



Endotyping Cellular and Humoral Cross-Reactivity between *Blomia tropicalis* and *Farfantepenaeus brasiliensis* in Patients with Allergic Multimorbidity

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Authors' contributions

This work was carried out in collaboration among all authors. Author CEO conceptualized the study, did data curation, formal analysis, literature review, and wrote original draft of the manuscript. Authors DGP, APMT, CSM, NSR, JLSS and RPSL performed laboratory procedures. Author ESM cultured the *Blomia tropicalis* specimens and prepared their extract. Author RAPGS performed cutaneous tests. All authors read and approved the final manuscript

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ABSTRACT

Background: Panallergens, such as tropomyosin, produce cross-reactivity between indoor allergens and food allergens, including the house dust mite *Blomia tropicalis* and the Brazilian pink shrimp *Farfantepenaeus brasiliensis*, and are responsible for the clinical symptoms present in patients with allergic multimorbidity phenotypes.

Aim: To evaluate the potential of the Tube Titration of Precipitins (TTP) and the Leukocyte Adherence Inhibition Test (LAIT) to discriminate and correlate cellular and humoral immunoreactivity against protein extracts of *B. tropicalis* and *F. brasiliensis* in patients with non-IgE-mediated allergic multimorbidity phenotypes.

Study Design: We examined retrospectively the medical charts of two cohorts of patients clinically diagnosed with non-IgE-mediated allergic multimorbidity phenotypes related to inhalation or contact with house dust and/or consumption of shrimps, who were concomitantly investigated for these allergens with the help of TTP or LAIT.

Methodology: The registered results of the TTP and LAIT against protein extracts of *B. tropicalis* and *F. brasiliensis* were distributed in ranges through cascade distribution charts. The correlation between the paired assays was calculated using Pearson's methodology and demonstrated by dispersion graphs.

Results: The TTP for the *B. tropicalis* extract showed a distribution concentrated on the higher dilutions. The mean was 1:353; the median was 1:256; the standard deviation was 1:163; the mode was 1:512 (appeared 49 times). The TTP for the *F. brasiliensis* extract showed a distribution concentrated on the higher dilutions. The mean was 1:380; the median was 1:512; the standard deviation was 1:157; the mode was 1:512 (which appeared 57 times). The LAIT for the *B. tropicalis* extract showed a wide distribution range of results. The LAI ranged from 0% to 100%. The mean was 49.8%; the median was 51.5%; the standard deviation was 30.0%; the mode was 0% (appeared eleven times). The LAIT for the *F. brasiliensis* extract showed a wide distribution range of results. The LAI ranged from 0% to 100%. The mean was 55%; the median was 56%; the standard deviation was 28.8%; the mode was 0% (appeared five times). There was no significant correlation between *B. tropicalis* and *F. brasiliensis* when analyzed by TTP or LAIT results.

Conclusion: Our preliminary results suggest that the TTP and LAIT may reveal humoral and cellular immunoreactivity in patients with allergic multimorbidity phenotypes using protein extracts of *B. tropicalis* and *F. brasiliensis*.

Keywords: *Blomia tropicalis*; *Farfantepenaeus brasiliensis*; hypersensitivity; house dust mite; leukocyte adherence inhibition test; Non-IgE-mediated immunoreactivity; shrimp; precipitins.

ABBREVIATIONS

LAI : Leukocyte Adherence Inhibition

LAIT : Leukocyte Adherence Inhibition Test

TTP : Tube Titration of Precipitins

1. INTRODUCTION

Blomia tropicalis is an arthropod (phylum) of the subphylum Chelicerata; class Arachnida; subclass Acari; superorder Acariformes; order Sarcoptiformes; suborder Astigmata; superfamily Glycyphagoidea; family Echimyopodidae (Schoch et al. 2020a).

Farfantepenaeus brasiliensis (*Penaeus brasiliensis*) is an arthropod (phylum) of the

subphylum Crustacea; class Malacostraca; order Decapoda; suborder Dendrobranchiata; superfamily Penaeoidea; family Penaeidae (Schoch et al. 2020b).

The first suspicion and evidence that house dust mites (HDM), from the genus *Dermatophagoides*, were causing respiratory allergies were publicized in the mid-1960s (Voorhorst et al., 1964; Fain, 1966). The allergenicity of HDM from the *Blomia* genus and its cross-reactivity with other HDM species were studied using *in vitro* neutralization of skin-sensitizing antibodies, as described in Japan in the late 1960s (Miyamoto et al. 1969). The species *Blomia tropicalis* was described in the early 1970s as a dust and storage mite found in tropical and subtropical regions (van Bronswijk et al., 1973). Soon,

several reports on the allergenicity of *Blomia tropicalis* in Western tropical countries were also published (Fernández-Caldas et al., 1990; Fernández-Caldas et al., 1993; Chew et al., 1999; Baqueiro et al., 2006).

Almost six decades after the discovery of their causality in respiratory allergies, several reports have indicated that food contamination with HDM, including *Blomia tropicalis*, also produces urticaria and anaphylaxis and is associated with hypersensitivity to non-steroidal anti-inflammatory drugs (Sánchez-Borges et al., 2013; Barrera-de-Pino, Murgas, and Miranda, 2012). After that, it was noticed that the HDM, including *Blomia tropicalis*, also produced Atopic Dermatitis (Emran et al. 2019).

The Allergen Nomenclature Sub-Committee of the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) has recognized twenty-six allergens to date, ranging in molecular weight from 7 to 110 kDa, which have been identified in *Blomia tropicalis* (Sub-Committee, 2025a; Chua et al., 2007).

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 (Bailey 1946) (Reese et al., 1999). Tropomyosin homologs are defined as the group 10 HDM allergens, and Blo t 10 is the *Blomia tropicalis* tropomyosin, sharing amino acid sequence homology with several forms of tropomyosins that are involved in cross-reactivity with mites, cockroaches, shrimps, snails, oysters, crabs, lobsters, squids, and other invertebrates (Martínez et al. 2024, Papia et al., 2021).

Tropomyosin is the major allergen of crustaceans from the genus *Penaeus* (Daul et al. 1994). Tropomyosin is considered a panallergen, a sensitizing protein acquired from different fonts, responsible for (apparently) unrelated clinical allergic reactions such as respiratory symptoms (elicited by inhalation of house dust mites) or systemic symptoms (elicited by the ingestion of edible invertebrates such as shrimps) (Wong et al., 2016, Yang et al. 2010).

Farfantepenaeus brasiliensis (pink shrimp) is the most exploited shrimp species along the Brazilian Coast (Carvalho et al., 2019). Shrimp's tropomyosin is a 34- to 38-kDa heat-stable allergen that can provoke IgE-mediated

immediate-type hypersensitivity reactions after ingestion (Shanti et al., 1993). Tropomyosin is considered the major allergen of shrimp and is usually classified as a group 1 allergen, following the group 1 allergens (Crac c 1, Exo m 1, Lit v 1, Met e 1, Pan b 1, Pen a 1, Pen i 1, Pen m 1) (Sub-Committee, 2025b). Nevertheless, uncharacterized, the *Farfantepenaeus brasiliensis* tropomyosin should receive the allergen nomenclature Far b 1). Besides the common major allergen, tropomyosin, several species-specific shrimp allergens may also participate in allergic reactions (Morgan et al. 1989).

Several symptoms have been attributed to shrimp allergy (urticaria, angioedema, chest tightness, shortness of breath, nausea, vomiting, diarrhea, fainting with documented hypotension, chills, fever, abdominal discomfort, abdominal pain, and finger stiffness) (Waring et al. 1985).

Allergic Multimorbidity is defined as the presence of concomitant or consecutive allergic phenotypes that may be IgE-mediated, partly IgE-mediated, or non-IgE-mediated (Bousquet et al., 2015; Bousquet et al., 2025). Besides the IgE-mediated hypersensitivity mechanism, several types of hypersensitivity mechanisms are associated with allergic phenotypes (Jutel et al., 2023). The humoral-dependent non-IgE-mediated allergic phenotypes may be evaluated by the research of Precipitins (Augustin 1953; Augustin et al., 1960; Cunningham-Rundles et al. 1978; Ferguson & Carswell 1972; Heiner et al., 1962). The Tube Titration of Precipitins (TTP) is routinely used at our facilities to evaluate humoral immunoreactivity against suspected allergens as a triage test prior to the exhaustive *in vivo* provocation tests, which define hypersensitivities diseases (Olivier et al. 2023e, Olivier et al. 2021e, Olivier et al. 2021d, Olivier et al. 2024e, Olivier et al. 2024c).

The Leukocyte Adherence Inhibition Test (LAIT) and its similar assay, the Leukocyte Migration Inhibition Test (LMIT), have traditionally been used to differentiate non-IgE-mediated immunoreactivity against microorganisms and aeroallergens (Fink et al. 1987, Kallen & Nilsson 1979, Kuratsuji 1981, Thomson, 1982). The LAIT and the LMIT have also been classically used to differentiate non-IgE-mediated immunoreactivity against food allergens (Allardyce & Shearman 1975, George & Vaughan 1962, Ashkenazi et al. 1980, Butler et al. 1981, Papageorgiou et al. 1983).

Non-IgE-mediated cellular immunoreactivity against food allergens had also been reported by our group using LAIT (Olivier et al. 2022b, Olivier et al. 2022a, Olivier et al. 2022c, Olivier et al. 2023a). Non-IgE-mediated cellular immunoreactivity against aeroallergens and microorganisms had also been reported by our group with the help of the LAIT (Olivier et al. 2023d, Olivier et al. 2023f, Olivier et al. 2023b, Olivier et al. 2023c, Olivier et al. 2024f). We also routinely employ the LAIT and TTP to endotype cellular and humoral non-IgE-mediated immunoreactivity against allergens already proved to produce allergic symptoms by *in vivo* provocation tests (Olivier et al. 2024b, Olivier et al. 2024d, Olivier et al. 2024g, Olivier et al. 2025)

To evaluate the potential of the LAIT and TTP to endotyping non-IgE-mediated cellular and humoral immunoreactivity against *Blomia tropicalis* and *Farfantepenaeus brasiliensis* extract, we retrospectively compiled the electronic medical charts of patients diagnosed with non-IgE-mediated Allergic Multimorbidity who were investigated for immunoreactivity simultaneously by one of these assays.

The present study provides proof of concept that hypothesizes LAIT and the TTP may demonstrate cellular and/or humoral immunoreactivity against *Blomia tropicalis* and *Farfantepenaeus brasiliensis* proteins in patients suffering from non-IgE-mediated Allergic Multimorbidity. This paper is a retrospective study of the results of the immunoassays, so we do not have access to the nutritional background or the genetic constitution of the patients. However, we are planning to address these issues in further prospective studies.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 04/2025), we reviewed the electronic chart of 10,500 outpatients who attended our facility from January 2018 to June 2025 selecting patients diagnosed with allergic multimorbidity who were evaluated simultaneously with LAIT or TTP against *Blomia tropicalis* or *Farfantepenaeus brasiliensis* extracts.

A cohort of 100 consecutive outside patients (TTP cohort) had been submitted to TTP with

Blomia tropicalis extract and *Farfantepenaeus brasiliensis* extract for presenting non-IgE-mediated Allergic Multimorbidity as defined by the concomitant or consecutive presence of at least two allergic phenotypes (allergic rhinoconjunctivitis, allergic bronchitis, atopic dermatitis, urticaria, gastrointestinal hypersensitivity, and/or anaphylaxis). This cohort counted 42 males; mean age 41.2 years; SD 18.4 years; range 10 to 82 years; median 39 years; mode = 36 (appeared five times); geometric mean = 36.5 years.

A cohort of 100 consecutive outside patients (LAIT cohort) had been submitted to TTP with *Blomia tropicalis* extract and shrimp extract for presenting non-IgE-mediated Allergic Multimorbidity as defined by the concomitant or consecutive presence of at least two allergic phenotypes (allergic rhinoconjunctivitis, allergic bronchitis, atopic dermatitis, urticaria, gastrointestinal hypersensitivity, and/or anaphylaxis). This cohort counted 30 males; mean age 36.4 years; SD 23.5 years; range 2 to 91 years; median 35 years; modes = 5 (appeared five times); geometric mean = 26.4 years.

This study did not include patients under biological and/or systemic anti-inflammatory therapy. These procedures were offered to patients with allergen multimorbidity related to house dust and/or shrimp consumption who had undetectable specific IgE against these suspected agents and demonstrated non-reactive or inconclusive skin tests against *Blomia tropicalis* and *Farfantepenaeus brasiliensis* extracts (Olivier et al., 2013).

2.2 Preparation of the *Blomia tropicalis* Extract

2.2.1 Origen of the *Blomia tropicalis* strain

The specimens of *Blomia tropicalis* were kindly provided by Prof. Dr. Jorge Martínez Quesada from the Faculty of Pharmacy at the University of the Basque Country, Spain. The culture medium was placed in 50 mL ThermoFisher® cell culture flasks (15 g per flask) in a sterile environment. 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.

2.2.2. Preparation of the culture medium

The culture and extraction of *Blomia tropicalis* 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 µm 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. The population peak (71,000 mites/g of culture) was reached after 90 days of culture.

2.2.3 Production of *Blomia tropicalis* allergenic 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 Na₂HPO₄) – 14.19 g/L; Monobasic Potassium Phosphate (KH₂PO₄) - 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 (Bradford, 1976). The solution was diluted in an antigen dilution solution (NaCl, 10g; KH₂PO₄, 0.72g; Na₃PO₄, 2.86g; methylparaben, 1g; propylparaben, 0.5g; glycerin, 400mL; H₂O, 600mL) to an estimated protein concentration of 1 mg/mL and stored at 4 °C in amber, opaque glass vials.

2.3 Shrimp Extract

Farfantepenaeus brasiliensis was acquired from the local market and identified based on its morphological characteristics (Silveira et al., 2022). The sample was grounded for 48 hours at 4 °C with a Coca-based extractor solution added to cover the amount of antigen. The sample was centrifuged (4,500 rpm for 10 min) and filtered. The protein concentration was estimated spectrophotometrically and diluted to 1 mg/mL in antigen dilution solution to perform allergic skin tests, TTP, and LAIT.

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

2.4.1 LAIT: procedure for allergen *Ex Vivo* challenging

We performed the LAIT as previously described (Olivier et al. 2012, Olivier et al. 2014, Olivier et al. 2021a, Olivier et al. 2021b, Olivier et al. 2021c). Shortly, each donor's fresh plasma was divided into two parts and used in parallel *ex vivo* challenging tests with the allergen extract 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).

2.4.2 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 allocated a clean coverslip over it. The remaining cells were counted in the same squares as previously examined.

2.4.3 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: $LAR = LA \text{ of the challenged sample} / LA \text{ of the unchallenged control plasma}$, 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 of which were performed using 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 (Olivier et al. 2024a). Shortly, the patient's blood was collected in a clot-activator collecting tube. After separation, the serum was centrifugated at 2,000 rpm for 10 minutes. Each allergen extract was allocated in sets of eleven glass tubes at progressively diluted serum concentrations. The progressive dilutions were combined with separated aliquots of 15 µL of the allergen extract with 250 µL 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, performed with 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 (Williams & Chase, 1971).

3. RESULTS

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

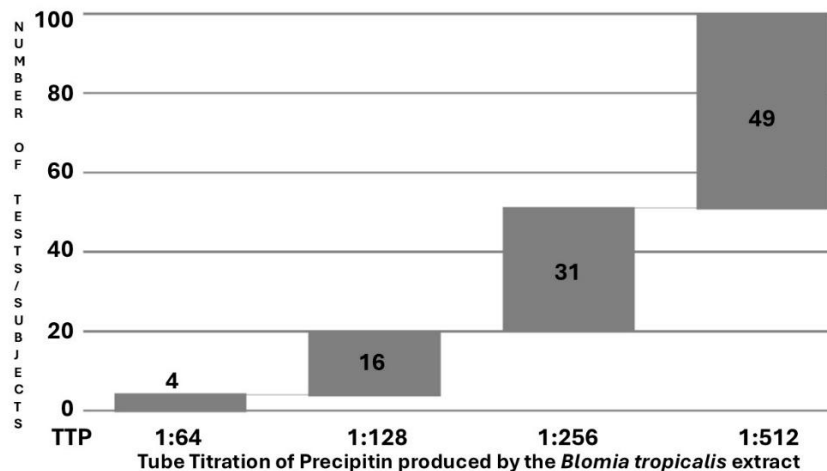


Fig. 1. Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the *Blomia tropicalis* extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

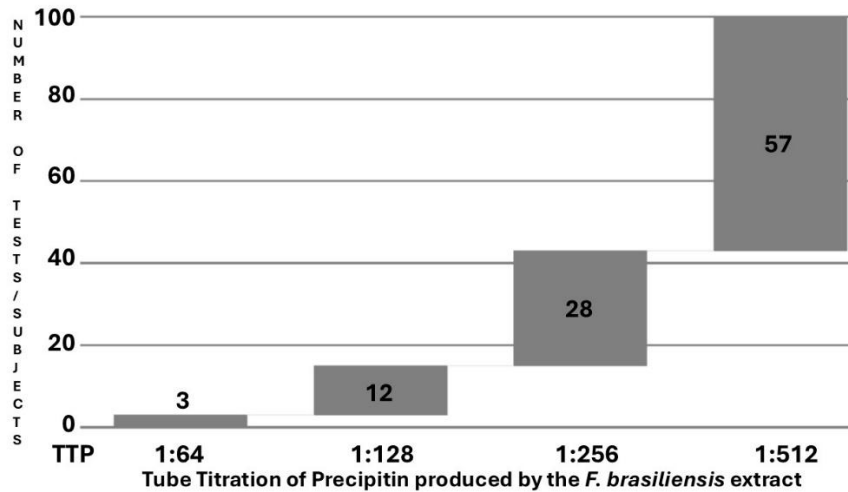


Fig. 2. Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the *Farfantepenaeus brasiliensis* extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

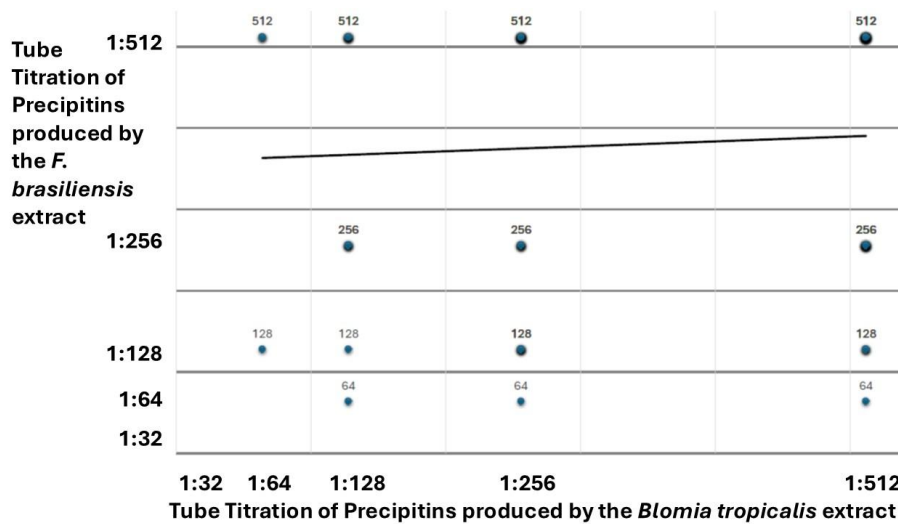


Fig. 3. Dispersion chart of the tube titration of precipitins results of the *ex vivo* challenge test against *Blomia tropicalis* extract (x-axis %), plotted against the Tube Titration of Precipitins results of the *ex vivo* challenge test against *Farfantepenaeus brasiliensis* extract (y-axis %)

The TTP for the *Blomia tropicalis* extract showed a distribution concentrated on the higher dilutions (Fig 1). There was no negative result. The mean was estimated at 1:353; the median was 1:256; the standard deviation was estimated at 1:163; the mode was 1:512 (appeared 49 times).

The TTP for the *Farfantepenaeus brasiliensis* extract showed a distribution concentrated on the higher dilutions (Fig 1). There was no negative result. The mean was estimated at 1:380; the median was 1:512; the standard deviation was estimated at 1:157; the mode was 1:512 (which appeared 57 times).

Pearson's correlation indicated a non-significant, small positive relationship between TTP results for *Blomia tropicalis* and *Farfantepenaeus brasiliensis*; $r(98) = 0.0629$, $p\text{-value} = 0.534$ (see Fig. 3).

The LAIT for the *Blomia tropicalis* extract showed a wide distribution range of results. The LAI ranged from 0% to 100%. The mean was 49.8%; the median was 51.5%; the standard deviation was 30.0%; the mode was 0% (appeared eleven times). The cascade distribution demonstrates a wide range of LAI results (Fig. 4). Some patients showed low or moderate immunoreactivity during

the ex vivo challenge test. In contrast, others displayed strong immunoreactivity, which could reflect the participation of *Blomia tropicalis* allergens in a non-IgE-mediated hypersensitivity condition in these patients (see Fig. 4)

The LAIT for the *Farfantepenaeus brasiliensis* extract showed a wide distribution range of results. The LAI ranged from 0% to 100%. The mean was 55%; the median was 56%; the standard deviation was 28.8%; the mode was 0% (appeared five times). The cascade distribution

demonstrates a wide range of LAI results (Fig. 5). Some patients showed low or moderate immunoreactivity during the ex vivo challenge test. In contrast, others displayed strong immunoreactivity, which could reflect the participation of shrimp allergens in a non-IgE-mediated hypersensitivity condition in these patients (see Fig. 5).

Pearson's correlation indicated a non-significant, small positive relationship between LAIT results for *Blomia tropicalis* and *F. brasiliensis*; $r(98) = 0.0713$, $p\text{-value} = 0.481$ (see Fig. 6).

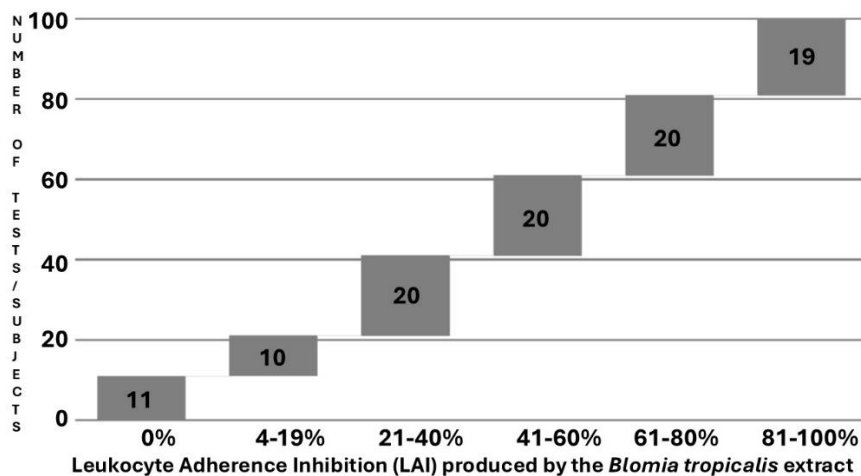


Fig. 4. Cascade distribution chart of the range groups of leukocyte adherence inhibition (LAI) results (x-axis %) of the ex vivo challenge test against the *Blomia tropicalis* extract monitored by the leukocyte adherence inhibition test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

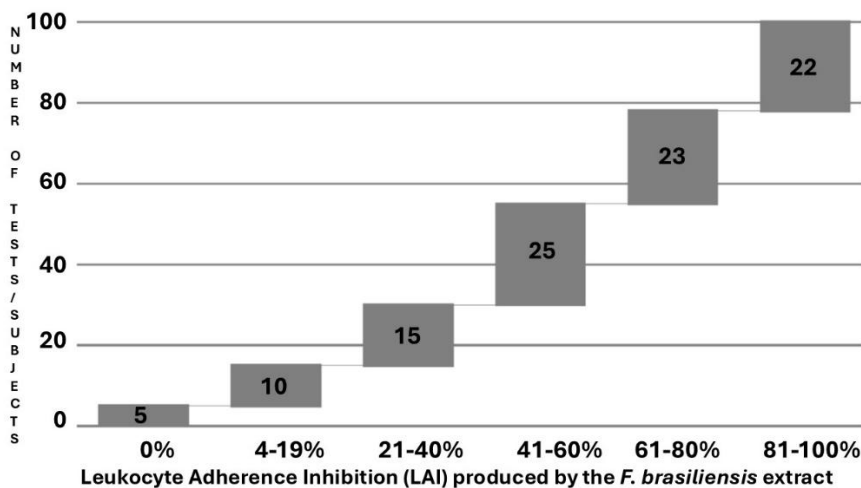


Fig. 5. Cascade distribution chart of the range groups of leukocyte adherence inhibition (LAI) results (x-axis %) of the ex vivo challenge test against the *Farfantepenaeus brasiliensis* extract monitored by the leukocyte adherence inhibition test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

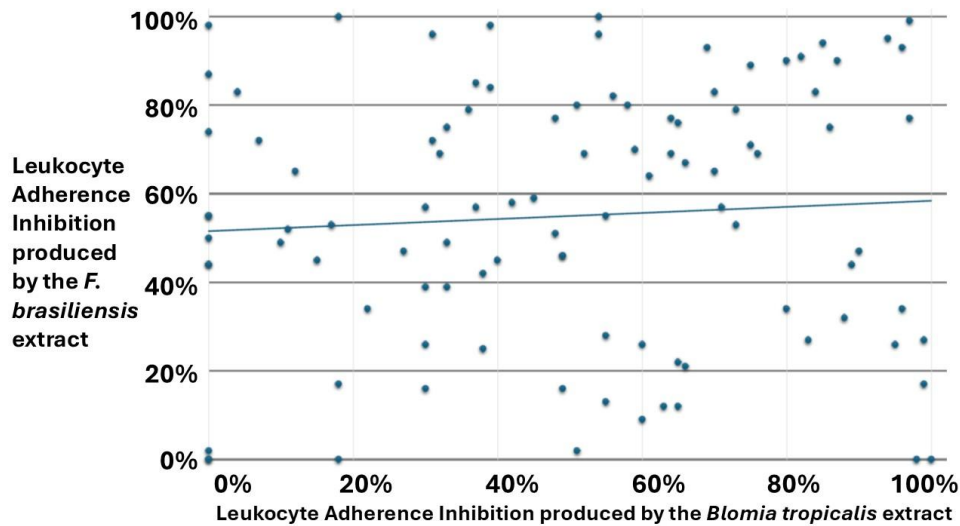


Fig. 6. Dispersion chart of the leukocyte adherence inhibition (LAI) results of the ex vivo challenge test against *Blomia tropicalis* extract (x-axis %), plotted against the LAI results of the ex vivo challenge test against *Farfantepenaeus brasiliensis* extract (y-axis %)

4. DISCUSSION

Polysensitization and cross-reactivity are related phenomena frequently observed in patients with allergic multimorbidity (Zhang *et al.*, 2025; Čelakovská *et al.*, 2024; Hasnain *et al.*, 2017). When investigating the etiology of allergic reactions, it is advisable to maintain an open mind regarding cross-reactivity among panallergens, especially in polysensitized patients presenting with Allergic Multimorbidity (Miltner *et al.*, 2024). The specificity and facility for researching precipitins transformed this immunoassay into a pioneering examination upon which the fundamentals of immunology were established (Hunter, 1905). Precipitins are yet used nowadays to monitor emerging diseases when more precise and sophisticated assays are inexistent or unavailable (Bellanger *et al.*, 2022). Precipitating antibodies indicate a humoral immune response against the tested antigens (Gell, Harington, and Rivers, 1946). Before the discovery of IgE, the research of precipitins was the only viable way to demonstrate immunoreactivity against allergenic agents (Augustin & Hayward, 1960) (Wells, 1911). Precipitin antibodies may belong to any Immunoglobulin class (except for IgG4), can mount immune complexes, and can participate in type II (antibody-dependent cell-mediated) and type III (immune complexes disease) Gell and Coombs hypersensitivity reactions, which may (theoretically) be demonstrated by the LAIT and the TTP assays, respectively (Gell & Coombs, 1968).

The LAIT is an *ex vivo* challenge test performed with live leukocytes, which allows the interaction of all immune-circulating cells with allergens. Several immune pathways can inhibit leukocyte adherence (Tong *et al.*, 1979; Halliday *et al.*, 1974).

The present study is a proof-of-concept that hypothesizes that LAIT and the TTP may differentiate diverse degrees of cellular and humoral immunoreactivity against *Blomia tropicalis* and *Farfantepenaeus brasiliensis* extracts among patients suffering from non-IgE-mediated Allergic Multimorbidity. As the tests were performed simultaneously using the same venous sample with both allergens, it was possible to calculate a correlation to distinguish any order of cross-reactivity between them.

The retrospective compilation of our data revealed a wide distribution of results when analyzing the LAIT results to explore cellular immunoreactivity. On the contrary, there was no such variability in the results of TTP, which demonstrated the presence of high titers of precipitin antibodies predominantly in the higher dilutions, suggesting the presence of humoral activity against these extracts in all selected patients, thereby limiting the analytical value of this immunoassay. Despite these immunoassays not precisely identifying the mechanisms responsible for the clinical condition, they provide general evidence about cellular and humoral immunoreactivity that may be involved in immune

tolerance, sensitization, and clinical hypersensitivity.

This preliminary retrospective survey yielded diverse results from the TTP and the ex vivo challenge test, monitored by LAIT, against *Blomia tropicalis* and *Farfantepenaeus brasiliensis* extracts in two cohorts of patients diagnosed with non-IgE-mediated Allergic Multimorbidity. 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 chemical and biological allergens, demonstrating positive results for some of them. Our results suggest that patients with allergies to HDM may exacerbate their symptoms by experiencing additional cross-immunoreactivity when consuming shrimp.

Assessing diverse pathways of immunoreactivity and hypersensitivity through tools such as TIAL and TTP against the whole extract of suspected allergens, as well as suspected molecular allergens, is a multi-omics approach that evaluates both diagnosis and treatment effectiveness in allergic patients (Hubert et al., 2023).

5. LIMITATIONS

This study is a retrospective analysis of data collected over a seven-year period 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 were unable to establish a cross-comparison between the positive and negative controls to validate the results. The number of subjects is suitable for a preliminary study; however, future studies should be more comprehensive. The lack of a research protocol implies the possibility of bias introduced by the physician's point of view (CEO) based on a clinical suspicion driven solely by the anamnesis and physical examination. The study lost many of these patients to follow-up, so it is not yet possible to ensure the relationship between the immunoassay results and the patient's clinical outcome. Unfortunately, it was impossible to compare the two procedures using paired statistics methodologies because they were derived from two distinct patient groups.

6. CONCLUSION

Our preliminary results suggest that the LAIT and TTP may distinguish between varying degrees of immunoreactivity against *Blomia tropicalis* and *Farfantepenaeus brasiliensis* extracts in patients clinically diagnosed with non-IgE-mediated Allergic Multimorbidity. Despite requiring trained personnel for execution, LAIT and TTP are inexpensive, can be performed with minimal laboratory equipment, and can be incorporated into strategies to address health disparities in diagnosing respiratory and food allergies (Anagnostou et al., 2025). As a preliminary report, the propaedeutic significance of the presented results and the potential interfering factors must be established (Anouar et al., 2024). More studies focused on the quality-by-design approach with larger, prospective, double-blind cohorts are needed to evaluate the potential contribution of LAIT and TTP for endotyping cellular and humoral immunoreactivity in patients suspected of hypersensitivity to aeroallergens and food allergens (Chiarentin et al., 2023).

7. FUTURE DIRECTIONS AND RECOMMENDATIONS FOR CLINICAL PRACTICE

The primary intended use of *in vitro* or *ex vivo* allergen challenge tests is to spare patients from undergoing unnecessary, exhaustive, and potentially 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, which clinicians often use as a decision support system to enhance diagnostic accuracy (Abers & Mathias, 2025).

ETHICAL APPROVAL

The authors have obtained and documented written ethical approval following international standards. As a retrospective survey of results recorded in cognito, consent was obtained collectively by the institution's ethics committee, following the principles outlined in the Declaration of Helsinki (World Medical Association, 2013).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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