ORIGINAL ARTICLE

LAD2 Mast Cell Activation Test Associates With the Reaction Severity and Diagnoses BAT Nonresponders in Hymenoptera Venom Allergy

Koren A[1], Dejanovic L[1], Kopac P[1,2], Erzen R[1,2,3], Bajrovic N[1], Zidarn M[1,2], Korosec P[1,4]

[1]University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia
[2]Medical Faculty, University of Ljubljana, Ljubljana, Slovenia
[3]Hospital Topolšica, Topolšica, Slovenia
[4]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

J Investig Allergol Clin Immunol 2025; Vol. 35(1): 24-31
doi: 10.18176/jiaci.0969

Abstract

Background: The usefulness of the mast cell activation test (MAT) in diagnosing patients with uninterpretable basophil activation test (BAT) results caused by nonresponding basophils has not been addressed. Our study evaluated whether the results of the MAT were associated with the severity of the allergic reaction.
Methods: We recruited 39 patients with Hymenoptera venom allergy (HVA), 22 nonsensitized controls, and 37 BAT nonresponding HVA patients. Specific IgE levels for honeybee venom (HBV) and yellow jacket venom (YJV) and total IgE were quantified using the IMMULITE® system. We performed a BAT and a MAT, which was based on the response of LAD2 cells to HBV and YJV.
Results: We first optimized the susceptibility of LAD2 cells to IgE-mediated degranulation in HVA and showed that prestimulation with IL-33 and IL-6 significantly increased the responsiveness of LAD2 cells to allergen stimulation ($P<.01$). The LAD2 MAT results correlated with the BAT results, and patients with severe sting reactions (Mueller grades III and IV) had a median 2-fold higher LAD2 MAT score than patients with nonsevere sting reactions (large local reaction or Mueller grades I and II) ($P<.05$). Furthermore, LAD2 MAT provided conclusive results in 20 of the 37 HVA patients (54.1%) with nonresponding basophils in the BAT.
Conclusions: The LAD2 MAT is a new diagnostic tool for HVA patients with nonresponding basophils. LAD2 MAT can identify patients at risk of severe sting reactions and can thus help guide recommendations for venom immunotherapy and improve the management of patients with HVA.

Key words: Basophil activation test nonresponders. Hymenoptera venom allergy. LAD2. Mast cell activation test. Reaction severity.

Resumen

Antecedentes: Aún no se conoce la utilidad del test de activación de mastocitos (MAT) en el diagnóstico de pacientes que tienen el test de activación de basófilos (BAT) no interpretable, debido a que sus basófilos son no respondedores, por lo que debería evaluarse si los resultados del MAT están asociados con la gravedad de la reacción alérgica.
Métodos: Se reclutaron 39 pacientes alérgicos al veneno de himenópteros (HVA), 22 controles no sensibilizados y 37 pacientes HVA que no respondieron a la BAT. Se cuantificaron los niveles de IgE específica frente all veneno de abeja melífera (VMH), el veneno de chaqueta amarilla (VJA) y la IgE tota mediante el sistema IMMULITE®. Se realizaron BAT y MAT con células LAD2 (MAT/LAD2) en respuesta al VHB y al YJV.
Resultados: En primer lugar, optimizamos la susceptibilidad de las células LAD2 a la degranulación mediada por IgE en HVA y demostramos que la preestimulación con IL-33 e IL-6 aumentaba significativamente la capacidad de respuesta de las células LAD2 a la estimulación con alérgenos ($p<0,01$). Los resultados del MAT/LAD2 se correlacionaron con los resultados de BAT, y los pacientes con reacciones graves a la picadura (grados IV o III de Mueller) tuvieron una mediana en el MAT/LAD2, 2 veces mayor que los pacientes con reacciones no graves a la picadura (grados II, I o LLR de Mueller) ($p<0,05$). Además, el MAT/LAD2 proporcionó resultados concluyentes en el 54,1% (20 de 37) de los pacientes HVA con basófilos no respondedores en el BAT.
Conclusiones: El MAT/LAD2 representa una nueva herramienta diagnóstica para pacientes HVA con basófilos no respondedores. Además, el MAT/LAD2 puede identificar a los pacientes con riesgo de reacciones graves a la picadura y, por tanto, puede ayudar a orientar las recomendaciones para la inmunoterapia con veneno y mejorar el tratamiento de los pacientes con HVA.

Palabras clave: Prueba de activación de basófilos no respondedores. Alergia al veneno de himenópteros. LAD2. Prueba de activación de mastocitos. Gravedad de la reacción.

Summary box

- **What do we know about this topic?**
  The MAT is a diagnostic tool that can facilitate diagnosis of food and drug allergies. However, data on the use of MAT, especially in patients with nonresponding basophils in the BAT, are lacking.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  This study showed that the LAD2 MAT can resolve diagnosis for most HVA patients with nonresponding basophils. The LAD2 MAT can also identify patients at risk of severe sting reactions and can thus improve the management of patients with HVA.

Introduction

Testing for serum IgE to recombinant venom components and the basophil activation test (BAT) can improve the diagnosis and monitoring of Hymenoptera venom allergy (HVA) [1-4]. The availability of venom allergen components has advanced our understanding of HVA and enabled molecular diagnostics of HVA. Molecular diagnostics can distinguish true allergy from cross-reactivity to honeybee and yellow jacket venoms, whereas BAT can confirm sensitization in the case of negative skin test and venom sIgE results and evaluate venom-specific clinical reactivity in the case of dual honeybee and vespid sensitization and unknown culprit history [3-9]. However, BAT has 2 inherent weaknesses that hinder its use in clinical practice [10]. First, it requires fresh blood for testing [11]. Second, approximately 10% of patients have basophils that do not react to an IgE-/FcεRI-mediated positive control or the allergen, but only to non–IgE-mediated stimulants, and are therefore designated *nonresponders* [1,10]. In this group of patients, the BAT results are uninterpretable.

We recently undertook the initial validation and assessment of a novel in vitro diagnostic tool, the mast cell activation test (MAT), using primary human blood–derived mast cells (pMCs) generated from $CD117^+$ peripheral blood precursors, which were passively sensitized with patients' sera and then incubated in vitro with an allergen [12]. Activation of mast cells (MCs) has been assessed using flow cytometry analysis of expression of the activation markers CD63 and CD107a, and the potential of the MAT has been assessed in patients with peanut allergy and HVA and as a diagnostic tool for peanut allergy compared with existing diagnostic tests [12]. The practical limitations of our initial approach [12] were that generation of pMCs is difficult to standardize for routine clinical laboratory use, as it is donor-dependent, with distinct combinatorial phenotypic profiles [12], and that different culture protocols can impact the result [14,15]. However, in comparison to the BAT, fresh blood is not required to perform the MAT. In addition, the MAT might be applicable in individuals with HVA who have uninterpretable venom BAT results caused by basophils that do not respond to either of the IgE-mediated positive controls (anti-IgE and anti-FcεRI) [11,16,17]. Routine maintenance of cell lines is more convenient than obtaining mast cells from the blood of multiple donors. LAD2 is a stem cell factor (SCF)–dependent human MC line that expresses FcεRI and was recently used in peanut LAD2 MAT studies [18,19]. However, pMCs are more susceptible to IgE-mediated degranulation than LAD2 cells [12].

The utility of venom MAT in diagnosing BAT nonresponders with HVA has not been broadly addressed, and it would be interesting to evaluate whether venom LAD2 MAT is associated with the severity of Hymenoptera sting reaction, as recently shown for venom BAT [7]. To explore these diagnostic techniques and the clinical goals of venom LAD2 MAT in HVA, we first optimized the susceptibility of LAD2 cells to IgE-mediated degranulation [12] in a group of well-defined HVA patients. We then determined the diagnostic utility of venom LAD2 MAT in HVA, assessed whether the LAD2 MAT was associated with the severity of sting reactions, and evaluated the utility of venom LAD2 MAT for diagnosis of BAT nonresponders.

Methods

Patients and Study Design

We prospectively recruited 39 HVA patients (64.1% females; age 21-77 years; median age, 46 years) and 22 non–Hymenoptera-sensitized nonallergic controls (81.8% females; age, 25-57 years; median age, 35 years). All patients were allergic to honeybee venom (HBV) or yellow jacket venom (YJV) only and had a single positive result in intradermal tests (IDTs), determination of sIgE, and BAT for the culprit allergen (HBV/YJV). All controls had negative sIgE and BAT results for both HBV and YJV. All participants were recruited prospectively between 2018 and 2019. BAT, sIgE, total IgE (tIgE), and tryptase measurements were taken at recruitment. The patient's plasma and serum samples were stored at –20°C, and MAT testing was conducted in 2020 and 2021. All LAD2 MAT optimization experiments were performed with up to 8 selected patients from this cohort. We evaluated the use of venom LAD2 MAT in patients with nonresponding basophils by retrospectively selecting a group of 37 HVA patients with nonresponding basophils in the BAT from 1839 patients in whom we routinely performed venom BAT between 2013 and 2021. Plasma samples were collected from the same heparinized blood used for BAT. All specimens were collected at the University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia. The study was approved by the National Medical Ethics Committee of the Republic of

Slovenia (N° 0120-188/2017/4). All patients provided their written informed consent.

LAD2 Mast Cell Activation Test

LAD2 cells were cultured in StemPro-34 complete media (CM) with 100 ng/mL SCF (STEMCELL Technologies) and sensitized with the participants' plasma overnight (1:10 dilution) at a final density of 2×10^5 cells/mL. Cells were washed, and 2×10^4 cells were stimulated with 0.01, 0.1, and 1 mg/mL HBV or YJV (both HalAllergie) for 15 minutes at 37°C. In the case of the controls, the cells were exposed to media alone (negative control) or 10 µg/mL anti-IgE mAb (positive control) (Sigma-Aldrich). Degranulation was stopped by chilling on ice, after which CD63-APC, CD107a-PE, CD107b-Alexa 700 (all BioLegend), and IgE-FITC (Miltenyi Biotec) were added, and the sample was incubated for 20 minutes. Finally, cell probes were washed twice and fixed before flow cytometry analysis using the FACSCanto II flow cytometer (BD Biosciences). We applied flow cytometry to measure degranulation based on the surface expression of CD63 on LAD2 cells, as previously described [12,18,19].

To ensure quality control throughout testing, we immunophenotyped LAD2 cells and determined FcεRI surface expression measurements weekly. In the case of reduced values for any of the markers, a new batch of LAD2 cells was thawed.

Optimization of Susceptibility of LAD2 Cells to IgE-Mediated Degranulation in MAT

IL-4 and human myeloma IgE

The effect of IL-4 and IgE treatment on FcεRI LAD2 expression was evaluated by treating LAD2 cells with 10-100 ng/mL of recombinant human IL-4 (Peprotech) or 1 µg/mL of human myeloma IgE (Millipore) for up to 9 days. We then evaluated FcεRI cell expression and the anti-IgE mAb response.

Stimulation media, IL-33, and IL-6

The stimulation media tested were StemPro-34 CM, Tyrode buffer (Sigma-Aldrich), RPMI-1640 medium (Sigma-Aldrich) with 0.1% bovine serum albumin (BSA) (Gibco), and Hank balanced salt solution buffer (Sigma-Aldrich). The effect of IL-33 and IL-6 on LAD2 IgE–mediated degranulation was evaluated by prestimulating LAD2 cells with 1-100 ng/mL of IL-33 (Peprotech) and 1-100 ng/mL of IL-6 (Peprotech) dissolved in StemPro-34 CM for 1 hour at 37°C and 5% CO_2 and then stimulating with the allergen/anti-IgE. The combined effect of IL-33 and IL-6 was observed at 1 ng/mL of IL-33 and IL-6.

Statistical Analysis

The data distribution was determined using the D'Agostino and Pearson omnibus tests. Since most data were not normally distributed, we used the Mann-Whitney test or Wilcoxon matched-pairs signed-rank test, as appropriate, unless stated otherwise. We used the Spearman rank correlation test to analyze the associations between variables. Flow cytometry was performed using BD FACSDiva (version 8.0.1) (BD Biosciences) or FlowJo (version 10.7.2) (BD Biosciences). The statistical analyses were performed using Prism 9 (GraphPad Software). A P value <.05 was considered statistically significant. All reported P values are 2-tailed.

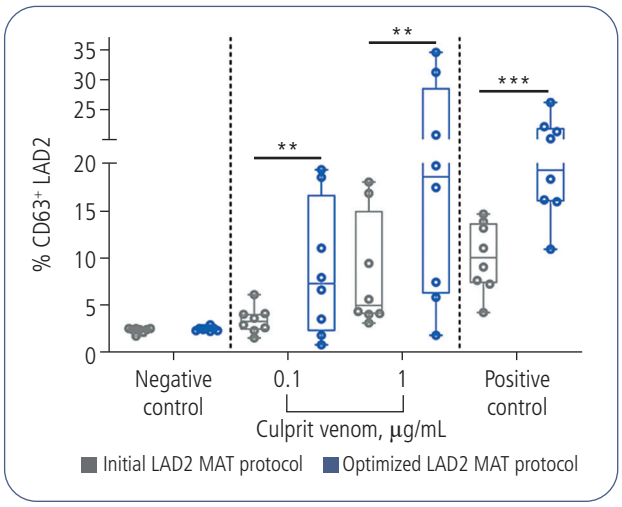

Figure 1. LAD2 degranulation capacity and susceptibility to IgE-mediated venom stimulation before and after the optimization of the LAD2 MAT protocol. Experiments were performed with 8 Hymenoptera venom–allergic patients (4 were allergic to honeybee venom and 4 were allergic to yellow jacket venom). See also figures S3 and S4 in the supplementary materials. ***P<.001 and **P<.01 (paired t test).

Results

Prestimulation Based on IL-33 and IL-6 in Tyrode Buffer With SCF Increases the Susceptibility of LAD2 Cells to IgE-Mediated Degranulation in MAT

We improved the LAD2 MAT by testing various stimulation media, allergen concentrations, and cytokine prestimulations. Our findings are explained in detail in the Supplementary Material. Briefly, the strongest specific response was obtained when using Tyrode buffer with SCF and stimulation with 1 µg/mL HBV/YJV allergen. This effect was further enhanced after prestimulation of LAD2 cells with IL-33 and IL-6, as previously demonstrated by Cop et al [20]. Consequently, for all further MATs, including those performed for basophil nonresponders, we used Tyrode buffer with SCF and prestimulation with IL-33 and IL-6 (Figure 1).

LAD2 MAT Results Correlated With BAT Results

We performed LAD2 MATs in a cohort of 39 HVA patients and 22 healthy controls and compared the results with those of the BATs. The demographic and clinical features of the study population are shown in Table S1. The receiver operating characteristic (ROC) curve analysis yielded an area under the curve (AUC) of 0.67 when prestimulation was applied based on Tyrode buffer with SCF and IL-33 and IL-6. Using the same approach, the optimal cut-off value for positive results was 5.5% of $CD63^+$ LAD2 cells (established using the Youden index, Table S2). The other protocols used (StemPro-34 CM and StemPro-34 CM with IL-33 and IL-6) yielded lower AUCs

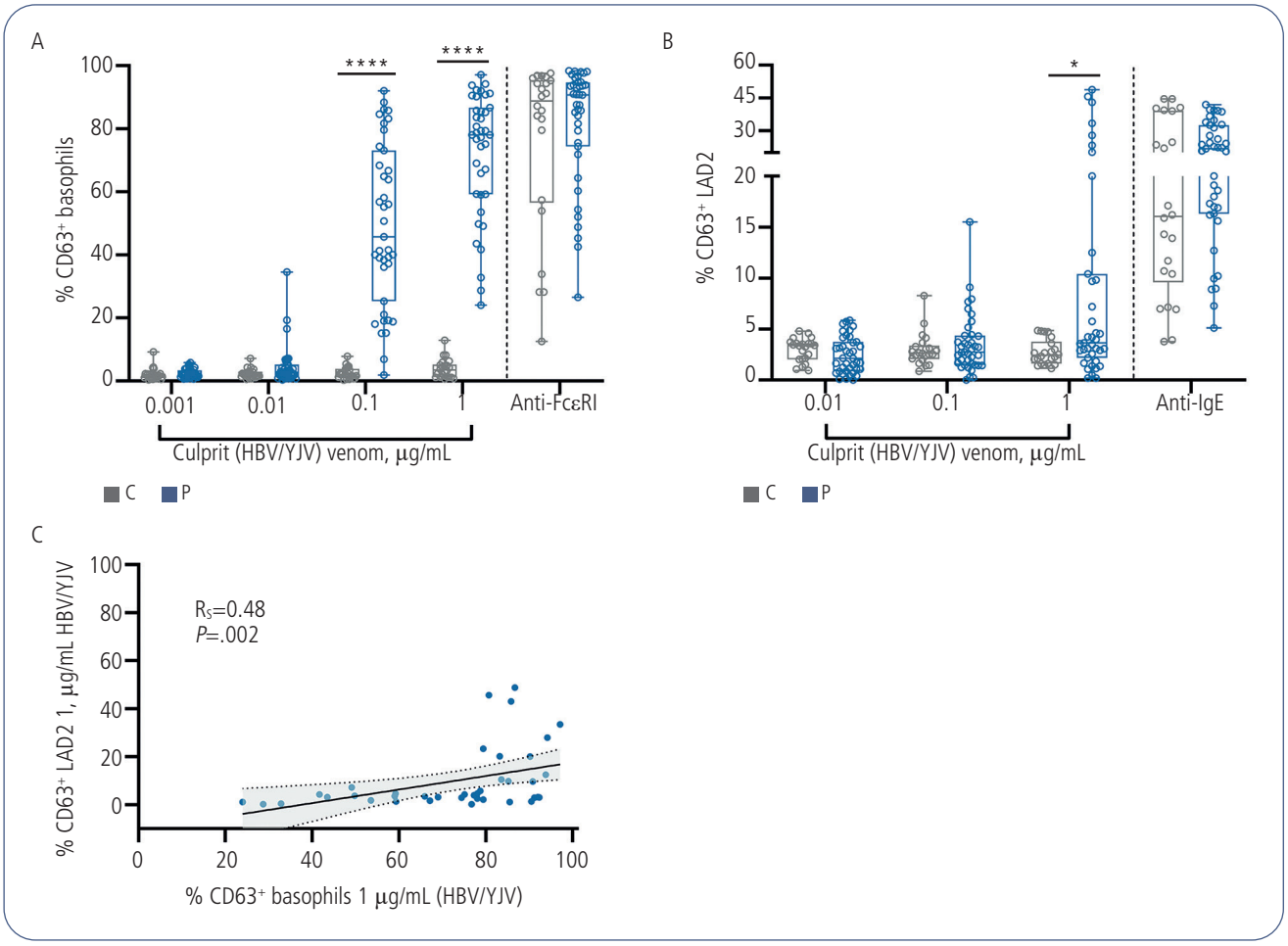

Figure 2. Dose-dependent A) basophil activation test (BAT) and B) mast cell activation test (MAT) in patients (P) with Hymenoptera venom allergy (HVA) and healthy controls (C) after stimulation with the culprit venom (honeybee venom [HBV] or yellow jacket venom [YJV]) ranging from 0.001 to 1 µg/mL or positive control anti-IgE/anti-FcεRI. C, Spearman correlation coefficient analysis of BAT and MAT results after stimulation with 1 µg/mL culprit venom in HVA patients. ****P<.0001 and *P<.05 (Mann-Whitney test).

(Figure S8). The LAD2 response was allergen-specific, and there was no evidence of LAD2 activation when we used sera from healthy controls, who were not sensitized to the allergens used for stimulation (Figure 2). Thus, all healthy controls had a negative LAD2 response to HBV or YJV stimulation (Table S2).

The comparison between LAD2 MAT and BAT results showed that patients had significantly higher results for LAD2 MAT (with HBV or YJV at 1 µg/mL; median, 3.6% vs 2.4%; P=.03) and BAT (with HBV or YJV at 0.1 µg/mL; median, 45.7% vs 1.8%; P<.0001; at HBV or YJV of 1 µg/mL; median, 78.0% vs 4.5%; P<.0001) than controls. Fifteen out of 39 patients with a positive BAT result also had a positive MAT result for the culprit allergen. LAD2 MAT results were correlated with those of the BATs (R_s=0.48, P=.002) (Figure 2). The correlation between LAD2 MAT and sIgE (R_s=0.34, P=.03) was comparable with the correlation between BAT and sIgE results (R_s=0.39, P=.01) (Figure S9).

The expression of CD63 in LAD2 MAT was strongly correlated with the expression of other lysosomal-associated membrane proteins, namely, CD107a (R_s=0.70, P=.0001) and CD107b (R_s=0.89, P<.0001) (Figure S10). There was a strong positive correlation between serum tIgE levels and the IgE sensitization rate (% of IgE+ LAD2 cells) (R_s=0.93, P<.0001), FcεRI LAD2 expression (R_s=0.64, P<.0001), and the CD63 LAD2 anti-IgE response (R_s=0.41, P=.0009) (Figure S11).

Patients With Severe Reactions Had Greater Percentages of Activated LAD2 Cells Than Patients With Mild-to-Moderate Reactions

Twelve (31%) patients experienced severe allergic reactions (7 Mueller grade IV and 5 grade III), whereas 27 (69%) patients experienced nonsevere allergic reactions (11 Mueller grade II, 12 grade I, and 4 large local reactions (LLR_s) (Table S1). Demographic, clinical, and laboratory features are presented in Table S3. There were no significant differences in sex or age between patients with severe and nonsevere allergic reactions. Similarly, there were no differences in baseline serum tryptase (BST), tIgE levels, levels of sIgE to HBV/YJV, or basophil CD63 expression (with HBV or YJV at 1 µg/mL or 0.1 µg/mL) and basophil response to the anti-FcεRI mAb positive control. Although not statistically significant, a trend toward a higher LAD2

response to the anti-IgE mAb–positive control was observed in patients with severe reactions (25.0% vs 19.1%; *P*=.07). Patients with severe allergic reactions exhibited higher LAD2 CD63 expression (with HBV or YJV at 0.1 µg/mL; median, 3.5% vs 2.4% [*P*=.04]; with HBV or YJV at 1 µg/mL, median, 7.8% vs 3.1% [*P*=.02]) (Figure 3).

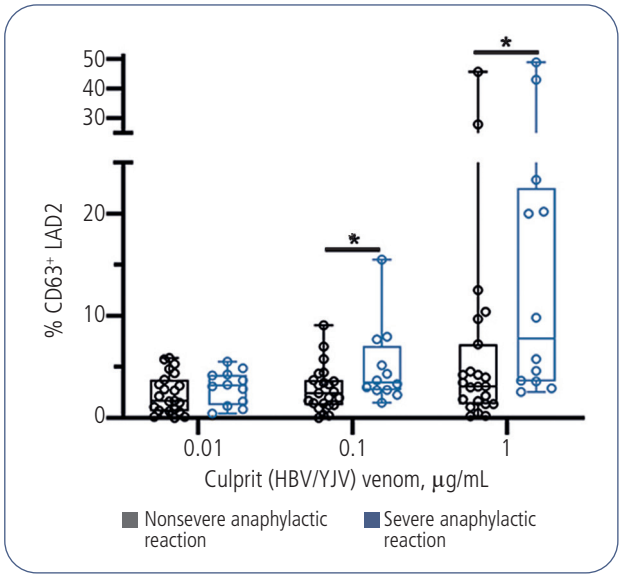

Figure 3. Activation of LAD2 mast cells in response to stimulation with culprit venom (0.01-1 µg/mL) in patients with nonsevere allergic sting reactions (n=27) vs those with severe allergic sting reactions (n=12). The severity was evaluated according to the Mueller scale. Large local and grade I-II reactions were considered nonsevere, while grade III and IV reactions were considered severe. HBV indicates honeybee venom; YJV, yellow jacket venom. **P*<.05 (Mann-Whitney test).

LAD2 MAT Provides Conclusive Results in More Than Half of Patients With Hymenoptera Venom Allergy and Nonresponding Basophils in BAT

From January 2013 to December 2021, a total of 1839 BAT tests were performed with Hymenoptera venoms. Of these, 57 yielded a negative anti-FcεRI response; therefore, the patients were considered BAT nonresponders. Plasma samples were available for 37 of the 57 tests (65%), and the 37 patients (median age, 51 years; age range, 24-80; 22 female) were analyzed further. The demographic and clinical features of the 37 patients are shown in Table S4. Most patients (26.7%) had severe allergic reactions (Mueller grade III-IV). Thirteen patients (35%) were allergic to HBV, 15/37 (41%) were allergic to YJV or hornet, and in 9/37 (24%) cases, the stinging insect was not identified. The median (IQR) CD63 basophil response to anti-FcεRI mAb was 5.0% (3.5%-6.9%). Three out of 37 patients had paired BAT results (median time after initial BAT, 13 months); the anti-FcεRI basophil response reversed to positive in 2 patients. The median CD63 LAD2 mast cell response to the anti-IgE mAb positive control was 16.4% (12.9%-21.1%). The median CD63 LAD2 mast cell response was 8.3% (5.1%-13.1%) in the HBV-allergic subgroup (at 1 µg/mL HBV) and 3.3% (2.4%-6.8%) in the YJV/hornet–allergic subgroup (at 1 µg/mL YJV). In the subgroup where the culprit insect was not identified, the median LAD2 mast cell response was 5.0% (3.3%-9.7%) to 1 µg/mL HBV and 2.85% (1.48%-4.02%) to 1 µg/mL YJV. Considering the previously defined cut-off value for positivity of 5.5% CD63+ cells, LAD2 MAT was positive in 20 of the 37 patients (54.1%) (Figure 4). In more than half of the LAD2 MAT–positive BAT nonresponders (11 of 20; Patient ID: 1, 6, 8, 9, 10, 13, 19, 20, 21, 28, and 37 in Table S5), who were all sensitized to both HBV and YJV, the LAD2 MAT results were positive to only 1 venom.

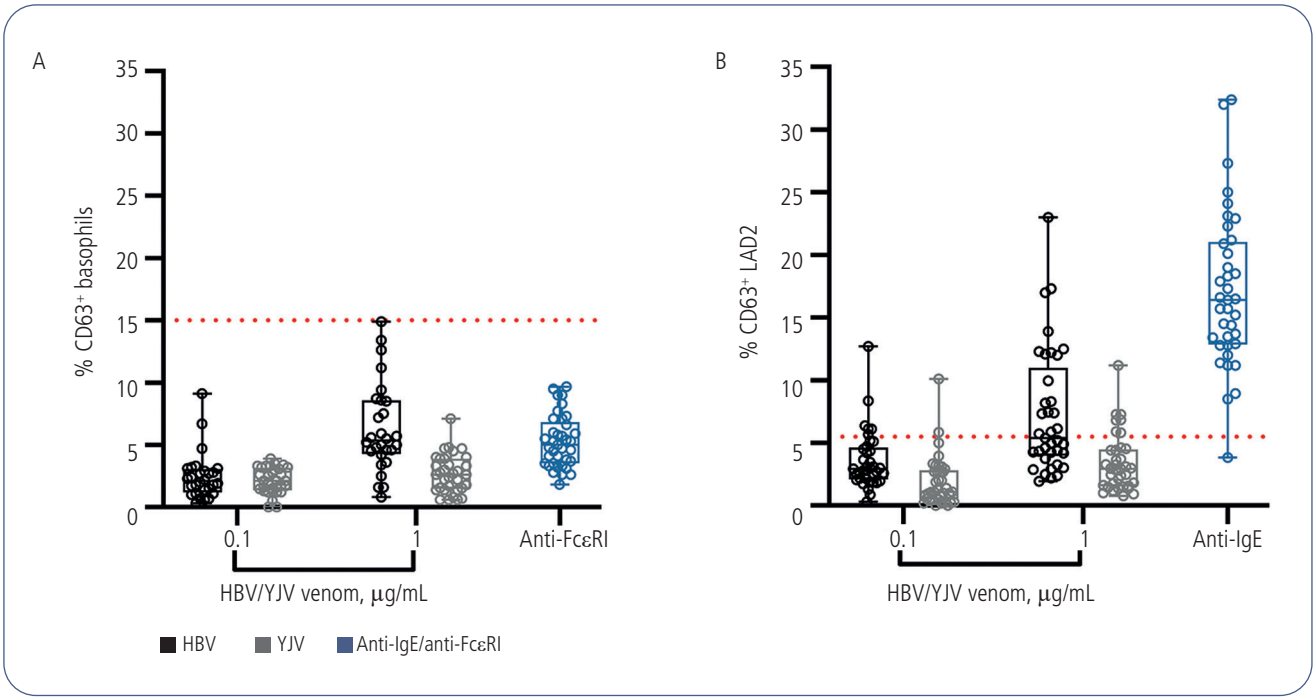

Figure 4. The results of A) the basophil activation test (BAT) and B) the LAD2 mast cell activation test in 37 patients with nonresponding basophils and, therefore, uninterpretable BAT results. The red dotted line indicates the cut-off for positivity. HBV indicates honeybee venom; YJV, yellow jacket venom.

The correlation between LAD2 MAT and sIgE results (R_s=0.33, P=.02) (Figure S12) was similar to the correlation between LAD2 MAT and sIgE results in BAT responders (Figure S9). Levels of sIgE to HBV and YJV were significantly higher in patients with positive LAD2 MAT results than in patients with negative LAD2 MAT results (median, 7.0 vs 1.4 kU/L; P=.002) (Figure S12). Detailed clinical and laboratory data of the 37 patients with nonresponding basophils are presented in Table S5.

Discussion

MAT is a new cellular assay. Recent studies have shown that it could be used as a diagnostic tool for food and drug allergies [12,18,12,22]. We evaluated LAD2 MAT in HVA and confirmed that the results correlated with the BAT results, as reported elsewhere [12,18]. Moreover, LAD2 MAT provided conclusive results for more than half of the participants with nonresponding basophils and, therefore, uninterpretable BAT results. Additionally, our results may suggest that the patients with severe reactions had higher percentages of activated LAD2 cells than patients with mild-to-moderate sting reactions.

In HVA, patients often have low venom-specific IgE levels [23,24], and it was previously shown that low sIgE can affect the results of the MAT [12,18,19]. To overcome this limitation, which is observed in HVA owing to lower sIgE levels (more critical than in peanut allergy [12]), we first optimized the susceptibility of LAD2 cells to IgE-mediated degranulation. We did so to improve cellular FcεRI density and increase the magnitude and specificity of the LAD2 IgE response.

Studies show it is possible to increase FcεRI expression with IL-4 and IgE pretreatment [25,26]. Our results revealed no increase in FcεRI surface expression after 9 days of treatment with IL-4 and an extensive rise in FcεRI expression after the first day of treatment with IgE, which coincided with the increased capacity of LAD2 cells to activate IgE. IL-33, on the other hand, potentiates IgE-mediated mast cell degranulation by increasing the number of responding cells and enhancing the response of individual mast cells [27]. In the MAT, IL-33 and IL-6 synergistically enhance the degranulation of pMCs [20]. We confirmed that treatment with IL-33 and IL-6 increased the responsiveness of LAD2 cells to IgE. This increase was allergen-specific and had no impact on baseline LAD2 activation.

LAD2 MAT in Hymenoptera Venom Allergy

Our results showed that in HVA, the LAD2 MAT result correlated with the BAT result; similar results have been reported elsewhere [12,18,19]. However, additional, larger-scale studies are necessary to evaluate this association. Furthermore, we showed that the LAD2 response was allergen (venom)-specific and concentration-dependent and that there was no response when we used sera from healthy controls (not sensitized to venom). Importantly, pMCs sensitized using sera from patients with positive skin test and BAT results for chlorhexidine showed drug-specific and concentration-dependent degranulation upon stimulation with chlorhexidine [21,22].

Several factors could significantly impact the performance of MAT. Some authors used patients' plasma [18], while others used serum [12,21,22]. According to our results, the choice of serum or plasma does not impact the MAT result. The factor with the greatest potential influence on MAT is the level of sIgE in plasma used for sensitization. In general, the sensitivity of MAT correlates with sIgE levels, meaning that it increases with higher sIgE levels, as observed in current and previous studies [12,18]. It is worth mentioning that our cohort of HVA patients had notably lower median levels of sIgE (4.1 kU/L) than patients with peanut allergy from the studies on MAT by Bahri et al [12], Santos et al [18], and Hemmings et al [19] (26, 14, and 151 kU/L, respectively).

Our previous study also showed that the response to MAT does not depend solely on sIgE levels [12] but might also depend on the functional characteristics of IgE antibodies (ie, clonality and affinity) [26]. In addition, individual differences in tIgE levels could also be a factor in the MAT response. We demonstrated a positive correlation between tIgEs and the percentage of IgE^+ LAD2, LAD2 FcεRI expression, and a more pronounced CD63 anti-IgE response. The positive correlation between FcεRI expression and plasma IgE concentration is well known [28]. Since basophils and MCs share the FcεRI degranulation pathway, the dynamics in FcεRI expression and the magnitude of CD63 activation associated with the patient's tIgE levels could also affect MAT results.

LAD2 MAT in HVA Patients With Nonresponding Basophils in BAT

To our knowledge, this is the first study to assess the performance of MAT in HVA patients with nonresponding basophils in BAT. To date, the utility of MAT in BAT nonresponders has only been reported by Santos et al [18], albeit in the individual cases of only 2 patients. Basophil nonresponsiveness is reported in up to 10% of patients [1,2], and its cause has not yet been uniformly explained. MCs are believed to play a more significant role in the allergic reaction than basophils [29] and could even be increased in the case of nonresponsive basophils. Evaluation of the activation of the patient's MCs could be an additional tool for diagnosing the IgE-mediated mechanism of allergic reactions. Still, the isolation of mature tissue-resident MCs is challenging. LAD2 MAT could therefore represent an alternative that would confirm the diagnosis in the case of BAT nonresponders, as shown with our data. LAD2 MAT confirmed the diagnosis in 11 cases with inconclusive results in the other tests. However, additional studies are needed to clarify the role of MAT in BAT nonresponders in daily clinical practice.

Previous studies suggested that MAT could identify patients at risk of severe peanut anaphylaxis [12,18] and that the MAT is a reliable diagnostic tool for chlorhexidine allergy [21,22]. Furthermore, our study demonstrated that LAD2 MAT might be associated with the severity of the sting reaction. These findings are similar to those of our previous study, where we showed that BAT was predictive of severe allergic sting reactions in HBV allergy [7]. However, in the current study, we could not confirm the association between age and baseline serum tryptase [7,30,31] and the severity

of the sting reaction, probably because of the relatively low sample size compared to previous studies.

In summary, the LAD2 MAT constitutes a new tool for sequential analysis if the HVA patient has nonresponding basophils in the BAT. In addition, LAD2 MAT may identify patients at risk of severe sting reactions and thus help guide recommendations for venom immunotherapy and improve the management of patients with venom allergy.

Funding

This work was funded by the Slovenian Research Agency through research grant P3-0360.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Hoffmann HJ, Santos AF, Mayorga C, Nopp A, Eberlein B, Ferrer M, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. Allergy Eur J Allergy Clin Immunol. 2015;70:1393-405.
2. Santos AF, Alpan O, Hoffmann HJ. Basophil activation test: Mechanisms and considerations for use in clinical trials and clinical practice. Allergy. 2021;76:2420-32.
3. Bonadonna P, Korosec P, Nalin F, Golden DBK. Venom Anaphylaxis: Decision Points for a More Aggressive Workup. J allergy Clin Immunol Pract. 2023;11:2024-31.
4. Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ. Hymenoptera venom allergy: taking the sting out of difficult cases. J Investig Allergol Clin Immunol. 2007;17:357-60.
5. Bokanovic D, Arzt-Gradwohl L, Schwarz I, Schrautzer C, Laipold K, Aberer W, et al. Possible utility of basophil activation test in dual honeybee and vespid sensitization. J allergy Clin Immunol Pract. 2020;8:392-4.e5.
6. Korosec P, Erzen R, Silar M, Bajrovic N, Kopac P, Kosnik M. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. Clin Exp Allergy. 2009;39:1730-7.
7. Kopač P, Custovic A, Zidarn M, Šilar M, Šelb J, Bajrović N, et al. Biomarkers of the Severity of Honeybee Sting Reactions and the Severity and Threshold of Systemic Adverse Events During Immunotherapy. J allergy Clin Immunol Pract. 2021;9:3157-63.e5.
8. Sturm GJ, Varga EM, Roberts G, Mosbech H, Bilò MB, Akdis CA, et al. EAACI guidelines on allergen immunotherapy: Hymenoptera venom allergy. Allergy. 2018;73:744-64.
9. Guerti K, Bridts CH, Stevens WJ, Ebo DG. Wasp venom-specific IgE: towards a new decision threshold? J Investig Allergol Clin Immunol. 2008;18:321-3.
10. Ebo DG, Bridts CH, Mertens CH, Sabato V. Principles, potential, and limitations of ex vivo basophil activation by flow cytometry in allergology: A narrative review. J Allergy Clin Immunol. 2021;147:1143-53.
11. Mukai K, Gaudenzio N, Gupta S, Vivanco N, Bendall SC, Maecker HT, et al. Assessing basophil activation by using flow cytometry and mass cytometry in blood stored 24 hours before analysis. J Allergy Clin Immunol. 2017;139:889-99.e11.
12. Bahri R, Custovic A, Korosec P, Tsoumani M, Barron M, Wu J, et al. Mast cell activation test in the diagnosis of allergic disease and anaphylaxis. 2018;142:485-96.e16.
13. Tam IYS, Lau HYA, Tam SY, Lee TH. Mast cell activation test using patient-derived mast cells exhibits distinct combinatorial phenotypic profiles among allergic patients. Allergy. 2020;75:1796-9.
14. Luo Y, Frischbutter S, Scheffel J, Siebenhaar F. Generation and Culture of Peripheral CD34+ Stem Cell-Derived Mast Cells (PSCMCs). Methods Mol Biol. 2020;2163:63-7.
15. Chirumbolo S, Bjørklund G, Vella A. Mast cell activation test versus basophil activation test and related competing issues. J Allergy Clin Immunol. 2018;142:1018-9.
16. Santos AF, Douiri A, Bécares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin Immunol. 2014;134:645-52.
17. Santos AF, Du Toit G, O'Rourke C, Becares N, Couto-Francisco N, Radulovic S, et al. Biomarkers of severity and threshold of allergic reactions during oral peanut challenges. J Allergy Clin Immunol. 2020;146:344-55.
18. Santos AF, Couto-Francico N, Bécares N, Kwok M, Bahnson HT, Lack G, et al. A novel human mast cell activation test for peanut allergy. J Allergy Clin Immunol. 2018;142:689-91.e9
19. Hemmings O, Du Toit G, Radulovic S, Lack G, Santos AF. Ara h 2 is the dominant peanut allergen despite similarities with Ara h 6. J Allergy Clin Immunol. 2020;146:621-30.e5.
20. Cop N, Ebo DG, Bridts CH, Elst J, Hagendorens MM, Mertens C, et al. Influence of IL-6, IL-33, and TNF-α on human mast cell activation: Lessons from single cell analysis by flow cytometry. Cytometry B Clin Cytom. 2018;94:405-11.
21. Elst J, van der Poorten MLM, Faber MA, Van Gasse AL, Garvey LH, Bridts CH, et al. Mast cell activation test in chlorhexidine allergy: a proof of concept. Br J Anaesth. 2020;125:970-5.
22. Elst J, Moonen N, van der Poorten MLM, Faber MA, Van Gasse AL, Garvey LH, et al. The passively sensitized mast cell activation test is a reliable diagnostic for chlorhexidine allergy. J Allergy Clin Immunol Pract. 2021;9:3826-8.e2.
23. Korošec P, Valenta R, Mittermann I, Čelesnik N, Eržen R, Zidarn M, et al. Low sensitivity of commercially available rApi m 1 for diagnosis of honeybee venom allergy. J Allergy Clin Immunol. 2011;128: 671-3.
24. Korošec P, Valenta R, Mittermann I, Čelesnik N, Šilar M, Zidarn M, et al. High sensitivity of CAP-FEIA rVes v 5 and rVes v 1 for diagnosis of Vespula venom allergy. J Allergy Clin Immunol. 2012;129:1406-8.
25. Gomez G, Jogie-Brahim S, Shima M, Schwartz LB. Omalizumab reverses the phenotypic and functional effects of IgE-enhanced Fc epsilonRI on human skin mast cells. J Immunol. 2007;179:1353-61.
26. Hjort C, Schiøtz PO, Ohlin M, Würtzen PA, Christensen LH, Hoffmann HJ. The number and affinity of productive IgE pairs determine allergen activation of mast cells. J Allergy Clin Immunol. 2017;140:1167-70.e2.
27. Joulia R, L'Faqihi FE, Valitutti S, Espinosa E. IL-33 fine tunes mast cell degranulation and chemokine production at the single-cell level. J Allergy Clin Immunol. 2017;140:497-509.e10.

28. Beck LA, Marcotte G V., MacGlashan D, Togias A, Saini S. Omalizumab-induced reductions in mast cell FcεRI expression and function. J Allergy Clin Immunol. 2004;114:527-30.
29. Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. J Allergy Clin Immunol. 2017;140:335-48.
30. Ruëff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase-a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol. 2009;124:1047-54.
31. Worm M, Francuzik W, Renaudin JM, Bilo MB, Cardona V, Scherer Hofmeier K, et al. Factors increasing the risk for a severe reaction in anaphylaxis: An analysis of data from The European Anaphylaxis Registry. Allergy. 2018;73:1322-30.

Manuscript received December 20, 2022; accepted for publication November 6, 2023.

Ana Koren

University Clinic of Respiratory and Allergic Diseases Golnik
Golnik 36, 4204 Golnik, Slovenia
E-mail: ana.koren@klinika-golnik.si