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Road map for the clinical application of the basophil activation test in food allergy

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Summary
The diagnosis of IgE-mediated food allergy based solely on the clinical history and the documentation of specific IgE to whole allergen extract or single allergens is often ambiguous, requiring oral food challenges (OFCs), with the attendant risk and inconvenience to the patient, to confirm the diagnosis of food allergy. This is a considerable proportion of patients assessed in allergy clinics. The basophil activation test (BAT) has emerged as having superior specificity and comparable sensitivity to diagnose food allergy, when compared with skin prick test and specific IgE. BAT, therefore, may reduce the number of OFC required for accurate diagnosis, particularly positive OFC. BAT can also be used to monitor resolution of food allergy and the clinical response to immunomodulatory treatments. Given the practicalities involved in the performance of BAT, we propose that it can be applied for selected cases where the history, skin prick test and/or specific IgE are not definitive for the diagnosis of food allergy. In the cases that the BAT is positive, food allergy is sufficiently confirmed without OFC; in the cases that BAT is negative or the patient has non-responder basophils, OFC may still be indicated. However, broad clinical application of BAT demands further standardization of the laboratory procedure and of the flow cytometry data analyses, as well as clinical validation of BAT as a diagnostic test for multiple target allergens and confirmation of its feasibility and cost-effectiveness in multiple settings.

1 | INTRODUCTION

The prevalence of IgE-mediated food allergy is increasing and so is the public awareness about food allergy, which together have resulted in a high demand for food allergy testing.1,2 Following the clinical assessment of patients, which includes the clinical history and a detailed dietary history, diagnosing IgE-mediated food allergy requires documentation of food-specific IgE using skin prick testing (SPT) and/or specific IgE testing.3 However, far more common than having food allergy is to have detectable food-specific IgE. Without a clear and recent history of an allergic reaction to the suspected food or alternatively a clear history of tolerating age-appropriate portions of the food, the interpretation of SPT or specific IgE results can be challenging.4 Therefore, food allergy testing is most useful when directed from the information collected from the clinical history.5 Patients with equivocal history and testing should be offered an oral food challenge (OFC), the current gold standard for diagnosis.3,6

2 | DO WE NEED IMPROVED DIAGNOSTIC TESTING FOR IGE-MEDIATED FOOD ALLERGY?

The diagnostic performance of SPT and specific IgE to whole extracts can vary depending on the food sources and the quality of
the allergen extracts.\textsuperscript{5} Allergen extracts usually contain the major and minor allergens that are relevant for the ability of the food to elicit allergic reactions. However, allergen extracts obtained from certain food sources, such as soya, wheat and certain nuts and seeds, may miss some important allergens (e.g., lipophilic proteins, such as oleosins,\textsuperscript{7} and other proteins that are lost during the process of producing the extracts), which can impair their diagnostic utility. Generally, when interpreting SPT and specific IgE as positive at the low limits of detection, SPT and specific IgE have a high sensitivity but poor specificity. Therefore, without a clinical history that is suggestive of allergy, the mere detection of sensitization by SPT or specific IgE leads to high false-positive rates and low positive predictive values (PPVs). When 95\% PPV value cut-offs are used (e.g., 8 mm for SPT to peanut and 15 KU/L for specific IgE to peanut\textsuperscript{8,9}), the specificity of these tests is enhanced but their sensitivity is reduced, resulting in many false negatives and low negative predictive value (NPV). Therefore, a large proportion of patients tested, particularly when the pre-test probability is low (e.g., no or remote history of known ingestion), have intermediate range results for SPT and specific IgE and require OFC to clarify whether or not they have food allergy.\textsuperscript{10}

These concepts also apply for specific IgE testing to individual food allergen components. The diagnostic utility of this “component testing” varies with the allergen in question. Some allergen components have shown to be more useful than the whole allergen extract in distinguishing allergic from non-allergic patients (e.g., Ara h 2 from peanut\textsuperscript{4,11} and Cor a 9 and Cor a 14 from hazelnut\textsuperscript{12,13}) as opposed to other components which do not seem to offer additional diagnostic accuracy compared to using whole allergen extracts (e.g., Jug r 1 in walnut allergy\textsuperscript{14}). Other examples of components which can support food allergy diagnosis are specific IgE to Bet v 1-homologues, such as Ara h 8 and Cor a 1, which can help to distinguish pollen-food syndrome (e.g., secondary to birch pollen allergy) from “true” plant food allergy (e.g., systemic peanut or hazelnut allergies).\textsuperscript{15-18} Specific IgE to cow’s milk allergens casein, alpha-lactalbumin and beta-lactoglobulin and specific IgE to the egg white allergens, ovalbumin and ovomucoid, do not seem to provide additional information compared to whole allergen extracts when diagnosing cow’s milk and egg allergies; however, casein and ovomucoid can be useful in identifying patients who are allergic to baked cow’s milk and baked egg, respectively, as well as patients with persistent cow’s milk and egg allergies.\textsuperscript{19-21} For the component-specific IgE that have shown additional diagnostic value compared to specific IgE to whole extracts, their enhanced diagnostic performance usually results from higher specificity with comparable sensitivity—for example, considering the cut-off of approximately 1 KU/L, the specificity of Ara h 2-specific IgE was 85\% (with 92\% sensitivity) and the specificity of specific IgE to peanut was 38\% (with 96\% sensitivity) in a Swedish study.\textsuperscript{22} In a Dutch study, Cor a 9-specific IgE and Cor a 14-specific IgE had higher specificity compared to specific IgE to whole hazelnut extract.\textsuperscript{12} Specific IgE to allergen components are now available from several foods and can be requested in addition or instead of specific IgE to allergen extracts. The results of specific IgE to allergen components need nevertheless to be interpreted in the light of the clinical history and even those components associated with the best test performance may not confirm or exclude food allergy with high enough certainty to forgo OFC in many cases. For instance, in the Healthnustudy, 18\% of infants with specific IgE to Ara h 2 lower than 0.35 KU/L reacted to peanut during the OFC, 5\% of infants with specific IgE greater or equal to 1 KU/L passed the OFC and a significant proportion (22\%) had results ranging between 0.1 and 1.0 KU/L, which were considered equivocal and an indication for OFC.\textsuperscript{23}

In specialized clinics, typically 20\%-50\%\textsuperscript{4,24,25} of patients undergoing OFC, and up to 70\% in some reports,\textsuperscript{26} develop an allergic reaction during the OFC. The proportion of positive OFC depends on the criteria chosen to refer patients for OFC. The severity of the allergic reactions is unpredictable and, while generally regarded as a safe procedure in qualified settings, severe reactions can and do occur during OFC to different foods in patients with varying degrees of IgE sensitization.\textsuperscript{27} The majority of severe reactions occur when less than half of the challenge food has been ingested.\textsuperscript{27} Furthermore, OFCs require significant resources and a highly skilled clinical team experienced in this procedure and prepared to treat severe allergic reactions including severe anaphylaxis.

Novel approaches with the potential to improve the accuracy of existing allergy tests and reduce the need for OFC, both for diagnostic and for monitoring purposes, especially given the multiple new therapeutic modalities now being assessed for approval, would be very useful in clinical practice and could have a measurable impact in the care for patients with suspected food allergy and for food-allergic patients.

3 \textbf{WHAT IS THE RATIONALE FOR BASOPHIL TESTING IN IGE-MEDIATED FOOD ALLERGY?}

Basophils like mast cells express the tetrameric form of the high-affinity IgE receptor and are thought to be directly involved in IgE-mediated acute allergic reactions and anaphylaxis. In fact, fatal and near-fatal cases of anaphylaxis have occurred without elevated tryptase, a mediator released by mast cells but not by basophils, raising the possibility that at least in some cases, reactions to foods may be driven primarily by basophils.\textsuperscript{28} Direct evidence of basophil activation during food allergy was recently provided by Commins et al.,\textsuperscript{29} who challenged patients with a form of allergy to red meat and showed that basophil activation coincided with the development of symptoms during the OFC in the majority of patients. In 9 of the 12 patients studied, no change in the tryptase level was observed at the different time points. This study reinforces the relevance of basophils in IgE-mediated reactions to foods, including anaphylaxis.

Whether or not basophils play a unique role in vivo, BAT may offer several advantages over skin testing as a biomarker for disease, including the determination of a dose-response for activation, the insensitivity to patient use of histamine blockers\textsuperscript{30} and the assessment of reactivity (when performed as is most common as a whole blood assay) in the presence of non-IgE allergen-specific antibodies (i.e IgG, IgA), which may be important negative regulators of
IgE-mediated reactivity. BAT also has the advantages of being possible to perform in children with extensive eczema and of not being an in vivo test, thus with a higher safety profile.

The basophil activation test (BAT) assesses the expression of activation markers such as CD63 and CD203c on the surface of basophils by flow cytometry following stimulation with food allergens and controls. The upregulation of CD63 or CD203c may not always correlate with the total histamine released, which has been suggested to be due to the fact that the two markers follow different pathways of basophil activation, with CD63 reflecting anaphylactic degranulation and CD203c piecemeal degranulation. CD63 is highly relevant to IgE-mediated allergic reactions as it directly correlates with histamine that is released and is in part inversely correlated with intracellular diaminoxidase, an enzyme responsible for patients allergic symptoms. CD63 expression is not always correlated with the total histamine released, which has been suggested to be due to the fact that the two markers follow different pathways of basophil activation.

Table 1: Basophil identification markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>IgE</th>
<th>CD123</th>
<th>CCR3</th>
<th>CRTH2</th>
<th>CD203c</th>
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<tr>
<td>Synonym</td>
<td>-</td>
<td>IL-3Rα</td>
<td>CD193</td>
<td>CD294</td>
<td>Neural cell surface differentiation antigen</td>
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<tr>
<td>Function</td>
<td>Defence against helminths, type I hypersensitivity</td>
<td>Low-affinity (α) subunit of IL-3 receptor</td>
<td>Receptor for C-C type chemokines</td>
<td>Receptor for prostaglandin D2</td>
<td>Unknown</td>
</tr>
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<td>Peripheral blood cells expressing the marker</td>
<td>Monocytes, dendritic cells, basophils, B cells and platelets</td>
<td>Basophils, monocytes, eosinophils, plasmacytoid dendritic cells, myeloid dendritic cells, and subsets of haematologic progenitor cells</td>
<td>Basophils, eosinophils, Th2 cells</td>
<td>Basophils, eosinophils, Th2 cells</td>
<td>Basophils</td>
</tr>
<tr>
<td>Markers to be used in combination</td>
<td>HLA-DRα</td>
<td>HLA-DRα</td>
<td>CD3β</td>
<td>CD3β</td>
<td>Noneβ</td>
</tr>
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Legend: CD3β is expressed on T cells and therefore allows the exclusion of this cell type when using CCR3 or CRTH2. The distinction between basophils and eosinophils can be carried out by size and granularity using forward scatter and side scatter. CD203c is specific for basophils and therefore can be used to identify basophils without other markers. Abbreviations: CCR3, C-C chemokine receptor type 3; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells.
65 patients prospectively recruited used to externally validate the findings. In this study, BAT showed 98% sensitivity and 96% specificity in the primary population and 83% sensitivity and 100% specificity in the second population to diagnose peanut allergy.

The diagnostic performance of BAT is allergen specific and can vary with the allergen preparation used for cell stimulation in the assay. The use of individual allergens in the BAT has also been tested, for example using lipid transfer proteins (e.g., Pru p 3 from peach62 and Ara h 9 from peanut67), seed storage proteins (e.g., Ara h 1, Ara h 2, Ara h 3 and Ara h 6 from peanut39) and Bet v 1 homologues (e.g., Ara h 8 from peanut67). In these studies, BAT using single allergen components showed to be advantageous compared to BAT using food extracts to diagnose allergy to some foods (e.g., BAT to Pru p 3 to diagnose peach allergy) but not to others (e.g., BAT to casein to diagnose cow’s milk allergy)—Table 2.

The enhanced specificity and retained sensitivity of BAT compared with SPT and specific IgE create the potential to reduce the number of patients referred for OFC compared to what is current practice. Santos et al.4 have documented a 67% reduction in the need for OFC in a peanut study. In a second population of patients, used for external validation of the test, the specificity of BAT reached 100%, meaning that a positive BAT would confirm the diagnosis of peanut allergy with a high degree of certainty. Therefore, the reduction in OFC was mostly a reduction in positive OFC, which would be most desirable in clinical practice.

**WHAT IS KNOWN ABOUT THE USE OF THE BASOPHIL ACTIVATION TEST TO ASSESS PROGNOSIS IN IGE-MEDIATED FOOD ALLERGY?**

Oral food challenge is currently the method of choice not only to diagnose food allergy but also to assess response to treatment. All the current interventional studies for food allergy have as their primary end-point the change in clinical sensitivity defined by the dose eliciting an objective reaction during a post-treatment food challenge from that at baseline. Although safe when carried out in qualified centres, these OFCs for individuals known or strongly suspected to be allergic are often particularly stressful experiences for both patients and their families as well as for the clinical team performing
the OFC. In addition, specific allergen immunotherapy-based inter-
ventions, which are those that are closest to FDA approval and
potential broad adoption in clinical practice, have been associated
with variable outcomes. While some individuals appear to achieve a
sustained long-term benefit without need of continuous treatment,
others appear to have rapidly waning benefit. Current testing does
not distinguish these individuals raising the potential need for peri-
odically repeated OFC unless other biomarkers can be identified.

The BAT has been used to monitor the acquisition of oral toler-
ance to foods over time, either naturally or under immunomodu-
ulatory interventions. Wanich et al.40 showed that basophil reactivity
following stimulation with a cow’s milk extract reflected different
phenotypes of cow’s milk allergy, with patients that tolerated heated
milk showing a degree of basophil reactivity that was intermediate
between that of patients allergic to all forms of cow’s milk and
patients that had outgrown their cow’s milk allergy. In another study,
BAT to cow’s milk showed to be useful in identifying patients who
had resolved their cow’s milk allergy.48 Basophil activation is modi-
fied during allergen-specific immunotherapy and has been shown to
be reduced in patients submitted to oral immunotherapy to foods such
as cow’s milk, peanut and egg.69-72 Basophil suppression during
desensitization can be observed not only following stimulation with
the implicated allergen but also with a bystander allergen and an
IgE-mediated positive control.73 It remains to be seen whether baso-
phil suppression persists following discontinuation of allergen-speci-
cific immunotherapy. Basophil activation in food-allergic patients is
reduced during treatment with omalizumab but increases after cessa-
tion of treatment.74 The reduction in basophil activation following
treatment with omalizumab seems to be dependent on low specific/
total IgE ratios and effects on the intrinsic basophil reactivity poten-
tially mediated by a variety of mechanisms.75-78

The results of the BAT have been associated with the severity
and the threshold of allergic reactions during the OFC. The pro-
portion of activated basophils in response to allergen in vitro, so-
called basophil reactivity, has been directly correlated with the
severity of symptoms experienced during OFC in studies of mostly
peanut and cow’s milk-allergic patients.48,79,80 Measures of in vitro
basophil sensitivity, such as “CD-sens,” in one study79 and the
ratio between activated basophils following stimulation with aller-
gen and an IgE-mediated control in another study80 have been
correlated with the threshold of reactivity during OFC. These data
suggest that BAT can provide information about the severity and
the threshold of allergic reactions that, in addition to other clinical
characteristics of the patients that have been identified as risk
factors (e.g., persistent asthma), might enable the clinician to iden-
tify high-risk allergic patients who require closer follow-up and
more intensified education.

Among the limitations of BAT for routine use are the proportion
of patients with non-responder basophils (ie basophils selectively
unresponsive to FcERI-mediated signalling, which has been reported
in as many as 17% of individuals49), the fact that the test requires
fresh blood (<24 hours since blood collection81) to run, the need for
standardized allergens whose optimal concentration is variable
depending on the specific allergen and the characteristics of the
extract. Furthermore, BAT requires expertise to perform a flow
cytometry-based assay that is not currently automated and to subse-
quently analyse the data.

6 WHAT WOULD BE THE VALUE OF
USING BAT IN CLINICAL PRACTICE?

The high specificity of BAT confers its greatest advantage compared
with tests that are currently used in clinical practice, such as SPT
and specific IgE.4,59 For the foods where component testing offers
added value compared to using whole allergen extracts, enhanced
specificity can be obtained with specific IgE to component allergens;
however, cases remain where specific IgE to individual components
is not enough to reach a clear definitive diagnosis of food allergy. An
example is Ara h 2-specific IgE which has shown high accuracy to
diagnose peanut allergy and higher than that of specific IgE to pea-
nut but not that of BAT to peanut.4,22 indicating that some
patients may benefit from BAT to peanut in addition to or as an
alternative to IgE to Ara h 2.4 Another example is the Cor a 9- and
Cor a 14-specific IgE which have shown to be more accurate than
specific IgE to hazelnut allergy12,13; however, in a study where these
two hazelnut components were tested together with BAT to hazelnut,
BAT to hazelnut showed higher specificity (97%) than specific IgE to Cor a 14 (94%) and specific IgE
to Cor a 9 (72%).58 The improved diagnostic performance of BAT
compared to specific IgE to allergen components which themselves
have shown to have very good diagnostic performance is probably
related to the fact that BAT is a functional assay, whose results
depend not only on the amount of IgE but also on other characteris-
tics of IgE (such as affinity and clonality) and possibly of antibodies
of other isotypes (such as IgG4) which together are responsible for
the ability of allergen to trigger effector cell activation.31

Basophil activation test can be performed using single allergen
components, which for some foods can be more accurate than using
allergen extracts in the BAT (Table 2). For example, BAT to ovomu-
coid and BAT to Pru p 3 showed improved diagnostic accuracy com-
pared to BAT to egg white and BAT to peach to diagnose egg
allergy (both baked and raw egg allergies) and peach allergy, respec-
tively.56,62 The use of single allergens has, however, the disadvan-
tage of missing the contribution of minor allergens that are clinically
relevant for some patients and of missing the combined effect of
multiple allergens to which polysensitized patients produce IgE and
which may increase the degree of basophil activation detected in
the BAT.

Due to its high specificity, which reaches 100% in some stud-
ies,4,48 a positive BAT allows confirming the diagnosis of IgE-
mediated food allergy with a high degree of certainty. Given the
practical implications involved in the performance of the BAT and
the fact that in some patients an allergy-focused clinical history
gether with the documentation of specific IgE to extracts or
components is sufficient to confirm or exclude the diagnosis of
IgE-mediated food allergy, the BAT is probably a test that is worth doing in selected patients with suspected food allergy. It could thus be used as a second step in the diagnostic process, in patients that would otherwise be referred for an OFC following the appropriate clinical assessment and the performance of SPT and/or specific IgE. This two-step approach has the advantage of requiring the performance of a smaller number of BAT. Patients with a positive BAT would see their OFC obviated and would be spared from experiencing an acute allergic reaction during the OFC. Patients with a negative BAT or non-responder basophils would need to be referred for an OFC (Figure 1). For example, in a peanut allergy study, using this two-step approach, BAT would need to be performed in less than 25% of assessed patients and allowed for a 67% reduction in peanut OFC.

Being a blood test that requires a small volume of blood (about 1 mL) and therefore is minimally invasive, BAT has a good chance to be well accepted by patients and families. However, because it requires fresh blood, it cannot be performed using stored frozen samples of blood and patients need to book an appointment to have the BAT done. To reduce the costs and the number of blood draws, it may be preferable to perform BAT following SPT, if available. SPT provides an immediate result and in cases for which the combination of history and SPT are equivocal, blood for BAT (and specific IgE) could be collected on the same day as the clinical appointment.

With the recent change in the guidelines for the prevention of peanut allergy, recommending introduction of peanut in the first year of life and between the age of 4 and 6 months in high-risk infants, a potential application of the BAT is the assessment of high-risk infants before introduction of peanut. BAT has shown to be more accurate than SPT and specific IgE to diagnose peanut allergy in children including children in the first year of life. Using BAT in peanut-sensitized infants could reduce the number of positive OFC.

Because acute allergic reactions and anaphylaxis are challenging to diagnose and to treat in infancy, BAT could reduce the number of adverse events and enhance patients’ comfort and safety at this young age. Recent guidelines contemplate only SPT and specific IgE in the assessment of patients prior to the introduction of peanut, as BAT is not yet established clinically.

7 | WHAT ARE THE NEXT STEPS TO BRING THE BAT TO THE CLINIC?

The performance of BAT to diagnose IgE-mediated food allergies can vary with different factors, some related to the study population, some related to the study design, some to the laboratory procedure selected for the BAT and some to the analyses of flow cytometry data. The clinical application of BAT would require, on the one hand, the standardization of the laboratory procedure and data analyses and, on the other hand, the clinical validation of the test, that could enable regulatory approval and eventual incorporation into guidelines, provided continued quality control and sufficient scientific evidence to support its clinical use was available (Figure 2).

Currently, significant heterogeneity exists in published studies with regard to most of these aspects. In terms of laboratory procedure, various methods have been developed in-house and kits are also commercially available. The latter may offer a greater sense of security to less experienced users and standardization across sites; however, the procedure does not differ significantly compared to in-house methods and has the disadvantage of not disclosing all information about the reagents included in the kit. The methods used for BAT can vary with regard to sample anticoagulation, the stimulation buffer used—especially the presence and concentration of IL-3, the time allowed between blood...
collection and performance of the assay, the allergens used for cell
stimulation, the antibodies used for cell staining, whether stimula-
tion and staining are done simultaneously or as separate steps,
buffer used for erythrocyte lysis and additional washing
steps. All these steps can affect the diagnostic perfor-
mance of BAT. The extracts or the allergens used for cell stimula-
tion can also impact the results and vary over time if there is no
standardization in place. In this regard, recombinant allergens offer
the highest stability and consistency, compared to purified allergens
or allergen extracts, but the potential disadvantage of failing to
account for the contributions of reactivity to minor allergens.

The methods used for flow cytometry data collection (e.g., flow
cytometry instrument and settings used over time) can also signifi-
cantly impact on the BAT results and must be standardized and
described. In addition, the criteria adopted for the analyses when
performed “manually,” as is still often the case for flow cytometry
data, are subject to significant subjectivity and poor reproducibility.
Automated analysis platforms and methods are being developed to
meet the need for more standardized data analyses. Automated
analyses approaches have significant advantages of being more time-
efficient, reproducible and high-throughput; however, they may be
more sensitive to the acquisition of high-quality flow cytometry data
(not necessarily a bad thing) and may be challenging to generalize to
multiple BAT assays that may employ different antigens, equipment
and other sources of variation. Finally, the “read-out” parameter(s)
selected to express the results of BAT, and the method used to calcu-
late it is another important aspect that needs to be standardized to
ensure consistency and reproducibility of BAT results. Ideally, multi-
centre studies should be performed to assess the reproducibility and
variability of the test procedure.

As with any allergy test, BAT requires clinical validation through
the performance of diagnostic studies in different food allergies, as
identified diagnostic decision levels are allergen specific. They are
also specific to the population where they are generated, as they
depend on the prevalence of food allergy in the population and can
vary with the geographical location and other factors that can affect
the clinical phenotype of patients being assessed for suspected food
allergy. While the identified diagnostic cut-offs can be extrapolated
to a population with similar characteristics to the one where they
were generated, the application of the test to a different patient
population would in principle require a separate clinical validation.

Before regulatory approval can be granted, continuous quality
control needs to be in place and evidence for cost-effectiveness and
beneficial effects on health and social outcomes should be gathered
(Figure 2). Although BAT is likely much cheaper and certainly safer
than OFC, it is probably more expensive than SPT or specific IgE.
Formal cost-effectiveness studies would be warranted.

Basophil activation test is a novel test which is quite distinct from
standard allergy tests currently in use. Its incorporation in clinical
practice requires dissemination of information about the test, namely
its methodology and interpretation of results, so that an improved
understanding of the assay can enhance confidence in its clinical use.
Once the BAT is being used in clinical practice, its impact on patients’
health outcomes and on the decision-making process involved in the
referral for OFC and in the confirmation or exclusion of the diagnosis
of food allergy should be assessed to sustain its clinical use. Another
important aspect that should be explored in future research is the
need for more sophisticated multi-dimensional diagnostic algorithms
using large sets of clinical and laboratory values to determine a com-
prehensive “post-test diagnostic probability.” Such analyses could pro-
vide valuable information on the combination of diagnostic tests for
optimal food allergy diagnosis.

8 | CONCLUSIONS

BAT is sensitive and more specific than standard testing and has the
potential to reduce the number of OFC currently needed to
diagnose IgE-mediated food allergy as well as to monitor clinical response to treatment and possible resolution of food allergy. Because of the better specificity, the most of the OFC avoided would be those that are positive, which would improve patient safety and comfort. However, in order to achieve a widespread adoption of BAT in clinical practice, it is necessary to achieve standardization of the laboratory procedure and data analyses and more rigorous validation. Finally, a comprehensive assessment of the impact of BAT on health and social outcomes and its cost-effectiveness would be warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


44. Chinumbolo S. Major pitfalls in BAT performance may be caused by gating protocols and CD63% cut off evaluation. Cytometry A. 2014;85:382-385.


74. Gernez Y, Tirouvanziam R, Yu G, et al. Basophil CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in subjects with nut allergy. *Int Arch Allergy Immunol*. 2011;154:318-327.


