In vitro diagnostic tests for perioperative hypersensitivity, a narrative review : potential, limitations, and perspectives

In vitro diagnostic tests for perioperative hypersensitivity: potential, limitations and perspectives

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Abstract

Correct diagnostic management of perioperative hypersensitivity (POH) aims at identifying the underlying mechanisms(s), responsible culprit(s) and safe alternatives. Although drug provocation tests are considered the gold standard, diagnosis of POH mainly relies on skin testing. Meanwhile, *in vitro* tests, such as quantification of specific IgE antibodies, serum tryptase and plasma histamine, and basophil activation tests have been developed and their use is becoming widespread. These tests have the advantage of having no risk of recurrence of immediate hypersensitivity reactions. In this review, we summarize the principles of *in vitro* tests, and the possibilities and limitations when these tests are used for testing sensitivity to substances with a high risk of causing POH. Hence, we particularly focus on neuromuscular blocking agents, antibiotics, natural rubber latex and opiates/opioids. Combination of multiple tests would allow us to diagnose POH with the right balance of safety and accuracy.

I Introduction

Perioperative hypersensitivity (POH), although rare, can have potentially life-threatening consequences due to the potential for diagnostic error. Hypersensitivity reactions in the perioperative period may be provoked by a variety of triggers, although, in most cases, POH reactions are triggered by drugs such as neuromuscular blocking agents (NMBAs) and antibiotics, natural rubber latex from *Hevea brasiliensis*, or related products such as chlorhexidine and dyes[1-4]. The standard reference test for accurate diagnosis of immediate hypersensitivity reactions (IHRs) to these substances is a controlled drug provocation test. However, drug provocation tests are not always possible for obvious ethical and practical reasons, and they might not always be predictive of the clinical outcome[5]. Therefore, in clinical practice, diagnostic workup of POH reactions generally starts with judicious skin testing[6, 7]. However, although skin tests still merit the status of the primary diagnostic test in the evaluation of POH reactions, we believe that there is room for additional *in vitro* tests for various reasons. First, skin test procedures have not been thoroughly validated for many compounds, since studies have mainly focussed on the determination of non-irritating concentrations in (exposed) control individuals[8]. Second, skin tests do not have absolute predictive value. For example, uncertainties remain for skin tests with potent non-specific histamine releasers, such as opiates[9] and fluoroquinolones[10], or with NMBAs that can elicit positive intradermal test responses independent of mast cell degranulation[11]. Alternatively, negative skin tests might not always guarantee safe re-exposure to the substance being evaluated[12]. Third, a positive skin test is not necessarily indicative of a specific immune-mediated pathomechanistic process, but could mirror off-target occupation of the MRGPRX2 receptor that is constitutively expressed on cutaneous mast cells[13]. Collectively, these observations indicate that *in vitro* tests would not only facilitate the diagnosis of POH, but might also deepen our knowledge and cause paradigm shifts in our understanding about the pathomechanisms of potentially life-threatening POH reactions[14]. Here, after stressing the need for acute serum effector cell mediator measurement, we will summarize the main principles, possibilities and limitations of quantification of specific IgE (sIgE) antibody assays and basophil activation tests (BATs).

II Quantification of serum tryptase and plasma histamine

The aetiologic diagnosis of POH relies on the presence of clinical, biological and allergological evidences[15]. Clinical evidence, including the features and severity of clinical signs, and the

interval between introduction of a suspected allergen and the onset of symptoms, should be the first line of evidence for initial diagnosis. However, since they are beyond the focus of this review, we will describe the second-most important evidence, *in vitro* primary testing.

Immediate hypersensitivity reactions, such as POH, result from activation of mast cells and basophils by the allergen, recognized through sIgE attached to the surface of these cells, resulting in the release of various inflammatory mediators[16] (cross-reference to manuscript on molecular mechanisms). Measurement of these inflammatory mediators is the basis of biochemical confirmation of the occurrence of such reactions. Of the several inflammatory mediators that are released in anaphylaxis, including tryptase, histamine, platelet-activating factor, prostaglandin D2, and leukotriene E4, tryptase is most widely assessed in blood tests used to confirm the occurrence of anaphylaxis, because of the advantage of a longer half-life than the other mediators. Indeed, tryptase levels peak between 60 and 120 minutes after onset of the reaction and return to baseline values within several hours. Since tryptase levels in basophils are <1% of those found in tissue mast cells[17], increases in tryptase concentrations are considered to be indicative of mast cell activation[18]. Several tryptase cut-off values, such as >25 µg/L[16] or >15.7 µg/L[19], have been proposed to identify mast cell activation. Alternatively, use of the ratio of peak to basal tryptase levels has been recently recommended to improve the diagnostic accuracy of anaphylaxis[19, 20]. Reportedly, the comparison between peak and baseline serum tryptase values provides more valuable information about mast cell activation than do absolute cut-off values. A consensus equation has been formulated for this[21]. According to this formula, mast cell activation is defined when peak tryptase levels exceed 2+1.2(baseline tryptase). This formula has recently been validated for POH with a sensitivity, specificity, positive predictive value and negative predictive value of 78%, 91%, 98% and 44%, respectively[22]. However, the sensitivity is not absolute and patients who present clinically with anaphylaxis but in whom serum tryptase concentrations are not increased still require investigation, as false negatives do occur[23].

Histamine is one of the important mediators in the early onset of anaphylaxis. It is produced by decarburization of histidine present in the Golgi apparatus of mast cells and basophils, and is rapidly metabolized by histamine transferase once it is released into the blood[24]. Therefore, plasma histamine begins to rise within 5 minutes after the onset of anaphylaxis, although its increase lasts for only 30 to 60 minutes. Hence, it is difficult to prove its presence more than 1 hour after the onset of hypersensitivity. The short half-life of histamine may prevent its use as a marker of anaphylaxis. **In addition, histamine assay has the following disadvantages: a) Since**

histamine is also produced by neurons and bacteria, increase of histamine does not necessarily indicate mast cell/basophil activation; b) histamine levels could be influenced by food and/or drug intake; c) measurement methods have specific requirements and are expensive. However, histamine assay at 30 min after a suspected hypersensitivity reaction is recommended by French guidelines[16]. This recommendation seems to be based on the evidence that the diagnostic accuracy of POH is increased when histamine and tryptase assay are combined. Hence, although the significance of histamine assay in the diagnosis of POH is controversial, there is no reason not to measure histamine levels if facilities for the measurement are available.

III Quantification of serum specific IgE

Principles

Quantification of drug-specific IgE (sIgE) with IgE immunoassays relies upon detection of a drug-(hapten)-carrier-antibody complex (Figure 1). Basically, the drug-(hapten)-carrier conjugate is coupled with a solid phase, which is incubated with patient serum. The amount of sIgE bound is subsequently detected with a secondary antihuman IgE antibody, labelled with a radioisotope in the older – largely abandoned – radioimmunoassays, or with an enzyme with colorimetric reading in the more recent enzyme-linked immunosorbent assay, or with fluorescence reading in the fluorescent enzyme immunoassay. Results of sIgE assays have been expressed in many ways. Today, results of most commercially available assays are expressed as arbitrary units, kUA/L. For years, the technical detection limit was established as 0.35 kUA/L. However, recently a new heterologous calibration scheme has been introduced, where quantification is based on the use of IgE antibody curves with a range of 0.00–100 kUA/L, with a detection limit set at 0.10 kUA/L and a cut-off value of 0.10 or 0.35 kUA/L for positive results. However, as will be addressed later, these decision thresholds have been set arbitrarily and the tests might benefit from allergen-specific cut-offs[25].

Clinical applications

<u>Neuromuscular blocking agents</u>

Currently, sensitisation to NMBAs is generally assessed serologically using various methods that measure drug-specific IgE antibodies, such as to suxamethonium, rocuronium and atracurium, or indirectly by measuring IgE reactivity to tertiary and quaternary substituted ammonium structures (NH4+) that are considered to be the major epitopes of NMBAs. Most

frequently employed is the morphine-based assay[25-27] or, in France, methods using choline chloride or a p-aminophenyl phosphoryl choline[28-30]. Overall, the sIgE assays for suxamethonium, rocuronium, atracurium and morphine that are available from Phadia Thermo Fisher (Uppsala, Sweden) display a specificity generally exceeding 85% and a sensitivity varying between 40% and 90%[31]. Importantly, the morphine-based assay, although valuable for depiction of sensitisation to suxamethonium and rocuronium, is unreliable for detection of antibodies to benzylisoquinolines[32, 33]. In addition, as IgE reactivity to tertiary and quaternary-substituted ammonium structures are frequent in the general population, the morphine-based test should not be used in isolation to diagnose NMBA hypersensitivity, nor should it be used to absolutely preclude use of an NMBA that tests negative in skin tests and BATs[34].

Antibiotics

The most studied antibiotic-sIgE assays and the only ones commercially available are those for β-lactams. Although, several cases of positive sIgE results in cases of IHRs with negative skin tests have been described[35, 36], sIgE for β-lactam assays generally exhibit a variably poor sensitivity (0-70%) that decreases over time[37]. Noteworthy, there is also increasing evidence supporting the low specificity of the tests due to non-specific binding of these antibodies in solid phase assay as a result of elevated total IgE titres or sIgE antibodies to phenylethylamine[38]. Therefore, sIgE antibodies to β-lactams seem of restricted utility and should ideally not be used in isolation to exclude or confirm IHRs to these antibiotics.

Natural rubber latex from *Hevea brasiliensis*

Although its use is apparently decreasing due to the application of other elastomers, natural rubber latex remains another significant cause of POH[1-4]. Diagnosis of natural rubber latex hypersensitivity is best documented by a positive result with both skin tests and measurement of sIgEs[39], since an isolated positive sIgE to latex – as seen in up to 25% of patients with a grass/weed pollen allergy and 20% of patients with an allergy to wasps/honey bees[40] – can easily be misleading and hide an alternative culprit. In cases with incongruent skin tests and sIgE results to latex, molecular diagnostics (reviewed in [41]) and/or BATs[42] might be required for correct diagnosis, as these techniques frequently enable identification of clinically irrelevant sIgE results due to sensitisation to cross-reactive carbohydrate determinants and profilins.

Opioids and opiates

Despite their ubiquitous use, genuine IgE-mediated reactions to opiates and (semi)synthetic opioids are exceedingly rare[43]. Moreover, hypersensitivity reactions to these substances often

result from alternative mechanisms, such as off-target occupation of the MRGPRX2 receptor[44] that is constitutively expressed on some mast cell subpopulations. Either way, correct diagnosis of hypersensitivity reactions to opiates and certain opioids is challenging mainly because of uncertainties associated with skin tests[9] and the absolute inadequacy of sIgEs to poppy seed (*Papaver somniferum*) and morphine[45].

Miscellaneous

A commercial assay of sIgE to chlorhexidine is available, although, to date, studies evaluating this assay in a large patient group are limited[46, 47]. For a traditional, arbitrarily-chosen threshold of 0.35 kUA/L, **the sensitivity and specificity of sIgE chlorhexidine is 84.2% and 93.7%, respectively**[47]. For a ROC-generated threshold of 0.20 kUA/L, the sensitivity is 94.1% and specificity is 90.7%[47]. The other compound that needs to be described here is bovine gelatine. Gelatine-containing products include certain plasma substitutes, haemostatic sponges and vaccines. To date, two distinct types of IgE-mediated bovine gelatine allergies have been recognized, namely, genuine gelatine allergy that results from sensitisation to the protein part of the molecule, and gelatine allergy resulting from sensitisation to the glycan moiety of the molecule, i.e. galactose-α-1,3-galactose (α-Gal)[48-50].

Another compound to which patients may demonstrate POH is ethylene oxide. Ethylene oxide is used for sterilization of many medical devices because it exerts sterilising effects even at low temperatures and has minimal effects on materials, despite the fact that it is toxic and suspected to be carcinogenic. Patients who frequently undergo surgery, such as those with spina bifida, reportedly have a high positivity rate for sIgE to ethylene oxide[51]. Interestingly, one-third of spina bifida patients with sIgE antibodies against latex also have those against ethylene oxide[51]. Since there are only few reports of IHRs to ethylene oxide, it suggests that patients with sIgE to ethylene oxide rarely show symptoms of IHR despite being positive for the antibodies. However, ethylene oxide should always be kept in mind when determining the cause of POH in patients who frequently undergo surgery[52].

IV Basophil activation tests

Principles

The foundations of current flow-assisted BATs were laid 25 years ago[53]; and the technique has largely supplanted older mediator release assays that rely upon difficult quantification of mediators released in the supernatant[54]. The technical principles and requirements of BATs have

been detailed elsewhere[55, 56]. As illustrated in Figure 2, traditional BATs rely upon flow cytometric analysis of various activation and degranulation markers on the surface membrane of basophils. These changes can be detected and quantified on a single-cell level using specific monoclonal antibodies conjugated with different LASER-excitable fluorochromes. **Although there are different ways to phenotype basophils, the following is a typical method: Cells are characterized according to scatters, presence of membrane-sIgE and CD203c. Activation is measured through appearance of CD63, up-regulation of CD203c and/or decrease of intracellular histamine content.**

Clinical applications

Neuromuscular blocking agents

Since it is impossible to perform full-dose drug provocation tests with NMBAs for obvious ethical and practical reasons, anaesthetists and immunologists/allergists mainly rely upon skin tests to confirm clinical suspicions of NMBA hypersensitivity. However, the predictive value of skin testing is not absolute, which leaves room for additional in vitro tests. As a matter of fact, BATs constituted the principal in vitro test to document hypersensitivity to curarizing neuromuscular blocking agents for a long time. This was probably due to the absence of specific IgE assays for many types of NMBAs. As reviewed elsewhere[31], the sensitivity of BATs for NMBAs varies between 36-92%, and the specificity varies between 81-100%. Most importantly, BATs not only complement skin tests in the diagnostic workup of patients with drug hypersensitivity, but also enable assessment of cross-reactivity between NMBAs[57].

Antibiotics

Most data about the usefulness of BATs to assess antibiotic hypersensitivity have been provided in the context of IHRs to β-lactams and quinolones (for review: [31]). Until now, studies that have investigated the BAT as a diagnostic tool in IHRs to β-lactams have mainly focused on amoxicillin. Compared with the quantification of sIgE antibodies, BATs show a higher sensitivity (about 50%) and specificity (approximately 90%). As with sIgE assessments, the sensitivity of BATs to β-lactams is rather low and decreases over time, although both sIgE antibody tests and BATs can remain positive for years. Regarding cefazolin-induced IHRs, a recent study demonstrated that the CD63-BAT attained a sensitivity of 38% and a specificity of 94%, whereas the CD203c read-out yielded a sensitivity of 67% and a specificity of 94%[58]. It has also been suggested that higher concentrations of cefazolin might increase the performance of BATs[59]. Studies on BATs with quinolones revealed quite divergent, but highly interesting, findings[60]. Most

CD63-based assays yielded poor or negative results, expect for the study by Aranda et al[61]. Alternatively, the more consistent results with CD203c upregulation could indicate that mediator release in response to quinolones could result from alternative degranulation pathways. Finally, the BAT could be useful for individual cases where other in vitro tests are not available and skin tests are not well validated[31].

Hevea latex

As already mentioned above, accurate diagnosis of natural rubber latex hypersensitivity has mainly been hindered by clinically irrelevant sIgE results. For long, the BAT proved highly accurate in discriminating between relevant and irrelevant results[42], especially for irrelevant IgE results due to sensitisation to cross-reactive carbohydrate determinants ubiquitously present in the plant kingdom. However, since 2018, the BAT has largely been supplanted by component resolved diagnosis using purified and/or recombinant *Hevea* proteins that are available in single and multiplexed tests[41, 62].

Opiates and opioids

As already described, accurate diagnosis of IgE-mediated opiate and (semi)synthetic opioid hypersensitivity is not always straightforward, mainly because of uncertainties associated with skin tests[9] and unavailability of reliable drug-sIgE assays[45]. On the other hand, the accumulated evidence has shown that the BAT is useful in the correct diagnosis of genuine IgE-mediated opiate hypersensitivity, since unlike cutaneous mast cells, basophils do not respond non-specifically to these substances. Moreover, we have demonstrated basophil activation experiments not only to differentiate between IgE-dependent and IgE-independent mast cell activation[43], but also to identify safe alternative drugs[63].

Miscellaneous

Since application of the BAT for chlorhexidine has not been studied extensively, its precise diagnostic accuracy is not known. However, a small-scale study reported that the sensitivity of the BAT for chlorhexidine was 50%[64]. Gelofusine®, a 4% w/v solution of succinylated gelatine used as an intravenous colloid was also targeted for studies on the outcomes of BATs. The sensitivity and specificity were observed as 100% and 87.5%, respectively[65]. Sugammadex, an agent for reversal of neuromuscular blockade, is not a common cause of POH in all countries. For example, in the UK, it is only used in less than 10% of reversed cases and there has been only one case of sugammadex-induced anaphylaxis in the UK[66]. In Japan, on the other hand, it is now the leading cause of POH, probably due to its high usage- an estimated 10% of the population

received sugammadex during an 8 year period from 2010 to 2018[67]. Similar to the above-mentioned drugs, the usefulness of the BAT for sugammadex-induced anaphylaxis has been shown[68, 69]. When using CD203c as the marker, the sensitivity of the BAT for sugammadex was 88% and specificity was 100%, while sensitivity and specificity for CD63 were 75% and 100%, respectively[69].

V Discussion

When conducting in vitro tests for POH, the order in which tests for hypersensitivity are conducted is extremely important. Although the test with higher diagnostic accuracy is obviously better, it is necessary to consider the risks and burden on the patient during testing. We will now discuss the points to be noted when using in vitro tests in clinical settings and future issues related to this.

Quantification of serum tryptase

As mentioned above, diagnosis of POH requires distinguishing it from other conditions that exhibit similar symptoms. Measurement of serum tryptase values is useful for establishing a differential diagnosis. Although the possibility of IHR increases if serum tryptase levels are elevated, an elevated tryptase measurement does not necessarily indicate mast cell activation[70, 71]. Indeed, elevated "peak" serum tryptase levels might result from mast cell hyperplasia due to slow elimination of stem cell factors. Tryptase can also be elevated in critically ill patients without anaphylaxis and in victims of trauma. Therefore, it is critical to measure both peak and baseline serum tryptase levels. Conversely, IHR cannot be excluded even if serum tryptase levels are not elevated.

Quantification of serum specific IgE

Since the commercial availability of the sIgE determination kit, the test can be carried out easily. However, sIgE tests generally have a low sensitivity and specificity and are only available for a limited number of drugs.

When an early surgical reintervention (<4 weeks) is necessary after POH, skin tests can have insufficient sensitivity to identify the culprit drug(s) and to rule out potential allergy to other drugs[72]. In such cases, sIgE determination can help to identify the culprit drug(s) and guide choices for alternatives soon after the event. Although it is known that the sIgE titre decreases over time, the attenuation over time of sIgE titres might be different depending on the causative agent and may vary between individuals. **Taken together, the above facts suggest that although**

determination of sIgEs can be performed soon after the reaction, the test might need to be repeated after 1-2 months if the test is negative in samples obtained at the time of the suspected hypersensitivity event.

Basophil activation tests

In vitro testing is often compared with in vivo testing. The BAT generally has high diagnostic accuracy to identify causative agents of POH with sensitivities between 50-90% and specificities exceeding 90% [31, 73, 74]. Yet, a recent study showed that the BAT allowed identification of the culprit antigen in only 80% of NMBA-allergic patients. Importantly, however, since negative skin tests do not always guarantee subsequent safe use of the NMBA[75], diagnosis of rocuronium-induced mast cell activation in patients with negative skin tests can be substantiated by the BAT[14]. Similarly, in a recent study of patients who presented anaphylaxis to amoxicillin-clavulanic acid, 30% of patients needed the drug provocation test, because they showed negative skin test results. Even in these patients, approximately 50% (15 out of 29) of patients had positive BAT results. The authors argue that BATs are particularly useful in patients with negative skin test results[76]. These evidences suggest that the vast majority of patients show the same results in skin tests and BATs, although a few patients show different results. In summary, since the positive predictive value of skin tests is not 100%, there seems to be room for other tests, including the BAT, in the diagnosis of POH. Finally, it should be kept in mind that BATs have been shown to complement skin tests in the identification of safe alternatives [57, 74].

Basophil activation tests require different considerations, including selection of the activation marker and determination of the threshold of positivity of the marker. At present, the most commonly used markers in basophil activation experiments are CD63 and CD203c. A few comparative studies showed that CD63 and CD203c are clearly different in their upregulation profile. Appearance of CD63 is generally bimodal, with a subpopulation of cells that express CD63 with high intensity versus a population with lower CD63 expression. Upregulation of CD203c expression is generally less prominent, but often occurs in almost all cells[77]. Since the marker with higher diagnostic accuracy varies depending on the drug of interest, future research to determine the ideal activation/degranulation marker will be necessary. The threshold for positivity is determined by two-graph receiver-operating characteristics analysis corresponding to the best sensitivity and specificity[78].

Diagnostic procedure in clinical settings

When POH is suspected, we propose the diagnostic algorithm shown in Figure 3. Since this

algorithm is designed for NMBAs, it is not necessarily applicable for all drugs. **For β-lactam antibiotics, for example, in vitro diagnostics should be carried out in the order of skin testing, specific IgE determination, and BAT[79, 80].**

VI Conclusion

In vitro diagnostic procedures, including quantification of sIgE and BAT, have several advantages over skin tests and drug provocation tests: They are less cumbersome for patients and do not carry the risk of precipitating IHRs. In addition, *in vitro* tests, besides being complementary diagnostic instruments to skin tests and drug provocation tests, can aid elucidation of mechanistic processes. Combination of these tests would allow us to diagnose POH with the right balance of safety and accuracy.

Figure legends

Figure 1: Quantification of drug-specific IgE with IgE immunoassays relies upon detection of a drug-(hapten)-carrier-antibody complex. **Cyanogen bromide (CNBr)-activated cellulose is a carrier used for binding the drug-(hapten)-carrier conjugate (allergen component). The binding of sIgE in the patients' serum to epitopes of the allergen component is evaluated with enzyme-linked immunosorbent assay. The amount of sIgE bound is detected with a secondary antihuman IgE antibody labelled with enzyme. Subsequently, fluorescence intensity generated by adding enzyme substrate is quantified. The results of this assay are usually expressed as arbitrary units, i.e. kUA/L.**

Figure 2: Basophil activation tests (BATs) rely upon a flow cytometric analysis of various activation and degranulation markers on the surface membrane of basophils. **A: Schematic diagram of a basophil with IgE-crosslinked FcεRI. B: Basophils are characterized by flow cytometry using forward scatter (FSC)/side scatter (SSC)(left), SSC/anti-IgE (middle), and anti-IgE/CD203c (right). Basophils are defined as anti-IgE and CD203c positive cells. C: Schematic diagram of a basophil before stimulation with allergen. D: Most basophils express CD203c but not CD63 on the cell surface (left). Most basophils have diamine oxidase (DAO), which is an enzyme involved in histamine metabolism (right). E: Schematic diagram of a basophil after stimulation by an allergen. Activation of basophils results in increased expression of CD203c/CD300a and novel expression of CD63 on the cell surface. F: These changes can be**

detected by flow cytometry. The number of cells positive for both CD203c and CD63 are increased (left). Since activated basophils release histamine by degranulation, histamine-releasing basophils are defined as DAO negative and CD63 positive cells (right).

Figure 3: Diagnostic algorithm for perioperative hypersensitivity (POH). Adapted from Ebo et al.[31] with permission from authors. **Since this algorithm is mainly designed for NMBAs, it is not necessarily applicable for all drugs.** *: Blood samples for baseline tryptase measurements should be obtained within 24 hours of the reaction, +: showing positive results, -: showing negative results.

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