In Vitro Diagnosis of Immediate Drug Hypersensitivity During Anesthesia: A Review of the Literature

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Quantification of specific IgE (sIgE) antibodies constitutes an important measure to document anesthesia-related immediate hypersensitivity reactions (IHRs). However, only a few drug-specific assays are available and their predictive value is not known. In cases of non-IgE mediated IHRs, diagnosis might benefit from cellular tests such as basophil mediator release tests and basophil activation tests (BATs). To review the potential and limitations of quantification of sIgE, mediator release, and BAT in anesthesia-related IHRs, a literature search was conducted using the key words allergy, basophil activation, CD63, CD203c, diagnosis, drugs, hypersensitivity, flow cytometry, MRGPRX2, specific IgE antibodies, leukotrienes, histamine, and tryptase; this was complemented by the authors' experience. The drugs and compounds that have predominantly been studied are neuromuscular blocking agents (NMBAs), β-lactams, latex, and chlorhexidine. For sIgE NMBA, sensitivity and specificity varies between 38.5% to 92% and 92% to 100%, respectively. For sIgE β-lactams, sensitivity varies between 0% to 85% and specificity between 52% to 100%. sIgE to morphine should not be used in isolation to diagnose IHRs to NMBAs or opiates. sIgE for latex, and, in difficult cases, molecular diagnosis with quantification of sIgE to *Hevea* components constitute reliable diagnostics. For drugs, the sensitivity of BAT varies between 50% and 60% and specificity reaches 80% to 90%. Basophil mediator release tests seem to be abandoned and supplanted by BATs. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1176-84)

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INTRODUCTION

The gold standard to ascertain correct diagnosis of immediate hypersensitivity reactions (IHRs) to drugs is a controlled drug provocation test (DPT) with the culprit compound(s). However, DPTs entail a risk of severe, life-threatening complications and can be contraindicated (eg, patients having suffered from life-threatening reactions) or impossible (eg, full-dose DPT in hypersensitivity to curarizing neuromuscular blocking agents [NMBAs]). Moreover, the predictive value of DPTs is not known and DPTs might yield false-negative results.[1] Therefore, the diagnostic approach of anesthesia-related IgE-mediated IHR generally starts with history taking, thorough review of anesthetic/surgical notes, complemented with skin testing and/or *in vitro* quantification of specific IgE (sIgE) antibodies. However, only a few drug sIgE assays are available, and most of them have not been clinically validated. Furthermore, IHRs might not *per se* involve IgE/high-affinity receptor for IgE (FcεRI)-cross-linking, but may also result from alternative pathways such as an off-target occupation 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 (BATs) would, to some extent, be promising in such cases.

The objective of this article was to review the literature on the value of serum tryptase, histamine, commercially available drug sIgE assays, and BATs such as mediator release tests in the diagnosis of anesthesia-related IHRs. 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 peak and baseline serum tryptase does not contribute to the identification of the culprit, serum tryptase has proven to be extremely valuable in diagnosing anesthesia-related IHRs, mainly to confirm mast cell degranulation and/or to rule out or confirm (clonal) mast cell disorders[4] and mast cell activation syndromes.[5] Currently, in the

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Abbreviations used
BAT- Basophil activation test
DPT- Drug provocation test
FcεRI- High-affinity receptor for IgE
IDHR- Immediate drug hypersensitivity reaction
IHR- Immediate hypersensitivity reaction
NMBA- Neuromuscular blocking agent
sIgE- Specific IgE

commercially available assay, total tryptase is quantified as the sum of continuously secreted baseline tryptase and β-tryptase released from degranulating mast cells (ImmunoCAP Thermofisher, Uppsala, Sweden). Because relevant increases have been observed way below the traditional decision threshold of 11.4 μg/L, it has been suggested to abandon this cutoff.[6-8] For example, an incremental threshold of 20% was shown to identify potential mast cell mediator release in an additional 14% of cases with peak tryptase between 5 and 14 μg/L and a further 15% with peak tryptase below 5 μg/L. Others have proposed that an increase in tryptase over baseline (24 hours after the acute event) levels is clinically relevant when it exceeds 2 + (1.2 × baseline).[9,10] Although in the study by Sprung et al, quantification of peak tryptase was performed between 30 minutes and 4 hours from the event, it is recommend to take the peak sample as close to 60 minutes after the reaction as possible, and if not possible later samples should still be taken and compared with a baseline taken at a later date.[11] Alternatively, by comparing the 2 measurements, anaphylaxis could be ruled out even for acute tryptase values of more than 11.4 μg/L in cases of baseline hypertryptasemia.[9] Quantifying baseline tryptase has another additional purpose, because elevated baseline levels might be indicative for underlying (clonal) mast cell disorders[4] that might underlie severe IHRs, particularly in men who do not demonstrate urticaria/angioedema.[12] β-Tryptase levels of more than 1 μg/L indicate mast cell degranulation. However, this test is not commercially available. Quantification of plasma histamine, although highly sensitive, was inferior to quantification of serum tryptase for discrimination between IgE-dependent and IgE-independent anesthesia-related IHRs.[13] Resuscitation maneuvers by themselves appear not to modify mediator concentrations.[14] Alternatively, it is important to stress that an elevated peak tryptase measurement does not necessarily indicate mast cell activation.[9,15] In chronic renal failure, elevated "peak" serum tryptase[15] might result from mast cell hyperplasia due to slow elimination of stem cell factor.[16] Note that tryptase is not cleared by the kidneys.[17] Tryptase can also be elevated in critically ill patients without anaphylaxis[18] and victims of trauma.[19] False-negative results mainly result from incorrect sampling time (ideally 60-90 minutes after onset of symptoms).

PRINCIPLES OF QUANTIFICATION OF DRUG sIgE ANTIBODIES AND BAT

Like tissue-resident mast cells, basophils can be triggered in IgE-dependent and various IgE-independent ways. Cross-linking of the surface-bound FcεRI generally occurs through (glyco) proteins, chemical allergens, or autoantibodies directed against FcεRI or membrane-bound sIgE antibodies. Quantification of sIgE antibodies predominantly relies on quantification of a drug-(hapten)-carrier antibody complex in which the secondary antihuman IgE is conjugated to an enzyme with colorimetric reading in the ELISA or with a fluorescence reading in the fluorescent enzyme immunoassay.[20] However, only a limited number of drug sIgE immunoassays are available and most of these assays have not been thoroughly validated, mainly as a result of the unavailability of sufficient numbers of patients and exposed or challenged control individuals.

An IgE-independent activation will mainly result from coupling of surface receptors with endogenous (eg, cytokines, anaphylatoxins, chemokines, IgG, and neuropeptides) or exogenous (eg, pathogen-associated molecular patterns) elements. Among these receptors is the Mas-related G-protein receptor MRGPRX2 that can lead to a quick but rather transient mast cell degranulation[21] and appears to be involved in different mast cell—associated conditions including nonimmune immediate drug hypersensitivity reactions (IDHRs).[22,23] Recently, McNeil et al[2] described the potential of MRGPRX2-related mast cell activation by various drugs containing a tetrahydroisoquinoline motif such as some fluoroquinolones and various NMBAs. The MRGPRX2 receptor has subsequently also been incriminated in reactions toward opioids[24] and vancomycin.[25] Alternatively, other largely unknown pathways might also induce degranulation.

The foundations of current flow-assisted BAT were laid 25 years ago[26] and in the meantime the technique has largely supplanted older mediator release assays that rely on difficult quantification of mediators released in the supernatant. Actually, the last reviews on mediator release tests date back to 2003.[27,28] To our knowledge, since then no large case-control studies including more than 15 patients and significant numbers of (exposed) control individuals on the application of the various mediator release tests have been published, except a report including patients who suffered from perioperative hypersensitivity reactions resulting from various causes.[29]

Traditional BAT relies on 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 mAbs conjugated with different LASER-excitable fluorochromes. The technical principles and requirements of BAT have been detailed elsewhere.[30] Basophils are traditionally identified by markers such as CCR3 (CD193)/CD3, CD123/HLA-DR, or IgE/CD203c. Of these markers, only CD203c is lineage specific. After activation, the appearance and/or upregulation of surface activation and/or degranulation markers such as CD203c and/or CD63 is quantified. For a review on the applications and limitations of the BAT in drug IHRs, see Mangodt et al.[31] Histamine release can also be quantified by flow cytometry[32] and the technique is applicable in IHRs to drugs.[33]

β-LACTAMS

The most studied sIgE assays are those for β-lactams, especially amoxicillin and benzyl penicilloyl. Although several cases of positive sIgE results in IHRs with negative skin test results have been described,[34-38] sIgE assays for β-lactams, as listed in Table I, generally exhibit a poor sensitivity that decreases over time.[45] Besides these disappointing sensitivity data, there is increasing evidence supporting low specificity of the tests.[35,37,42,43,46,47] In some studies, false positivity could have resulted from nonspecific binding in the solid-phase assay as a result of elevated total IgE titers.[42,43,47,48] An alternative