Original Research Article

Endotyping Non-IgE-Mediated Immunoreactivity to *Dermatophagoides farinae*: Implications for Allergic Patients.

ABSTRACT

Background: Several publications report *Dermatophagoides farinae* allergens as responsible for several types of non–IgE-mediated allergic reactions. There is no standardized lab exam to endotype (quantify the participation of these mechanisms) in the context of allergic disease pathophysiology.
Aims: To evaluate the potential of the Leukocyte Adherence Inhibition Test (LAIT) and the Tube Titration of Precipitins (TTP) on serum to discriminate non–IgE-mediated immunoreactivity against *D. farinae* in patients with non–IgE-mediated allergic phenotype with clinically suspected hypersensitivity to their allergens.
Study Design: We retrospectively examined the medical charts of 134 allergic patients investigated with LAIT and 100 allergic patients investigated with TTP against an extract of *D. farinae*.
Place and Duration of Study: Instituto Alergoimuno de Americana – São Paulo – Brazil – between January 2018 and May 2024.
Methodology: The registered results of the semi-quantitative serum TTP against *D. farinae* extract were distributed in ranges through a cascade distribution chart to outline the variability of the results. The registered results of the percentage of Leukocyte Adherence Inhibition (LAI) promoted by the *ex vivo* challenges with *D. farinae* extract were distributed in ranges through a cascade distribution chart to outline the variability of results. The statistical characteristics of these cohorts were calculated.
Results: The TTP showed a wide distribution range, with four negative and most positive results concentrating on the higher dilutions. The mean was estimated at 1:242; the median was 1:128; the standard deviation was estimated at 1:210; the mode was 1:512 (appeared 36 times). The LAI ranged from 0% to 100%. The mean was 52%; the median was 55.5%; the standard deviation was 23.6%; the mode was 63% (appeared seven times). The cascade distribution demonstrates a wide range of LAI results.
Conclusion: Our preliminary results support that the semi-quantitative TTP and the LAIT have the potential to endotype and clinically discriminate diverse degrees of cellular and humoral non–IgE-mediated immunoreactivity against *D. farinae* in allergic patients.

Keywords: Allergy; Asthma; Bronchitis; Dermatophagoides farinae; Diagnosis; Hypersensitivity; Leukocyte Adherence Inhibition Test; Non–IgE-mediated Immunoreactivity; Precipitins; Rhinitis.

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

1. INTRODUCTION

Storing food and sharing mattresses with mites exposed humanity to commensalism, which was only noticed about a hundred years ago [1]. Storage mites have been reported as causes of allergic diseases since 1924 when Willem Storm van Leeuwen described several cases of asthma in patients exposed to mite-infested wheat and oats [2]. Storage mites are eight-legged members of the Arachnid class and a significant cause of allergic diseases through atopic sensitization to their multiple allergens [3]. *Dermatophagoides farinae* is a storage mite belonging to the phylum Arthropoda, subphylum

Chelicerata, class Arachnida; subclass Acari; superorder Acariformes; order Sarcoptiformes; suborder Psoroptidia; family Pyroglyphidae; subfamily Dermatophagoidinae [4]. The allergic reactions produced by the ingestion of storage mites are frequently reported as "oral mite anaphylaxis" or "pancake syndrome" [5]. Several IgE-mediated and Non–IgE-mediated hypersensitivity mechanisms were associated with these conditions [6].

Besides a typical storage mite, *D. farinae* is also a house dust mite found in mattresses and pillows, which provides food, moisture, and a thermal source [7]. Besides corporal allergens, several allergens are identified in the feces, eggs, and excretions of *D. farinae,* which accumulate in the environment and are recognized as significant immunogens [8]. The Allergen Nomenclature Sub-Committee from the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) had already classified dozens of allergens belonging to *D. farinae* [9]. Most *D. farinae* allergens were identified by proteomics, using IgE-based techniques such as enzyme-linked immunosorbent assay inhibition tests, immunoblots, basophil activation tests, and skin prick tests [10]. Besides the IgE-mediated allergenicity, the immunoreactivity of the *D. farinae* allergens on the innate immune system and its contribution to the production of symptoms in allergic patients have also been studied [11]. Several IgG epitopes of *D. farinae* allergens were already identified by peptide microarray immunoassay [12]. Specific *D. farinae* allergens, such as the Der f 38, stimulate Innate immunoreactivity by binding to Toll-like receptors (such as TLR4), acting as allergy inducers [13]. *D. farinae* also stimulates the transcription of long noncoding RNAs (non-protein-coding RNA that exert transcriptional and post-transcriptional regulation on messenger RNAs and microRNAs), causing cell dysfunction and dysregulation of circulating $CD8^+$ T cells of patients with asthma [14]. Some *D. farinae* allergens, besides acting as IgE ligands, also stimulate Innate immune cells, such as the α-Tubulin (Der f 33), which increases the expression of interleukin-4 and upregulates CD80 and TNF-α levels in dendritic cells [15]. Exosomes from *D. farinae* induce immunogenic inflammation by stimulating epithelial cells and macrophages to release inflammatory-related cytokines such as interleukin-33, thymic stromal lymphopoietin, TNF-alpha, and IL-6 [16]. *D. farinae* induces innate inflammation via Interleukin-33 via Receptor-interacting protein kinase signaling [17].

Besides their allergens, mites also harbor a microbiome that produces toxins and a diversified set of allergens that can trigger innate and adaptive allergic responses [18]. Environmental allergens such as mites and microorganisms may produce innate immune dysregulation, resulting in epithelial damage, inadequate adaptive response, and a persistent inflammatory state [19]. The mite's microbiome is rich in pathogen-associated molecular patterns (PAMPs), which are also involved in mast cell activation and non–IgE-mediated allergic responses [20]. The microbial Damage-Associated Molecular Patterns (DAMPs) promote type IVc hypersensitivity reactions through Th17 cell cytokines and Group 3 Innate Lymphoid Cells, leading to the liberation of Neutrophil Extracellular Traps (NETs), stimulating the innate immune response and inflammation [21, 22]. DAMPs trigger the type VII Hypersensitivity reaction through Pattern Recognition Receptors (PRRs) [23]. Non–IgE-mediated hypersensitivity to mite bacterial microbiome is documented in patients with acne rosacea [24, 25].

To endotype non–IgE-mediated immunoreactivity against suspected allergens, we routinely employ the semi-quantitative Tube Titration of Precipitins (TTP) and the Leukocyte Adherence Inhibition Test (LAIT), an *ex vivo* challenge immunoassay made with viable leukocytes already reported to demonstrate allergen-specific immunoreactivity against *D. farinae* in allergic patients [26]. To evaluate the potential of these procedures to discriminate non–IgE-mediated immunoreactivity against *D. farinae,* we retrospectively compiled the electronic medical charts of patients with non–IgE-mediated allergic rhinitis, allergic bronchitis, allergic conjunctivitis, and/or atopic dermatitis who were investigated with them. Patients diagnosed with these allergic conditions were eligible for this investigation after demonstrating non-reactive or inconclusive skin tests against *D. farinae* extract, a normal range total IgE, and undetectable specific IgE for *D. farinae*.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 04/2024), we proceeded with the electronic chart review of 8,800 allergic patients who attended our outpatient facility from January 2018 to May 2024.

A cohort of 134 patients had been submitted to an *ex vivo* allergen challenge test with *D. farinae* extract monitored with LAIT for presenting non–IgE-mediated allergic conditions. The LAIT cohort

counted 44 males; mean age 42.9 years; SD 17.8 years; range 17 to 89 years; modes = 19 and 25 (each appeared seven times); geometric mean = 39.3 years.

A cohort of 100 patients had been submitted to TTP with *D. farinae* extract for presenting non–IgE-mediated allergic conditions. The TTP cohort counted 35 males; mean age 40.8 years; SD 20.5 years; range 3 to 86 years; modes = 94 (appeared five times); geometric mean = 33 years.

These procedures were offered to patients with allergic rhinitis, allergic bronchitis, allergic conjunctivitis, atopic dermatitis, and/or urticaria with a normal range total IgE, undetectable specific IgE against *D. farinae* (investigated through ImmunoCAP®), and a non-reagent or inconclusive investigation performed with allergic skin tests done with the *D. farinae* extract [27].

2.2 Antigen preparation

The D. farinae extracts were obtained from frozen cultures. The contents of the bottles containing mites (adults, nymphs, larvae, feces, and eggs) and culture medium were weighed and left at a rate of 10 ml of PBS buffer per gram of material (10%) by gentle magnetic stirring (1,000 rpm) for four hours at 4ºC. The material was centrifuged at 5,000 rpm for 30 minutes. The supernatant was kept apart. The sediment was resuspended in the same conditions as the previous step and stirred at four °C for 24 hours. The centrifugation was repeated, and the supernatants were mixed into an Erlenmeyer flask. The solution was filtered through a double paper filter and later through a 0.2 µm pore-size filter with the help of a vacuum flask and a vacuum pump. The extract was dialyzed with the aid of the Thermo Fisher Scientific SnakeSkin™ 88244 Dialysis Tubing in distilled water (1:50 ratio) with three water changes for 24 hours to eliminate molecules of low molecular mass (< 5,000 Da). After dialysis, the extract was centrifuged at 10,000 rpm for 30 minutes at 4ºC and frozen at -40 ºC. The protein concentration was estimated spectrophotometrically and diluted to 1 mg/mL in saline (NaCl 0.9%) to perform the allergic skin tests, LAIT, and TTP [28].

2.3 *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test (LAIT)

LAIT was performed as previously described [29-39]. Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with *D, farinae* extract, and the unchallenged plasma assay. We collected the 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 kept under agitation for 30 minutes (200 rpm at 37 °C) with antigen extract (10µL of a solution with 1mg/mL and pH 7.5) or with the antigen dilution solution (when used as control). After incubation, the 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 the humidified atmosphere of the covered water bath to allow leukocytes to adhere to the glass. Next, we counted the leukocytes, removed the coverslip, and washed the chamber by immersion in a beaker with 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. 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 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 unchallenged control plasma multiplied by 100 (%). To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from 100 (%). We employed the LAI results for the cascade distribution chart and the statistics calculations, both performed with the help of the Microsoft Excel® statistical package.

2.4 *In vitro* Investigation: Tube Titration of Precipitins (TTP)

As previously reported, the semi-quantitative tube titration of precipitins (TTP) against D. farinae extract was performed in a transparent vitreous tube [40]. 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. The allergen extracts were allocated in sets of eleven glass tubes at progressive duplicated

serum dilutions. The progressive dilutions were combined with the 15 µL of the antigen (1 mg/mL) 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 done with the water and serum to observe occasional spontaneous precipitation (Sia Test) to detect circulating immune complexes [41]. After 24 hours, one of us examined the tubes, and the titers (the highest dilution factor yielding a positive reading) were recorded [42].

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 showed a wide distribution range, with four negative results and most positive results concentrated on the higher dilutions (Fig 1). The mean was estimated at 1:242; the median was 1:128; the standard deviation was estimated at 1:210; the mode was 1:512 (appeared 36 times). All Sia tests were negative.

The LAI ranged from 0% to 100%. The mean was 52%; the median was 55.5%; the standard deviation was 23.6%; the mode was 63% (appeared seven times). The cascade distribution demonstrates a wide range of distribution of LAI results (Fig.2). Six patients ignored the presence of the allergen on the plasma and presented no inhibition of leukocyte adherence (LAI = 0%) after contact with *D. farinae* extract (4.5% of the tests). Some patients showed low or moderate immunoreactivity during the *ex vivo* challenge test, while others displayed strong immunoreactivity, which could reflect the participation of *D. farinae* allergens in a non–IgE-mediated hypersensitivity condition.

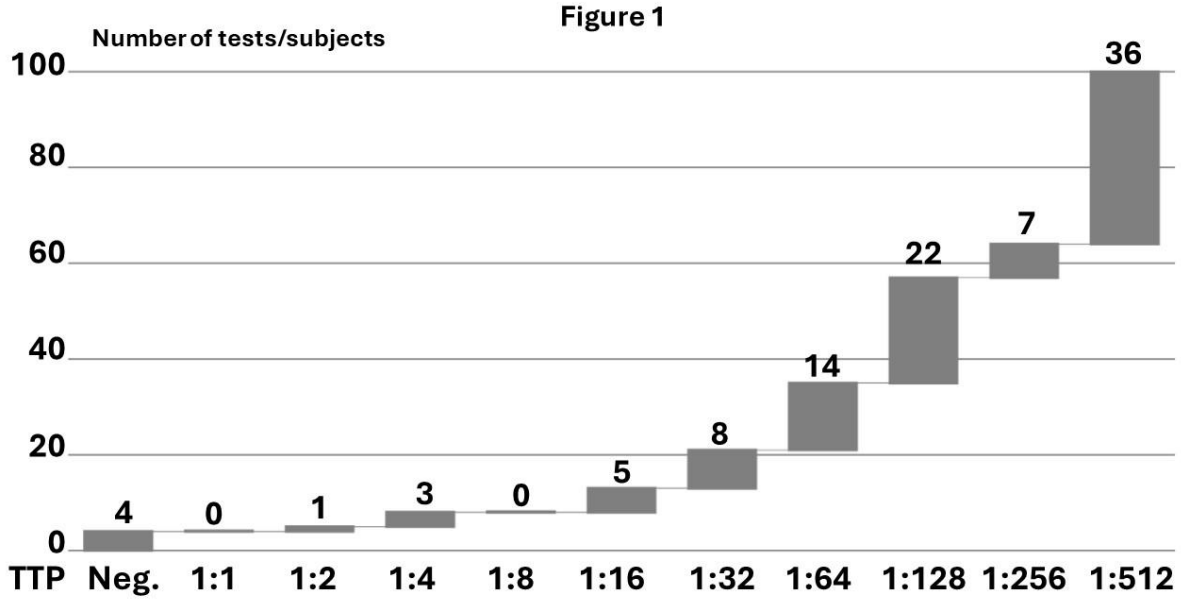

Fig. 1. Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the *D. farinae* extract against the serum of a cohort of 100 tests/subjects (y-axis).

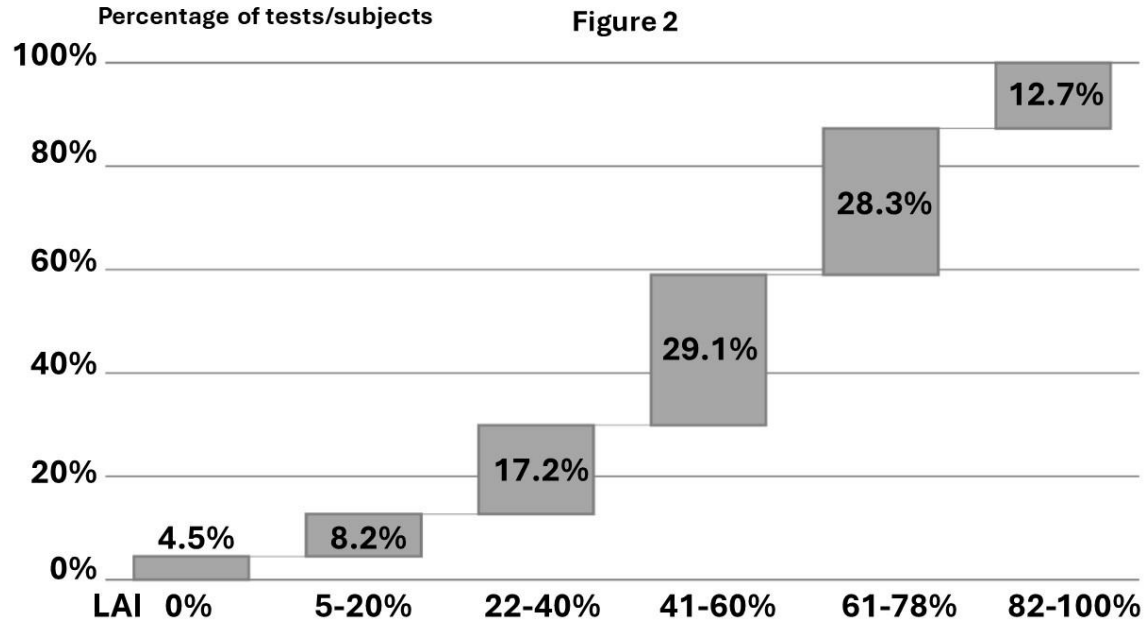

Fig. 2. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo D. farinae* extract challenges monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over a cohort with 134 tests/subjects (y-axis).

4. DISCUSSION

Diagnosing hypersensitivity reactions is essential in increasing patients' awareness of their sensitivities and commitment to avoiding allergens. The development of several lab methods to detect specific IgE has raised concerns about the eviction of house dust mite allergens, mainly by chemical treatment and encasement methods for pillows and bed mattresses [43]. However, non-IgE-mediated food-storage mite hypersensitivity may be a challenging diagnosis in the field of food allergies [44].

Significant mite mortality and reduction of Der f 1 fecal allergen were observed in carpets and mattresses after the treatment with hard surface steam cleaners [45]. Some studies demonstrated that negative ions produced by ionizers kill dust mites and can be used to reduce mite populations on surfaces such as floors and clothes [46]. Sublingual immunotherapy drops are a highly effective treatment for *D. farinae* allergy by increasing the subsets of T immune cells, specifically Th17 cells and $CD4^{+}CD25^{+}$ regulatory T cells (Treg cells), in peripheral blood [47].

Before the discovery and dissemination of lab methods to detect allergen-specific IgE, endotyping of allergic diseases was performed with the help of allergic skin tests, Complement fixation assays, and the research of precipitins [48]. However, diagnosing non-IgE-mediated hypersensitivity is difficult in medical practice since clinical analysis diagnostic laboratories do not offer specific tests to support this diagnosis, which is only possible in academic research institutions.

An *ex vivo* challenge test with a viable leukocyte buffy coat, the LAIT allows the living interaction of all immune-circulating participants with the tested allergen, such as the innate and adaptive immune cells, cytokines, alarmins, and antibodies [49]. However, as an observant of the final phenomenon, the LAIT does not indicate which pathways were involved [26, 50-52].

The TTP classically proves that patients produce circulating antibodies against the specific allergen added to the serum in sufficient quantities to produce immune complexes large enough to precipitate visibly at the bottom of the tubes. TTP is the most basic laboratory exam upon which Immunology was established as a science [53, 54].

This preliminary retrospective survey demonstrated extensive results from the *ex vivo* challenge test monitored by LAIT and the TTP with *D. farinae* in two heterogeneous cohorts of allergic patients.

These exams mostly demonstrate that IgE-mediated hypersensitivity is not responsible for the allergic diseases in these patients. These allergic diseases should instead be viewed as a conjoint of intricate immune hypersensitivity mechanisms that act conjointly to produce damage. Endotyping these mechanisms helps to clarify the individual's immune response to better prescribe the management and the treatment in personalized medical care.

We routinely employ the LAIT and the research of tube precipitins as a complementary triage test to select worthwhile antigens to proceed with more laborious *in vivo* provocation tests when the specific IgE is undetectable. Our results suggest that most allergic patients present some immunoreactivity against *D. farinae* allergens, while some do not. However, the complete significance of these results is not yet fully established. As an isolated piece of information, the LAIT immunoreactivity or the presence of precipitins do not prove that the tested antigens explain the allergic symptoms exactly. Indeed, the clinical diagnosis must be accomplished by the *in vivo* challenges, the degree of colonization of the patient's environment, and the benefits of a change of ambient and an occasional desensitization treatment. More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of these methods to diagnosing patients suspected of *D. farinae* non–IgE-mediated hypersensitivity.

5. CONCLUSION

Our preliminary results support that the semi-quantitative TTP and the LAIT have the potential to endotype and clinically discriminate diverse degrees of cellular and humoral non–IgE-mediated immunoreactivity against *D. farinae* in allergic patients. The propaedeutic meaning of these results, however, must be better established, as well the effects of possible interferents [55]. More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of LAIT and TTP for endotyping immunoreactivity of patients with allergic phenotypes suspected of presenting *D. farinae* non-IgE-mediated hypersensitivity.

CONSENT

As a retrospective survey of results recorded *incognito*, consent was given collectively by the institution's ethics committee following the principles of the Declaration of Helsinki [56].

ETHICAL APPROVALS

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

REFERENCES

1. Vackova T, Pekar S, Klimov PB, Hubert J. Sharing a bed with mites: preferences of the house dust mite *Dermatophagoides farinae* in a temperature gradient. Exp Appl Acarol. 2021;84(4):755-767.

2. Spieksma FT, Dieges PH. The history of the finding of the house dust mite. J Allergy Clin Immunol. 2004;113(3):573-6.

3. Miller JD. The Role of Dust Mites in Allergy. Clin Rev Allergy Immunol. 2019;57(3):312-329.

4. Schoch CL *et al.* NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford). 2020: baaa062. PubMed: 32761142 PMC: PMC7408187. https://130.14.29.110/Taxonomy/Browser/wwwtax.cgi (accessed 02/11/2023).

5. Sánchez-Borges M, Suárez-Chacon R, Capriles-Hulett A, Caballero-Fonseca F, Iraola V, Fernández-Caldas E. Pancake syndrome (oral mite anaphylaxis). World Allergy Organ J. 2009:2(5):91-96.

6. Sánchez-Borges M, Fernandez-Caldas E. Hidden allergens and oral mite anaphylaxis: the pancake syndrome revisited. Curr Opin Allergy Clin Immunol. 2015;15(4):337-43.
7. Vackova T, Pekar S, Klimov PB, Hubert J. Population growth and respiration in the dust mite *Dermatophagoides farinae* under different temperature and humidity regimes. Exp Appl Acarol. 2023;89(2):157-169.
8. Erban, T.; Hubert, J., Two-dimensional gel proteomic analysis of Dermatophagoides farinae feces. Exp Appl Acarol 2015, 65 (1), 73-87.
9. World Allergen Nomenclature Sub-Committee from the World Health Organization and the International Union of Immunological Societies (WHO/IUIS) https://allergen.org/search.php?allergensource=Dermatophagoides+farinae&searchsource=Search.
10. An S, Chen L, Long C, Liu X, Xu X, Lu X, et al. *Dermatophagoides farinae* allergens diversity identification by proteomics. Mol Cell Proteomics. 2013:12(7):1818-28.
11. Cao H, Liu Z. Clinical significance of dust mite allergens. Mol Biol Rep. 2020;47(8):6239-6246.
12. Teng F, Han F, Zhu X, Yu L, Gai D, Xu C, et al. Identification of continuous immunoglobulin G epitopes of *Dermatophagoides farinae* allergens by peptide microarray immunoassay. IUBMB Life. 2020:72(9):1976-1985.
13. Kim G, Hong M, Kashif A, Hong Y, Park BS, Mun JY, et al. Der f 38 Is a Novel TLR4-Binding Allergen Related to Allergy Pathogenesis from Dermatophagoides farinae. Int J Mol Sci. 2021;22(16).
14. Wang L, Zhou Y, Cui Y. lncRNA profiling in NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts. Int J Immunopathol Pharmacol. 2018;32:394632017750997.
15. Wang H, Lin J, Liu X, Liang Z, Yang P, Ran P, et al. Identification of α-tubulin, Der f 33, as a novel allergen from Dermatophagoides farinae. Immunobiol. 2016;221(8):911-7.
16. Yang T, Xu Z, Yu J, Liu J, Wang W, Hong S. Exosomes Derived from *Dermatophagoides farinae* Induce Allergic Airway Inflammation. Microbiol Spectrum. 2023;11(4):e0505422.
17. Jin M, Bang JS, Ha DL, Kim JY, Park KD, Lee WJ, et al. *Dermatophagoides farinae* Extract Induces Interleukin 33-Mediated Atopic Skin Inflammation via Activation of RIP1. Int J Mol Sci. 2023;24(6):5228.
18. Lee J, Kim JY, Yi MH, Hwang Y, Lee IY, Nam SH, et al. Comparative microbiome analysis of *Dermatophagoides farinae, Dermatophagoides pteronyssinus*, and *Tyrophagus putrescentiae*. J Allergy Clin Immunol. 2019;143(4):1620-1623.
19. Kuo IH, Yoshida T, Benedetto AD, Beck LA. The cutaneous innate immune response in patients with atopic dermatitis. J Allergy Clin Immunol. 2013;131(2):266-78.
20. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast Cell: A Multi-Functional Master Cell. Front Immunol. 2016;6(6):620.
21. Keir HR, Chalmers JD. Neutrophil extracellular traps in chronic lung disease: implications for pathogenesis and therapy. Eur Resp Rev. 2022;31(163):1-19.
22. Croxatto D, Micheletti A, Montaldo E, Orecchia P, Loiacono F, Canegallo, F, et al. Group 3 innate lymphoid cells regulate neutrophil migration and function in human decidua. Mucosal Immunol. 2016:9(6):1372-1383.
23. Jutel M, Agache I, Zemelka-Wiacek M, Akdis M, Chivato T, Giacco SD, et al. Nomenclature of allergic diseases and hypersensitivity reactions: Adapted to modern needs: An EAACI position paper. Allergy. 2023:78(11);2851-2874.
24. Lacey N, Delaney S, Kavanagh K, Powell FC. Mite-related bacterial antigens stimulate inflammatory cells in rosacea. Br J Dermatol. 2007;157(3):474-81.
25. Roihu T, Kariniemi AL. Demodex mites in acne rosacea. J Cutan Pathol. 1998;25(10):550-2.
26. Fink A, Heller L, Eliraz A, Weisman Z. Miskin A. Schlezinger, et al. Allergen-specific leukocyte adherence inhibition (LAI) assay: sensitivity, specificity and mechanism. Immunol Lett. 1987;16(1):65-70.

27. Olivier CE, Argentão DGP, Santos RAPG, Silva MD, Lima RPS, Zollner RL. Skin scrape test: an inexpensive and painless skin test for recognition of immediate hypersensitivity in children and adults. Open Allergy J. 2013:6:9-17.
28. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976:72:248-54.
29. Olivier CE, Lima RPS, Pinto DG, Santos RAPG, Silva GKM, Lorena SLS, et al. In search of a tolerance-induction strategy for cow's milk allergies: significant reduction of beta-lactoglobulin allergenicity via transglutaminase/cysteine polymerization. Clinics. 2012;67(10):1171-1179.
30. Olivier CE, Santos RAPG, Lima RPS, Argentão DGP, Silva GKM, Silva MD. 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. J Cell Adhesion. 2014:1-6 Article ID 860427 (http://dx.doi.org/10.1155/2014/860427).
31. Olivier CE, Pinto DG, Lima RPS, Silva MD, Santos RAPG, Teixeira, et al. Assessment of Immunoreactivity against Therapeutic Options Employing the Leukocyte Adherence Inhibition Test as a Tool for Precision Medicine. Eur J Clin Med. 2021;2(3):40-45.
32. Olivier CE, Pinto DG, Santos RAPG, Lima RPS. Dextran's interference over the Leukocyte Adherence Inhibition Test. Academia Letter 2021, Article 3792.
33. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Immunoreactivity against Dermatophagoides pteronyssinus Assessed by the Leukocyte Adherence Inhibition Test in Patients with Intrinsic Atopic Dermatitis and Correlated "Intrinsic" Non–IgE-mediated Allergic Conditions. Eur J Clin Med. 2021;2(6):45-50.
34. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. 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. Eur J Clin Med. 2022;3(1):11-17.
35. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test for the evaluation of immunoreactivity against gluten extracts in non—IgE-mediated / non-autoimmune Gluten-Related Disorders. Eur J Clin Med. 2022;3(2):1-7.
36. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Leukocyte Adherence Inhibition Test to the Assessment of Immunoreactivity Against Cow's Milk Proteins in Non—IgE-Mediated Gastrointestinal Food Allergy. Eur J Clin Med. 2022;3(2):38-43.
37. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Evaluating Non-IgE-mediated Allergens' Immunoreactivity in Patients with "Intrinsic" Persistent Rhinitis with Help of the Leukocyte Adherence Inhibition Test. Eur J Med Health Sci. 2023;5(1):17-22.
38. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Evaluating Non-IgE-Mediated Allergens' Immunoreactivity in Patients Formerly Classified as "Intrinsic" Asthmatics with Help of the Leukocyte Adherence Inhibition Test. Eur J Clin Med. 2023:4(2):1-7.
39. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Immunoreactivity against Cobalt. Asian J Immunol. 2023;6(1):174-184.
40. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Intrinsic Atopic Dermatitis: Titration of Precipitins in the Screening of Food Allergens for Prescription of Elimination Diets and Desensitization Strategies. Eur J Clin Med. 2021;2(6):1-9.
41. Keystone E, Pruzanski W. Immunochemical and physical studies of the Sia test. Am J Med Sci. 1976;271(2):151-7.
42. Williams CA, Chase MW. Chapter 13 - Precipitation Reactions. In: Reactions of Antibodies with Soluble Antigens. Academic Press: 1971;3:1-102.

43. Marks GB, Tovey ER, Green W, Shearer M, Salome CM, Woolcock AJ. House dust mite allergen avoidance: a randomized controlled trial of surface chemical treatment and encasement of bedding. Clin Exp Allergy. 1994;24(11):1078-83.
44. Venter C, Vieira MC, Fleischer D. Tolerance development in non-IgE mediated food allergies: lessons from Brazil. Jornal de Pediatria. 2023:2(30):1-4.
45. Glass EV, Needham GR. Eliminating *Dermatophagoides farinae* spp. (Acari: Pyroglyphidae) and Their Allergens Through High Temperature Treatment of Textiles. J Med Entomol. 2004;41(3):529-532.
46. Abidin SZ, Ming HT. Effect of a commercial air ionizer on dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Acari: Pyroglyphidae) in the laboratory. Asian Pacific J Trop Biomed. 2012:2(2):156-8.
47. Tian M, Wang Y, Lu Y, Jiang YH, Zhao DY. Effects of sublingual immunotherapy for *Dermatophagoides farinae* on Th17 cells and CD4(+) CD25(+) regulatory T cells in peripheral blood of children with allergic asthma. Int Forum Allergy Rhinol. 2014:4(5):371-5.
48. Walker IC. Study XII: Complement Fixation and Precipitin Reactions with the serum of Bronchial Asthmatics who are sensitive to the proteins of wheat, horse dandruff, cat hair, and bacteria, using these proteins as antigens, and the cutaneous reaction as an index of sensitization. J Med Res. 1917;36(2):243-66.
49. Olivier CE, Lima RPS, Pinto DG, Santos RAPG. 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. Biom J Sci Tech Res. 2021;36(3):28647-28655.
50. Thomson DMP. Assessment of immune status by the leukocyte adherence inhibition test. Academic Press: New York. 1982;XVII:380p.
51. Tong AW, Burger DR, Finke P, Barney C, Vandenbark AA, Vetto RM. Assessment of the mechanism of the leukocyte adherence inhibition test. Cancer Res. 1979:39(2):597-603.
52. Halliday WJ, Maluish A, Miller S. Blocking and unblocking of cell-mediated anti-tumor immunity in mice, as detected by the leucocyte adherence inhibition test. Cell Immunol. 1974;10(3):467-475.
53. Gell PGH, Harington CR, Rivers RP. The antigenic function of simple chemical compounds; production of precipitins in rabbits. British Journal of Experimental Pathology 1946, 27 (5), 267-286.
54. Wells, H. G., Studies on the chemistry of anaphylaxis (III). Experiments with isolated proteins, especially those of the hen's egg. J Infec Dis. 1911;9:147-171.
55. Anouar S. Hazim R. Brahim A. Interferences in Immunological Assays: Causes, Detection, and Prevention. Asian J Immunol. 2024;7(1):71-78.
56. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.