL* pholcodin, *QAM* quaternary ammonium morphine, *QAS* quaternary ammonium structure, *ROC* receiver operating curve, *RAST* radio allergosorbent test, *Ref.* reference, *RIA* radio immunoassay, *ROCU* rocuronium, *ST* skin tests, *SUC* succinyl choline, *SUXA* suxamethonium

[a] Applying ROC-generated drug-specific thresholds

[b] For a ROC-generated threshold of 0.11 kUA/L for SUXA and 0.13 kUA/L for ROCU

[c] For a traditional threshold of 0.35 kUA/L

[d] 'Optimized' MOR-based assay

causative compound(s) is impeded because of simultaneous intake or administration of different agents, 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

Table 5 BAT in immediate NMBA hypersensitivity

Stimulus	Ref. test	Activation marker	Sensitivity (%)	Specificity (%)	*N*	Ref.
Various NMBA	H	CD63	64	81	26	[123]
		CD45	43	96		
Various NMBA	H + ST	CD63	54	100	56	[12]
Various NMBA	H	CD63	79	100	31	[124]
		CD203c	36	100		
Various NMBA	H + ST	CD63	36–86[a]	93	92	[125]
Rocuronium	H + ST	CD63	92[b]	100	22	[71]
Various NMBA	H + ST + IgE	CD63	60	100	49	[126]
Rocuronium	H	CD63	80	96	104	[68]
Various NMBA	H + ST	CD63	68	100	56	[127]
Atracurium	H + ST	CD63	71[c]	100	75	[72]

BAT basophil activation test, *H* history, *IgE* immunoglobulin E, *N* number of patients and control individuals, *NMBA* neuromuscular blocking agent, *Ref.* reference, *ST* skin test

[a] Increasing sensitivity when only the reactions that occurred during the 3 years were taken into account

[b] Taking into account the non-responders, sensitivity is 76%

[c] Taking into account the non-responders, sensitivity is 63%

Table 6 BAT in immediate NSAID hypersensitivity

Stimulus	Ref. test	Activation marker	Sensitivity (%)	Specificity (%)	Number of patients and controls	Ref.
Metamizol	H + DPT	CD63	42	100	56	[73]
Metamizol	H	CD63	42.3	100	56	[74]
Various NSAID	H + DPT	CD63	15–55	74–100	90	[128]
Diclofenac	H	CD63	No significant difference in CD63 expression between patients and controls (IgE-independent basophil degranulation)		26	[129]
Various NSAID	H	CD63	43	100	72	[130]
Pyrazolones	H + IDT + DPT	CD63	55	86	107	[75]
ASA	H + DPT	CD63	34	79	42	[131]
		CD203c	17	100		
Diclofenac		CD63	17	92		
		CD203c	22	100		
ASA	H + DPT	CD63	30	40	20	[132]
		CD203c	70	45		
Diclofenac	H + DPT	CD63	0	–	22	[133]
Aspirin	H + DPT	CD63	80[a]	83[a]	59	[134]
			78[b]	50[b]		
Various NSAID	H + DPT	CD63	61	91	29	[135]
Various NSAID	H	CD63	37	90	80	[136]
Metamizol	H	CD63	0	–	6 patients, no controls	[76]
Metamizol	H + ST	CD63	70	100	30	[77]
Various NSAID	H + DPT	CD63	100	20	91	[137]

ASA aspirin acetyl salicylic acid, *BAT* basophil activation test, *DPT* drug provocation test, *H* history, *IDT* intradermal test, *IgE* immunoglobulin E, *NSAID* non-steroidal anti-inflammatory drug, *Ref.* reference, *ST* skin test

[a] For anaphylaxis

[b] For asthma/rhinoconjunctivitis

rocuronium or suxamethonium. For the time being, the sole in vitro method to document opiate allergy is BAT, as these cells, unlike cutaneous mast cells, are unresponsive to non-specific stimulation with opiates [81, 82] (see also Fig. 2). Moreover, negative BAT, along with negative skin testing for different NMBA and negative provocation tests for the

structurally almost similar opiates, suggest these drugs are probably safe in pholcodine hypersensitivity [82].

8 Iodinated Contrast Media

IDHR to radio contrast media (RCM) have been described, but their prevalence is low and estimated to be between 0.02% for non-ionic RCM and 0.4% for ionic RCM. Non-specific RCM binding to surface receptors on mast cells or basophils can result in direct histamine release and indirect cell activation by means of the complement or kinin cascade. These alternative pathways outnumber the genuine IgE-mediated reactions and might be overlooked by skin testing [83, 84]. IgE-mediated reactions are believed to account for approximately 4% of the IDHR to RCM. Up to now, three studies reported on the value of BAT in the diagnosis of IDHR to RCM [83, 85, 86]. These studies demonstrate a sensitivity of 46–63% depending on the chosen threshold, and a specificity of 89–100%. Furthermore, it seems that the results of BAT and skin testing are complementary [83] (Table 7).

9 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 [87], IDHR (including life-threatening anaphylaxis) [88–91] and even a combination of both contact dermatitis and IDHR [92]. For a traditional arbitrarily chosen decision threshold of 0.35 kUA/L, the sensitivity of sIgE chlorhexidine varied between 84.2 and 91.6% and the specificity between 93.7 and 100%. For a ROC-generated threshold of 0.20 kUA/L, sensitivity was 94.1% and specificity 90.7% [90, 91]. Like for β-lactam [29–31] and NMBA [65], 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 2000 kU/L [91]. Recently, it was demonstrated the optimal sampling time for sIgE chlorhexidine is between 1 and 4 months [93], but sIgE might persist for years [46].

10 Miscellaneous

Bovine gelatin constitutes the active component in certain plasma substitutes and haemostatic sponges, and can be present in various other drugs such as vaccines. Since the first descriptions of the allergenicity of gelatin [94], IgE-mediated IDHR to this compound, including fatal anaphylaxis, have been increasingly reported. Today, two distinct types of IgE-mediated bovine gelatin allergy are recognized: genuine gelatin allergy that results from sensitization to the protein part of the molecule; and gelatin allergy resulting from a sensitization to a glycan moiety of the molecule, i.e. galactose-α [1, 3] -galactose (α-gal) [95–97], as first described by Chung et al. [98] and Commins et al. [99]. 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 α-gal sensitization are generally overlooked by traditional gelatin-sIgE assay and need additional testing including quantification of α-gal-sIgE antibodies and gelatin skin testing [95–97].

11 Quantification of Serum Tryptase

Although quantification of acute and baseline serum tryptase does not add to the identification of the culprit, serum tryptase has proven to be of additional value in diagnosing IDHR, mainly to confirm mast cell degranulation and/or to rule out or confirm (clonal) mast cell disorders [100] and mast cell activation syndromes [101]. Currently, in the commercially available tryptase assay, total tryptase is quantified as the sum of continuously secreted baseline tryptase and β-tryptase released from degranulating mast cells (ImmunoCAP, Thermo Fisher, Uppsala, Sweden). It has been suggested that the recommended decision threshold of 11.4 μg/L be abandoned, since increases in serum tryptase might often be relevant even when values are below this cut-off [102, 103].

Table 7 BAT in immediate hypersensitivity to iodinated RCM

Stimulus	Ref. test	Activation marker	Sensitivity (%)	Specificity (%)	Number of patients and controls	Ref.
Various RCM	H + ST	CD63	100	100	3 patients, unknown number of controls	[85]
Various RCM	H + ST	CD63	46–62%[a]	89–100%[a]	40	[83]
Various RCM	H + ST + DPT	CD63	63	100	28	[86]

BAT basophil activation test, *DPT* drug provocation test, *H* history, *RCM* radiocontrast media, *Ref.* reference, *ST* skin test

[a] Depending on the cut-off value

Furthermore, a new algorithm for interpretation of serum tryptase has been proposed in which the minimal elevation of acute tryptase (within 30–240 min from the event) over baseline (24 h after the acute event) levels is suggested to be clinically relevant, and is calculated as at least $2 + 1.2 \times$ baseline [104]. Importantly, the sensitivity of this approach seems higher if basal (post-reaction) levels are obtained within 2 months from the acute event [105]. Alternatively, by comparing the two measurements, anaphylaxis could be ruled out even for acute tryptase values of >11.4 μg/L in cases of baseline hypertryptasaemia due to non-allergic causes [104]. Quantifying baseline tryptase has another additional purpose, as elevated baseline levels might be indicative for underlying (clonal) mast cell disorders [100]. Hypotension without urticaria and angioedema in patients suffering from severe IDHR warrants further diagnostics to rule out a mast cell disorder, particularly in men [106]. Levels of mature tryptase of >1 μg/L indicate mast cell degranulation. However, this test is not commercially available.

12 Commentaries and Perspectives

From this review, it appears that drug-sIgE antibody testing can provide useful information, but can rarely be applied as a solitary diagnostic test to exclude or document IDHR, as these tests lack absolute predictive values. For β-lactam determinants, the main issue is low sensitivity, which could not be increased without significant loss of specificity [29]. For NMBA, drug-sIgE tests seem to attain acceptable sensitivity and specificity, provided drug-specific cut-offs are applied [65, 91]. Although quantification of sIgE to morphine appears a reliable biomarker of sensitization to tertiary and quaternary ammonium structures, IgE reactivity to this compound in general and in an 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 unsatisfactory sensitivity of some tests, it has been argued that this observation relates to 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 European Network on Drug Allergy and European Academy of Allergy and Clinical Immunology (ENDA/EAACI) Drug Allergy Interest Group. Based upon a single publication about negativation of sIgE to β-lactam antibiotics [26], in their position paper [107], further use of drug-sIgE is dissuaded when the time interval exceeds 3 years. However, this is not our experience [68], and drug-sIgE may persist as long as 5–30 years [108, 109]. With respect to the low specificity of some tests, it is re-emphasized that correct interpretation of sIgE results requires taking into account total IgE values [29, 65, 91]. Whether the introduction of sIgE/total IgE ratios increases specificity [29] remains to be confirmed.

Since the earliest days of BAT, it was obvious that this technique would become an asset in the diagnostic instrumentation to document IDHR, particularly when diagnosis cannot be established by other means. However, additional collaborative large-scale studies are needed to verify whether BAT lives up to its promise, to optimize and harmonize the protocols, to avoid instigation of cynicism and scepticism, and to enable and justify its entrance in routine diagnostic application.

Acknowledgements ALVG is a fellow of the FWO (1113617N). DGE is a senior clinical researcher of the FWO (1800614N).

Compliance with Ethical Standards

IID, EAM, ALVG, KC, AU, MF, VS, CHB, CM, MMH, LSDC and DGE declare they have no conflict of interest. No funding for this review was provided.

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