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REVIEW

Biomarkers of immediate drug hypersensitivity

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Abstract

Immediate drug hypersensitivity reactions (IDHRs) are a burden for patients and the health systems. This problem increases when taking into account that only a small proportion of patients initially labelled as allergic are finally confirmed after an allergological workup. The diverse nature of drugs involved will imply different interactions with the immunological system. Therefore, IDHRs can be produced by a wide array of mechanisms mediated by the drug interaction with specific antibodies or directly on effector target cells. These heterogeneous mechanisms imply an enhanced complexity for an accurate diagnosis and the identification of the phenotype and endotype at early stages of the reaction is of vital importance. Currently, several endophenotypic categories (type I IgE/non-IgE, cytokine release, Mast-related G-protein coupled receptor X2 (MRGPRX2) or Cyclooxygenase-1 (COX-1) inhibition and their associated biomarkers have been proposed. A precise knowledge of endotypes will permit to discriminate patients within the same phenotype, which is crucial in order to personalise diagnosis, future treatment and prevention to improve the patient's quality of life.

KEYWORDS
biomarker, drug, hypersensitivity, immediate

1 | INTRODUCTION

Drug hypersensitivity reactions (DHRs) are a burden for patients and the health systems, not only due to the increasing prevalence (10%–20% of hospitalised patients and up to 25% of outpatients) but also to the complexity and severity of the reactions.[1]

Labelling a patient as allergic represents not only a health problem but also a significant financial burden for affected individuals and health systems, with high medical costs mainly on inpatient care. This problem increases taking into account that only a small proportion of patients initially labelled as allergic are finally confirmed as such after an allergological workup.[2] DHR diagnosis has as main consequence the interruption of treatment and the switch to second-line therapeutic alternatives, which may be less effective, and more toxic and costly, usually causing a negative impact on life quality and expectancy of these patients, in addition to increasing the costs to the health system.[3]

From DHRs, immediate DHRs (IDHRs) are those occurring within 1–6 h after drug administration and the clinical symptoms range from mild/moderate as urticaria or angioedema to more severe ones like anaphylaxis, which can be life threatening.[4] The symptoms in IDHRs appear after drug-induced activation of effector cells, mast cells and basophils or activation of inflammatory pathways and the mediators release. Classical drugs triggering these reactions are non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, radiocontrast media (RCM), neuromuscular blocking agents (NMBAs) and anaesthetic agents drugs.[1,5] But in the last decades, other new drugs for the treatment of oncologic or autoimmune diseases such as chemotherapy agents, monoclonal antibodies and biological agents have also demonstrated to trigger these reactions, increasing their complexity. Therefore, all this highlights the need for identifying the specific biomarkers for accurately labelling patients and performing the right future recommendations.[5]

The diverse nature of these drugs will imply different interactions with the immunological system having mast cells as main effector cells (Figure 1); therefore, although IDHRs have been classically considered type I IgE-mediated reactions according to Gells and Coombs classification, other mechanisms, such as immunological non-IgE-mediated or non-immunological mechanisms due to off-target interactions with effector cells receptor or enzymes have been demonstrated.[5–7]

The identification of the phenotype and endotype at early stages of the reaction is of vital importance. Phenotypes are characterized by clinical features, the time elapsed between drug exposure and the onset of symptoms and appearance after the first administration or repeated doses. The endotypes that accompany these phenotypes are defined by the mechanisms involved and by the molecular mediators released by the effector cells used as biomarkers. Currently, several endophenotypic categories (type I IgE/non-IgE, cytokine release, Mast-related G-protein coupled receptor X2 (MRGPRX2) or Cyclooxygenase-1 (COX-1) inhibition and their associated biomarkers have been proposed, being tryptase, platelet activating factor (PAF) and interleukin 6 (IL-6) some of the most clinically relevant markers in diagnosis.[8] (Figure 2).

It is important to highlight that specific endotypes can be associated with (i) high reoccurrence rate as it happens in urticaria angioedema after low doses of rocuronium in IgE-mediated reactions; (ii) dose effect, since lower in IgE-mediated reactions compared to other mechanisms. Also, MRGPRX2-mediated reactions can be prevented by lowering the dose/infusion rate and (iii) role of cofactors like infectious diseases that are especially important in hypersensitivity to betalactams, …[9–11]

Therefore, clarification of these aspects will definitely help in the correct management of these patients. Consequently, the identification of specific biomarkers would be the first milestone in this process (Box 1).

2 | MECHANISMS ON DRUG-INDUCED HYPERSENSITIVITY

The mechanism involved in IDHRS has been classically referred to as an immune response mediated by drug-specific IgE (sIgE) antibodies. However, the fact that sIgE levels are not detectable may indicate that another mechanism may be involved; in this sense, IgG-mediated mechanisms have been also reported by the low-affinity IgG receptors (FcγRIII) on the surface of basophils, macrophages or neutrophils that specifically need high amount of drug typically used in biological agents treatment.[12]

Another mechanism involved in chemotherapy or biological agent hypersensitivity is the cytokine release reaction (CRR) that can be produced by a direct activation or lysis of target cells by these drugs resulting in a massive secretion of cytokines in serum.[12,13]

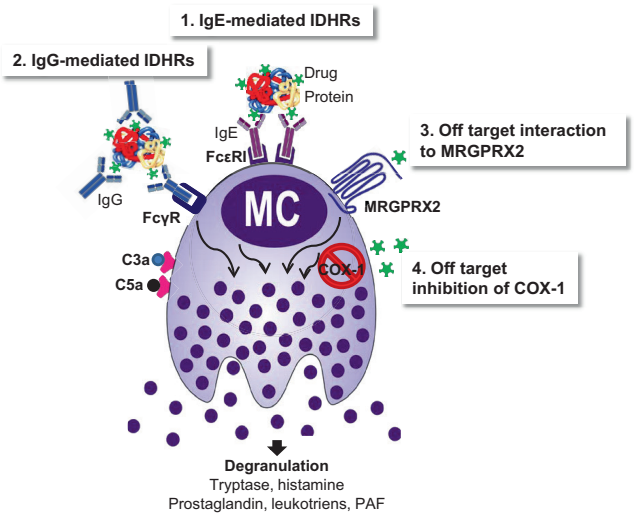


FIGURE 1 Mast cells as central effector cells on immediate drug hypersensitivity reactions (IDHRs). 1 and 2 are immunological mechanisms that need the drug binding to carrier molecules forming adducts; 3 and 4 are non-immunological mechanisms produced by the direct interaction of the drug to either receptors or enzymes.

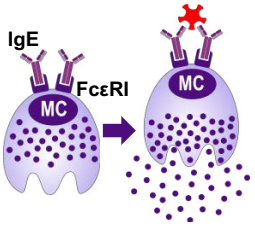
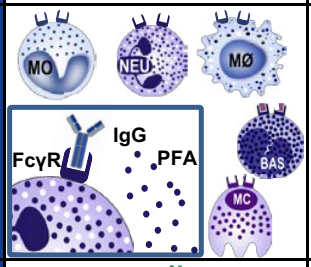
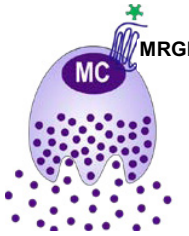
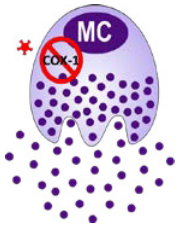
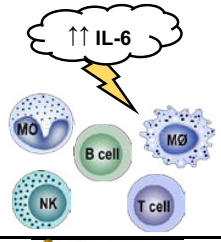
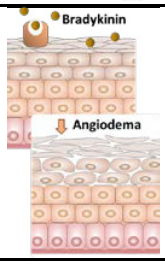
		Effector cells	Phenotyping biomarkers	Endotyping biomarkers	Effect of the dose
IgE-mediated reactions		Mast cells, basophils	Pruritus, erythema, urticaria, angioedema, rhinitis broncospasm, abdominal pain, vomiting, diarrhea, cardiovascular colapse	Tryptase, histamine, histamine metabolites, sIgE, basophil activation markers; skin tests	• From second dose • Low dose
IgG-mediated reactions		Mast cells, basophils, neutrophils, macrophages, monocytes		PAF, PAF-AH, Anaphylotoxin C3a, C5a	• From second dose • Higher dose than IgE-mediated reactions
MRGPRX2 mediated reactions		Mast cells, (conditioned) basophils		Tryptase, histamine, MRGPRX2 polymorphisms Skin tests	• From first dose • Higher dose than IgE-mediated reactions
NSAIDs cross-hypersensitivity		Mast cells		LTC4, LTD4, LTE4, 9a,11b-PGF2 NERD biomarkers: IL-5, ECP, IFN-γ, TGF-β1, DDP10, SPD, foliculin	• From first dose • Higher dose than IgE-mediated reactions
Cytokine release syndrome		Monocytes, macrophages, T cells, B cells and NK cells	Fever, chills, rigor, nausea, pain, headache, hypotension, oxygen desaturation (in mixed reactions with mast cells and basophils involving, also flushing, urticaria and cardiovascular collapse)	IL-6, IL-8, IL-10, IL-1, TNF-α, IFN-γ (in mixed reactions: tryptase, histamine)	From first dose
Bradykinin mediated angioedema		Damaged tissues	Angioedema	Serum BK, plasma activity of DPP-IV protein and APP, serum endothelial-selectin, angiopoietin-2, 6-keto-PG F1α	Higher dose than IgE-mediated reactions

FIGURE 2 Different mechanisms involved in IDHRs indicating the effector cells, phenotyping and endotyping biomarkers and the effect of the drug dose and the sensitization need.

Furthermore, drugs-induced reactions after the first exposure, suggesting that non-immunologically mediated mechanism might be involved by off-target interaction with receptors on effector cells, that is, fluoroquinolones (FQs), NMBA and some other drugs interact with MRGPRX2 on mast cells inducing their activation and inflammatory mediators release.[14] Another possible mechanism is through

BOX 1 Relevance of endophenotyping immediate drug hypersensitivity reactions

- Precise diagnostic: Avoid false-labelled patients and also causing harm in patients with reaction
- Desensitization procedure application
- Future management of patients
- Accuracy of drug avoidance recommendations
- Future alternative drug recommendations and understanding the role of comorbidities

the inhibition of enzymes in immunological cells, that is, COX-1 inhibition by NSAIDs modifying the arachidonic acid metabolism.[15]

Finally, there is a group of drugs like angiotensin-converting enzyme inhibitors (ACEI) drugs, dipeptidyl peptidase IV (DPPIV) inhibitors (gliptin drugs),[16] neutral endopeptidase P/neprilysin inhibitors, fibrinolytic agents and oestrogen that produce angioedema mainly because they are involved in the decreased degradation of bradykinin (BK) and other vasoactive substances[17] (Figure 2).

These arrays of mechanisms can be associated with similar symptoms and therefore the diagnosis of patients with IDHRs with correct endophenotyping is currently a major challenge during diagnosis. A proper knowledge of endotypes based on specific biomarkers will permit discriminating patients within the same phenotype. This will be important to personalise diagnosis, which will be crucial for future treatment as well as possible alternatives, and prevention to improve the patient's quality of life. Moreover, endotyping patients could also be relevant during the clinical decision of performing drug desensitizations since, as reported, these procedures are effective in IgE- or IgG-mediated reactions and some cases of CRR but not in those produced after off-target interaction with MRGPRX2.[18,19]

3 | BIOMARKERS IN IGE-MEDIATED REACTIONS

IgE-mediated mechanism has been classically accepted as the underlying mechanism in IDHRs.[1] In this case, drugs, frequently low molecular weight compounds, act as haptens needing to bind to macromolecules and form adducts that interact to IgE bound to high-affinity receptor (FcεRI) on the surface of effector cells, mast cells and basophils (Figure 1). Then, after subsequent contact, adducts cross-link with two or more adjacent sIgE molecules, triggering the activation and degranulation of mast cells and basophils, with the release of preformed inflammatory mediators, and the synthesis and secretion of lipid mediators and cytokines.[20] Drugs typically involved are antibiotics, beta-lactams (BLs) or FQs, RCM, NMBAs, carbamazepine, sulphanilamides or pyrazolones.[5,20] Furthermore, in the last decades, other drugs like platins used in chemotherapy treatments and monoclonal antibodies used for the treatment of different immunological diseases have been reported as producing IgE-mediated reactions.[21,22] However, it seems that cross-linking of IgE is not a condition sine qua non for degranulation of mast cell and basophils because monovalent complexes can also induce degranulation.[23] Future studies are needed to evaluate the role of this phenomenon in IgE-mediated drug allergy.

Identification of IgE-mediated IDHRs is mainly based on skin tests and the quantification of drug-sIgE in serum as a main biomarker.

Skin tests normally include skin prick tests and intradermal tests at immediate readings and have been used for a large range of drugs like BLs, perioperative drugs, heparins, platinum salts and RCM.[24] A positive skin test suggests an IgE-mediated reaction to the incriminated drug.[24] Their sensitivity depends on the drug but is generally more sensitive compared to sIgE determination.[24,25]

sIgE determination by in vitro methods is classically done by immunoassays (commercial or in-house)[1] as reported to BLs,[26] FQs,[27] NSAIDs[28] and NMBAs.[29] The most used commercial method for detecting drug-sIgE is the fluoroimmunoassay ImmunoCAP®, however, the availability is limited to only a few drugs and sensitivity and usefulness depend on analysed drug. For penicillins, sensitivity has been reported to be low and variable (0%–50%) depending on the clinical symptoms[26] and showing false-positive results to penicillin G in an important percentage.[30] Higher sensitivity values have been reported for NMBAs[31]: rocuronium (83%–92%), morphine (78%–84%) and suxamethonium (44%). Moreover, high-sensitivity values (84%–97%) for sIgE to chlorhexidine, an increasingly relevant element in perioperative hypersensitivity, should be noted.[32] Despite the increasing prevalence of hypersensitivity to biologicals, there are no commercial kits available. Interestingly, it has been shown that cetuximab can induce IDHRs even at first administration due to cross-reactivity with galactose-α-1,3-galactose (α-gal) by natural exposure indicating that α-gal-sIgE detection can predict cetuximab-induced anaphylaxis prior to first administration.[33]

Moreover, in-house methods, mostly using radiolabeled anti-IgE, are indispensable to overcome sensitivity limitations of the fluoroimmunoassays and analyse the immunological recognition of new chemical structures. In this sense, recent studies have shown the relevance of the inclusion of different determinant antigens for the detection of drug sIgE to cephalosporins, carbapenems and monobactams[34,35] as well as the beta-lactamase inhibitors clavulanic acid (CLV)[36,37] and tazobactam.[38] All these findings suggest the need to include different antigenic structures in the same assay for diagnosing the maximal number of patients, ensuring the detection of different patterns of recognition. On the other hand, great efforts are being made in the implementation of more sensitive detection methods by using ultra-sensitive chemiluminescence immunoassay,[39] and a multiplex microimmunoassay,[40] both of them reported being used for the detection of sIgE to penicillin. Additionally, the use of synthetic structures that mimic carrier molecules, instead of classically used poly-L-lysine, and new solid phases can provide interesting alternatives to improve the in vitro clinical diagnostic practice. Indeed, the use of nanoparticles decorated with BL-dendrimers shows promising results for detecting sIgE to BLs, with a preliminary study ($N = 21$) showing higher sensitivity values for the detection of sIgE

to BP and/or AX with nanoparticles as solid phase (100%) compared with traditional cellulose discs (83% for AX, 78% for BP), and interestingly, the detection of false-positive results of BP sIgE for confirmed AX-selective patients decreased from 41% for cellulose discs to 0% for nanoparticles.[41] This method provides high reproducibility due to the homogeneous composition of nanoparticles and facilitates the effective exposure of drugs to sIgE improving sensitivity.

Basophil activation test (BAT) is a useful additional tool for diagnosing IgE-mediated reactions[1,42] and has been recommended for diagnosing BLs, NMBAs, FQs, RCM and pyrazolone allergies.[1] However, due to sensitivity limitations, different studies are focused on improving it through the use of new chemical structures derived from the parent drug or new methodological approaches. In this sense, comparisons of basophil activation biomarkers, CD63 and CD203c, in a prospective evaluation of amoxicillin (AX) and CLV allergic patients showed that the best sensitivity and specificity was obtained for CD203c (46.6% and 94.6%), with good positive predictive value and like-hood ratio.[43] These results are in line with those obtained by Abuaf et al.[44] However, another recent study in IDHRs to AX obtained lower sensitivity (23%) when selecting a high specificity (95%).[45] All this suggests that methodological or analytical variations in the classical procedure could be useful to improve the diagnostic value of BAT.

Basophils can be also activated through non-IgE-mediated mechanisms,[46] limiting the capacity of BAT to differentiate between the endotypic mechanism underlying the IDHR. So in order to consider basophil activation as a biomarker for IgE-mediated reactions, the involvement of the FcεRI-mediated pathway should be confirmed. Studies have performed BAT inhibition by using phosphatidylinositol 3-kinase (PI3K) inhibitors[47] to determine if basophil activation was mediated by sIgE instead of other mechanisms such as MRGPRX2-mechanisms. However, since IgG-mediated basophil activation has been also proposed as an alternative pathway,[48] which would be also inhibited by PI3K inhibitors, other approaches should be applied to confirm in vitro basophil activation mediated by IgE. This is the case of designed ankyrin repeat proteins (DARPin), disruptive IgE inhibitors that are able to desensitise in vitro allergic effector cells by actively removing IgE from cell surfaces.[49,50] In the same line, humanised monoclonal anti-IgE antibodies, such as omalizumab[51] or ligelizumab,[52] with high potency to block IgE/FcεRI signalling have been applied in both in vitro and in vivo studies and eventually, bruton tyrosin kinase inhibitors[53] could also be used.

Another approach to indirectly confirm IgE-mediated reactions is the negativisation in long-term studies due to the clearance of sIgE if patients are not exposed to the culprit drug.[54,55] These changes in the in vitro results do not happen when the activation is due to non-immunological mechanisms like the off-target interaction to MRGPRX2 or enzyme inhibition, COX-1 for NSAIDs.

Finally, IgE-mediated reactions are also characterised by the release of inflammatory mediators that can be measured as biomarkers, although they are not limited to IgE-mediated mechanisms. Serum tryptase is the most commonly analysed mediator in the acute phase to confirm anaphylaxis,[1,56] with recent studies in perioperative hypersensitivity showing that high tryptase values determined as >11.4 ng/mL[57] or >(1.2 × baseline-tryptase) + 2 μg/L[58] were more frequent in life-threatening reactions. On the other hand, histamine is the most abundant inflammatory mediator for acute anaphylaxis; however, limitations for the detection exist due to its short half-life in serum (20 min), therefore, detection of the metabolites N-methylhistamine and N-methylimidazole acetic acid in urine samples (24 h) is an alternative indirect method for the determination of histamine. It should be highlighted that the use of histamine metabolites as a biomarker requires the avoidance of microbially processed foodstuffs, which can contain large amounts of histamine,[59] as the oral administration of histamine has been reported to increase the 24 h excretion of N-methilimidazole acetic acid.[60]

4 | BIOMARKERS IN IGG-MEDIATED REACTIONS

Although the IgE-mediated pathway is classically considered the main underlying mechanism of human IDHRs,[61] the evidence supporting the existence of alternative mechanisms has grown in the last years. In this sense, new data regarding IgG-mediated mechanism, complement and coagulation-dependent activation, and MRGPRX2-induced reactions has arisen.[14,62]

IgG-mediated reactions are well studied and documented in mice, and the evidence in humans is frequently limited and extrapolated from these animal models.[48,63,64] IgG immunocomplexes (IC) that engage low-affinity IgG receptors (FcγR) in different myeloid cells such as macrophages/monocytes and neutrophils, but also basophils and mast cells, with the release of PAF as a major mediator, are considered the main actors in these reactions.[48,64] Interestingly, a higher amount of antigen/drug is required to induce IgG-mediated anaphylaxis, reflecting the much higher affinity of IgE binding by high-affinity IgE receptor (FcεRI) than IgG binding by FcγR.[9] For this reason, IgG-mediated IDHRs are mainly related to parenteral administration and a high amount of drug, whereas food, absorbed in a smaller amount, is more likely an IgE-dependent pathway.[9] However, recent evidence shows that the most severe food anaphylaxis may be also related to the simultaneous activation of both IgE and IgG mechanisms.[65]

Understanding the physiopathology of IDHRs may help to identify potential biomarkers that could differentiate between IgE and IgG reactions. Considering that a high rate of patients suffering an acute IDHR may not show any biomarker of an IgE-mediated reaction, the identification of the underlying mechanism is of the utmost interest to stratify the risk before attempting a drug challenge and/or give avoidance recommendations.[48]

In a recent study, Jönsson et al.[48] have shown the utility of neutrophils, FcγR and PAF acetylhidrolase (PAF-AH) as potential biomarkers of IgG-mediated reactions using a cohort of patients with IHRs to NMBA. They observed that no IgE-related biomarkers could be found in about 26% of cases that were classified as potential IgG-mediated reactions. These individuals showed higher

values of neutrophil activation markers and lower FcγR expression on neutrophils surface, which is related to IgG-mediated activation.[64] Interestingly, even those with suspected IgE-mediated reactions showed signs of neutrophil activation through an IgG mechanism, and lower values of PAF-AH, which correlates with higher serum PAF values, although these biomarkers had lower values when compared to those patients with suspected IgG-mediated reactions. Indeed, they observed a correlation between severity and the presence of both IgE and IgG biomarkers, suggesting a double mechanism in most severe reactions, as previously suggested in a model of food allergy.[65] In this same line, PAF directly correlated, whereas PAF-AH inversely correlated with anaphylaxis severity in a cohort of patients with anaphylaxis of different aetiologies, including drugs.[66] Considering that neutrophils are one of the main sources of PAF, and express FcγRI but not FcεRI,[67] we may understand that these reactions may be also IgG-related.

In a study,[64] it has been concluded that a decreased expression of FcγRIII in neutrophils, without an increase of the expression of IL-4Rα in T cells, IL-4 or IL-4α soluble receptor levels in serum (markers of IgE reactions) would likely indicate an IgG-dependent mechanism. This hypothesis, based on mice experiments, is supported by some observations in human models; IgE but not IgG receptors engagement in human basophils induces the activation of IL-4 pathway.[68] Moreover, human neutrophils incubated for 4 h with serum-containing drug-specific IgG-IC show a decrease of 60% in the expression of FcγRIII compared with the same conditions but without drug-specific IgG-IC.[64]

Finally, the complement system, through the generation of anaphylatoxins such as C3a, can also activate mast cells and basophils upon engagement with its receptor. Interestingly, C3a has demonstrated a synergistic effect with IgG activation, increasing up two-fold the intensity of the reaction.[69] In the same line, complement can be activated by IgG IC. Regarding its relationship with DHRs, it has been demonstrated that drugs solubilized in therapeutic liposomes or lipid-based excipients, as well as intravenous iron preparations, are able to induce complement activation.[70] Serum C5b-9, final product of the complement activation cascade, may be used as a biomarker of complement-related DHRs. Actually, some authors have observed the correlation between complement levels increase and symptom duration, although the supporting evidence is based on in vitro and ex vivo studies, and animal models.[71,72]

5 | BIOMARKERS IN CYTOKINE RELEASE REACTIONS (CRR)

A mechanism of IDHRs recently associated with new treatments such as chemotherapy and biological agents is the massive cytokine release by different cells, including monocytes or macrophages, T cells, B cells and natural killer cells. This CRR is a type of IDHR that can occur at the first dose of the drug. These mechanisms can be induced after the direct interaction or through FcγRIII on target cells.[73] Although also FcεRI engagement can have a role in CRR amplifying the classical IgE-mediated anaphylaxis. The pro-inflammatory mediators, like TNF-α, IFN-γ, IL-8 IL-10 and IL-1 and especially IL-6, are considered as biomarkers for these CRR.[13,74] However, measurement of these cytokines is not routinely performed.

6 | BIOMARKERS IN MRGPRX2-MEDIATED REACTIONS

Different observations like the lack of detection of drug sIgE or the appearance of IDHRs after the first drug administration suggest the involvement of a non-immunological mechanism, with the involvement of MRGPRX2 in the mast cell activation reinforcing it.[75] Indeed, cationic peptidergic drugs such as NMBAs, FQs and icatibant can have an off-target interaction with the MRGPRX2.[75] Moreover, resting basophils barely express MRGPRX2 on their surface, but this expression can be quickly upregulated after stimulation, making 'conditioned' cells responsive to MRGPRX2 occupation.[76]

The MRGPRX2 mechanism seems to display its own peculiarities and its diagnostic and therapeutic approach most likely differs from the IgE-mediated process.[14,53,77] However, there is no irrefutable evidence for its clinical relevance. The most relevant limiting aspects relate to (i) the oversimplified dichotomy IgE vs MRGPRX2, (ii) the possible additive effect of IgE- and MRGPRX2-pathway[78] and (iii) the possibility of some drugs (e.g. FQs, NMBAs) possibly triggering both pathways. Therefore, the identification of specific clinical, diagnostic and susceptibility biomarkers is pivotal for the comprehensive elucidation of the role of the MRGPRX2 endotype. Potential clinical biomarkers include drug naivety, the requirement of higher doses than for IgE-mediated reactions, and the impact of comorbidities. A cardinal point is whether the different spatio-temporal dynamics of IgE and MRGPRX2 engagement[79] are clinically discernible. IgE and MRGPRX2 endotypes can cause the entire spectrum of clinical manifestations, including anaphylaxis. Onset and duration cannot practically be used to discriminate IgE and putative MRGPRX2 anaphylaxis mainly because both require prompt therapy. On the other hand, it is tempting to speculate that in case of MRGPRX2 occupation: (a) anaphylaxis would invariantly present with cutaneous manifestations and (b) resolution of isolated cutaneous symptoms should be faster.

Diagnostic biomarkers comprise assessment of mast cell activation and mechanistic studies. Paired analyses of acute and baseline serum tryptase are prerequisites for an appropriate assessment of mast cell activation.[80] Recent in vitro data pointed out that tryptase levels cannot be used for differentiating between the IgE and MRGPRX2 endotype, for that, PBMCs were activated via sIgE-mediated mechanism and via MRGPRX2-mediated pathway and then, tryptase levels in the collected supernatants were determined by ImmunoCAP, and no significant difference in the variation of the tryptase concentration (before and after stimulation) was observed.[81] In the same line, as shown by a retrospective analysis of rocuronium hypersensitivity, although the significant rise in tryptase is observed more frequently in IgE endotype,

there is no difference in acute titres between presumed IgE and alleged MRGPRPX2 phenotypes.[82] It is appropriate to conclude that no acute biomarker is available to ascertain of MRGRPX2-induced mast cell degranulation. Identification of potential new acute biomarkers can benefit from transcriptomics, metabolomics and proteomics. Thus far, no mechanistic diagnostic tool is available to document with certainty an MRGPRX2 endotype. Skin test positivity is expected for potent MRGPRX2 agonists and cannot be used to differentiate an IgE from an MRGPRX2 mechanism. Theoretically, bruton tyrosin kinase inhibitors[53] could be used during skin testing to document IgE-mediated endotypes. Although sIgE detection can be considered the most valuable discriminator, as mentioned above, sIgE assays are available only for a limited number of drugs, and their accuracy is suboptimal. For the time being there is no specific test able to demonstrate unambiguously an MRGPRX2-mediated mechanism. Identification of a putative MRGPRX2-mediated IDHR remains a diagnosis by exclusion when in vitro or in vivo mast cell activation is not associated with a specific immune response (IgE or specific T cells). 'MRGPRX2 assays' should include functional studies on cultured non-sensitised mast cells (direct mast cell activation test—dMAT) with measurement of membrane markers,[83] mediator release, G-protein dependent (Ca^{++} endpoint) and independent (beta-arrestin endpoint) pathways read-outs.[84] In vitro analysis of 'conditioned' basophils can serve as an additional model for exploring MRPGPRX2 agonism. However, none of these assays alone is diagnostic. Assays to document IgE endotype include passive mast cell activation tests (based on sensitised, MRGPRX2-silenced mast cells)[85] BAT and T-lymphocyte activation test. A theoretical algorithm to discriminate MRGPRX2 and IgE endotype has been recently published.[77]

Finally, polymorphisms of MRGPRX2 can significantly impact the responsiveness of the receptor to agonistic drugs.[86] This might explain why MRGPRX2-mediated reactions occur only in a minority of the population and why an individual patient reacts only to some agonists and tolerates others. Therefore, it is anticipated that analysis of MRGPRX2 polymorphisms can provide significant advances in the identification of susceptibility biomarkers.

7 | BIOMARKERS IN NSAIDS CROSS-HYPERSENSITIVITY

Another type of non-immunologically mediated IDHRS is those induced by drugs which inhibit enzymes such as COX-1 in mast cells, deviating the synthesis of prostaglandins (PGs) and thromboxanes towards overproduction of cysteinyl-leukotrienes (CysLTs; LTC4, LTD4 and LTE4).[15] Most studies have focused on NSAIDs-exacerbated respiratory disease (NERD). NERD is a sub-endotype of T2 asthma, and therefore, inflammatory biomarkers eosinophils, fractional exhaled nitric oxide (FENO) and serum IgE are typically elevated and directly related to exacerbation risk.[87–92] However, levels of these biomarkers and cut-off values are not clinically fixed and vary according to external agents such as corticosteroid treatment, tobacco smoke or alcohol.[93,94]

The total IgE in serum ≥100 IU/mL confirms the adaptive immune system involvement in T2 asthma, although this biomarker has little diagnostic or prognostic value.[94] However, IgE level in NP tissue from NERD patients has been associated with faster recurrence of NP compared to aspirin tolerants.[95]

Recently, serum periostin has been proposed as an NERD biomarker because it contributes to airway remodelling connected to T2 inflammation, although the clinical utility and cut-off points remain to be established.[90,96] Moreover, plasma eosinophil-derived neurotoxin, L-plastin, serum sphingosine-1-phosphate and urine sphingosine have been also proposed as an NERD biomarker[97] although clinical validation is required.

Increased expression of type 2 cytokines, including IL5, and eosinophil cationic protein (ECP) levels has been found to be higher in the NP tissue and in nasal secretions of patients with NERD compared with aspirin tolerant asthmatics (ATA).[98,99] Additionally, type 1 inflammatory cytokines such as interferon (IFN)-γ has been found to be elevated in the nasal tissue of NERD compared to chronic hyperplastic eosinophilic sinusitis.[100] Serum levels of TGF-β1 were significantly higher in NERD patients than in ATA and positively correlated with urinary LTE4 levels.[101] Moreover, serum levels of dipeptidyl peptidase 10 (DDP10) may be also a potential biomarker to distinguish NERD from ATA and predict disease severity as a positive correlation has been found with TGF-β1 in patients with NERD.[102]

Surfactant protein D (SPD) interacts with phagocytic cells attenuating airway inflammation and remodelling. Therefore, serum SPD levels were lower and negatively correlated with FEV1% decrease after aspirin challenges in patients with NERD than with ATA.[103]

Folliculin, an intracellular protein that regulates cell–cell adhesion, is increased in the sera of NERD patients compared to ATA.[104]

The provocation with aspirin is the most accurate biomarker to diagnose NSAID hypersensitivity, being typically performed orally but in NERD, it can also be done intranasally or inhaled. However, this is a costly non-free-risk test that requires trained personnel and resources.[15,105]

NERD patients experience respiratory reactions after NSAID intake due to dysregulation of arachidonic acid (AA) metabolism with an overproduction of LTs.[15,106] Therefore, LTE4 serum levels and the ratio LTE4/9a,11b-PGF2,[107] as well as urinary and salival LTE4 have been found to be increased in NERD and could potentially be used to identify the risk of aspirin hypersensitivity in asthmatics.[108,109] Recently, in sputum inflammatory cell distribution, the concentrations of LTE4 and the LTE4/logPGE2 ratio have been used for subphenotyping NERD patients.[110] Additionally, an increased concentration of LTE4 in urine, and nasal and bronchoalveolar lavage fluids have been found after oral aspirin challenge, being associated to the severity of the respiratory reaction during challenge.[111]

The pathogenic model proposed for NERD has been extended to NSAID hypersensitivity manifested with the exclusively cutaneous symptom, and urinary LTE4 have been proposed as a biomarker for NSAID-exacerbated cutaneous disease (NECD).[112] Similarly to NERD, LTE4 and 9α,11β-PGF2, the main metabolite of PGD2, are increased after aspirin challenge in NECD,[113] NSAID-induced urticaria/angioedema (NIUA)[113] and blended reactions patients.[114]

Genetic variants have been associated with NSAID hypersensitivity, being distinct in patients experiencing respiratory or cutaneous symptoms although most of them have not been replicated. Most studies have focused on evaluating AA pathway-related variants or immune cell activation.[115,116] In NERD, variants in genes associated with LT production (5-Lipoxygenase, 5-LOX) and LTC4 synthase and PGs production (COX) pathways were reported.[115] Available data show that NECD and NIUA share similar genetic backgrounds; nevertheless, different gene polymorphisms have been reported.[117,118]

8 | BIOMARKERS IN BRADYKININ-MEDIATED ANGIOEDEMA

Another non-immunologically mediated IDHR is BK-mediated angioedema, which has been associated with angiotensin-converting enzyme inhibitor (ACEI) drugs,[119] DPPIV inhibitors (gliptin drugs),[16] neutral endopeptidase P/neprilysin inhibitors,[120] fibrinolytic agents and oestrogen,[121] although most studies focused on ACEI-induced angioedema. In these reactions, angioedema is believed to be the consequence of the decreased degradation of BK and other vasoactive substrates, such as substance P[16] leading to a rapid local increase of vascular permeability and extravasation of fluid into the interstitial space in the dermis and subcutis.[17]

Serum BK levels have been proposed as a biomarker as it has been found to be elevated during acute attacks of ACEI-induced angioedema compared with remission.[17] However, its clinical utility is questioned because of BK short half-life and technically challenging measurement, and additionally, the role of BK as the main mediator of this angioedema subtype has recently been questioned.[122] In addition, laboratory parameters associated with coagulation and fibrinolysis including cleaved high molecular weight kininogen, plasma kallikrein and activated coagulation factor FXII are also tedious.[123]

The normal level of complement component 4 and C1-inhibitor (C1-INH) allows differentiating from hereditary angioedema (HAE).[124] Recently, an increase in C1-INH activity during acute ACEI-induced AE attacks has been reported.[125] In cases of ACEI-induced angioedema, C-reactive protein serum level has been reported to be significantly increased by 7.3-fold compared to normal values in patients with angioedema of unknown cause,[126] and leukocytosis, especially in those patients with abdominal symptoms,[127] has been reported.

Plasma activity of DPP-IV protein and aminopeptidase P (APP), which catabolises BK, has been shown to be decreased in patients with ACEI-associated angioedema compared to patients on ACEI therapy and with no angioedema history.[128]

Recently, serum endothelial-selectin and angiopoietin-2 have shown to be increased in BK-induced angioedema compared to histamine-mediated angioedema, showing the role of endothelial dysfunction and serine proteases in this angioedema subtype.[129]

The determination of 6-keto-PG F1α has also been proposed as a biomarker for assessing the risk of developing ACE-induced angioedema, as it is increased in patients experiencing angioedema under ACEI therapy.[130]

9 | CONCLUSIONS

IDHRs can be produced by a wide array of mechanisms after the drug interaction with specific antibodies bound on their receptors or directly on effector target cells on their receptor. There is a need to accurately endophenotyping the patients for a precise diagnosis. This is crucial for further drug prescription and alternative drug recommendations since readministration and cross-reactivity should be managed in a different way when immunological or non-immunological mechanisms are involved. To discriminate these reactions, we can use both clinical and laboratory biomarkers. From them, clinical biomarkers are difficult to manage and regarding diagnostic biomarkers, besides drug-sIgE, recent studies are indicating many others that can characterize the different endotypes of IDHRs, although further analyses are necessary to precisely indicate whether we are dealing with a specific endotype or several of them occurring simultaneously. The presence of simultaneous endotypes that could increase the severity of the reaction could not be ruled out, especially for those drugs that could induce a reaction by different mechanisms; however, further studies addressing this issue in drug hypersensitivity are required.

AUTHOR CONTRIBUTIONS

MJT, CM and AA designed the review and coordinated the work. Authors contributed to different sections: CM 'Mechanisms on drug-induced hypersensitivity'; AA 'Biomarkers in IgE mediated reactions'; RMC 'Biomarkers in IgG-mediated reactions'; VS 'Biomarkers in MRGPRX2 mediated reactions'; ID 'Biomarkers in NSAIDs cross-hypersensitivity and Biomarkers in Bradykinin mediated angioedema'; MJT 'Introduction and Conclusions'. All authors reviewed and accepted the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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