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A quarter of a century fundamental and translational research in perioperative hypersensitivity and anaphylaxis at the Antwerp university hospital, a Belgian Centre of Excellence of the World Allergy Organization

Didier G. Ebo, MD, PhD[a,b]*, Nils Vlaeminck, MD[a,c], Marie-Line M. van der Poorten, MD[a,d], Jessy Elst, MSc, PhD[a], Alessandro Toscano, MD[a,e], Athina L. Van Gasse, MD, PhD[a,d], Margo M. Hagendorens, MD, PhD[a,d], Sophie Aerts, MD[c], Ine Adriaensens, MD[c], Vera Saldien, MD, PhD[c] and Vito Sabato, MD, PhD[a,b]

ABSTRACT

Perioperative hypersensitivity constitutes an important health issue, with potential dramatic consequences of diagnostic mistakes. However, safe and correct diagnosis is not always straightforward, mainly because of the application of incorrect nomenclature, absence of easy accessible *in-vitro/ex-vivo* tests and uncertainties associated with the non-irritating skin test concentrations. In this editorial we summarize the time line, seminal findings, and major realizations of 25 years of research on the mechanisms, diagnosis, and management of perioperative hypersensitivity.

Keywords: Perioperative hypersensitivity, Basophils, Mast cell, MRGPRX2, sIgE

INTRODUCTION

Immediate perioperative hypersensitivity (POH) reactions start within 2 hours (usually within minutes), and the clinical presentation can vary from single organ system features (eg, cutaneous, respiratory) to anaphylaxis.[1] POH constitutes a rare but important health issue with serious, potentially life-threatening and fatal consequences of diagnostic error. Unfortunately, correct diagnosis can be challenging. The possible pharmacological effects of anaesthetics, the surgical procedure, simultaneous exposure to several drugs and related compounds, hidden exposures and several differential diagnoses complicates the investigation of perioperative events. In a pursuit to unravel the complex molecular pathomechanisms and pathophysiology of POH and development of new diagnostics, the departments of Immunology - Allergology and Paediatric Immunology - Allergology of the Antwerp University Hospital, together with the laboratory of Immunology of the Antwerp University have, for the past 25 years,

[a]Faculty of Medicine and Health Science, Department of Immunology – Allergology – Rheumatology, Antwerp University Hospital and the Infla-Med Centre of Excellence, University of Antwerp, Antwerp, Belgium
*Corresponding author. Laboratory of Immunology, University of Antwerp, CDE T5.95 Universiteitsplein 1, 2610 Antwerpen, Belgium.
E-mail: didier.ebo@uantwerpen.be
Full list of author information is available at the end of the article.

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studied the mechanisms that culminate in basophil and mast cell (MC) degranulation, and the diagnostic techniques for POH. As shown in Fig. 1, our research has led to seminal findings and important breakthroughs contributing to the recognition, diagnosis, and management of immediate POH reactions.

Molecular mechanisms and pathophysiology: complex and still incompletely appreciated

The molecular pathomechanisms and pathophysiology of immediate POH reaction have been extensively reviewed elsewhere.[2] Immediate drug hypersensitivity reactions (IDHRs) can be either IgE-dependent or IgE-independent. POH usually involves activation of T- and B-lymphocytes through the adaptive immune system leading to allergen-specific immune responses with cross-linking of specific immunoglobulin E (sIgE) antibodies on MCs and basophils, as we observed with certain neuromuscular blocking agents (NMBAs) such as rocuronium[3] and atracurium,[4] β-lactams such as cefazolin,[5] chlorhexidine[6] and *Hevea* latex.[7] However, POH can also result from various alternative specific and non-specific effector cell activation/degranulation processes such as via the complement-derived anaphylatoxins C5a and C3a and off-target occupancy of MC and/or basophil surface receptors such as the activation of the mas-related G-protein coupled receptor X2 (MRGPRX2) by certain NMBAs and opiates such as morphine.[8–10] Using MCs cultured from healthy donors and patients, we investigated MC activation/degranulation by staining for intracellular calcium and CD63 upregulation. Using MRGPRX2-silencing, via electroporation with Dicer-substrate short interfering RNAs, and individual cell flow cytometric analyses, we showed atracurium, but not rocuronium or sux-amethonium, to trigger degranulation as reflected by CD63 upregulation. This degranulation was observed only in MRGPRX2^{+ve} and not MRGPRX2^{-ve} or -silenced MCs.[11,12] The MCs of patients with a possible MRGPRX2-dependent anaphylaxis to rocuronium were similar in their MRGPRX2 expression and function to those of patients with an IgE-mediated anaphylaxis.[11]

However, as indicated by these reviews,[8–10] evidence for activation of the MRGPRX2 receptor has mainly been gathered in heterogenous mutated animal or *in vitro* studies. Unfortunately, translation of these preclinical findings into clinical relevance is not simple and data should be critically appraised. So while a reclassification of NMBA-induced hypersensitivity reactions is emerging, many unknowns and uncertainties remain.[13,14] Therefore, we think that such a generalized pathomechanistic reclassification focusing on MRGPRX2 activation and signalling is currently unjustified and needs further studying. A particular risk for patients is that it has been suggested that in MRGPRX2-mediated reactions one could consider re-administration of the relevant drug by lowering administration speed or dose which might be dangerous in patients who experienced an IgE-mediated reaction (eg, anaphylaxis). Moreover, in the absence of a reference standard to document MRGPRX2-dependent

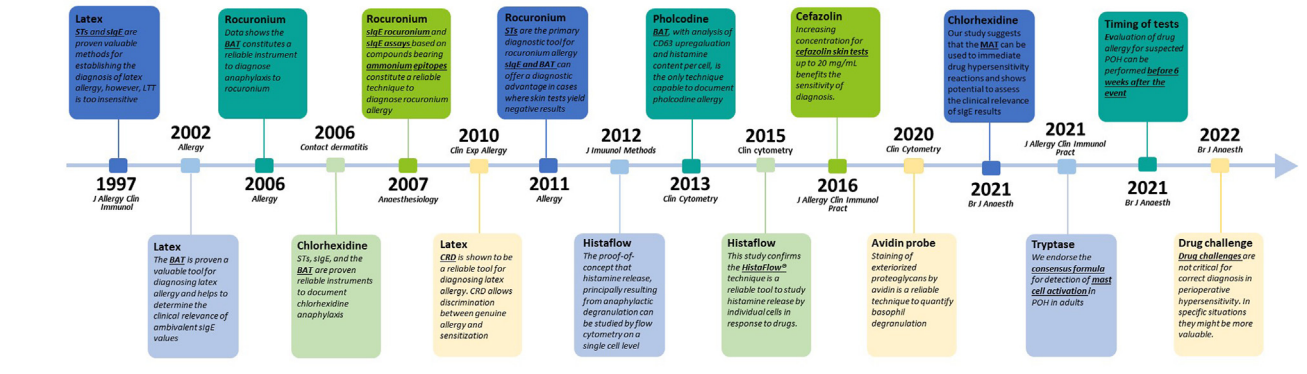

Fig. 1 Studies by our research group representing important milestones in the diagnosis of perioperative hypersensitivity. ST = skin test, sIgE = specific IgE quantification, BAT = basophil activation test, CRD = component resolved diagnosis, POH = perioperative hypersensitivity.

anaphylaxis, it cannot be excluded that some patients with (false) negative *in vitro/ex vivo* tests for IgE-dependent POH (ie, the putative MRGPRX2 patients) did in fact experience an IgE-dependent reaction. Hence we firmly dissuade any re-administration of MRGPRX2 agonists in skin-test-positive patients. This position is irrespective of the outcomes of *in vitro/ex vivo* tests.

Another mechanism independent from sIgE includes cross-linking of IgG/Fc-gamma receptor (FcγR) complexes by a specific allergen.[9] However, evidence for IgG-mediated anaphylaxis is mostly provided by animal models, and there have been no unequivocal examples of IgG-mediated POH reported. Moreover, POH can occur independent from degranulation of MCs and basophils. Further mechanistic research in this area is needed.

Description and management of POH: a plea for correct nomenclature, consensus clinical scoring, and a robust diagnostic algorithm

Unlike many other hypersensitivity reactions, POH reactions, defined in Sabato et al,[1] are witnessed by a physician, specifically the anaesthetist and surgical staff. This certainly benefits prompt recognition and adequate treatment; but while anaesthetists and surgical staff are theoretically trained to identify and treat hypersensitivity reactions, gaps in knowledge beyond the immediate management of POH persist and often documentation is sub-optimal for later diagnostic insights to be made. This is likely due to the limited exposure of anaesthetists and surgeons to the diagnostic workup of POH and the limited availability of clinical decision support tools. Therefore, we urge use of standardized reporting based upon the consensus clinical scoring for suspected immediate POH validated by a Delphi consensus process by the International Suspected Perioperative Allergic Reaction (ISPAR) working group formed in 2018 and consisting of 26 experts across specialties.[15] This score is capable of optimizing the estimation of the likelihood that a particular clinical scenario is an immediate POH reaction. It should, thus, as part of a close collaboration between anaesthetist and allergist, help to advance individual diagnostic capabilities and enable objectivity and uniformity in the determining of the sensitivity of diagnostic tests. To ensure a correct approach to gathering the relevant and complete information, deciding on whether POH is likely and how to identify the potential culprit agents to investigate, we refer to a practical algorithm by Garvey et al which has been prospectively validated elsewhere.[16,17]

As shown by a POH survey we developed, it is clear that the manifestations of POH mainly affect the cardiovascular, respiratory, and integumentary systems.[18] If anaphylaxis occurs, treatment with epinephrine together with intravenous fluid substitution is essential for successful resuscitation. It was, therefore, concerning to observe that in life-threatening anaphylaxis, intravenous adrenaline and fluids were administered in 75.7% and only 31%, respectively.[18] For an overview of knowledge on the immediate and postoperative management of suspected POH reactions we refer to Garvey et al[19]

Paired tryptase sampling

Tryptases are trypsin-like proteases predominantly expressed by MCs, and at a 200-fold lower level by circulating basophils.[20,21] Synthesis of α- and β-protryptase monomers takes place continuously in MCs, with a fraction being spontaneously released by resting MCs *in vitro*[22] and likely also *in vivo*. This secretion accounts for nearly all of the tryptase measured in baseline samples at a level that remains quite constant for a given individual over time and is dependent primarily on genetic factors.[23] Another portion of α- and β-protryptases are processed into mature forms that spontaneously form tetramers; α-tryptase homotetramers, β-tryptase homotetramers, and α/β-tryptase heterotetramers. These are stored in the secretory granules in a complex with heparin proteoglycan, awaiting exteriorization during degranulation. Quantification of serum tryptase is performed using a commercially available immunoassay that measures the mature and pro forms of α- and β-protryptases, referred to as "total tryptase" (ImmunoCAP Tryptase, Thermo Fisher Scientific, Uppsala, Sweden). The potential of serum tryptase as a biomarker of MC degranulation during anaphylaxis dates back to 1989.[24] Using an experimental anaphylaxis model, it was demonstrated that in a majority of the patients, the tryptase level peaks 1–2 hours after the

precipitating event. The first tentative judgment criterion for mast cell activation (MCA) was an acute serum tryptase (aST) equal to at least double the baseline serum tryptase (bST).[25,26] Twenty years later, an aST value exceeding [1.2xbST + 2] was proposed as a consensus threshold to define clinically significant MCA.[27] In line with others, based on measurements from 296 patients and 75 control individuals who had uneventful anaesthesia, we have shown that the use of a single aST value does not accurately represent MCA during a POH. Paired sample analyses of aST and bST are necessary to document MCA during POH, as is reflected by the higher accuracy of the paired sampling thresholds (Δ tryptase >3.2 ng/mL; the consensus formula; >85% increase).[28] For an historical overview of tryptase as a biomarker we refer to Vitte et al.[29] Of note, an apparently normal aST, that is, a value below the manufacturer's upper reference value of 11.4 ng/mL, does not exclude MCA and changes in acute tryptase are not discriminative between sIgE- and MRGPRX2-mediated POH, including anaphylaxis.[30,31] Finally, it has to be kept in mind that most of the data about paired sample analysis of aST and bST have been gathered in adults, and it remains elusive whether these findings can readily be translated to a paediatric population, especially in infants.[32]

Confirmatory testing

Providing an updated systematic approach to identify the culprit(s) and safe alternatives for the future when investigating a patient with a suspected POH is far beyond the scope of this article. However, a brief summary of confirmatory testing can be found in Fig. 2 and below. Starting from our clinical expertise and experience with flow-assisted functional analyses of basophil and cultured human MCs, we reflect on some of the challenges one could encounter whilst investigating a POH.

Skin tests

For most drugs/compounds used in the perioperative period, serial skin prick tests (SPTs), and if required, adjunct intradermal tests (IDTs)

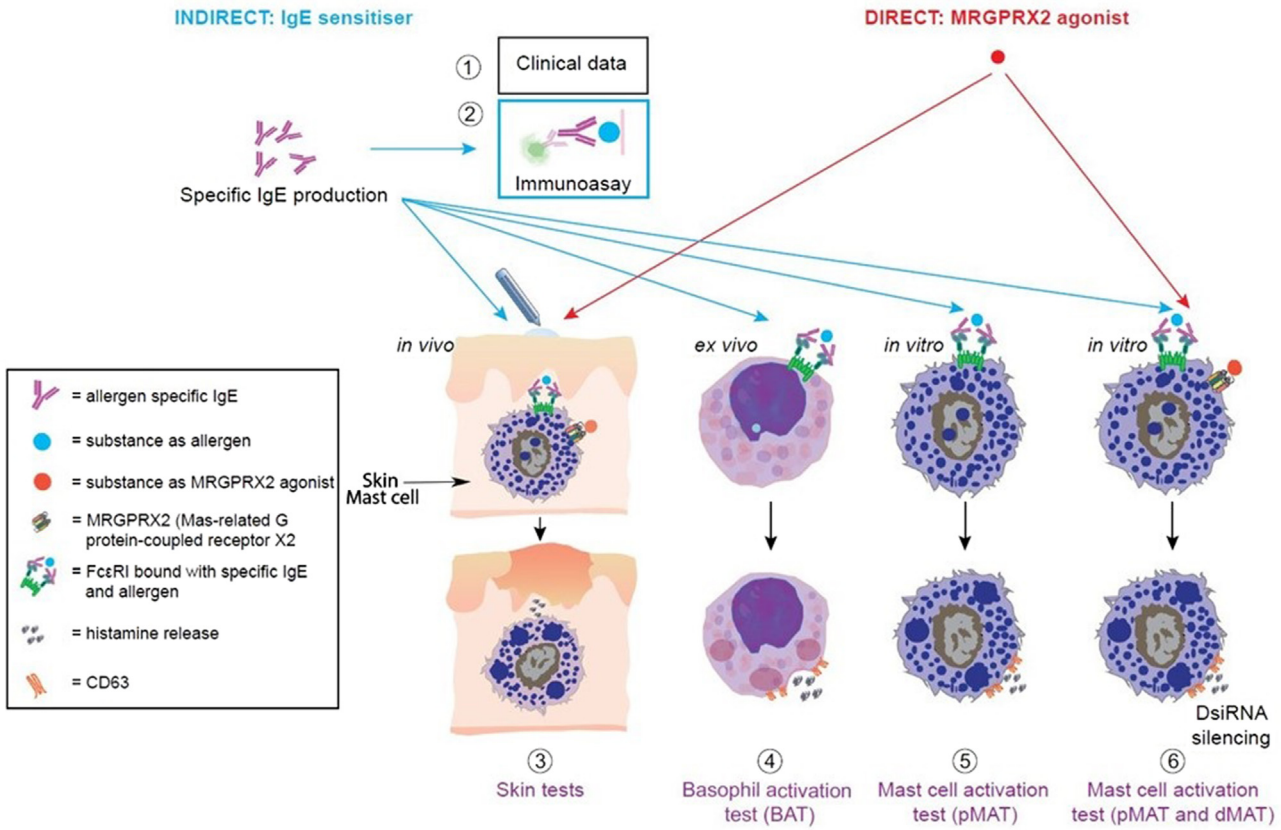

Fig. 2 Diagnostic techniques in perioperative hypersensitivity.

constitute the primary confirmatory diagnostic. However, there remain many unknowns and uncertainties associated with skin testing. First, for several drugs skin tests are not entirely standardized and optimal skin test concentrations are yet to be established. We showed that for cefazolin, which together with amoxicillin/clavulanic acid, constitutes the third most common cause of POH in Belgium, increasing IDT concentration up to 20 mg/mL benefits the sensitivity of the test without compromising its specificity.[33] Second, with respect to NMBAs, especially rocuronium, the predominate cause of POH in Flanders, it was shown that negative skin test responses might not necessarily give the green light for a safe re-exposure.[34,35] In cases with ambiguous and negative skin tests, adjunct *in vitro* and/or *ex vivo* tests can advance diagnosis and reduce the need for drug challenges that are not systematically performed in the context of POH.[36]

Furthermore, our study in rocuronium hypersensitivity challenges the dogma that a positive skin test is specific for sIgE-mediated reaction.[31] Like for other potent MRGPRX2 agonists, summarized by McNeil,[37] skin test responses cannot discriminate between sIgE- and MRGPRX2 reactions.[31]

Total and specific IgE immunoassay

In the diagnostic algorithm, for some drugs, quantification of serum drug-reactive sIgE antibodies can be part of the evaluation. Unfortunately, drug-specific sIgE tests are only available for a limited number of compounds and a critical review reveals their accuracy to be suboptimal and highly dependent on the studied drug (class).[38] For penicillins and cefaclor the sensitivity and specificity of the sIgE assays vary significantly, between 0-85% and 52–100%, respectively. For cefazolin we showed that, unlike sIgE cefazolin, the sIgE-to-total IgE ratio was discriminative between patients and control individuals. For a cutoff ratio of 1.42×10^{-3} a sensitivity of 49% and a specificity of 94% were found.[5] Moreover, the optimal cutoff for positivity remains a matter of debate. For non-life-threatening immediate penicillin allergy we showed that diagnosis should not rest upon low sIgE results between 0.10 and 0.35 kUA/L.[39] For other beta-lactams, no sIgE assays are available.

Today, sensitization to NMBAs is serologically primarily assessed by quantification of IgE reactivity to tertiary and quaternary substituted ammonium structures that are known to be the dominant epitopes of NMBA.[40,41] The most frequently applied methods are a choline chloride, a paminophenyl phosphoryl choline (PAPPC), and/or morphine-based solid-phase assays. Crucially, it has been demonstrated that the commercially available morphine-based assay reliably depicts suxamethonium and rocuronium-reactive antibodies. However, it fails to detect atracurium-reactive antibodies.[4,42,43] Alternatively, quantifying IgE reactivity to substituted ammonium structures to systematically screen patients at risk for NMBA hypersensitivity is not advisable.[43–45] In addition, a positive sIgE to substituted tertiary or quaternary ammonium structures in isolation should not be considered as diagnostic and does not necessarily preclude further administration of an NMBA.[46] Note that the sIgE morphine and poppy seed assays cannot be used to reliably document opiate allergy[47] and that sIgE to substituted tertiary or quaternary ammonium structures, like skin testing with NMBAs and sugammadex, might not depict reactions to the sugammadex-rocuronium inclusion complex.[48]

As indicated in international consensus recommendations, all patients who experienced a suspected POH should be tested with *Hevea* latex and antiseptics (chlorhexidine, povidone iodine).[16,19,49] Along with skin tests, diagnosis of both these allergies can be documented by measurement of sIgE, but prudence is advised in the interpretation of incongruent positive sIgE and negative skin tests.[50,51] In such difficult cases adjunct tests such as component resolved diagnosis and/or *ex vivo* basophil activation tests or *in vitro* mast cell activation test might be warranted.[6,52–55] For latex, component resolved diagnosis (CRD) might depict clinically irrelevant sIgE-reactivity due to sensitization to pollen profilin and/or pollen or Hymenoptera venom cross-reactive carbohydrate determinants.[50] In contrast, sIgE to ethylene oxide should not be

systematically tested in POH, mainly because of the risk for false positive results.

Basophil activation tests (BAT)

The major challenge of drug hypersensitivity/allergy diagnosis in patients with equivocal and/or negative conventional tests lies in the development of safe, accessible, and reliable diagnostics enabling the correct prediction of the clinical outcome following exposure to the offending allergen(s) and cross-reactive structures. Historically, *in vitro* and *ex* vivo drug allergy diagnosis have focused on 2 techniques. The first technique, used mainly in the context of nonimmediate reactions that occur later than 2 hours after the exposure (often 48–72 h later),[1] uses T-lymphocyte transformation based upon incorporation of [methyl-3H] thymidine[56,57] and release of various cytokines.[58,59] The second technique, used for sIgE-mediated reactions, is based on basophil histamine as well as sulphidoleukotriene release.[60] However, the time- and cost-consuming two-step approach, ie, (a) cell incubation and (b) quantification of mediators, has restricted their application.

For almost 25 years, we have shown that flow-based analysis and quantification of *ex vivo*-activated basophils (BAT) might meet the requirements to safely document sIgE-dependent allergy to *Hevea* latex, different NMBAs, chlorhexidine, β-lactam antibiotics and many other, more rare, causes of POH.[61,62] With respect to *Hevea* latex, the most significant contribution of BAT was its capability to be discriminative between clinically relevant and irrelevant sIgE results;[52] the latter mainly due to the sensitization to ubiquitous structures such as cross-reactive carbohydrates (CCDs) and profilin.[50] However, today BAT latex has been largely supplanted by single or multiplexed CRD,[63,64] a technique more amenable to routine use, provided components are available.[53] The added value for obtaining BAT in NMBA, which was validated for rocuronium[3,65,66] and atracurium,[67] is dual. It can benefit diagnosis in difficult cases with equivocal or negative ST results[35] and advance resolving the sIgE vs. MRGPRX2 conundrum.[31,35] For chlorhexidine allergy,[68] BAT can advance diagnosis in patients with incongruent ST and sIgE results[6,55,69] and can help with the identification of the cross-reactivity profile.[70] With respect to β-lactam antibiotics, BAT was shown to be of poor performance to document cefazolin allergy and amoxicillin.[71] Additionally, BAT can be helpful to diagnose IgE-mediated allergies for antibiotics such as rifamycin and clindamycin/lincomycin or dyes such as patent blue, for which no sIgE assay is available.[72–74] Other so-called nondiagnostic applications of BAT include therapeutic monitoring, follow-up of natural histories, and identification of allergenic recognition sites, eg, allergenic changes at the sugammadex primary rim because of inclusion of rocuronium.[48] For more nondiagnostic applications of BAT in drug hypersensitivity see Elst et al and Ebo et al.[75,76]

However, to guarantee optimal results it is recommended to perform the analyses within 4 hours after sampling, and, importantly, the technique does not advance diagnosis in patients with non-responsive cells. Furthermore, BAT is difficult to standardize mainly because of the difficulty of performing large batches that might require repetitive analyses spanning over several days.

Mast cell activation tests

While BAT over the last decades has indisputably contributed to our knowledge of the oftentimes complex mechanisms that govern drug-driven activation/degranulation of basophils and advanced diagnosis in POH patients in whom identification of the culprit was not straightforward, its use is still not generalized. Although offering many advantages over traditional mediator release tests,[60] it leaves us with some unanswered questions hindering a wider application and entrance in mainstream use. For example, the time-sensitivity of the tests and 15% of patients showing a non-responder status. One of the strategies to resolve these limitations is the mast cell activation test (MAT) that is reviewed elsewhere.[69] Briefly, in MAT, both MC lines and/or cultured donor MCs are stimulated directly (eg, by MRGPRX2 agonists in the dMAT) or indirectly after passive sensitization of the donor MCs with patients' sera or plasma to depict serum sIgE antibodies (pMAT). It is of note that the pMAT can still be of use to depict sIgE-mediated activation of a drug also capable of inducing MRGPRX2 activation, as MRGPRX2 can be silenced. In the

context of POH, we have currently applied the dMAT and pMAT to unveil the MRGPRX2 agonistic capacity of NMBAs and opiates[11,12] and investigated its potential as an adjunct test to diagnose sIgE-dependent chlorhexidine allergy and to explore the chlorhexidine IgE-cross-reactivity profile.[51,55,77] Admittedly, additional comprehensive explorations are required to establish the utility of dMAT and pMAT in fundamental and translational research on suspected POH.

Drug challenge tests

Drug challenge tests (DCT) are considered the reference test in drug hypersensitivity investigation. However, DCTs have not been systematically recommended in the investigation of POH, mainly because of the pharmacological effects of drugs such as induction agents and NMBAs.[36] In an attempt to provide insights in the potential benefit of DCTs with hypnotics, opioid analgesics, and NMBAs, we reviewed the anaesthetic notes and surgical reports of 344 patients who had anaesthesia after diagnostic investigation comprising skin testing, sIgE, and BAT for an earlier suspected POH reaction.[78] Our data show that subsequent reactions after allergic work-up are rare at <1%, which includes accidental re-exposure to a known culprit and which matches the incidences reported by Fisher et al, Guyer et al, and Miller et al.[78–81] Consequently, DCTs would not have benefited diagnosis in the majority of our cases. It is of note, however, that many anaesthetists opted to not re-administer rocuronium despite negative work-up, especially if no other culprit had been found. Theoretically, this group could have the highest risk of false negative work-up and potentially benefit from DCT. Moreover, there are some specific situations in which DCTs with these compounds might be recommended to advance diagnosis, especially in centres depending solely on STs that do not have access to sIgE and/or BAT and MAT. First, STs can sometimes be unreliable, such as in patients with skin anergy or dermographism. Second, DCT studies might be warranted in research settings, such as for rapid and standardized development of novel diagnostics. Third, we could have offered DCTs to cases with clinical anaphylaxis and increased tryptase but in whom no culprit could be identified by conventional tests as indications and contraindications for DCTs in POH might differ from those applicable to drug hypersensitivity reactions unrelated to anaesthesia. Finally, it is important to remember that DCT do not permit unambiguous discrimination between sIgE-dependent and putative MRGPRX2 POH reactions.[31]

Perspectives

Despite significant progress in the understanding, diagnosis, and management of POH, there is still room for improvement. First, many knowledge gaps and uncertainties remain concerning the phenotypes and endotypes of POH. Mast cell activation via the MRGPRX2 receptor provides a novel paradigm in our knowledge of sIgE-independent POH. However, current proof for activation of the MRGPRX2 receptor comes almost exclusively from preclinical or *in vitro* studies, and translation of these findings into clinical relevance in humans is difficult. Unfortunately, this translation poses a significant challenge, mainly because of the absence of a reliable diagnostic to irrefutably document a MRGPRX2 reaction. Currently, diagnosis of MRGPRX2 reactions can only be established indirectly by exclusion of other, mainly sIgE-mediated, mechanisms. Given the unavailability and shortcomings of sIgE assays[38] and the inability of STs to reliably discriminate between endotypes,[31] it seems that *ex vivo* basophils and *in vitro* mast cell activation/degranulation experiments could play a more prominent role.[75] However, there is still far to go from our current observations to more universal adoption and clinical use of these effector tests. In sIgE-mediated POH, unlike in MRGPRX2-mediated reactions, CD4+Th2-lymphocytes are essential for isotype-switching to drug reactive-sIgE. We think that further research to unveil the underlying pathomechanisms of POH and to optimize the diagnostic approach could also benefit from *ex vivo* T-lymphocyte experiments with analyses of drug-reactive memory T cells.[57] Diagnosis of POH could benefit from new diagnostic techniques, such as MAT. MAT overcomes some of the limitations of traditional BAT and might be a useful added diagnostic tool in difficult cases and centres in which no BAT is available. Additional explorations are required to establish the place

of MAT in fundamental and translational research in suspected POH.

Except for some human leukocyte antigen (HLA) associations, genetic mutations seem of little importance in sIgE-mediated reactions.[82] In contrast, receptor polymorphisms of MRGPRX2 related to gain-of-function mutations appear to contribute in MRGPRX2-mediated reactions.[83,84] Therefore, it is attractive to speculate that genetic studies could also help to resolve the sIgE vs MRGPRX conundrum and fill in the missing link in MRGPRX2 diagnosis.

Challenges remain regarding accurate and timely diagnosis and referral, including a complete description of the reaction, together with paired acute and basal serum tryptase analyses. We urge implementation of a standardized reporting tool based upon the Consensus Clinical Coring for Suspected Immediate POH that was formed by ISPAR working group.[15]

Over the past few years, anaesthetists more commonly acquire acute tryptase samples; however, the optimal formula for mast cell activation is still a matter of debate. The current consensus formula has proven to be a valuable diagnostic method in adults; further studies exploring the optimal formula in a paediatric population are needed.

Abbreviations
aST, acute serum tryptase; BAT, basophil activation test; bST, basal serum tryptase CRD, component resolved diagnosis DCT, direct challenge test POH, perioperative hypersensitivity IDT, intradermal test ISPAR, international suspected perioperative allergic reaction MAT: mast cell activation test (dMAT = direct MAT, pMAT = passive MAT) MC, mast cell MCA: mast cell action NMBA: neuromuscular blocking agent MRGPRX2, mas-related G-protein coupled receptor X2 sIgE, specific IgE SPT, skin prick test ST, skin test.

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The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials or can be made available upon request.

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Creation figures: MVP, NV, JE.
All authors approved the final text.

Ethics approval
All studies discussed within this review were approved by the ethical committee of the Antwerp University hospital as well as by the local committees of all participating centres. Individual approvals can be found in the referenced published articles.

Author consent
All authors agree to the submission of this article to the World Allergy Organization Journal and, if accepted, to its publication in this journal. We warrant that this article is original, does not infringe on any copyright or other proprietary right of any third party, is not under consideration by another journal and has not been previously published. Ethical approval has been sought and obtained as necessary and any conflicts of interest stated.

Competing interests
The authors declare no conflict of interest.

Author details
[a]Faculty of Medicine and Health Science, Department of Immunology – Allergology – Rheumatology, Antwerp University Hospital and the Infla-Med Centre of Excellence, University of Antwerp, Antwerp, Belgium[b]Department of Immunology and Allergology, AZ Jan Palfijn Gent, Ghent, Belgium[c]Faculty of Medicine and Health Science, Department of Anaesthesiology, Antwerp University Hospital, University of Antwerp, Antwerp, Belgium[d]Faculty of Medicine and Health Science, Department of Paediatrics, University of Antwerp, Antwerp University Hospital, Antwerp, Belgium[e]Post-graduate School of Allergology and Clinical Immunology, University of Milan, Milan, Italy.

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