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Research Article

Endotyping Cellular and Humoral cross-reactivity among *Aedes spp* and *Dermatophagoides spp* in patients with Allergic Multimorbidity

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Abstract

Background: Sensitization to panallergens is associated with allergic multimorbidity and polysensitization. Mosquito bites may produce several allergic phenotypes related to specific or combined humoral and cellular hypersensitivity endotype mechanisms. Mosquito-derived debris in house dust and air also function as inhalant allergens and contain several allergens that cross-react with house dust mite allergens.
Study Design: We examined retrospectively the medical charts of two cohorts of patients clinically diagnosed with non–IgE-mediated multimorbidity allergic phenotypes related to inhalation of house dust and/or papular urticaria who were investigated by the Tube Titration of Precipitins (TTP) and the Leukocyte Adherence Inhibition Test (LAIT) simultaneously against *Aedes spp* and *Dermatophagoides spp* extracts.
Methodology: The registered results of the TTP and LAIT against Aedes spp and *Dermatophagoides spp* extracts were plotted in ranges using a cascade distribution chart to illustrate the variability of the results within the first cohort. The registered results of the Leukocyte Adherence Inhibition (LAI) percentage and precipitin titration were distributed in ranges using a cascade distribution chart to outline the variability of results. The correlation between the paired assays was calculated using Pearson's methodology and demonstrated by dispersion graphs.
Results: The paired t-test indicated no significant difference between *Aedes spp* and *Dermatophagoides spp* LAIT results (p-value = 0.72). Pearson's correlation indicated a significantly moderate positive relationship between *Aedes spp* and *Dermatophagoides spp* LAIT results: r(98) = 0.37, p-value < 0.001. The paired t-test indicated a non-significant difference between *Aedes spp* and *Dermatophagoides spp* TTP results (p-value = 0.08). Pearson's correlation indicated a non-significant relationship between TTP results of *Aedes spp* and *Dermatophagoides spp*; r(98) = 0.06 p-value = 0.55.
Conclusion: The preliminary results suggest that the TTP and LAIT may discriminate between diverse humoral and cellular immunoreactivity levels in patients with various allergic phenotypes, due to *Aedes spp* and *Dermatophagoides spp* hypersensitivity. There was a significant correlation between *Aedes spp* and *Dermatophagoides spp* cellular immunoreactivity, as evaluated by LAIT, but there was no correlation between the titration of antibodies, as evaluated by TTP.

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Abbreviations

LAI: Leukocyte Adherence Inhibition
LAIT: Leukocyte Adherence Inhibition Test
TTP: Tube Titration of Precipitins

1. Introduction

Severe reactions to mosquito bites (Skeeter syndrome) are common among young children, immunocompromised persons, and tourists visiting areas with indigenous mosquitoes to which they are not habituated [1].

Mosquito bites may produce several types of allergic phenotypes: A) immediate local reactions; B) delayed local reactions; C) regional reactions; D) long-distance reactions; E) anaphylactic reactions; F) scarring reactions [2]. These reactions are related to specific or combined humoral and cellular hypersensitivity mechanisms classified as types I, II, III, or IV by Gell and Coombs [3]. Recently, this classification has been expanded to seven types [4]. Allergic reactions to mosquito bites may involve IgE, IgG, and/or T-lymphocyte-mediated hypersensitivities to allergens in mosquito saliva [5].

The leading causes of respiratory allergies are environmental aeroallergens such as mites, pollens, and molds, which promote Th2-skewed immune dysregulation through oxidative stress, epithelial barrier dysfunction, and inflammation [6]. However, mosquito-derived debris in house dust and air also function as an inhalant allergen, inducing allergic respiratory symptoms in sensitized patients [7]. The whole-body extracts of Aedes species contain variants of tropomyosin which mediate IgE cross-reactivity with tropomyosins from house dust mites, shrimps, and nematodes [8]

Mosquito salivary preparations for skin, *ex vivo*, and in vitro tests are usually unavailable because obtaining mosquito saliva or salivary glands is laborious and time-consuming. Most allergists use the whole-body extract to perform diagnostic tests, so allergic reactions to mosquito bites are usually underdiagnosed. However, whole body mosquito extracts may be associated with respiratory allergies [9]. Some recombinant allergens from mosquito saliva have been elaborated to surpass the technical difficulties [10].

The Allergen Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) has recognized so far eleven allergens weighing from 23 to 68 KDa, identified from the Aedes aegypti, and four allergens, weighing from 27 to 34 KDa, from *Aedes albopictus* [11].

The Allergen Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) has recognized so far more than seventy allergens weighing from 12 to 177 KDa, identified from the *Dermatophagoides* species [12].

Tropomyosin is a phylogenetically conserved heat-stable alpha-helical coiled-coil dimeric protein found in vertebrates and invertebrates that interacts with actin, producing muscle contraction [13, 14]. Tropomyosin homologs are defined as the group 10 allergen (Der p 10; Der f 10 and Aed a 10) sharing amino acid sequence homology with several forms of tropomyosins that are involved in cross-reactivity with mites, mosquitoes, cockroaches, shrimps, snails, oysters, crabs, lobsters, squids, and other invertebrates [15, 16].

Tropomyosins are panallergens widely distributed throughout nature and may be found in different allergenic sources [17]. Sensitization to panallergens is a condition associated with allergic multimorbidity and polysensitization [18]. Allergic Multimorbidity is defined in patients with concomitant or consecutive allergic phenotypes with endotypes that may be IgE-mediated, partly IgE-mediated, or non-IgE-mediated [19–21].

Besides tropomyosins, several Aedes allergens also cross-react with arthropod proteins, and four of them were identified as odorant binding protein, mitochondrial cytochrome C, peptidyl-prolyl cis-trans isomerase, and a protein with a hypothetical magnesium ion binding function [22].

Humoral immunoreactivity against food allergens and aeroallergens has been classically evaluated by research on Precipitins [23–27]. Among the techniques for researching precipitating antibodies, the Tube Research of Precipitins (TTP) is the most straightforward to evaluate humoral immunoreactivity against suspected antigens [28–32].

The Leukocyte Adherence Inhibition Test (LAIT) and its similar assay, the Leukocyte Migration Inhibition Test (LMIT), have been used to differentiate Non–IgE-mediated immunoreactivity against microorganisms and aeroallergens [33–36]. The LAIT and the LMIT have also been used to differentiate Non–IgE-mediated immunoreactivity against food allergens [37–41].

Non–IgE-mediated cellular immunoreactivity against food allergens had also been reported by our group employing the LAIT [42–45]. Non–IgE-mediated cellular immunoreactivity against aeroallergens and microorganisms had also been reported by our group employing the LAIT [46–50]. The primary use of TTP is as a triage to evaluate non–IgE-mediated immunoreactivity against suspected allergens before performing more exhaustive *in vivo* provocation tests [51–54].

To evaluate the potential of the LAIT and TTP to endotype cross-reactive cellular and humoral non–IgE–mediated immunoreactivity against Aedes species and *Dermatophagoides* species (*D. pteronyssinus or D. farinae*), we retrospectively compiled the electronic medical charts of patients diagnosed with non–IgE–mediated allergic multimorbidity involving respiratory and dermatological symptoms (such as allergic rhinitis, allergic conjunctivitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, atopic dermatitis, and/or papular urticaria) who were investigated for immunoreactivity against these allergens simultaneously using one of these assays.

The present study was designed as a proof-of-concept, hypothesizing that LAIT and the TTP may demonstrate a correlation between cellular and/or humoral immunoreactivity against *Dermatophagoides* species and Aedes species in patients suffering from non–IgE–mediated Allergic Multimorbidity.

2. Materials and Methods

2.1. Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 07/2025), we reviewed the electronic chart of 11.000 outpatients who attended our facility from January 2018 to October 2025, selecting those diagnosed with allergic

multimorbidity who were evaluated simultaneously with LAIT or TTP against *Aedes spp* and *Dermatophagoides spp* extracts.

A cohort of 100 consecutive outside patients (TTP cohort) had been submitted to TTP with *Aedes spp* and *Dermatophagoides spp* for presenting non–IgE-mediated Allergic Multimorbidity as defined by the concomitant or consecutive presence of at least two allergic phenotypes such as allergic rhinitis, allergic conjunctivitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, atopic dermatitis, and/or papular urticaria. This cohort counted 29 males; mean age 34.3 years; SD 19.4 years; range 1 to 81 years; median 33 years; modes = 8; 28; 43 (each appeared 5 times); geometric mean = 26.9 years.

A cohort of 100 consecutive outside patients (LAIT cohort) had been submitted to LAIT with *Aedes spp* and *Dermatophagoides spp* extracts for presenting non–IgE-mediated Allergic Multimorbidity as defined by the concomitant or consecutive presence of at least two allergic phenotypes such as allergic rhinitis, allergic conjunctivitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, atopic dermatitis, and/or papular urticaria. This cohort counted 37 males; mean age 32.4 years; SD 23.6 years; range 1 to 86 years; median 29.5 years; mode = 5 (appeared six times); geometric mean = 21.2 years.

This study excluded patients receiving biological and/or systemic anti-inflammatory therapy. These procedures were offered to patients with clinical suspicion of chicken meat hypersensitivity who demonstrated a non-reactive or inconclusive skin test against *Aedes spp* and *Dermatophagoides spp* extracts [55].

2.2. Preparation of the Dermatophagoides extract

Alive samples of Dermatophagoides pteronyssinus and D. farinae were kindly provided by Prof. Dr. Jorge Martínez Quesada from the Faculty of Pharmacy at the University of the Basque Country, Spain. In a sterile environment, the culture medium was placed in 50 mL ThermoFisher® cell culture flasks (15 g per flask). These flasks were placed in an airtight container containing a saturated solution of distilled water and sodium chloride for at least 24 hours to maintain the humidity of the culture medium. They were brought to Brazil inside airtight bags containing cotton soaked in the saturated NaCl solution, thus maintaining humidity during the flight.

Preparation of the culture medium

The culture and extraction of *Dermatophagoides spp* proteins were performed at the BioAllergy laboratory (Álvaro de Carvalho, São Paulo, Brazil). The culture media (yeast extract and Tetramin®) were weighed on Thermomix's built-in scale (Vorwerk, TM6®), then crushed using the device's primary function for 30 seconds at maximum speed (10), and this step was repeated three times until a fine, homogenized powder was obtained. After the grinding step in the Thermomix®, all culture media were sieved through a 60-mesh sieve (250 μ m opening) to ensure standardization and granulometry of the medium. The sieved culture medium was then weighed (10 g) and placed in Erlenmeyer flasks with a cotton plug and gauze to be transferred to a sterilization and drying oven (Ethik Technology, 400D®), previously heated, where it remained for 1 hour at 110°*C*. After cooling, the contents of the Erlenmeyer flasks were transferred to 50 mL cell culture flasks using a previously sterilized plastic funnel, which was performed under unidirectional airflow. The flasks had been previously treated with ultraviolet light for 15 minutes to prevent contamination by microorganisms and other mite species. The 50 mL culture flasks containing only the culture medium were kept in airtight boxes with a saturated sodium chloride solution and distilled water for at least 48 hours to humidify them before inoculating the mites.

Production of Dermatophagoides extract

The samples of whole culture (mites and culture medium) underwent the grinding process to improve the exposure of allergens to the extraction buffer. Next, they were subjected to the defatting process (using ethyl ether), which favored the loss of non-protein molecules. The material was sonicated to facilitate disruption. The sample was solubilized in Phosphate-Buffered Saline (pH 7.2; Sodium Chloride (NaCl) – 81.9 g/L; Potassium Chloride (KCl) – 1.87 g/L; Dibasic Sodium Phosphate (Anhydrous Na_2HPO_4) – 14.19 g/L; Monobasic Potassium Phosphate (KH_2PO_4) - 2.38 g/L). The extraction was performed at a rate of 10 mL of buffer per gram of raw material (10%), using gentle magnetic stirring (1,000 rpm) for 4 hours at 4°*C*. The material was centrifuged at 5,000 rpm (3,020g for 30 minutes, and the supernatant was preserved. The sediment was resuspended under the same conditions as in the first step and kept under stirring for 2 hours. The centrifugation was then repeated, and the supernatants were mixed. The final extract was dialyzed against the buffer to eliminate low-molecular-weight proteins (<5,000 Da) for 24 hours, with three buffer changes and a 1:50 dilution ratio. The process was continued by centrifugation at 10,000 rpm (12,100 g) for 30 minutes at 4°*C*. Protein concentration was determined using the Bradford method [56]. The solution was diluted in an antigen dilution solution (NaCl, 10g; KH_2PO_4, 0.72g; Na_3PO_4, 2.86g; methylparaben, 1g; propylparaben, 0.5g; glycerin, 400mL; H_2O, 600mL) to an estimated protein concentration of 1 mg/mL and stored at 4°*C* in amber, opaque glass vials.

2.3. *Aedes spp* extract

Whole-body extracts of *Aedes spp* were prepared by crushing and grinding whole-body parts of frozen mosquitoes, after which allergen extraction was performed in PBS buffer (pH 7.4) for 24 hours and centrifuged at 8,820 g for 30 min. The supernatant was collected and used for the subsequent procedures. The protein quantification of the allergen extracts was done according to Bradford's protein-dye binding methodology. The solution was diluted in an antigen dilution solution to an estimated protein concentration of 1 mg/mL and stored at 4°*C* in amber, opaque glass vials. The *Aedes spp* extract solution was used to perform allergic skin tests, TTP, and LAIT. All relevant and mandatory laboratory health and safety measures have been complied with during the experiments.

2.4. LAIT: *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

LAIT: Procedure for allergen *ex vivo* challenging

We performed the LAIT as previously described [57–61]. Shortly, each donor's fresh plasma was divided into two parts and used in parallel *ex vivo* challenging tests with the allergen extracts and the unchallenged plasma (added with antigen dilution solution as a control). We

collected plasma with high leukocyte content (buffy coat) from the heparinized tube after one hour of sedimentation at 37°*C*. Then, we distributed aliquots of 100 μL into Eppendorf tubes with (or without) the challenging extract and kept them under agitation for 30 minutes (200 rpm at 37°*C*).

LAIT: Procedure for adherence assay

After incubation, the challenged plasma was allocated into a standard Neubauer hemocytometer counting chamber with a plain, non-metallic glass surface and left to stand for 2 hours at 37°*C* in a humidified atmosphere of the covered water bath, allowing leukocytes to adhere to the glass. Next, we counted the leukocytes, removed the coverslip, and washed the chamber by immersing it in a beaker containing phosphate-buffered saline (PBS) at 37°*C*. Then, we added a drop of PBS to the hemocytometer's chamber and placed a clean coverslip over it. The remaining cells were counted in the same squares as previously examined.

LAIT: Procedure for calculation

The percentage of Leukocyte Adherence (LA) of each assay was estimated as: (the number of leukocytes observed on the hemocytometry chamber after washing divided by the number of leukocytes observed on the hemocytometry chamber before washing) and multiplied by 100%. The Leukocyte Adherence Ratio (LAR) was estimated based on the ratio between the Leukocyte Adherence (LA) from the antigen-specific challenged plasma and the LA from the unchallenged control plasma: LAR = LA of the challenged sample divided by LA of the unchallenged control plasma, multiplied by 100%. To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from 100%. We utilized the LAI results for the cascade distribution chart and the statistical calculations, both performed with the assistance of the Microsoft Excel statistical package.

2.5. TTP: In vitro Investigation: Tube Titration of Precipitins

As previously reported, the semi-quantitative TTP was performed in a transparent vitreous tube array [62]. Shortly, the patient's blood was collected in a clot-activator collecting tube. After separation, the serum was centrifuged at 2,000 rpm for 10 minutes. Each allergen extract was allocated in sets of eleven glass tubes at progressively diluted serum dilutions. The progressive dilutions were combined with separated aliquots of 15 μL of the allergen extract with 250 μ L of the patient's serum, progressively diluted into physiological saline solution (NaCl 0,9%) in the dilution ratios of 1:1; 1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256; and 1:512. One tube was a blank control done with the water and serum to observe occasional spontaneous precipitation (Sia Test). After 24 hours, the tubes were examined, and the titers (the highest dilution factor that yields a positive reading) were recorded [63].

3. Results

As a retrospective survey, there was no research protocol; therefore, we report the incidental immune investigation as registered in the digital medical charts.

The TTP cascade graph for the *Aedes spp* extract showed a distribution concentrated on the higher dilutions Figure 1. The mean was estimated at 1:272; the median was 1:256; the standard deviation was estimated at 1:169; the modes were 1:256 and 1:512 (each appeared 30 times); the geometric mean was estimated at 1:213 see Figure 1.

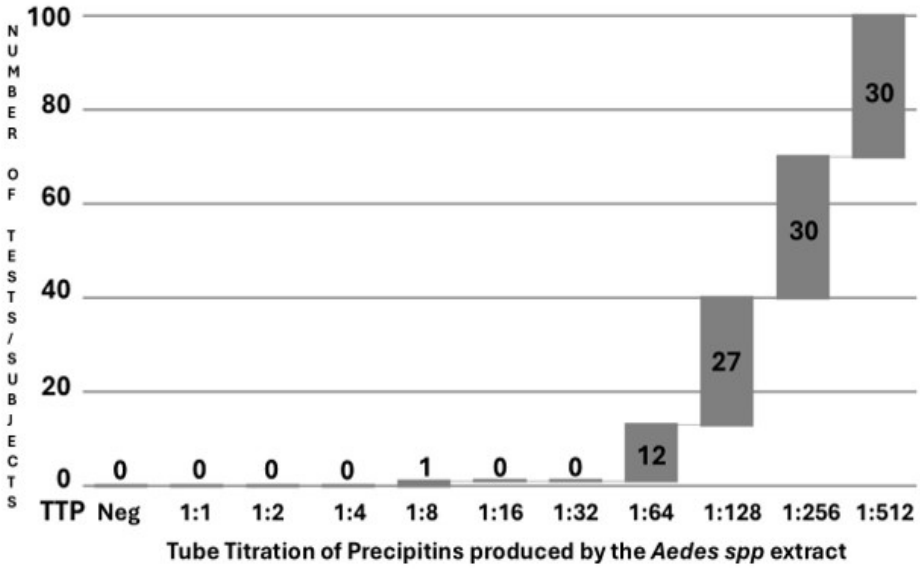

Figure 1: Cascade distribution chart of the Tube Titration of Precipitins (TTP on x-axis%) resulting from the *Aedes spp* extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

The TTP cascade graph for the *Dermatophagoides spp* extract showed a distribution concentrated on the higher dilutions Figure 2. The mean was estimated at 1:317; the median was 1:256; the standard deviation was estimated at 1:175; the mode was 512 (appeared 42 times); the geometric mean was estimated at 1:254 see Figure 2.

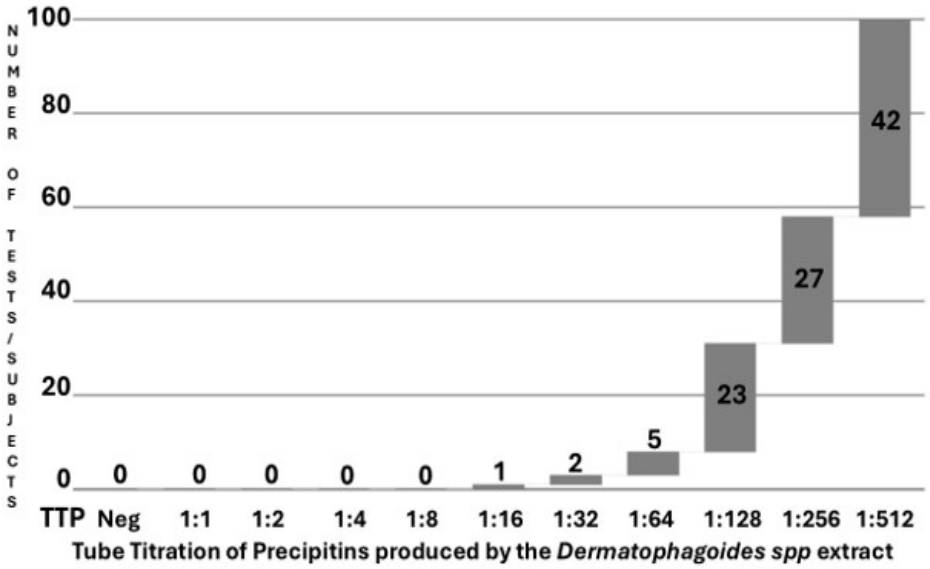

Figure 2: Cascade distribution chart of the Tube Titration of Precipitins (TTP on x-axis %) resulting from the *Dermatophagoides spp* extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

The paired t-test indicated a non-significant difference between *Aedes spp* and Dermatophagoides spp TTP results (p-value = 0.08). Pearson's correlation indicated a non-significant relationship between TTP results of *Aedes spp* and *Dermatophagoides spp*; r(98) = 0.06 p-value = 0.55 and see Figure 3.

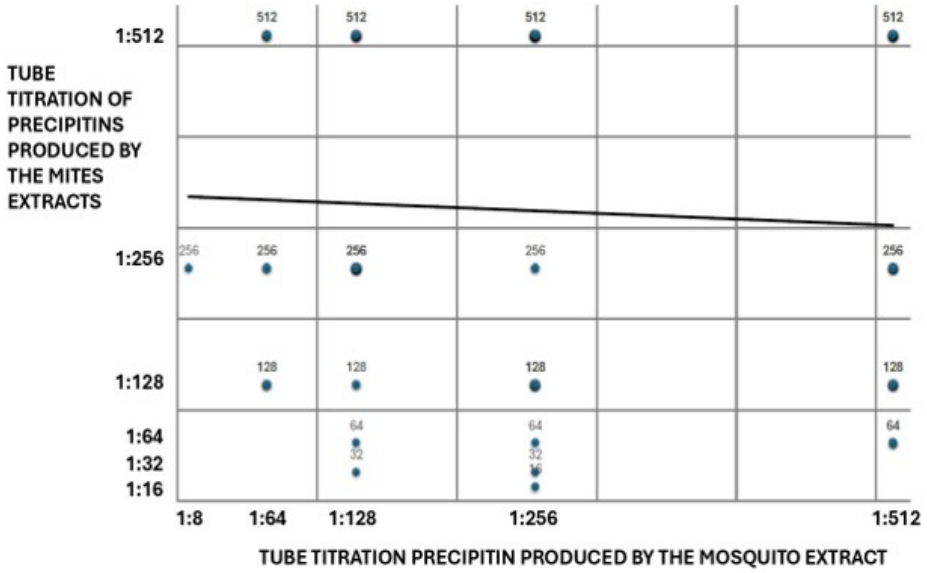

Figure 3: Dispersion chart of the Tube Titration of Precipitins results of the in vitro serum challenge against *Dermatophagoides spp* (mites) extract (x-axis %), plotted against the in vitro serum challenge against *Aedes spp* (mosquito) extract (y-axis %)

The LAIT cascade graph for the *Aedes spp* extract showed a wide distribution range of results. The LAI ranged from 0% to 98%. The mean was 59.6%; the median was 63.5%; the standard deviation was 26.1%; the mode was 0% (appeared six times). The cascade distribution demonstrates a wide range of LAI results. Most patients showed a strong immunoreactivity, which could reflect the participation of *Aedes spp* allergens in a Non–IgE-mediated hypersensitivity condition in these patients and see Figure 4.

The LAIT cascade graph for the *Dermatophagoides spp* extract showed a wide distribution range of results. The LAI ranged from 0% to 99%. The mean was 60.7%; the median was 65%; the standard deviation was 26.9%; the modes were 78% and 87% (each appeared six times). The cascade distribution demonstrates a wide range of LAI results. Most patients showed a strong immunoreactivity, which could reflect the participation of mite allergens in a Non–IgE-mediated hypersensitivity condition in these patients and see Figure 5.

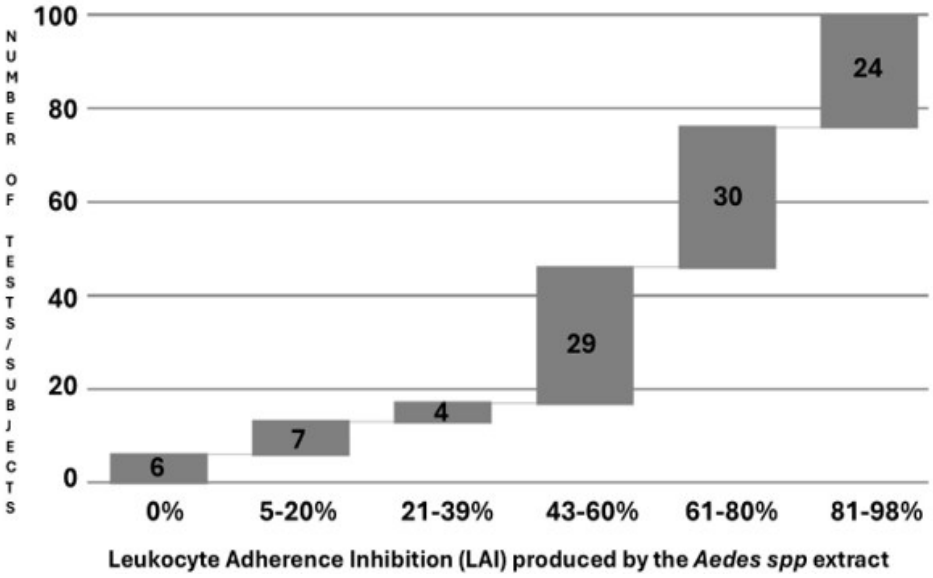

Figure 4: Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the *ex vivo* challenge test against *Aedes spp* extract monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

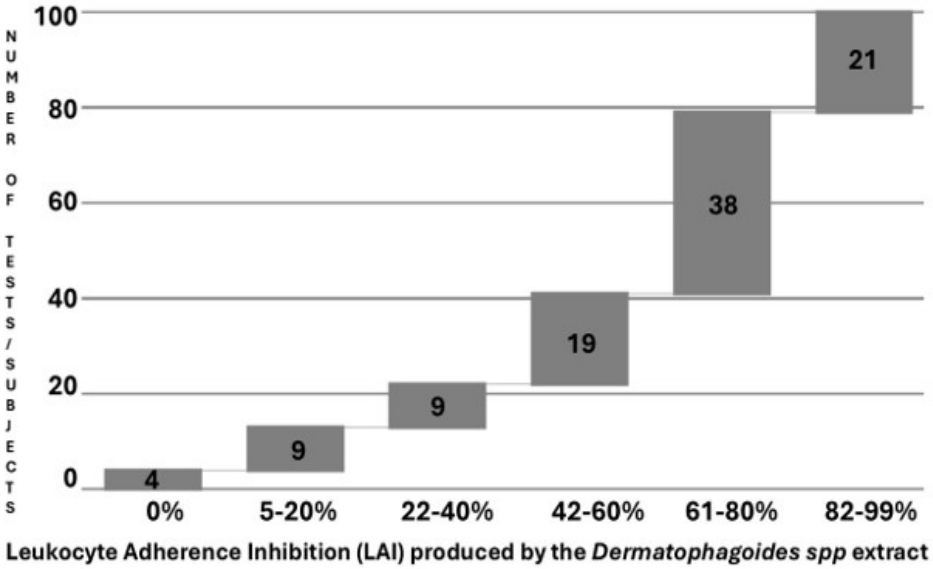

Figure 5: Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the *ex vivo* challenge test against *Dermatophagoides spp* extract monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

The paired t-test indicated no significant difference between *Aedes spp* and Dermatophagoides spp LAIT results (p-value = 0.72). Pearson's correlation indicated a significantly moderate positive relationship between *Aedes spp* and *Dermatophagoides spp* LAIT results: $r(98) = 0.37$, p-value < 0.001 and see Figure 6.

4. Discussion

Allergic diseases exhibit a complex pathogenesis involving multiple inflammatory cells, cytokines and mediators [64].

Reactions to mosquito bites were classified in five stages: Stage I presents just an immediate papular reaction; Stage II is characterized only by the delayed reaction; Stage III presents both immediate and delayed reactions; Stage IV presents just the immediate reaction; and Stage V does not present any reaction [65].

Local reactions to mosquito saliva components usually cause pruritus. Although the exact pathophysiology is not well-understood, immediate reactions are associated with histamine through IgE-mediated hypersensitivity reactions, while delayed reactions are associated with humoral (IgG) and cellular non-IgE-dependent hypersensitivity pathways [66].

Mosquito allergens may be an unsuspected cause of respiratory hypersensitivity conditions; similarly, mite allergens may be an unsuspected cause of dermatologic hypersensitivity conditions [67]. Isolated delayed cutaneous allergic reactions (Stage II) may not be easily associated with mosquito bites, due to the false layperson's idea that allergic reactions are always immediate. Multimorbidity patients, especially those with respiratory and cutaneous allergic conditions, may inadvertently suffer from delayed and non-IgE-mediated mosquito's

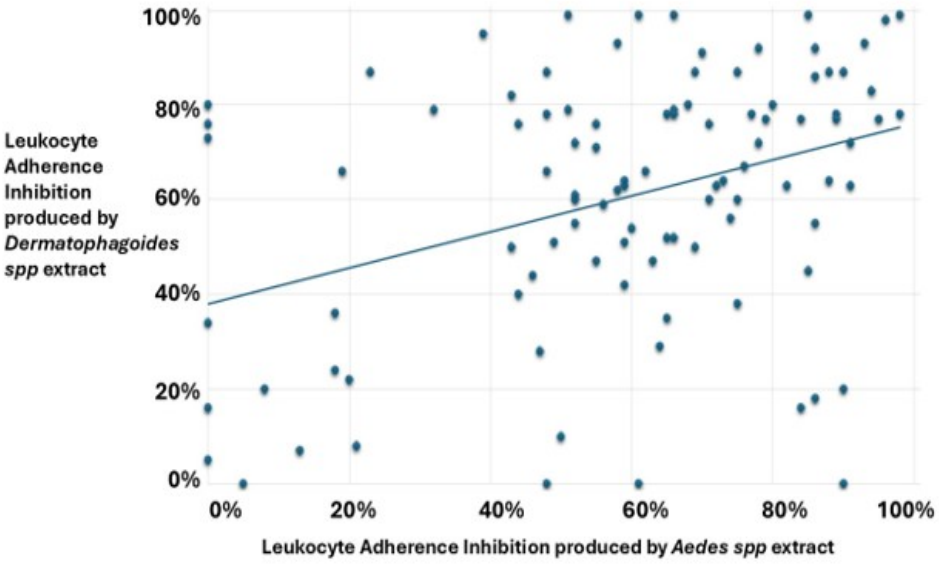

Figure 6: Dispersion chart of the Leukocyte Adherence Inhibition (LAI) results of the *ex vivo* challenge test against *Dermatophagoides spp* extract (x-axis %), plotted against the LAI results of the *ex vivo* challenge test against *Aedes spp* extract (y-axis %)

bites hypersensitivities, undiagnosed due to technical difficulties in establishing a laboratory confirmation.

Atypical hypersensitivity responses to mosquito bites (intense, bullous, necrotic skin reactions) may be associated with systemic immune diseases such as the Epstein-Barr virus infection, natural killer cell lymphoproliferative disorder, eosinophilic panniculitis (Wells' syndrome), chronic lymphocytic leukemia, mantle cell lymphoma, B cell lymphoblastic lymphoma, and others [68].

Atopic dermatitis is a Th2-skewed disorder that may present (or not) high serum IgE levels and blood eosinophilia, usually associated with hypersensitivity to mosquito bites [69].

Using nonspecific cellular inflammatory markers has proven helpful for evaluating allergic conditions, demonstrating cross-reacting reactions among allergens of distinct fonts, eliciting different allergic phenotypes [70–73]. In cross-sensitization, where several allergens manifest clinical symptoms, a strategic treatment consists of using group-specific multiallergen desensitization immunotherapy by the sublingual-swallow route, where the collateral effects are minimal [74–76].

Non-conventional diagnostic approaches, such as LAIT and TTP against allergen extracts, are proposed not to produce a molecular diagnosis of the responsible factor for the allergic phenotype but to obtain an idea of the intensity of the patient's immunoreactivity against the specific extract.

The present study assumes that LAIT and the TTP may differentiate diverse cellular and humoral immunoreactivity degrees against *Aedes spp* and *Dermatophagoides spp* allergens among non–IgE-mediated allergic multimorbidity patients. As the tests were performed simultaneously with the same collected venous sample with the two allergens, it was possible to calculate a correlation to quantify cross-reactivity between them.

The retrospective compilation of our data showed a large distribution of results when we ascertained the results of TTP and LAIT to explore humoral and cellular immunoreactivity against the studied allergens. These immunoassays did not precisely identify the mechanisms responsible for clinical condition. Instead, they provide evidence about cellular and humoral immunoreactivity distributed into an extensive spectral range that may suggest immune tolerance or hypersensitivity.

This preliminary survey demonstrated extensive results from the TTP, and the *ex vivo* challenge test monitored by LAIT against *Aedes spp* and *Dermatophagoides spp* allergens in two cohorts of non–IgE-mediated allergic multimorbidity patients. TTP and LAIT are complementary triage tests used at our facilities to select worthwhile antigens to proceed with more laborious *in vivo* provocation tests when the specific IgE is undetectable. None of our patients presented an exclusive reaction to these allergens. Every patient was simultaneously evaluated for several known allergens, demonstrating positive results for some of them. Our results suggest that when simultaneously in contact with both allergens, allergic patients may synergistically impair their symptoms through cross-reactivity hypersensitivity mechanisms.

Limitations

This study is a retrospective analysis of data collected over seven years since our facility began employing laboratory immune assays. There was no protocol research, and the subject's data was limited to the essentials available on our electronic sheets. Therefore, we could not establish a cross-comparison between positive and negative controls to validate the results. The number of subjects is appropriate for preliminary study; however, future studies must be more comprehensive. The lack of a research protocol implies the possibility of a bias produced by the point of view of the physician who indicated the exam (CEO) based on a clinical suspicion led purely by the anamnesis and physical examination. The study lost many of these patients to follow-up, so assuring the relationship between the immunoassay results and the patient's clinical outcome is not possible yet. Unfortunately, it was impossible to compare the two procedures with paired t-tests because they were taken from distinct groups of patients.

5. Conclusion

The preliminary results support that the TTP and LAIT performed with *Aedes spp* and *Dermatophagoides spp* allergens may discriminate diverse humoral and cellular immunoreactivity degrees in patients suffering from Allergic Multimorbidity. There is a significant association between *Aedes spp* and *Dermatophagoides spp* allergens immunoreactivity. LAIT and TTP are inexpensive, can be performed with minimum laboratory equipment, and can be incorporated into strategies to address health disparities in multimorbidity allergies [77]. As a preliminary report, the propaedeutic meaning of the presented results and the possibility of interferents must be established [78]. More studies focused on the quality-by-design approach with prospective and larger double-blind cohorts need to evaluate the potential contribution of LAIT and TTP for endotyping cellular and humoral immunoreactivity in patients suspected of hypersensitivity against *Aedes spp* and *Dermatophagoides spp* allergens [79].

Future Directions and Recommendations for Clinical Practice

The primary intended use of in vitro or *ex vivo* allergen challenge tests is to spare the patients from being submitted to unnecessary, exhaustive, and dangerous *in vivo* challenge tests. Exploring the humoral and the cellular arms of immune systems, the TTP and LAIT alone or combined may represent, in the near future, a tool for allergists to construct an etiologic diagnosis from their patients, as well as determine the endotypes (mechanisms) of hypersensitivity, in order to choose more convenient and personalized therapies for them. Adding data provided by TTP and LAIT may also contribute to streamlining biomedical research and improving tools such as Large Language Models, usually used by clinicians as a decision support system to enhance diagnostic accuracy [80].

Article Information

Consent: As a retrospective survey of results recorded in cognito, consent was given collectively by the institution's ethics committee following the principles of the Declaration of Helsinki [81].

Ethical Approvals: The authors have collected and preserved written ethical approval per international standards.

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References

[1] F. E. R. Simons and Z. Peng. Skeeter syndrome. *Journal of Allergy and Clinical Immunology*, 104:705–707, 1999.

[2] J. F. Cantillo, E. Fernández-Caldas, and L. Puerta. Immunological Aspects of the Immune Response Induced by Mosquito Allergens. *International Archives of Allergy and Immunology*, 165:271–282, 2015.

[3] P. G. H. Gell and R. R. A. Coombs. Classification of Allergic Reactions Responsible for Clinical Hypersensitivity and Disease. In P. G. H. Gell and R. R. A. Coombs, editors, *Clinical Aspects of Immunology*, pages 575–596. Blackwell Scientific Publications, Oxford, 1968.

[4] M. Jutel, I. Agache, M. Zemelka-Wiacek, M. Akdis, T. Chivato, and S. Del Giacco et al. Nomenclature of allergic diseases and hypersensitivity reactions: Adapted to modern needs An EAACI position paper. *Allergy*, 78:2851–2874, 2023.

[5] Z. Peng, M. Yang, and F. E. R. Simons. Immunologic Mechanisms in Mosquito Allergy: Correlation of Skin Reactions with Specific IgE and IgG Antibodies and Lymphocyte Proliferation Response to Mosquito Antigens. *Annals of Allergy, Asthma Immunology*, 77: 238–244, 1996.

[6] Z. Kanannejad, W. R. Taylor, M. Mohkam, and M. A. Ghatee. Urban Lifestyle and Climate-Driven Environmental Exposures: Immunological Consequences for Pediatric Respiratory Allergies. *Immun Inflamm Dis*, 13:e70248, 2025.

[7] M. A. Kausar, V. K. Vijayan, S. K. Bansal, B. K. Menon, M. Vermani, and M. K. Agarwal. Mosquitoes as sources of inhalant allergens: Clinicoimmunologic and biochemical studies. *Journal of Allergy and Clinical Immunology*, 120:1219–1221, 2007.

[8] J. F. Cantillo, L. Puerta, S. Lafosse-Marin, J. L. Subiza, L. Caraballo, and E. Fernández-Caldas. Identification and Characterization of IgE-Binding Tropomyosins in Aedes aegypti. *International Archives of Allergy and Immunology*, 170:46–56, 2016.

[9] Z. Peng and F. E. R. Simons. Advances in mosquito allergy. *Current opinion in allergy and clinical immunology*, 7, 2007.

[10] F. E. R. Simons and Z. Peng. Mosquito Allergy: Recombinant Mosquito Salivary Antigens for New Diagnostic Tests. *International Archives of Allergy and Immunology*, 124:403–405, 2012.

[11] A. N. Sub-Committee. *The official list of allergens Aedes ssp*. WHO/International Union of Immunological Societies, 2025.

[12] A. N. Sub-Committee. *The official list of allergens Dermatophagoides ssp*. WHO/International Union of Immunological Societies, 2025.

[13] K. Bailey. Tropomyosin: a new asymmetric protein component of muscle. *Nature*, 157:368, 1946.

[14] G. Reese, R. Ayuso, and S. B. Lehrer. Tropomyosin: an invertebrate pan-allergen. *International Archives of Allergy and Immunology*, 119:247–258, 1999.

[15] D. Martínez, L. Fang, C. Meza-Torres, G. Garavito, G. López-Lluch, and E. Egea. Toward Consensus Epitopes B and T of Tropomyosin Involved in Cross-Reactivity across Diverse Allergens: An In Silico Study. *Biomedicines*, 12, 2024.

[16] F. Papia, C. Bellia, and C. G. Uasuf. Tropomyosin: A panallergen that causes a worldwide allergic problem. *Allergy Asthma Proc*, 42: e145–e151, 2021.

[17] M. Hauser, A. Roulias, F. Ferreira, and M. Egger. Panallergens and their impact on the allergic patient. *Allergy Asthma Clin Immunol*, 6:1, 2010.

[18] O. E. McKenna, C. Asam, G. R. Araujo, A. Roulias, L. R. Goulart, and F. Ferreira. How relevant is panallergen sensitization in the development of allergies? *Pediatr Allergy Immunol*, 27:560–568, 2016.

[19] V. Siroux, N. Ballardini, M. Soler, C. Lupinek, A. Boudier, I. Pin, J. Just, R. Nadif, E. Melen, R. Valenta, M. Wickman, and J. Bousquet. Polysensitization and allergic multimorbidity: the extreme allergy phenotype from childhood to adulthood. *European Respiratory Journal*, 50:A3503, 2017.

[20] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, C. S. Miguel, J. L. S. Santana, R. P. S. Lima, N. S. Rocha, R. A. P. G. Santos, and E. S. Monteiro. Endotyping Cellular and Humoral Cross-reactivity among Canine, Feline, and Swine Allergens in Patients with Allergic Multimorbidity. *Asian Journal of Immunology*, 8:142–162, 2025.

[21] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, C. S. Miguel, J. L. S. Santana, R. P. S. Lima, N. S. Rocha, R. A. P. G. Santos, and E. S. Monteiro. Endotyping Cellular and Humoral Cross-Reactivity between Blomia tropicalis and Farfantepenaeus brasiliensis in Patients with Allergic Multimorbidity. *Asian Journal of Immunology*, 8:163–178, 2025.

[22] J. F. Cantillo, L. Puerta, S. Lafosse-Marin, J. L. Subiza, L. Caraballo, and E. Fernandez-Caldas. Allergens involved in the cross-reactivity of Aedes aegypti with other arthropods. *Ann Allergy Asthma Immunol*, 118:710–718, 2017.

[23] R. Augustin. Precipitins to grass pollen proteins. *Nature*, 172:307, 1953.

[24] R. Augustin, B. J. Hayward, and J. L. Longbottom. Isolation and physico-chemical characterization of reagins, blocking antibodies and precipitins to grass pollens. 1960.

[25] C. Cunningham-Rundles, W. E. Brandeis, R. A. Good, and N. K. Day. Milk precipitins, circulating immune complexes, and IgA deficiency. *Proceedings of the National Academy of Sciences of the United States of America*, 75:3387–3389, 1978.

[26] A. Ferguson and F. Carswell. Precipitins to dietary proteins in serum and upper intestinal secretions of coeliac children. *British medical journal*, 1:75–77, 1972.

[27] D. C. Heiner, J. W. Sears, and W. T. Kniker. Multiple Precipitins to Cow's Milk in Chronic Respiratory Disease: A Syndrome Including Poor Growth, Gastrointestinal Symptoms, Evidence of Allergy, Iron Deficiency Anemia, and Pulmonary Hemosiderosis. *American Journal of Diseases of Children*, 103:634–654, 1962.

[28] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. S. Santos, and R. P. S. Lima. Anti-Saccharomyces cerevisiae antibodies (ASCA) researched by tube precipitins are elevated in patients with dermatologic and gastrointestinal non—IgE-mediated hypersensitivity. *European Journal of Clinical Medicine*, 4:25–30, 2023.

[29] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. S. Santos, R. P. S. Lima, and Intrinsic Atopic Dermatitis. Titration of Precipitins in the Screening of Food Allergens for Prescription of Elimination Diets and Desensitization Strategies. *European Journal of Clinical Medicine*, 2:1–9, 2021.

[30] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, and R. P. S. Lima. Self-imposed food restriction and oral food challenges are correlated with precipitin's accuracy in the diagnosis of non-IgE-mediated food-related adulthood acute episodes of urticaria. *Journal of Allergy Therapy*, 12:1–8, 2021.

[31] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, and R. P. S. Lima. Endotyping Non-IgE-mediated Immunoreactivity to Polyethylene Glycol: Implications for Allergic Patients. *Asian Journal of Immunology*, 7:100–111, 2024.

[32] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, C. S. Miguel, R. A. P. G. Santos, J. L. S. Santana, and R. P. S. Lima. Endotyping Cellular and Humoral Immunoreactivity Against Tartrazine in Allergic Patients. *A Retrospective Study, Asian Journal of Immunology*, 7: 217–227, 2024.

[33] A. Fink, L. Heller, A. Eliraz, Z. Weisman, A. Miskin, M. Schlezinger, H. Bibi, and Z. Bentwich. Allergen-specific leukocyte adherence inhibition (LAI) assay: sensitivity, specificity and mechanism. *Immunology Letters*, 16:65–70, 1987.

[34] B. Kallen and O. Nilsson. Effect of encephalitogenic protein. PPD and tetanus toxoid on leukocyte migration in agarose. *A study of "cross-reactivity", Allergy*, 34:97–102, 1979.

[35] T. Kuratsuji. Studies on leukocyte adherence inhibition test. Part II, Clinical applications of LAI test to detect delayed type hypersensitivity in infants and children. *Keio Journal of Medicine*, 30:65–69, 1981.

[36] D. M. P. Thomson. *Assessment of immune status by the leukocyte adherence inhibition test*. Academic Press, New York, 1982.

[37] R. A. Allardyce and D. J. Shearman. Leukocyte reactivity to alpha-gliadin in dermatitis herpetiformis and adult coeliac disease. *Int Arch Allergy Appl Immunol*, 48:395–400, 1975.

[38] M. George and J. H. Vaughan. In vitro cell migration as a model for delayed hypersensitivity. In *Proceedings of the Society for Experimental Biology and Medicine*, pages 514–521, N. Y.), 111, 1962. Society for Experimental Biology and Medicine (New York.

[39] A. Ashkenazi, S. Levin, D. Idar, A. Or, I. Rosenberg, and Z. T. Handzel. In Vitro Cell-Mediated Immunologic Assay for Cow's Milk Allergy. *Pediatrics*, 66:399–402, 1980.

[40] H. L. Butler, W. J. Byrne, D. J. Marmer, A. R. Euler, and R. W. Steele. Depressed Neutrophil Chemotaxis in Infants with Cow's Milk and/or Soy Protein Intolerance. *Pediatrics*, 67:264–268, 1981.

[41] N. Papageorgiou, T. H. Lee, T. Nagakura, O. Cromwell, D. G. Wraith, and A. B. Kay. Neutrophil chemotactic activity in milk-induced asthma. *J Allergy Clin Immunol*, 72:75–82, 1983.

[42] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. S. Santos, and R. P. S. Lima. Contribution of the Leukocyte Adherence Inhibition Test to the Evaluation of Cellular Immunoreactivity against Latex Extracts for Non—IgE-Mediated Latex-Fruit-Pollen Syndrome in Allergic Candidates to Exclusion Diets and Allergic Desensitization. *European Journal of Clinical Medicine*, 3: 11–17, 2022.

[43] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. S. Santos, and R. P. S. Lima. Contribution of the Leukocyte Adherence Inhibition Test for the evaluation of immunoreactivity against gluten extracts in non—IgE-mediated / non-autoimmune Gluten-Related Disorders. *European Journal of Clinical Medicine*, 3:1–7, 2022.

[44] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. S. Santos, and R. P. S. Lima. Leukocyte Adherence Inhibition Test to the assessment of Immunoreactivity Against Cow's Milk Proteins in Non-IgE-Mediated Gastrointestinal Food Allergy. *European Journal of Clinical Medicine*, 3:38–43, 2022.

[45] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, and R. P. S. Lima. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non–IgE-mediated Immunoreactivity against Saccharomyces cerevisiae. *Asian Journal of Immunology*, 6:234–241, 2023.

[46] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, R. P. S. Lima, and E. S. Monteiro. Evaluating Non-IgE-mediated Allergens' Immunoreactivity in Patients with "Intrinsic" Persistent Rhinitis with Help of the Leukocyte Adherence Inhibition Test. *European Journal of Medical and Health Sciences*, 5:17–22, 2023.

[47] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. S. Santos, R. P. S. Lima, and E. S. Monteiro. Evaluating Non-IgE-Mediated Allergens' Immunoreactivity in Patients Formerly Classified as "Intrinsic" Asthmatics with Help of the Leukocyte Adherence Inhibition Test. *European Journal of Clinical Medicine*, 4:1–7, 2023.

[48] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, R. P. S. Lima, and E. S. Monteiro. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non–IgE-mediated Immunoreactivity against Alternaria alternata. *Asian Journal of Immunology*, 6:243–251, 2023.

[49] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, R. P. S. Lima, and E. S. Monteiro. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Non–IgE-mediated Immunoreactivity against Candida albicans in Patients with Atopic Dermatitis. *Asian Journal of Immunology*, 6:268–276, 2023.

[50] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, R. P. S. Lima, and E. S. Monteiro. Contribution of the Leukocyte Adherence Inhibition Test in Diagnosing Non–IgE-Mediated Immunoreactivity against Aspergillus fumigatus in Patients with Allergic Rhinitis and Asthma. *Asian Journal of Immunology*, 7:12–20, 2024.

[51] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, C. S. Miguel, R. A. P. G. Santos, J. L. S. Santana, and R. P. S. Lima. Endotyping Cellular and Humoral Immunoreactivity against Allium spices and Sulfites preservatives in Allergic Patients. A Retrospective Study. *Asian Journal of Immunology*, 7:185–200, 2024.

[52] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, and R. P. S. Lima. Endotyping Cellular and Humoral Immunoreactivity against Aluminum in Allergic Patients. *A Retrospective Study, Asian Journal of Immunology*, 7:149–158, 2024.

[53] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, R. A. P. G. Santos, R. P. S. Lima, and E. S. Monteiro. Endotyping Non-IgE-Mediated Immunoreactivity to Dermatophagoides farinae: Implications for Allergic Patients. *Asian Journal of Immunology*, 7:90–99, 2024.

[54] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, C. S. Miguel, R. A. P. G. Santos, and N. S. Rocha. Et-al, Endotyping Cellular and Humoral Immunoreactivity against Pollen and Citrus Fruits in Patients with Non–IgE-mediated Rhinoconjunctivitis. *Asian Journal of Immunology*, 8:8–25, 2025.

[55] C. E. Olivier, D. G. P. Argentão, R. A. P. G. Santos, M. D. Silva, R. P. S. Lima, and R. L. Zollner. Skin scrape test: an inexpensive and painless skin test for recognition of immediate hypersensitivity in children and adults. *The Open Allergy Journal*, 6:9–17, 2013.

[56] M. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemical*, 72:248–254, 1976.

[57] C. E. Olivier, R. P. S. Lima, D. G. Pinto, R. A. P. G. Santos, G. K. M. Silva, S. L. S. Lorena, M. B. Villas-Boas, F. M. Netto, and R. L. Zollner. In search of a tolerance-induction strategy for cow's milk allergies: significant reduction of beta-lactoglobulin allergenicity via transglutaminase/cysteine polymerization. *Clinics*, 67:1171–1179, 2012.

[58] C. E. Olivier, R. A. P. G. Santos, R. P. S. Lima, D. G. P. Argentão, G. K. M. Silva, and M. D. Silva. A Novel Utility for an Old Method: The Leukocyte Adherence Inhibition Test Is an Easy Way to Detect the Immunoreactive Interference of the Collection Tube Anticoagulant on Cellular Immunoassays. *Journal of Cell Adhesion*, pages 1–6, 2014. Article ID 860427.

[59] C. E. Olivier, R. P. d. S. Lima, D. G. Pinto, and R. A. P. G. d. Santos. The Plasma Preincubation with Papain Before the Assay Suggests that a Gell and Coombs Type II Reaction is Been Demonstrated by the Leukocyte Adherence Inhibition Test. *Biomedical Journal of Scientific Technical Research*, 36:28647–28655, 2021.

[60] C. E. Olivier, D. G. Pinto, R. P. S. Lima, M. D. d. Silva, R. A. P. G. Santos, A. P. M. Teixeira, and P. U. Simioni. Assessment of Immunoreactivity against Therapeutic Options Employing the Leukocyte Adherence Inhibition Test as a Tool for Precision Medicine. *European Journal of Clinical Medicine*, 2:40–45, 2021.

[61] C. E. Olivier, D. G. Pinto, R. A. P. G. Santos, and R. P. S. Lima. Dextran's interference over the Leukocyte Adherence Inhibition Test. *Academia Letter*, 2021. Article 3792.

[62] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, C. S. Miguel, R. A. P. G. Santos, J. L. S. Santana, and R. P. S. Lima. Cellular and Humoral Immunoreactivity against Hen's Egg White: Relevance in Allergic Patients. *Asian Journal of Immunology*, 7:274–284, 2024.

[63] C. A. Williams and M. W. Chase. Precipitation Reactions, Reactions of Antibodies with Soluble Antigens. *Academic*, Press1971: 1–102.

[64] Z. M. S. R. Network Pharmacology Combined with Animal Experiments Elucidates the Mechanism of Effect of Xin'an Formula in Treating Allergic Rhinitis. *Journal of Inflammation Research*, 18:15357–15378, 2025.

[65] K. Mellanby. Man's Reaction to Mosquito Bites. *Nature*, 158:554–554, 1946.

[66] A. Vander Does, A. Labib, and G. Yosipovitch. Update on mosquito bite reaction: Itch and hypersensitivity, pathophysiology, prevention, and treatment. *Front Immunol*, 13, 2022. Article 1024559.

[67] R. S. Bumbacea, S. L. Corcea, S. Ali, L. C. Dinica, I. S. Fanfaret, and D. Boda. Mite allergy and atopic dermatitis: Is there a clear link? *Review), Experimental and therapeutic medicine*, 20:3554–3560, 2020.

[68] K. Tatsuno, T. Fujiyama, H. Matsuoka, T. Shimauchi, T. Ito, and Y. Tokura. Clinical categories of exaggerated skin reactions to mosquito bites and their pathophysiology. *J Dermatol Sci*, 82:145–152, 2016.

[69] C. E. Olivier, D. G. Pinto, A. P. M. Teixeira, J. L. S. Santana, G. S. Brito, R. A. P. G. Santos, R. P. S. Lima, and E. S. Monteiro. Papular Urticaria: Insights into Non—IgE-mediated Hypersensitivity Reactions Produced by Stings of Culex spp and Aedes spp in Patients with Intrinsic Atopic Dermatitis. *Asian Journal of Immunology*, 6:139–147, 2023.

[70] H. Shen, P. Zhao, L. Shang, and S. Kuai. The association of peripheral blood inflammatory markers with the distribution patterns of IgE in children with allergen sensitization. *BMC Immunology*, 26:75, 2025.

[71] J. Zhang, Y. Tang, D. Yang, and J. Yu. Investigating allergen-specific IgE distribution and correlations in chronic urticaria: a retrospective study in Shanghai. *China, European Journal of Medical Research*, 30:182, 2025.

[72] J. Čelakovská, E. Čermákova, C. Andrýs, P. Boudkova, and J. Krejsek. Sensitization to latex and food allergens in atopic dermatitis patients according to ALEX2 Allergy Xplorer test. *Molecular Immunology*, 175:89–102, 2024.

[73] S. M. Hasnain, A. Alqassim, and A. Al-Frayl. Diagnosis of allergy: IgE mediated cross-reactions amongst selected asthma elicitors. *Journal of Disease and Global Health*, 10:111–122, 2017.

[74] C. E. Olivier, R. P. S. Lima, D. G. P. Argentão, M. D. d. Silva, R. A. P. G. Santos, M. Pensuti, and T. H. Piai de Morais. Group-specific Multi-allergen Sublingual/Swallow Immunotherapy Improves the Quality of Life of Polysensitized Children and Adults with Allergic Rhinitis. *Journal of Allergy Therapy*, 4:148, 2013. doi:110.4172/2155-6121.1000148.

[75] M. M. Khan, Allergic Disease. In M. M. Khan, editor, *Immunopharmacology*, pages 197–225. Springer International Publishing, Cham, 2016.

[76] C. E. Olivier. The use of allergoids and adjuvants in Allergen Immunotherapy. *Archives of Asthma Allergy and Immunology*, 1:40–60, 2017.

[77] A. Anagnostou, J. Wang, S. Chinthrajah, R. Gupta, C. M. Davis, C. Parrish, R. Lo, M. Groetch, L. Herbert, J. Shroba, S. Sansweet, M. Shaker, C. Rolling, J. Tam, and M. Greenhawt. Addressing health disparities in food allergy: A Position Statement of the AAAAI Prior Authorization Task Force. *Journal of Allergy and Clinical Immunology*, 155:53–61, 2025.

[78] S. Anouar, R. Hazim, and A. Brahim. Interferences in immunological assays: Causes, detection, and prevention. *Asian Journal of Immunology*, 7:71–78, 2024.

[79] L. Chiarentin, C. Gonçalves, C. Augusto, M. Miranda, C. Cardoso, and C. Vitorino. Drilling into "Quality by Design" Approach for Analytical Methods. *Critical Reviews in Analytical Chemistry*, pages 1–42, 2023.

[80] M. S. Abers and R. A. Mathias. Novel applications of large language models in clinical research. *Journal of Allergy and Clinical Immunology*, 155:813–814, 2025.

[81] WMA and World Medical. Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *Journal of the American Medical Association*, 310:2191–2194, 2013.