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ORIGINAL ARTICLE

Flow-based basophil activation test in immediate drug hypersensitivity. An EAACI task force position paper

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Abstract

Diagnosing immediate drug hypersensitivity reactions (IDHRs) can pose a significant challenge and there is an urgent need for safe and reliable tests. Evidence has emerged that the basophil activation test (BAT), an in vitro assay that mirrors the in vivo response, can be a complementary test for many drugs. In this position paper, members of Task Force (TF) "Basophil activation test in the evaluation of Drug Hypersensitivity Reactions" from the European Academy of Allergy and Clinical Immunology (EAACI) present the data from a survey about the use and utility of BAT in IDHRs in Europe.

Abbreviations: ADRs, adverse drug reactions; AX, amoxicillin; BAT, basophil activation test; BLs, beta-lactam antibiotics; CHX, chlorhexidine; COX-1, cyclo-oxygenase; DAIG, drug allergy interest group; DHRs, drug hypersensitivity reactions; EAACI, European Academy of Allergy and Clinical Immunology; EDTA, ethylenediaminetetraacetic acid; FcεRI, high-affinity IgE receptor; FQs, fluoroquinolones; GR, grade of recommendation; GRADE system, grading of recommendations, assessment, development, and evaluations; IDHRs, immediate DHRs; IDTs, intradermal tests; IGAD, allergy diagnosis interest group; IL, interleukin; LE, level of evidence; MCs, mast cells; MRGPRX2, mas-related G-protein coupled receptor X2; NIDHRs, nonimmediate DHRs; NMBAs, neuromuscular blocking agents; NPV, negative predictive value; NSAIDs, nonsteroidal anti-inflammatory drugs; pBAT, passive BAT; PEG, polyethylene glycol; PG, penicillin G; PPV, positive predictive value; PV, penicillin V; RCM, radio contrast media; SI, stimulation index; sIgE, specific IgE; SPTs, skin prick tests; SSC, side scatter; STs, skin tests; TF, task force.

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The survey results indicate that there is a great interest for using BAT especially for diagnosing IDHRs. However, there are still main needs, mainly in the standardization of the protocols. Subsequently consensus-based recommendations were formulated for: (i) Technical aspects of BAT in IDHRs including type of sample, management of drugs, flow cytometry protocols, interpretation of the results; and (ii) Drug-specific aspects that should be taken into account when performing BAT in relation to betalactams, neuromuscular blocking agents, fluoroquinolones, chlorhexidine, opioids, radio contrast media, chemotherapeutics, biological agents, nonsteroidal anti-inflammatory drugs, COVID vaccine, and excipients. Moreover, aspects in the evaluation of pediatric population have also been considered. All this indicates that BAT offers the clinician and laboratory a complementary tool for a safe diagnostic for IDHRs, although its place in the diagnostic algorithm depends on the drug class and patient population (phenotype, geography, and age). The standardization of BAT is important for generalizing this method beyond the individual laboratory.

KEYWORDS
basophil, drug, flow cytometry, hypersensitivity, IgE-mediated reactions

1 | INTRODUCTION

Drug hypersensitivity reactions (DHRs) account for about 10% of all adverse drug reactions (ADRs). DHRs are unpredictable, reproducible, often severe, and may be caused by distinct immunologic and nonimmunologic mechanisms.[1] Allergic drug reactions are immunologic DHRs that are mostly mediated either by drug-specific IgE (sIgE) antibodies (Type I) with immediate onset, or drug-specific T-lymphocytes (Type IV) with nonimmediate onset.[2] However, immediate DHRs (IDHRs) may also be nonallergic and occur independently of sIgE. IgE-mediated IDHR are initiated by the interaction between a hapten/drug covalently bound to autologous proteins (e.g., serum albumin) and the immune system, resulting in production of sIgEs that bind to tissue resident mast cells (MCs) and circulating basophils (sensitization). Upon re-exposure, cross-linking of drug adducts to surface-bound sIgE leads to MC and basophil activation/degranulation with release of mediators, producing the clinical manifestations of an IDHR, including anaphylaxis.[2]

Nonallergic IDHRs, previously called "pseudo-allergy", present similar clinical pictures to IgE-mediated IDHRs, but without specific immunologic mechanism.[2] The mechanisms involved in nonallergic IDHRs are not completely understood. Some can be related to a deviated cysteinyl-leukotriene/prostaglandin balance through inhibition of cyclo-oxygenase (COX)-1 by nonselective nonsteroidal anti-inflammatory drugs (NSAIDs).[1] Others are likely due to an off-target occupation of the Mas-related G-protein coupled receptor X2 (MRGPRX2) as suggested for fluoroquinolones (FQs), some neuromuscular blocking agents (NMBAs) and opiates such as morphine.[1]

Diagnosing IDHRs can pose a challenge that ideally starts with a detailed history, paired tryptase measurements, complemented with confirmatory diagnostics such as skin tests (STs), in vitro/ex vivo assays and, eventually, drug challenge. The choice of investigations are guided by the history (chronology and morphology of the reaction)[3] and the suspected underlying immune mechanisms.[4,5] STs, that is, skin prick tests (SPTs) and intradermal tests (IDTs) are often primary means for detecting alleged allergic IDHRs. However, their diagnostic value in IDHRs varies among drug(s) (classes) and is not validated for many drugs. Importantly, a positive ST and drug challenge does not per se reflect an IgE-mediated reaction. Additionally, for some drugs a full-dose drug challenge might be difficult mainly because of pharmacologic activity[4]; moreover, it might be contraindicated in patients who experienced a life-threatening reaction. In such difficult cases, in vitro/ex vivo tests might offer a safer option to confirm or refute a diagnosis of IDHRs and influence medical decision-making.[5]

In vitro assays that focus on measuring serum sIgE as main biomarker are only available for a limited number of drugs, and not all are commercialized.[5,6] Furthermore, accuracy of these assays is far from optimal with frequent false negative but also false positive results.[7,8] The basophil activation test (BAT), in which fresh patient's whole blood is incubated with a suspected drug or its metabolite(s), mirrors the in vivo response more closely than serum sIgE measurement and could thus fill this gap.[9,10]

There is a consensus on BAT's utility, and recommendations on correct use of BAT to evaluate IDHRs to many drugs have been published.[5,9,10] However, BAT protocols are still not fully standardized in terms of cellular identification and activation markers, ideal timing, factors influencing activation, and drug concentrations and management. There is still a need for further validation with larger numbers of well-characterized patients and exposed control subjects.[9,10] Data in nonallergic IDHRs indicate that BAT is not useful for NSAIDs hypersensitivity evaluation and in the case of off-target interaction to

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MRGPRX2, there are few studies. Therefore further analysis with modifications of the method are necessary to establish the role of BAT.[9,11–13]

Given the difficulties in the diagnosis of IDHRs by STs, sIgE, and drug challenge, BAT has been proposed as a complementary test.[5,14] However, it is not known how, and to which extent, the above indicated recommendations have been translated into daily practice. Hence, a survey about the use and utility of BAT in allergic and nonallergic IDHRs was conducted. Based on the results of this survey, a literature search, and the expert opinion of the members of the European Academy of Allery and Clinical Immunology (EAACI) Task Force (TF) "Basophil activation test in the evaluation of Drug Hypersensitivity Reactions", several recommendations for BAT in IDHRs have been established in this position paper. It is important to emphasize that the main limitation about the establishment of certain recommendations is the absence of strong endorsement since they can be based on low/moderate evidence obtained from a low number of reports and low numbers of cases.

2 | METHODS

2.1 | Survey

The survey addressed current clinical practice, questions, and unmet needs in Europe. The web-based survey about use of BAT in the diagnosis of IDHRs (Google® platform) was emailed to all members of the EAACI Drug Allergy (DAIG) and Allergy Diagnosis Interest Group (IGAD) and also to different members of National Allergy Societies between March and May 2021. The survey included 15 multiple-choice and free-text questions grouped into four main domains: (i) indications; (ii) value in IDHR diagnosis; (iii) limitations; and (iv) limitations of the current literature. The questionnaire was previously agreed upon by all TF members. The similar open-answer questions have been clustered (Survey details in Appendix S1).

2.2 | Development of position paper with recommendations

Based on the needs and limitations identified in the survey, we performed a literature search and gathered the experience of the task force members in order to formulate consensus-based recommendations. The literature search was performed using electronic databases (MEDLINE and PubMed) and a systematic review database (Cochrane library). Keywords were drug hypersensitivity reactions, allergy, in vitro tests, IgE, drugs, basophil activation, and MRGPRX2. Key statements were provided with a level of evidence (LE) and grade of recommendation (GR) according to Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) system. Briefly, quality of evidence was assessed by the group and recommendations were defined. The strength of recommendation was defined as "strong", "weak", or "no recommendation". We used wording of "recommend" for strong recommendation whereas "suggest" for weak recommendations.[15,16] Finally, a voting was performed to establish the agreement status on recommendations. When evidence was lacking, a consensus was reached among the task force experts.

3 | RESULTS

3.1 | Survey results: Use of the basophil activation test in Europe

One hundred and six responders from 14 countries (mainly from Turkey 38/106 (36%) and Spain 30/106 (28%)) completed the survey. Most responders were allergists 79/106 (74%) (Figure 1A,B). BAT was mainly used in the evaluation of IDHRs to beta-lactam antibiotics (BLs) and NSAIDs (Q1; Survey) (Figure 1C). A total of 61/106 (58%) use BAT in their clinical practice (Q3; Survey) (Figure 1D) and all of them for evaluating IDHRs (100%) among other applications (Figure 1E) and mainly in adults (Q5; Survey) (Figure 1F). However, only 34% of participants had access to BAT in their own centre (Figure 1D).

The clinicians stated to mainly use in vivo tests for evaluating mild/moderate IDHRs (Q6; Survey) with an increase in using BAT for severe IDHRs (Q7; Survey) (Figure 2). Importantly, 63% agree or strongly agree that BAT can be/is useful for evaluating IDHRs (Q8; Survey) (Figure 3) Box 1.

BOX 1 | Main results from survey.

IDHRs evaluation in clinical practice	Surveyed appreciations about BAT
• Beta-lactams and NSAIDs (single NSAID-induced hypersensitivity) are the most frequently evaluated drugs • 58% use BAT during their clinical practice, all of them for DHR although also for other allergies to some extent • Of the BAT users 94% were for evaluating adults, whilst 45% for pediatric population • When evaluating nonsevere IDHRs, BAT is moderately used and mainly for BLs and NMBAs • When evaluating severe IDHRs, BAT is increasingly used and mainly for BLs and NMBAs	• 63% of responders agree about the usefulness of BAT for evaluating IDHRs • They agree that BAT is useful, complementary to STs, mainly for BLs • They agree that BAT is useful when STs are negative in severe reactions, mainly for BLs • Main BAT limitations: • Lack of funding • Availability of a flow cytometer • Experienced personnel • Lack of standardized protocols

The responders' expectations of BAT are displayed in Box 1 and the survey responders main needs in Box 2 indicating a demand for guidance on correct execution and interpretation of BAT. The complete description of the survey and its results are given in the Appendix S1. All the results from the survey, especially the requests on technical issues as well as clinical aspects for each drug were discussed by the TF member in order to be addressed as recommendations.

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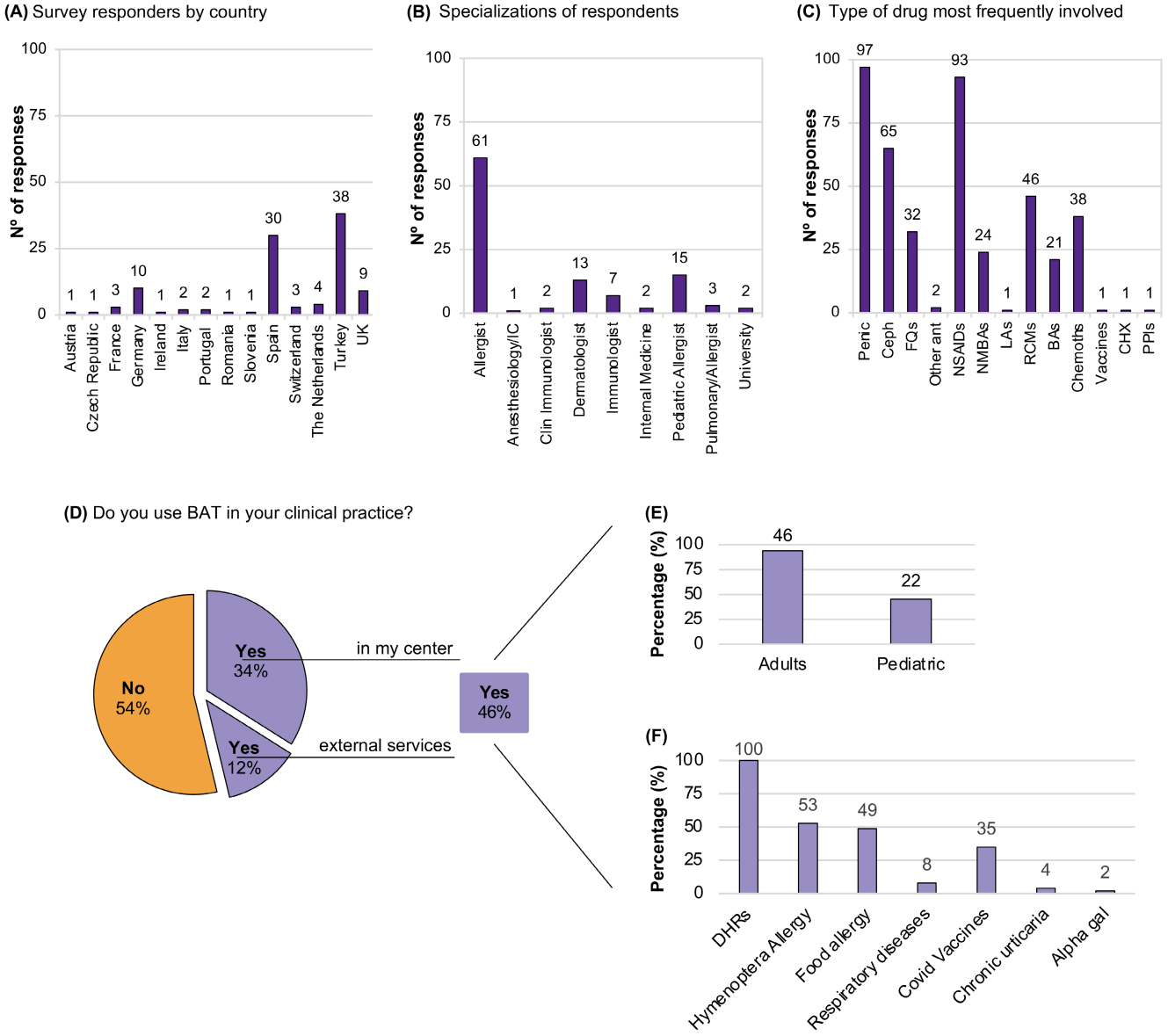

FIGURE 1 Survey results from 106 responders. (A) Number of survey responders in each country; (B) Specializations of responders; (C) Type of drug most frequently involved in allergic reactions that the professionals attend in their clinics. Survey results about use of BAT: (D) Do you use BAT in your clinical practice? (106 responses); (E) For which population do you use BAT? (49 responses); (F) For which type of allergy would you use BAT? (49 responses). Antibiotics; BAs, biological agents; Ceph, cephalosporins; Chemoths, chemotherapeutics; CHX, chlorhexidine; FQs, fluoroquinolones; LA, local anesthetics; NMBAs, neuromuscular blocking agents; NSAIDs, nonsteroidal anti-inflammatory drugs; Other ant, other antibiotics; Penic, penicillins; PPIs, proton plumb inhibitors; RCMs, radio contrast media.

BOX 2 | Main needs identified in survey.

- Funding, availability of multicolour flow cytometry, and experienced personnel
- Standardization (methods and drug concentrations)
- Validation of protocols. Intra- and inter-assay differences (round robin tests). Interpretation of results
- Settings of threshold for positivity and diagnostic indexes (likelihood ratios, ROC, etc)
- Increase in sensitivity and specificity, as well as PPV and NPV
- BAT data in pediatric populations
- Prospective studies with well-characterized patients (large sample size and multi-centric)

3.2 | Recommendations and unmet needs

BAT is a flow cytometric assay that measures the expression of activation/degranulation markers on blood basophils before and after incubation with drug/allergen. It could represent a safer, gentler, and cheaper alternative to drug challenge and, in particular cases, be the only available diagnostic method, especially in life-threatening reactions. However, its utility should consider several critical technical and clinical aspects ensuring correct execution and interpretation. IgE-mediated IDHRs present some particularities such as their haptenic nature (low molecular weight compounds) for most drugs, and

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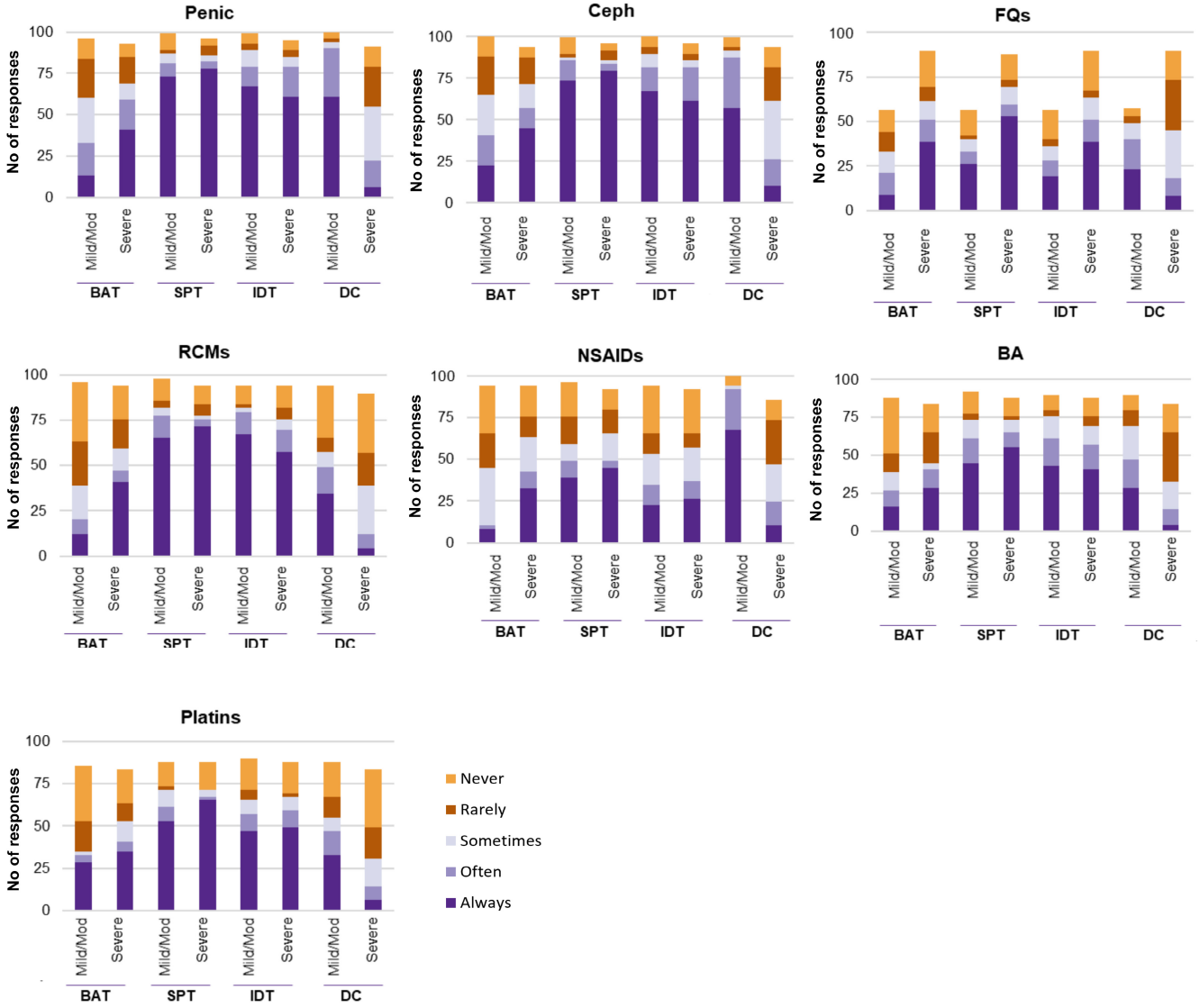

FIGURE 2 Survey results about use of different in vivo and in vitro tests for evaluating IDHRs. Rank (from never [1] to always [5]) the use of the following tests for evaluating mild/moderate (Mild/Mod) or severe IDHRs to BA, biological agents (49 responses); BAT, basophil activation test; Ceph, cephalosporins; DC, drug challenge; FQs, fluoroquinolones; IDT, ntradermal test; NMBAs, neuromuscular blocking agents; NSAIDs, nonsteroidal anti-inflammatory drugs; Penic, penicillins; RCMs, radio contrast media; SPT, skin prick test.

low level of serum sIgE or basophil activation induction capacity. Therefore, an optimal analytical sensitivity is mandatory.

3.2.1 | Technical aspects of BAT in the evaluation of IDHRs

Several technical issues are critical in the evaluation of IDHRs with BAT, with some of them strongly differing from those for evaluating allergy to allergenic proteins (Table 1). The recommendations for technical aspects with the corresponding grades are shown in Table 2.

Use of fresh blood

Since BAT is performed using whole blood basophils, an anticoagulant is needed. The most used are endotoxin-free heparin and ethylenediaminetetraacetic acid (EDTA), with the latter having a calcium chelation effect that influences cell degranulation and thus the expression of activation markers. Note, basophils are delicate cells that can suffer in terms of viability or spontaneous activation due to different factors, that is, time from collection, vibration, and temperature.[17] See recommendations Q1–4; Table 2.

Management of drugs for basophil stimulation

Some drugs are unstable or degrade in solution depending on factors like ambient temperature, pH, or exposure to light. This later factor is critical for photolabile drugs, such as FQs (i.e., moxifloxacin).[33,41] This is very important since optimal drug concentration(s) or even metabolites involved in the reaction should be used in BAT.[10,42] See recommendations Q5–9 in Table 2.

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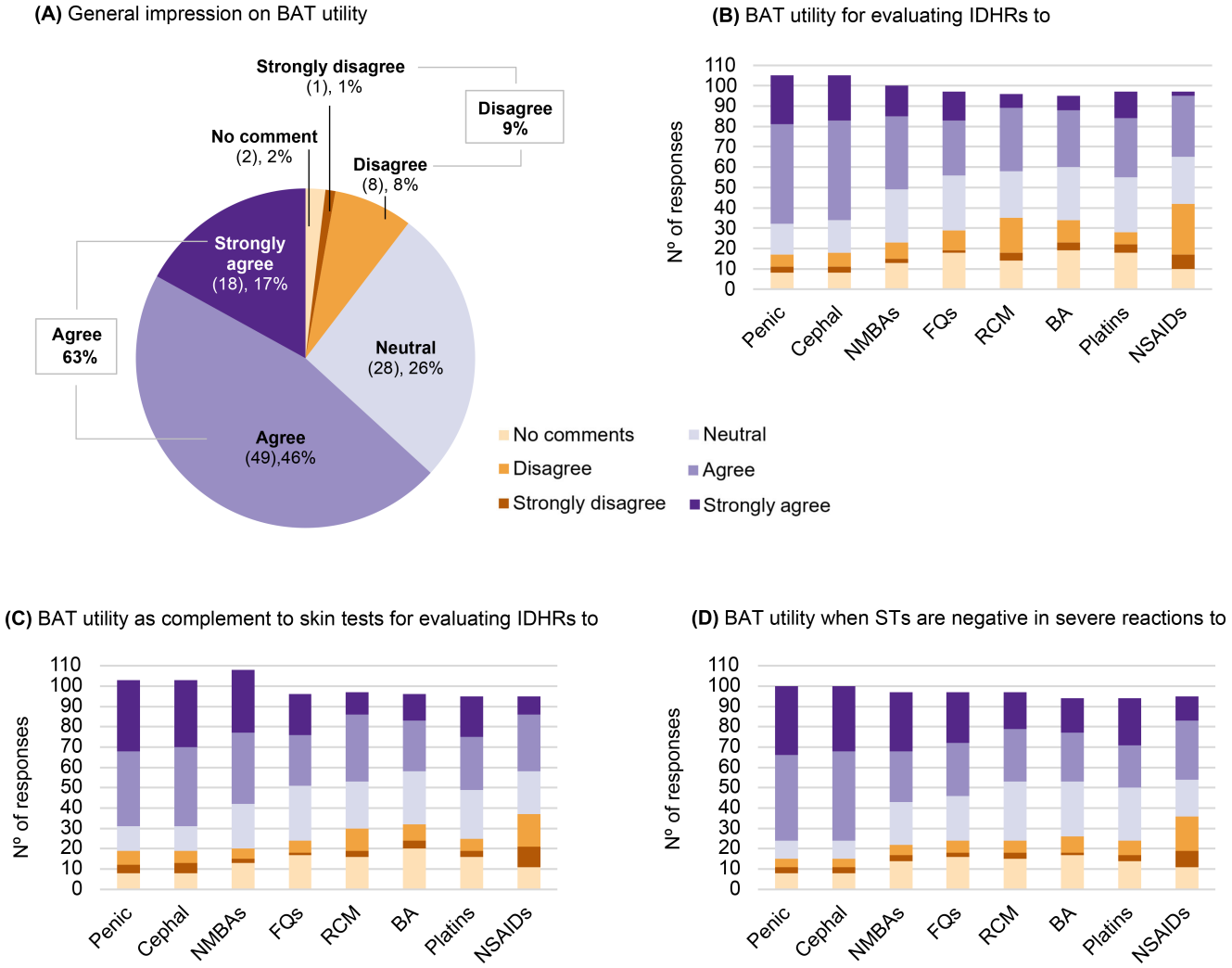

FIGURE 3 Survey results about: (A) General impressions on the utility of BAT for evaluating IDHRs (106 responses); (B) Impressions about the BAT utility for evaluating IDHRs to penicillins, cephalosporins, NMBAs, fluoroquinolones, RCMs, NSAIDs, biological agents, or platins (106 responses); (C) Impressions about BAT utility as complement to skin tests for evaluating IDHRs to penicillins, cephalosporins, NMBAs, fluoroquinolones, ICMs, NSAIDs, biological agents, or platins (106 responses); (D) Impressions about BAT utility when STs are negative in severe reactions to penicillins, cephalosporins, NMBAs, fluoroquinolones, RCMs, NSAIDs, biological agents, or platins (106 responses). Answers range from no comments to strongly agree. BA, biological agents; Ceph, cephalosporins; FQs, fluoroquinolones; NMBAs, neuromuscular blocking agents; NSAIDs, nonsteroidal anti-inflammatory drugs; Penic, penicillins; RCMs, radio contrast media.

Concentrations of drugs for basophil stimulation

In BAT, drugs are generally tested in high concentrations in the mg/mL range that might cause false negative results by cytotoxicity or unspecific/false positive results. This should be controlled from results in small windowed dose-finding curves. These concentrations depend on the drug included in the test. Table 3 summarizes the current estimates of the optimal concentrations for the drugs most commonly studied in BAT. See recommendations Q10-11 in Table 2.

Basophil selection

Basophils can be selected through their low side scatter (SSC), intermediate between lymphocytes and monocytes, and a number of surface markers such as high-affinity IgE receptor (FcεRI), CD203c, CCR3(CD193), $CD45^+/CD3^-/CRTH2^+/CD203c^{low}$, or $CD123^+/HLA\text{-}DR^-$. Among these, only CD203c is lineage-specific and constitutively expressed on resting basophils (although also on pluripotent progenitors of MCs) and $CD193^+$ is also on SSC^{high} eosinophils. See recommendations Q12-13 in Table 2.

Basophil activation

It is mostly detected through selected surface proteins (i.e., activation markers). Amongst these, the lysosomal membrane protein CD63 is most commonly used and with the most published evidence. The ectonucleotide pyrophosphatase/phosphodiesterase CD203c is also used as activation marker and upregulated slightly earlier than CD63. Additional activation markers have been reported (CD107a, CD107b, CD164, and CD13) although not widely used for routine testing yet.[55] Data from avidin/DAO-histamine experiments nicely show CD63, but not CD203c, to be associated with compounded degranulation. In fact, CD63 shows strong correlation with

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TABLE 1 Differential aspects of performing BAT for allergy evaluation to nondrug allergens versus drugs.

	Allergy to allergens	Drug hypersensitivity
Type of sample	Whole blood: • Heparin • EDTA	
Selection markers	• $IgE^+CD203c^+$ • $CD193^+$and/or$CD203^+$ • $CD123^+HLA\text{-}DR^-$	
Activation markers	• $CD63^+$	• $CD63^+$ • $CD203c^+$
Use of IL-3	Recommended (4.5-2 ng/mL)	
Range of allergen/drug concentrations	μg-ng/mL	mg/mL
Interpretation of results	• %$CD63^+$ cells • CD-sens	• %$CD63^+$ or $CD203^+$cells • SI (cutoff to be established after ROC curves)
Patient's' treatment	• Antihistamines and topical steroids do not affect BAT • Systemic immunosuppressants (i.e., oral steroids can influence test results)	
Stimulus	• Whole extracts • Allergen components	• Native drug • Drug metabolites
Time interval to avoid anergy	Best not before 3–4 weeks	
Time interval to avoid sIgE clearance	Unknown	Close to reaction

exteriorization of the granular content with histamine secretion.[24,56] There is some evidence demonstrating the interest of the determination of CD203c overexpression[30] and especially for some drugs that induce very poor CD63 expression such as FQs, particularly moxifloxacin.[25] However, others questioned the utility of CD203c for evaluating this drug.[32] See recommendations Q14–16 in Table 2.

Use of IL-3 for increasing activation

Priming with IL-3 can increase test sensitivity depending on the activation marker used.[33,57] IL-3 enhances the allergen-specific CD63 upregulation, in fact allergen reactivity may increase by 25% and sensitivity by twofold when using 4.5 ng/mL of IL-3.[33] For CD203c, IL-3 dependent upregulation has been demonstrated in a slower process (about 90 min) compared to the FcεRI-mediated.[58] See recommendations Q17-18 in Table 2.

Interpretation of the results

BAT results can be referred to in terms of reactivity or sensitivity. Basophil reactivity[10] refers to the percentage of gated basophils that express activation markers at a given drug concentration. BAT outcome should always be reported as the percentage of basophils expressing activation markers (e.g., % $CD63^+$ cells). In addition, the results can be given as stimulation index (SI) which is the proportion of activated basophils after drug stimulation compared to nonstimulated basophils. Regarding the determination of the cutoff for positive results, there is great variability in the different studies (Table 5). Basophil sensitivity (CD-sens)[10] is measured with a dose–response curve, and defined as the lowest allergen concentration giving 50% of maximum upregulation of CD63.[59] However, in IDHRs, achieving these conditions, that is, high activation levels or sigmoidal curve, is infrequent. Moreover, CD-sens cannot be calculated in nonallergic individuals; therefore, no comparisons between healthy controls and allergic patients can be performed.[59] See recommendations Q19–21 in Table 2.

Patient's medication

Being a cellular test, BAT can be affected by patient's regular medication. Noteworthy, treatment with antihistamines and topical steroids do not seem to influence BAT outcomes.[35] Nevertheless, treatment with systemic immunosuppressant might affect BAT results.[33,35] See recommendation Q22 in Table 2.

False negative results in BAT

These can be produced by different causes: (i) Temporal basophil anergy and sIgE consumption, thus, to avoid this effect, BAT should be performed ideally 3–4 weeks after the reaction occurrence.[36] (ii) Given that exposure to drugs is infrequent and sIgE levels decline over time, the test can show false negative results if the evaluation is too long after the index reaction (over 1 year for penicillins).[37] (iii) In nonresponders (around 10%–15% of cases), basophils can be unresponsive (neither CD63 nor CD203c activation) to drug stimulation and to positive controls through anti-IgE and/or FcεRI.[10] In these cases, results are interpretable (invalid). This is attributed to differences in the intracellular signalling pathway of this receptor, particularly in the expression of Syk.[40] (iv) Moreover, a negative test with a parent drug does not rule out its metabolite being the real sIgE inductor.[18,19] See recommendations Q23–25 in Table 2.

3.2.2 | Drug-specific aspects of BAT in the evaluation of DHRs

Current experience with BAT in IDHRs diagnosis has focused on hypersensitivity to NMBAs, antibiotics (BLs and FQs), chlorhexidine (CHX), opiates, and iodinated radio contrast media (RCM). As already exemplified in some reviews, the performance of BAT in IDHRs varies significantly; mainly according to the drug (class), applied protocol and decision threshold, clinical presentation, and time elapsed

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TABLE 2 Recommendations on technical aspects of BAT in the evaluation of IDHRs.

	Definition	Grade recommendation	Comments	Agreement		
1.1. Use of fresh blood	Q1. We *recommend* BAT to be analysed in fresh blood within 24 h	Strong	BAT is recommended to be performed early after sampling in diagnosis of drug hypersensitivity reactions [17]	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
	Q2. We *recommend* blood to be stored at room temperature until BAT execution	Strong		Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
	Q3. We *recommend* to use either Heparin or EDTA as anticoagulant	Strong	· Heparin is recommended when BAT will be performed close to blood collection · EDTA when it will be performed after 4h	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
	Q4. When using EDTA as anticoagulant, we *recommend* blood must be processed	Strong	Blood should be processed by adding $CaCl_2$ at 1mM final concentrations and heparin [17]	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
1.2. Management of drugs for basophil stimulation	Q5. We *recommend* to prepare drugs fresh for each test	Strong	This is important to avoid drug degradation	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
	Q6. We *recommend* the use of the parenteral injectable drugs or pure substance/drug the patient reacted to	Strong	For some drugs the excipient included in injectable form could be important and, in this case pure, substance could be useful	Agree Not agree Abstention	11/12 1/12 0/12	92% 8% 0%
	Q7. We *suggest* use of drug form the patients reacted to (injectable, tablets, Pepys principal)	Weak	When using this form, it is important to be sure of using homogeneous solution	Agree Not agree Abstention	3/12 9/12 0/12	25% 75% 0%
	Q8. We *suggest* use of crushed tablet drugs only when pure substance/drug or injectable drug is not available	Weak	When using this form, it is important to be sure of using homogeneous solution	Agree Not agree Abstention	9/12 3/12 0/12	75% 25% 0%
	Q9. We *suggest*, when available, the inclusion of drug metabolites known to be recognized by sIgE	Weak	As has been shown for pyrazolones and clavulanic acid[34,35]	Agree Not agree Abstention	11/12 1/12 0/12	92% 8% 0%
1.3. Concentrations of drugs for basophil stimulation	Q10. We *recommend* non-cytotoxic concentrations to be used in BAT	Strong	A dose-response curve to at least 5 sequential dilutions of drug should be performed	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
	Q11. We *recommend* to ensure specificity	Strong	The same sequential dilutions of drug (at least 3) should be performed in uneventfully exposed donors	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
1.4. Basophil selection	Q12. We *recommend against* using the receptor FcεRI for basophil selection	Strong	The receptor FcεRI expression varies on basophil surfaces from individuals or during their activation hampering the basophil identification sometimes [98–101]	Agree Not agree Abstention	10/12 1/12 1/12	84% 8% 8%
	Q13. We *recommend* to select basophils by using CCR3 (CD193) alone or together with either CD123 or CD203c, or CD123 together with lack of HLA-DR	Strong	It should be aware that CD193 is also expressed on eosinophils	Agree Not agree Abstention	10/12 1/12 1/12	84% 8% 8%

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TABLE 2 (Continued)

	Definition	Grade recommendation	Comments	Agreement		
1.5. Basophil activation	Q14. We *recommend* the use of CD63	Strong	For particular drugs BLs, NMBA, proton pump inhibitors, ciprofloxacin[22,25,52,68,102,103]	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
	Q15. We *suggest* the use of CD203c	Strong	For BLs [24,42] Also for some FQs like moxifloxacin that do not induce the expression of CD63[25] although other authors questioned its utility for evaluating this drug[26]	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
	Q16. We *suggest* using both CD63 and CD203c for assessment of basophil activation induced with some drugs	Strong	The combination of the results from each marker increases the sensitivity with no changes in specificity[24]	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
1.6. Use of IL-3	Q17. We *recommend* to add IL3 in the stimulation buffer when CD63 is used as activation marker	Strong	It could be included 2-4.5 ng/mL of IL-3 at the stimulation buffer[19,104]	Agree Not agree Abstention	9/12 0/12 3/12	75% 0% 25%
	Q18. We *recommend* against adding IL3 in the stimulation buffer when CD203c is used as activation marker	Strong	It can upregulate expression in a non-specific way[19,104]	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
1.7. Interpretation of the results	Q19. We *recommend* the use of ROC curve analyses for each drug to define the threshold (cut-off) for positivity after critically balancing sensitivity and specificity	Strong	For this, a higher number of patients (>10) is preferable.	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
	Q20. In case of low number of cases, we *recommend* to define positivity based on comparison with uneventfully exposed controls (at least 3)	Strong	The higher expression of basophil activation markers in patients as compared to (exposed) controls is a hallmark of sensitization in BAT. The higher number of patients and controls analyzed, the more robust is the probability for a clinical relevance.	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
	Q21. When expressing the results as SI, we *recommend* that spontaneous basophil activation must be greater than 1%	Strong	To minimise the risk of false positive results.	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
1.8.Patient's medication	Q22. We *recommend* stopping treatment with systemic immunosuppressants i.e. oral steroids 3 weeks prior to testing	Strong	If this is not possible, BAT could be performed but being aware of false negative results. In that case, it is preferable to retest.[19,30]	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
1.9. False negative results in BAT	Q23. We *suggest* that BAT should preferentially be performed after 3–4 weeks post index reaction	Weak	To avoid false negative results[31]	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
	Q24. We *recommend* that BAT should preferentially be performed within one year when possible	Strong	To avoid false negative results [32,39,85]	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
	Q25. We *suggest* to re-test non-responder patients in BAT after 6 months	Weak	Identify non-responders by checking activation and positive controls (Anti-IgE or Anti-FcεRI). Even in these non-responders, BAT can be positive with drugs and therefore the results can be considered as diagnostic[33]	Agree Not agree Abstention	10/12 0/12 2/12	84% 0% 16%

Note: Purple colours means recommendations/suggestions in favour of; Orange colours means recommendations/suggestions against of

Abbreviations: BAT, basophil activation test; BLs, betalactams; EDTA, Ethylenediaminetetraacetic acid; FQs, fluoroquinolones; IDHRs, immediate drug hypersensitivity reactions; NMBA, neuromuscular blocking agents; ROC, Receiver operating curves.

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TABLE 3 Optimal end concentrations for drugs used in basophil activation test.

Group	Drug	Concentration range		Reference
		mg/mL	mM	
Beta-lactams	Benzylpenicillin	3.9–0.4	11.7–1.2	[20,34,37,43,105,106]
	Amoxicillin	4–0.01	10.9–0.03	[20,31,34,37,38,42–44,105,106,107]
	Clavulanic acid	1.25–0.05	6.3–0.25	[19,38,107]
	Ampicillin	2.5–0.01	7.2–0.03	[20,31,34,43,105]
	Cefuroxime	1.25–0.01	2.9–0.02	[20,31,34]
	Cefazolin	10–0.006	22–0.013	[34,45,108,109]
Quinolones	Ciprofloxacin	2–0.1	6.03–0.30	[25,26,41,46,110,111]
	Moxifloxacin	2–0.1	4.98–0.25	[25,26,41,46,110,111]
	Levofloxacin	4–0.1	11–0.28	[26,46,110,111]
	Norfloxacin	2–0.1	6.3–0.31	[110,111]
	Ofloxacin	4–0.1	11.07–0.28	[110]
	Lomefloxacin	4–0.1	11.38–0.28	[110]
RCM	Lodixanol	3–0.3	1.9–0.19	[112]
	Lomeprol	3.5–0.01	4.5–0.013	[112]
	Lohexol	6–0.006	7.3–0.007	[112,113]
	Loxaglate	6–0.006	4.1–0.004	[112,113]
NSAIDs	Metamizole	5–0.00025	15–0.00075	[11,18,39,47,48,105,114,123]
NMBA	Atracurium	5–0.000025	5.4–0.000027	[27,28,48,49,115,116]
	Mivacurium	0.02–0.00004	0.018–0.000036	[115]
	Vecuronium	2–0.00008	3.14–0.00012	[28,49–51,115,117]
	Pancuronium	0.5–0.0005	0.87–0.00087	[48,51,116]
	Rocuronium	5–0.0002	9.4–0.0004	[28,48–51,115,116,117]
	Suxamethonium	5–0.00004	13.8–0.00011	[28,48,49,51,115,116,117]
	Cisatracurium	1–0.5	1.08–0.54	[28,49,51,117]
Chemotherapeutic agents	Platins	0.5–0.0005	1.35–0.000125	[52,118,119]
	Paclitaxel	0.05–0.000005	0.06–0.000006	[53]
Biological agents	Rituximab	2–0.25	0.014–0.0017	[54]
Others	Pump proton inhibitors	2–0.02	5.8–0.05	[29]
	Codeine	1–0.001	3.3–0.003	[50]
	Chlorhexidine	0.001–0.0001	0.002–0.0002	[120]
	Alexidine	0.001–0.0001	0.002–0.0002	[120]
	Metronidazole	5–0.005	29.21–0.029	[121]
	Ornidazole	5–0.005	22.8–0.023	[121]
	Pristinamycin	1–0.1	1.15–0.115	[122]

Abbreviations: NMBA, neuromuscular blocking agents; NSAIDs, nonsteroidal anti-inflammatory drugs; RCM, radio contrast media.

between the index reaction and testing.[9,60] BAT might mainly benefit diagnosis in cases where a safe alternative diagnostic is unavailable, for example, when ST is not providing a clear diagnosis or when full-dose challenges are difficult due to the pharmacologic properties of the investigated drug(s) and severity of the symptoms. See recommendation Q26 in Table 4.

Some important aspects should be taken into account to guarantee correct execution and interpretation of BAT. In validation studies, it is important a correct inclusion of patients, which should not be based on the clinical history alone but supported by other diagnostic tests. Moreover, when possible, studies should also include data from paired tryptase measurements (indicative for mast cell activation) and a sample size large enough (i.e., at least 10 patients with clear history—expert opinion) to ensure statistical comparisons and the conclusion accuracy, to form a representative study population.

The place of BAT in the diagnostic algorithm of IDHRs is not uniform and sometimes controversial. This should be discussed for each drug independently. The recommendations for each drug evaluated for its use in BAT with the corresponding grades are shown in Table 4. Moreover, data on sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of BAT to different drugs from literature are included in Table 5.

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TABLE 4 Recommendations for drug-specific aspects of BAT in the evaluation of DHRs.

	Definition	Grade recommendation	Comments	Agreement		
2. Severity of the reactions	Q26. We *recommend* the use of BAT as a first step in cases of life-threatening anaphylaxis to drugs such as cardiac arrest	Strong	In the context of a convincing clinical history[39,40]	Agree Not agree Abstention	10/12 1/12 1/12	84% 8% 8%
2.1. Betalactams	Q27. We *recommend* the use of BAT for diagnosing BLs IDHRs	Strong	For penicillin and considering the limitations of sIgE determinations[8], BAT although with moderate[24]/ low[43] sensitivity, it shows high specificity. Thus, its placing as a first step in the diagnostic procedure could be an option to reduce the need of performing an allergological work-up and diminishing the risk of re-inducing allergic reactions [24].	Agree Not agree Abstention	11/12 1/12 0/12	92% 8% 0%
2.2. NMBA	Q28. We *recommend* the use of BAT for diagnosing NMBA IDHRs.	Strong	Dual staining for CD63 and CD203c showed a sensitivity around 60%[91]. BAT can help to discriminate clinically relevant sensitization to tertiary and quaternary substituted ammonium structures	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
2.3. FQs	Q29. We *suggest* the use of BAT for diagnosing FQs IDHRs with the exception of moxifloxacin.	Weak	BAT can be suggested in FQ allergies particularly to avoid unnecessary drug challenge[53]	Agree Not agree Abstention	9/12 0/12 3/12	75% 0% 25%
	Q30. We *suggest* the use of different activation markers depending on the FQ included in the test	Weak	It might be suggested CD63 for ciprofloxacin although with no optimal sensitivity; for moxifloxacin, CD203c could be used although, it is still too insensitive[25,26,51-53,63]	Agree Not agree Abstention	10/12 0/12 2/12	84% 0% 16%
2.4. Chlorhexidine	Q31. We *suggest* the use of BAT for diagnosing CHX IDHRs	Strong	BAT in CHX allergy can be used as a supplement when other tests are equivocal or suspected false negative[59-61]	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
2.5. Opioids	Q32. We *suggest* the use of BAT for diagnosing opiate/opioid IDHRs.	Weak	Genuine opiate/opioid allergy is exceedingly rare. In contrast to nonspecific mediator release likely by occupation of the MRGPRX2 receptor.	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
2.6. RCM	Q33. We *suggest* the use of BAT for the diagnosis of IDHR to RCM.	Weak	BAT can be considered as a complementary tool and especially useful in cases with severe reaction where drug challenge is contraindicated.	Agree Not agree Abstention	11/12 1/12 0/12	92% 8% 0%
2.7. Chemotherapeutics	Q34. We *suggest* the use of BAT for diagnosing hypersensitivity to chemotherapeutics	Weak	BAT might have a role not only in diagnosing but also as a predictor of severe reactions and monitoring rapid drug desensitization.	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
2.8. Biological Agents	Q35. We *suggest* the use of BAT for diagnosing hypersensitivity to biological agents	Weak	CD63 BAT can be helpful in evaluating hypersensitivity to biological agents, if no other diagnostic tests are available. BAT might have a role in monitoring rapid drug desensitization.	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
2.9. NSAIDs	Q36. We *recommend against* the use of BAT for the evaluation of nonallergic IDHR to NSAIDs.	Strong	BAT show very low sensitivity and specificity even including different NSAIDs and analysing the results from the expression of two activation markers[5,9-12]	Agree Not agree Abstention	11/12 1/12 0/12	92% 8% 0%

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TABLE 4 (Continued)

	Definition	Grade recommendation	Comments	Agreement		
2.10. COVID Vaccine	Q37. We *recommend against* the use of BAT with COVID-19 vaccine (mRNA vaccines and vaccines not based on mRNA technology) in the diagnosis of patients with reaction to COVID-19 vaccine.	Strong	The possibility of positive results related to a past COVID-19 disease has to be taken into account[88]	Agree Not agree Abstention	12/12 0/12 0/12	100% 0% 0%
2.10. PEG containing drugs	Q38. We *suggest* to perform BAT with PEG in patients with suspected PEG allergy	Weak	It should be included a range of PEG molecular weight (>2.000 Da)	Agree Not agree Abstention	10/12 1/12 1/12	84% 8% 8%
3. BAT in the evaluation of paediatric population	Q39. We *suggest* to perform BAT in paediatric population following the same principles rules and protocols as in adults	Weak	Although further studies are needed in large population in different age groups with different viral disease implications	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%
4. Direct versus passively sensitized BAT	Q40. We *recommend against* the use of passively sensitized BAT for evaluating IDHRs	Weak	Currently, passively sensitized BAT shows several limitations over direct BAT.	Agree Not agree Abstention	11/12 0/12 1/12	92% 0% 8%

Abbreviations: BAT, basophil activation test; BLs, betalactams; CHX, chlorhexidine; FQs, fluoroquinolones; IDHRs, immediate drug hypersensitivity reactions, MRGPRX2, Mas-related G-protein coupled receptor X2; NMBA, neuromuscular blocking agents; NSAIDs, non-steroidal anti-inflammatory drugs; PEG, Polyethilenglycol; RCM, radiocontrast media.
Note: Purple colours mean recommendations/suggestions in favour of; Orange colours means recommendations/suggestions against.

Betalactams

In a recent EAACI position paper on the diagnosis of hypersensitivity to BLs there seems to be little place, if at all, for BAT, as in most cases diagnosis can be readily made by ST, sIgE, or drug challenge.[8,14] Moreover, with the increasing knowledge of nonirritating concentrations for SPT and IDT,[72] and optimized clinical risk-stratification for drug challenge,[73] it seems unlikely that BAT should be able to substitute in vivo tests. However, BAT can be indicated in severe cases when STs and quantification of sIgE yield negative results and when a drug challenge is contraindicated, for example, due to life-threatening anaphylaxis such as cardiac arrest.[38,43] Moreover, the role of in vitro specific IgE determination has been recently questioned in a well-defined population with confirmed BL allergy. Indeed a combined Spanish and Italian study showed a low sensitivity to penicillin V (PV), penicillin G (PG), and amoxicillin (AX), as well as false-positive results to PV and PG, suggesting relevant limitations of sIgE determination by fluoroimmunoassays[8] and opening room for the use of BAT for evaluating IDHRs to penicillins. Moreover, BAT has shown usefulness for evaluating BLs, such as clavulanic acid, which is not possible in other in vitro tests.[38,44] Regarding patients with anaphylaxis, it has been shown that AX induced upregulation of CD203c in 60% patients and BAT sensitivity increased to 70% when combining CD63 and CD203c as activation markers.[31] There are two recent manuscripts that show different results and give BAT a different value: in a prospective study, although CD203c had a rather low sensitivity (47%), it displayed a high specificity (95%).[30] However, in the other study where a higher specificity was selected (98%), a poor sensitivity was obtained (23%)[61] (Table 5). There are several reasons that can explain these discrepant results: (i) the inclusion of patients diagnosed by STs to PG and sIgE to PG and PV, that has been shown to induce false positive results,[8,14] (ii) the time interval between reaction and study that can decrease sensitivity[37]; (iii) the sensitivity-specificity balance chosen to select the cut-off point for positive results; and (iv) the drug concentration used in BAT. Furthermore, in cefazolin-induced severe reactions, six out of eight patients (75%) with negative STs and positive drug challenge had a positive BAT to cefazolin.[45] Regarding specificity, it ranges between 79% and 100% depending on the drug and study (Table S1). See recommendation Q27 in Table 4.

Neuromuscular blocking agents

BAT seems to merit the status of secondary diagnostic tool before ST but after quantification of sIgE.[74,75] Actually, it has been shown that negative ST to NMBAs might not always give the green light for safe re-exposure[75,76] and that sIgE (either for NMBAs or morphine) has a limited use on the diagnosis of NMBA allergy.[77] BAT may be a useful complementary test for evaluating NMBA hypersensitivity with no sensitization on STs. Actually, sIgE to morphine is frequent in the general population[78] and does not capture sensitization to benzylisoquinolines.[79] Furthermore, because resting basophils barely express the MRGPRX2, BAT might help to discriminate between

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TABLE 5 Sensitivity, specificity, NPV, and PPV of basophil activation test to different drugs.

	Drugs	Patients	Thresholds	Diagnosis	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Ref
Beta-lactams	PG, AX, AMP, CEFU, and CEFAZ	58 Pat/30 Cont	SI ≥2	ST	50.0	93.3	49.1	93.5	[34]
	PG, AMP, and AX	23 Pat/30 Cont	SI ≥2	ST and DC	39.1	93.3	–	–	[123]
	PG, AX, AMP, CEFU, CEFAZ, and CEFAC	70 Pat/40 Cont	SI ≥2	ST and DC	48.6	93	50.8	92.4	[43]
	AX, AMP, and CEFU	27 Pat/40 Cont	2 SD (6%)	ST	63	79	52.5	85.2	[31]
	BP, AX, AMP, and CEFs	178 Pat/81 Cont	SI >2 and ≥ 5%	ST and DC	48.3	88.9	43.8	90.5	[105]
	PG, PV, AMP, AX, and CEFU	24 Pat/15 Cont	SI ≥2 and ≥ 5%	ST	55	80	52.6	81.5	[20]
	AX	55 Pat/30 Cont	SI ≥2	ST	52.7	90	50.9	90.6	[42,44]
	CEF	16 Pat/17 Cont	5%	ST	33 (CD63) 67 (CD203c)	94 (CD63) 94 (CD203c)	–	–	[124]
	AX and CLAV	57 Pat/58 Cont	SI ≥s1.5 and ≥ 5%	ST and DC	47.0 33.0	93 94	–	–	[38]
	PG, PV, AX, and AMP	25 Pat 25 Pat	SI ≥2 and >5%	ST	8.0	100	–	–	[125]
	AX and CLAV	105 Pat 74 Cont	SI ≥1.2 and >2.5%	ST, DC	47	95	55.6	92.5	[30]
	AX	66 Pat 70 Cont	9%	ST, sIgE, and DC	13 (CD63) 23 (CD203c)	100 (CD63) 98 (CD203c)	–	–	[61]
	CEF	23 Pat/20Non allergic	SI >2, required background >2.5%	ST and DC	43.5 (CD63) 50 (CD203c) 66.7 (CD63 and CD203c)	100	60.6 (CD63) 40 (CD203c) 50 (CD63 and CD203c)	100	[45]
Quinolones	CIPRO, MOXI, and LEVO	38 Pat/25 Cont	SI >2 and > 5% above negative control value	DC	71.1	88	66.7	9ß.1	[26]
	CIPRO, MOXI, LEVO, OFLOX, LOME, FLUME, NORFLO, and PiPEMI	17 Pat/15 Cont	At least two sequential drug dilutions >10% above negative control value	ST and DC	76.5	100	78.9	100	[62]
	CIPRO and MOXI	28 Pat/20 Cont	SI >3	DC	57.1	90	59.9	88.9	[41]
	CIPRO and MOXI	17 Pat 18 Cont	SI >3 (better than SI >2)	DC	83.3 (CD63) 0 (CD203c) 9.1 (CD63) 36.4(CD203c)	88.9 (CD63) 94.4(CD203c) 77.8 (CD63) 94.4 (CD203c)	66.7	81.8	[25]

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TABLE 5 (Continued)

	Drugs	Patients	Thresholds	Diagnosis	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Ref
	MOXI	15 Pat/9 Cont	15.5%	–	13.3 (CD63) 46.7 (CD203c)	100 (CD63) 100 (CD203c)	–	–	[32]
	CIPRO, LEVO, MOXI, NORFLO, OFLOX, and LOME	76 Pat	SI >2 and >5% above negative control value	ST and DC	89.5	–	–	–	[110]
	CIPRO, LEVO, MOXI, and NORFLO	19 Pat	SI ≥2 and ≥5%	ST and DC	47.0	–	–	–	[46]
Radio Contrast Media	IOXIT, IOPR, IOPA, IOH, and IOB	26 Pat 43 Cont	9.3% SI >7.3	ST	57.7	97.7	79.3	93.8	[69]
	GADO	33 Pat 14 Con	6%	ST and DC	69.0	93.0	–	–	[70]
NMBA	META	26 Pat 30 Cont	SI ≥5 and ≥5%	ST and DC	42.3	100	66.7	100	[47]
	META	26 Pat 30 Cont	SI ≥5 and ≥5%	ST	42,3	100	99,4	100	[126]
	SUX, GALLA, VECU, and PAN	21 Pat 29 Cont	>15%	ST	64.0	93.0	78.1	86.9	[127]
	ROCss, SUX, and ATRAC	39 Pat 17 Cont	>10% at least two sequential drug dilutions	ST	54.0	100	48.6	100	[115]
	ROC, ATRAC, SUX, and VECU	14 Pat 10 Cont	>10% at least two sequential drug dilutions	ST	78.6	100	76.9	100	[128]
	ROC	14 Pat 8 Cont	4%	ST	91.7	100	87.3	100	[28,49]
	ROC, VECU, ATRA, PAN, and SUX	47 Pat 45 Cont	15%	ST, DC	36.1	93.3	58.3	84.9	[129]
	ROC	41 Pat 25 Cont	4%	ST	80.5	96.0	74.5	97.0	[130]
	ATRAC, ROC, SUX, and PAN	22 Pat 34 Cont	SI ≥1.71 and >5%	ST	68.2	100	82.9	100	[48]
	ROC	10 Pat 3 Cont	Not mentioned (lowest positive value: 11%)	ST	100	100	75	100	[131]
	ROC, VECU, CIS, SUX, and PAN	61 Pat	CD63: 4.45%, SI =1.44 CD203c: 8.8%, SI = 1.49	ST	48 (CD63) 58 (CD203c)	87 (CD63) 89 (CD203c)	–	–	[51]

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TABLE 5 (Continued)

	Drugs	Patients	Thresholds	Diagnosis	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Ref
Chemotherapeutic agents	Platins	15 Pat 12 Cont	SI>2	ST	40 (CD63) 73 (CD203c)	100	-	-	[52]
	Cisplatin	16 Pat 20 Cont	3.5%	-	56.3	86.7	-	-	[71]
Biological agents	Rituximab	18 Pat 18 Cont	Not mentioned Activation: 6.75% in patients vs. 1.92% in controls	-	-	-	-	-	[54]

Note: In this table only studies with a sample size of at least 10 patients with have been included.

Abbreviations: AMP, ampicillin; ATRAC, atracurium; AX, amoxicillin; CEFAC, cefaclor; CEFAZ, cefazoline; CEFs, cephalosporins; CEFU, cefuroxime; CIPRO, ciprofloxacin; CIS, cisatracurium; CLAV, clavulanic acid; Cont, controls; DC, drug challenge; FLUME, flumequin; GADO, gadolinium; GALLA, gallamine; IOB, iobitridol; IOH, iohexol; IOPA, iopamidol; IOPR, iopromide; IOXIT, ioxithalamate;LEVO, levofloxacin; LOME, lomefloxacin; META, metamizol; MOXI, moxifloxacin; NORFLO, norfloxacin; NPV, negative predictive value; OFLOX, ofloxacin; PAN, pancuronium; Pat, patients; PG, penicillin G; PiPEMI, pipemidic acid; PPV, positive predictive value; PV, penicillin V; ROC, rocuronium; SI, stimulation index; ST, skin tests; SUX, suxamethonium; VECU, vecuronium.

IgE-dependent and MRGPRX2-dependent reactions to NMBA.[13] See recommendation Q28 in Table 4.

Fluoroquinolones

For FQs correct determination of the position of BAT as a complementary diagnostic tool might be more problematic and not find universal acceptance, mainly because of conflicting findings on different studies.[25,26,32,46,62,63,80] The most likely reason for this is that FQ-related IDHRs are believed to result from MRGPRX2-signalling, a process that cannot be captured by traditional BAT using resting basophils as a starting point.[32] Although some authors state a poor utility of BAT for FQs,[32] other studies suggest that for FQ-IgE mediated reactions, the use of CD63 or CD203c, depending on the culprit FQ, could help increase BAT global sensitivity.[25,26,32,62,63] BAT has good negative predictive value helping avoid the performance of drug challenge.[62] Considering severe IDHRs to FQs, in patients with anaphylactic shock to moxifloxacin, an increase in cells that upregulate CD203c was observed.[25] Moreover, we must be aware of possible photodegradation of FQs that could affect BAT sensitivity.[41] See recommendations Q29-30 in Table 4.

Chlorhexidine

It is a popular biguanide antiseptic that has evolved to a significant (hidden) cause of sometimes dramatic anaphylaxis with serious consequences of misdiagnosis.[81,82] Generally, diagnosis of CHX-allergy rests upon an evocative history complemented with STs and CHX-sIgE.[83] However, in the absence of a CHX challenge test, difficult cases with negative or equivocal test outcomes can benefit from cellular tests such as in vitro basophil activation[49,65–67] or MCs activation, the latter using passively sensitized donor MCs and offering an attractive alternative for stripped donor basophils.[64,84] See recommendation Q31 in Table 4.

Opioids

Although frequently used, genuine IgE-mediated reactions to opiates and (semi)synthetic opioids seem to be exceedingly rare and their diagnosis can be challenging because of their potent non-specific histamine releasing capacity by skin MCs.[85] By contrast, evidence has emerged that BAT might advance correct diagnosis of IgE-mediated reactions to these compounds.[50,86] Indeed, unlike skin MCs, and likely reflecting differences in MRGPRX2 surface expression, basophils do not respond to MRGPRX2-signalling to opiates and other MRGPRX2 agonists (e.g., atracurium) in traditional BAT.[27,86] See recommendation Q32 in Table 4.

Iodinated and gadolinium-based radio contrast media

The exact mechanisms of IDHRs to RCM are a matter of controversy. IgE-mediated reactions to RCM have been reported in different populations but have been only found in the minority of patients (17%) with IDHRs to RCM.[87,88] A study featuring patients with mostly mild reactions showed sensitivity values for BAT ranging between 46% and 62%, depending on the threshold, and a specificity of

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89%–100%.[69] BAT shows good correlation with ST and drug challenge results[89]; however, predictive values have not been clearly determined.[87] In IDHRs to gadolinium-based contrast agents, sensitivity of BAT was 69% and specificity 93% in one study.[70] See recommendation Q33 in Table 4.

Chemotherapeutics

The use of BAT to study chemotherapy IDHRs is limited by turnaround times (patients usually need chemotherapy urgently) and issues regarding hazardous drugs handling. However, the lack of commercialized sIgE assays for chemotherapy drugs makes BAT a potentially useful tool. Seminal investigations used CD63 BAT for the three main platinum drugs (cisplatin, carboplatin, and oxaliplatin), docetaxel, and paclitaxel.[53,71,90] A prospective case–control study of patients receiving carboplatin showed that CD203c BAT is useful to predict carboplatin-related IDHRs and severe anaphylaxis[71] whereas in another study increased CD63 expression tended to be associated with more severe initial reactions.[52] However, data of BAT to assess IDHRs with other chemotherapy drugs are scarce and only based on case report studies.[91]

Additionally, BAT can be used to monitor desensitization procedure showing CD203c BAT as a possible predictor for severe reactions during desensitization to platins.[52,90] See recommendation Q34 in Table 4.

Biological agents

There is limited data available for the use of BAT in IDHRs to biological agents. BAT with CD63 was helpful in a series of 18 rituximab-reactive patients.[54] In two cases with a strongly positive BAT to adalimumab, a reduction in CDsens (a parameter correlated with basophil sensitivity) was observed during a rapid drug desensitization protocol.[92] However, in a case report of a confirmed DHR to infliximab (positive drug challenge), with negative ST results, BAT results were negative for both infliximab and adalimumab.[89] Alpha-gal syndrome was originally detected by anaphylaxis to cetuximab, because cetuximab carries the alpha-gal epitope due to its production in mouse myeloma cell line. BAT with cetuximab confirmed IgE-mediated mechanisms in patients with alpha-gal syndrome.[93] Also, other alpha-gal containing drugs could be detected by BAT, for example, antivenins against snake or scorpion venoms, porcine enzymes and gelatin in volume colloids or vaccines.[94–96] See recommendation Q35 in Table 4.

Nonsteroidal anti-inflammatory drugs

Positive results have been obtained for IgE-mediated IDHRs especially reported for metamizole with a sensitivity range of 42% to 65% and complementary to STs results with a specificity ranging from 83.3% to 100%.[39,47,48] However, in nonallergic DHR, BAT has shown a low sensitivity when including one NSAID in the test[11,12] and although sensitivity could increase when including several NSAIDs, the specificity decreased dramatically.[11] See recommendation Q36 in Table 4.

COVID vaccine and excipients

Very recently, BAT has been used in the evaluation of adverse reactions due to mRNA COVID-19 vaccine. BAT with these vaccines has shown unspecific positive results in patients recovering from COVID-19 infection and therefore has limited usefulness in evaluating reactions to the vaccine itself; however, more promising results have been found in cases of very rare IDHRs to the excipient polyethylene glycol PEG using PEGs ≥2000 MW and PEG-containing medicines in the BAT[68,97] See recommendations Q37-38 in Table 4.

3.2.3 | BAT in the evaluation of pediatric population

Compared to adults, differences in the management of DHRs in children have been highlighted, mainly due to a higher frequency of viral-induced skin eruptions and a lower frequency of real IDHRs. However, in principle, BAT in selected children with a suspicion of IDHRs should work as in adults.

In the pediatric population, BAT has been mainly evaluated in the diagnostic management of perianesthetic anaphylaxis and was shown to be useful as an additional test to diagnose NMBA allergy.[27,51,77,98] However, most of these studies include mixed populations, that is, adults and children. BAT has also been evaluated in few studies for the diagnosis of antibiotic allergy in children.[99–101] Although some authors found that BAT is an additional valuable and sensitive diagnostic test for IDHRs to antibiotics, others did not find an increase in sensitivity.[99–101] Those differences might be explained by geographical variations, different phenotypes, and ages of the patients, but also by inclusion of Non-Immediate DHRs (NIDHRs) in which BAT is not useful. In addition, BAT has been scarcely investigated (case reports) for the diagnosis of allergy to vaccines and corticosteroids.[102,103] Although results suggest that BAT may be useful also in children, this needs confirmation in larger focused studies. Moreover, it would be interesting to evaluate the value of BAT in different ages group with different viral disease implications. See recommendation Q39 in Table 4.

3.2.4 | Direct versus passively sensitized BAT

Over the last two decades the flow-based ex vivo BAT has become a pervasive test in allergy diagnosis, especially in IDHRs.[56,60] However, the technique leaves us with some shortcomings and weaknesses such as the necessity for analyses within 4 h after sampling and the nonresponder status as seen in 10%–15% of patients.

To circumvent these issues, different groups have focused on the development of passive BAT (pBAT) where stripped donor basophils are sensitized with patients' sera. Although the pBAT is a step forward, some limitations remain since it is: (i) less sensitive than traditional BAT; (ii) highly dependent on the basophil donor whose status can only be determined ad hoc; (iii) strongly influenced by the serum sIgE level of the patient.

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Moreover, there is currently no single test that enables documentation of IDHRs from MRGPRX2 occupation unambiguously.[60] Indeed, BAT and pBAT do not enable direct cell activation by occupation of the MRGPRX2, as resting basophils barely express this receptor.[13] See recommendation Q40 in Table 4.

4 | CONCLUSIONS

Although BAT offers the clinician and laboratory a valuable adjunct safe diagnostic for IDHRs, its position in the diagnostic algorithm strongly varies depending on the studied drug class and patient population (phenotype, geography, and age). Evidence that in IDHRs the BAT might be more than a diagnostic aid is accumulating.[56,64] From these reviews it seems that the technique, might also deepen our insights into immune (allergic) and nonimmune (nonallergic) mechanistic processes of IDHRs, benefit the identification of antibody recognition sites, and advance our understandings on desensitization strategies. The standardization of BAT and its analysis is important if we want to generalize beyond the individual laboratory. Indeed, very recently within a Task force from the EAACI, a BAT protocol has been identified and consensuated that gives acceptable inter- and intra-laboratory variability (according to accepted standards), indicating that it could be implemented across Europe.[104]

AUTHOR CONTRIBUTIONS

Mayorga C and Ebo DG should be considered joint senior author, Mayorga C designed the position paper and the survey and wrote the different versions to be discussed; Çelik GE designed the recommendation grade; Pascal M, Hoffmann HJ, Eberlein B, and Ebo DG were in charge of the Technical aspects of BAT; Torres MJ, Çelik GE, Brockow K, Garvey LH, Barbaud A, Madrigal-Burgaleta R, Caubet JC, and Ebo DG were involved in writing the Drug specific aspects of BAT; all authors discussed the survey to get an agreement for the final version as well as the different versions of the position paper and voted the recommendations.

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CONFLICT OF INTEREST STATEMENT

None of the authors declare any conflict of interest in relation to this work.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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