Asian Journal of Immunology

Volume 7, Issue 1, Page 12-20, 2024; Article no.AJI.112345

Contribution of the Leukocyte Adherence Inhibition Test in Diagnosing Non–IgE-Mediated Immunoreactivity against *Aspergillus fumigatus* in Patients with Allergic Rhinitis and Asthma

Celso Eduardo Olivier [a*], Daiana Guedes Pinto [a], Ana Paula Monezzi Teixeira [a], Jhéssica Letícia Santos Santana [a], Raquel Acácia Pereira Gonçalves Santos [a], Regiane Patussi Santos Lima [b] and Everton Salgado Monteiro [c]

[a] Instituto Alergoimuno de Americana, Brazil.
[b] Lavoisier's laboratories, São Paulo, Brazil.
[c] Department of Allergy and Immunopathology, Faculty of Medicine, São Paulo University, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. 'All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/112345

Original Research Article

Received: 15/01/2024
Accepted: 27/01/2024
Published: 30/01/2024

**Corresponding author: E-mail: celso@alergoimuno.med.br;*

ABSTRACT

Aims: To evaluate the potential of the Leukocyte Adherence Inhibition Test (LAIT) to discriminate non–IgE-mediated immunoreactivity against *Aspergillus fumigatus* in patients with non–IgE-mediated Allergic Rhinitis and/or Asthma.
Study Design: We retrospectively examined the medical charts of 100 patients diagnosed with respiratory allergy with clinical suspicion of non–IgE-mediated fungal hypersensitivity who were investigated with an *ex vivo* challenge monitored by LAIT against an extract of *A. fumigatus.*
Place and Duration of Study: Instituto Alergoimuno de Americana – São Paulo – Brazil – between January 2018 and January 2024.
Methodology: The percentage of Leukocyte Adherence Inhibition (LAI) promoted by the *ex vivo* challenges with *A. fumigatus* extract was distributed in ranges through a cascade distribution chart to outline the variability of the results.
Results: The LAI ranged from 0% to 90%; the mean was 52%; the median was 55.5%; the standard deviation was 21%; the Mode was 61% (appeared six times), showing a Gaussian Distribution. This extensive distribution of LAI results suggests that some patients had non–IgE-mediated immunoreactivity against *A. fumigatus* allergens while others did not.
Conclusion: Our preliminary results support that the LAIT performed with *A. fumigatus* may discriminate diverse degrees of *ex vivo* immunoreactivity against this airborne allergen in patients suffering from respiratory allergies.

Keywords: Allergy; Aspergillus fumigatus; aspergillosis; asthma; bronchitis; diagnosis; hypersensitivity; leukocyte adherence inhibition test; non–IgE-mediated immunoreactivity; rhinitis.

ABBREVIATIONS:

ABPA : Allergic Bronchopulmonary Aspergillosis
DAMPs : Damage-Associated Molecular Patterns
LAI : Leukocyte Adherence Inhibition
LAIT : Leukocyte Adherence Inhibition Test
LAI : Leukocyte Adherence Ratio
NETs : Neutrophil Extracellular Traps
PAMPs : Pathogen-Associated Molecular Patterns
PRRs : Pattern Recognition Receptors

1. INTRODUCTION

Anemophilous fungi are airborne inhabitants of the wild, rural, and urban environments able to produce allergic respiratory diseases through IgE-mediated and non–IgE-mediated hypersensitivity mechanisms [1]. Fungi are recognized as causes of reactive airway diseases such as allergic bronchitis, allergic rhinitis, allergic sinusitis, and hypersensitivity pneumonitis [2]. Environmental occurrence of fungi species may produce contamination of stored food (peanuts, maize, mushrooms, rice, spices) with toxins, such as aflatoxins (named after *Aspergillus flavus*), and be highly hazardous for humans and animals [3]. *Aspergillus spp* are soil saprophytic filamentous microfungi that can also be found indoors with the appearance of black molds [4]. *Aspergillus fumigatus* is an airborne species that can become allergenic and pathogenic upon inhalation from sensitive and immunocompromised individuals [5]. The *A. fumigatus* species comprises several strains classified in the subgenus *Fumigati*, from the genus *Aspergillus*, belonging to the family *Aspergillaceae*, from the order *Eurotiales*, from the class *Eurotiomycetes*, from the subphylum *Pezizomycotina*, from the clade *Sacharomyceta*, from the phylum *Ascomycota*, from the subkingdom *Dikarya* of the Fungi's kingdom [6,7]. *A. fumigatus* spreads through air by conidia (asexual spores) that usually does not cause damage to immunocompetent or non-sensitive subjects. However, in immunocompromised hosts, the conidia may develop into a living, invasive filamentous form, producing a respiratory disease called Aspergillosis [8]. Allergic Bronchopulmonary Aspergillosis (ABPA) is an invasive clinical syndrome characterized by a peculiar combination of innate immune

incompetence and IgE-Mediated hypersensitivity that complicates previous respiratory conditions such as tuberculosis, bronchiectasis, lung abscess, and cystic fibrosis, clinically manifesting as asthma and respiratory failure [9]. However, *Aspergillus*-sensitized asthma and ABPA are very distinct conditions [10]. Rarely does a patient sensitized to A. fumigatus develop ABPA. The combination of previous pulmonary damage associated with laboratory parameters such as total IgE, specific IgE, *A. fumigatus* precipitins, *A. fumigatus* skin test, pulmonary imaging, fractional exhaled nitric oxide, and eosinophil count (on blood and sputum) are necessary elements to define a differential diagnosis for these conditions [11,12].

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 *A. fumigatus* [13,14]. Several allergens and antigens of *A. fumigatus* are present in the cell wall and bind to IgE and IgG of allergic patients, producing Gell & Coombs type I and type III reactions [15]. The secretome analyses of *A. fumigatus* revealed that the Asp-hemolysin is a major secreted toxin among 63 other proteins [16]. For some time, Asp-hemolysin, a 30 KDa-glycoprotein, was thought to be the major virulent agent from *A. fumigatus* [17]. However, the main allergen (Asp f1) is a highly cytotoxic toxin with a ribonuclease activity that inhibits protein synthesis, resulting in cellular death [18]. The immunological characterization of the (yet unnominated) second major *A. fumigatus* allergen (Asp f2) suggests its role in fungal adherence and morphogenesis [19]. *A. fumigatus* also produces two forms of melanin (a complex polyketide involved in virulence and resistance mechanisms to stress): dihydroxy-naphthalene melanin (DHN-melanin) and pyomelanin [20]. The research of specific antigens to diagnose IgE-mediated hypersensitivity or IgG-mediated immunoreactivity to *A. fumigatus* is hampered by the fact that not all strains produce every specific antigen, which must be first characterized by recombinant technology before being converted into a standard diagnostic lab kit [21].

Besides the specific antigens, as any fungi, *A. fumigatus* strains also express at their cell membranes Pathogen-Associated Molecular Patterns (PAMPs), which are recognized by host innate Pattern Recognition Receptors (PRRs) [22]. The phagocytosis of *A. fumigatus* conidia by macrophages involves recognition by the Dectin-1 (beta-glucan receptor) and Toll-like receptor 2 [23]. Neutrophils have a critical role in host defenses against invasive Aspergillosis; however, resting conidia of *A. fumigatus* are relatively resistant to cell-free killing by oxidants generated by neutrophils [24].

The innate immune responses can develop hypersensitivity reactions by cellular activity or antigen presentation to the adaptive immune arm, which produces specific non-IgE antibodies, resulting in symptomatic clinical allergies [25]. Beyond FcεRI receptors, Mast Cells also express Fc receptors for IgG and receptors for pathogen-associated molecular patterns (PAMPs), also involved in mast cell activation and immune responses to fungi [26]. The innate hypersensitivity hypothesis was first proposed by Rajan in 2003 [27]. Fungal components can promote type IVc hypersensitivity reactions through Th17 cell cytokines and Group 3 Innate Lymphoid Cells, leading to the liberation of Neutrophil Extracellular Traps (NETs), which trap extracellular pathogens stimulating immune response and inflammation [28,29]. Nowadays, it is recognized that microbial Damage-Associated Molecular Patterns (DAMPs) are triggers of the type VII Hypersensitivity reaction through Pattern Recognition Receptors (PRRs) [30].

To evaluate non–IgE-mediated immunoreactivity against suspected allergens, we routinely employ the Leukocyte Adherence Inhibition Test (LAIT), an *ex vivo* immunoassay made with viable leukocytes, able to demonstrate immunoreactivity against diverse kinds of allergens, including fungi [31-36]. To evaluate the potential of the LAIT to discriminate non–IgE-mediated immunoreactivity against *A. fumigatus,* we retrospectively compiled the electronic medical charts of patients with non–IgE-mediated allergic rhinitis, allergic sinusitis, and/or asthma who were investigated with this procedure. These patients were selected after demonstrating non-reactive or inconclusive skin tests against *A. fumigatus* extract, a normal range total IgE, and undetectable specific IgE for *A. fumigatus*.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de

Americana (Brazil; 01/2024), we proceeded with the electronic chart review of 8,200 allergic patients who attended our outpatient facility from January 2018 to January 2024. A cohort of 100 patients had been submitted to an *ex vivo* allergen challenge test with *A. fumigatus* extract monitored with LAIT for presenting non–IgE-mediated allergic rhinitis, allergic sinusitis, and/or asthma. The cohort counted 28 males; mean age 40.8 years; SD 21.7 years; range 2 to 81 years; modes = 51 and 58 years (each appeared five times); geometric mean = 32 years. This procedure was offered to patients with respiratory allergy with a normal range total IgE, undetectable specific IgE against *A. fumigatus* investigated through ImmunoCAP®, and a non-reagent or inconclusive investigation performed with allergic skin tests done with an *A. fumigatus* extract [37].

2.2 Antigen Preparation

The strains of *A. fumigatus* were cultivated in Czapek medium during three weeks of incubation at 28ºC. The fungal culture was filtered through a 0.45µm filter to obtain the fungal mass from which the micellar molecules were extracted. Extraction was performed at 4ºC for 24 hours, using a 0.125M ammonium bicarbonate extraction buffer, pH 7.5, with a high-speed stirrer. After 24 hours of extraction, the content was filtered through a coarse and 0.45 µm filter. The protein concentration was estimated spectrophotometrically and diluted to 500 µg/mL in antigen dilution solution (NaCl 10 g, KH_2PO_4 0.72 g, Na_3PO_4 2.86 g, methylparaben 1 g, propylparaben 0.5 g, glycerin 400 mL, H_2O 600 mL) to perform the LAIT and skin allergic tests.

2.3 *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

We performed the LAIT as previously described [32-46]. “Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with *A. fumigatus* 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)”. [47] 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 calculate the Leukocyte Adherence Inhibition (LAI) further, 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.

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 LAI ranged from 0% to 90%; the mean was 52%; the median was 55.5%; the standard deviation was 21%; the mode was 61% (appeared six times), showing a Gaussian distribution.

The cascade distribution demonstrates a wide range of distribution of LAI results (Fig.1). Two patients ignored the presence of the allergen on the plasma and presented no inhibition of leukocyte adherence (LAI = 0%) after contact with the *A. fumigatus* extract (2% of the tests). Some patients showed low or moderate immunoreactivity during the *ex vivo* challenge test, while others displayed strong immunoreactivity, which could possibly reflect the participation of *A. fumigatus* allergens in a theoretical non–IgE-mediated hypersensitivity condition.

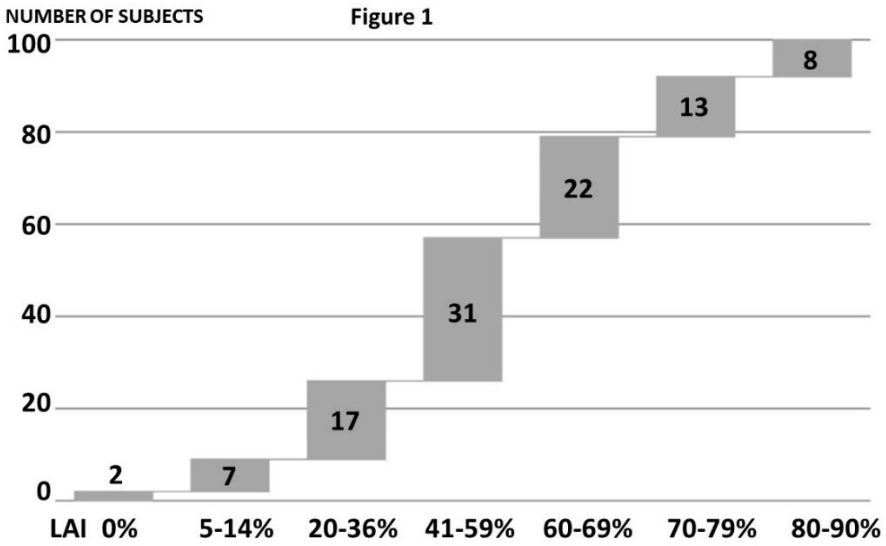

Fig. 1. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo A. fumigatus* extract challenges monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over 100 tests/subjects (y-axis)

4. DISCUSSION

“Airborne allergenic fungi have been recognized as allergens by allergists and allergic patients (colloquially named "mold allergy") for a long time” [48]. Respiratory fungal allergy, including allergic rhinitis, allergic bronchitis, allergic conjunctivitis, and allergic sinusitis, usually results from exposure and hypersensitivity to airborne spores with the involvement of both arms of immunity [49].

The non-IgE-mediated clinical hypersensitivities are a heterogeneous group of diseases that mainly present in common, just a technically challenging diagnosis due to the lack of an easy-to-execute, automatable, pathognomonic laboratory marker [50]. “Long before the discovery of the reaginic activity of IgE, patients with asthma were classified into two overlapping categories: A) "extrinsic" when there was substantial evidence of hypersensitivity against at least one allergen, and B) "intrinsic" when there was no clear association with any suspected allergen” [51]. “The diagnosis of sensitization before the "IgE epoch" was performed by allergic skin tests, Complement fixation assays, and the research of precipitins” [52]. “Further, this classification was oversimplified by the research of specific IgE. Following this trend, the immunoassay investigation of immunoreactivity against *A. fumigatus* is mainly limited to the research of specific IgE and, sometimes, scarcely supplemented by the research of precipitins since this procedure is well-documented by the literature in patients with ABPA” [53]. However, the most significant activity against fungal antigens comes from the Immune System's Innate arm, represented by PAMPs and PRRs, which routine medical laboratories do not assay [54]. An *ex vivo* challenge test with a viable leukocyte buffy coat can theoretically explore every immune pathway. 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 [55]. However, as an observant of the final phenomenon, the LAIT does not indicate which pathways were involved [56-59].

This preliminary retrospective survey demonstrated an extensive range of results from the *ex vivo* challenge test monitored by LAIT against *A. fumigatus* extract in a group of patients with AR and/or Asthma. The condition presented by our patients is just the opposite of the conditions presented by patients with ABPA. While ABPA patients present innate immune incompetence and adaptive hypersensitivity, our patients appear to present innate immune hypersensitivity and adaptive immune incompetence to produce an active state of immunotolerance without medical help [60].

We routinely employ the LAIT 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 LAIT results suggest that most allergic patients present some immunoreactivity against *A. fumigatus* antigens, 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 does not prove that the complaints presented by the patient are due to the tested antigen. Indeed, the clinical diagnosis is performed by the responses to the *in vivo* challenges, the degree of colonization of the patient's environment, and the benefits of a change of ambient, an occasional antifungal or a desensitization treatment. More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of LAIT in diagnosing patients with *A. fumigatus* non–IgE-mediated hypersensitivity.

5. CONCLUSION

Our preliminary results show that the LAIT may differentiate diverse degrees of *ex vivo* immunoreactivity against the *A. fumigatus* extract in patients clinically diagnosed with non–IgE-mediated fungal respiratory allergies. The propaedeutic meaning of these results, however, must be established. More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of LAIT for the etiologic diagnosis of patients with fungal respiratory allergy suspected of presenting *A. fumigatus* 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 [61].

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhang Z, Reponen T, Hershey GKK. Fungal Exposure and Asthma: IgE and Non-IgE-Mediated Mechanisms. Curr Allergy Asthma Rep. 2016;16(12):86-86.
2. Chaudhary N, Marr KA. Impact of *Aspergillus fumigatus* in allergic airway diseases. Clin Transl Allergy. 2011;1(1):4.
3. Sarma UP, Bhetaria PJ, Devi P, Varma A. Aflatoxins: Implications on Health. Indian J Clin Biochem: IJCB. 2017;32(2):124-133.
4. Plascencia-Jatomea M, Susana M, Gómez Y, Velez-Haro JM. Chapter 8 - *Aspergillus spp*. (Black Mold). In Postharvest Decay, Bautista-Baños, Ed. Academic Press: San Diego. 2014:267-286.
5. O'Dea EM, Amarsaikhan N, Li H, Downey J, Steele E, Van Dyken SJ, et al. Eosinophils are recruited in response to chitin exposure and enhance Th2-mediated immune pathology in *Aspergillus fumigatus* infection. Infect Immun. 2014;82 (8):3199-205.
6. Spatafora JW, Aime MC, Grigoriev IV, Martin F, Stajich JE, Blackwell M. The fungal tree of life: From molecular systematics to genome-scale phylogenies. Microbiol Spectrum. 2017;5(5):10.
   DOI: 10.1128/microbiolspec.funk-0053-2016
7. Schoch CL, et al.NCBI Taxonomy: A comprehensive update on curation, resources and tools. Database (Oxford). 2020: Baaa062.
   Available:https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Tree&id=746128&lvl=3&lin=f&keep=1&srchmode=1&unlock
   Accessed on: 02/11/2023.
   PubMed: 32761142 PMC: PMC7408187.
8. Latgé JP. *Aspergillus fumigatus* and Aspergillosis. Clin Microbiol Rev. 1999; 12 (2):310-50.
9. Greenberger PA. Allergic bronchopulmonary Aspergillosis. J Allergy Clin Immunol. 2002;110(5):685-692.
10. Agarwal R, Bansal S, Chakrabarti A. Are allergic fungal rhinosinusitis and allergic bronchopulmonary aspergillosis lifelong conditions? Med Mycol. 2017;55 (1):87-95.
11. Chen H, Zhang X, Zhu L, An N, Jiang Q, Yang Y, et al. Clinical and immunological characteristics of *Aspergillus fumigatus*-sensitized asthma and allergic bronchopulmonary Aspergillosis. Front Immunol. 2022;13:939127.

12. Agarwal R, Maskey D, Aggarwal AN, Saikia B, Garg M, Gupta D, et al. Diagnostic Performance of Various Tests and Criteria Employed in Allergic Bronchopulmonary Aspergillosis: A Latent Class Analysis. PLOS ONE. 2013;8(4): e61105.
13. Pomés A, Davies JM, Gadermaier G, Hilger C, Holzhauser T, Lidholm J, et al. WHO/IUIS Allergen Nomenclature: Providing a common language. 2018;100: (3-13)
DOI: 10.1016/j.molimm.2018.03.003.
14. Allergen nomenclature Sub-committee from the World Health Organization and the International Union of Immunological Societies (WHO/IUIS)
Available:https://allergen.org/index.php
15. Latgé JP, Debeaupuis JP, Sarfati J, Diaquin M, Paris S. Cell wall antigens in *Aspergillus fumigatus*. Arch Med Res. 1993;24(3):269-74.
16. Wartenberg D, Lapp K, Jacobsen ID, Dahse H-M, Kniemeyer O, Heinekamp T, et al. Secretome analysis of *Aspergillus fumigatus* reveals Asp-hemolysin as a major secreted protein. Int J Med Microbiol. 2011;301(7):602-611.
17. Yokota K, Shimada H, Kamaguchi A, Sakaguchi O. Studies on the toxin of *Aspergillus fumigatus*. VII. Purification and some properties of hemolytic toxin (asp-hemolysin) from culture filtrates and mycelia. Microbiol Immunol. 1977;21(1): 11-22.
18. Madan T, Arora N, Sarma PU. Ribonuclease activity dependent cytotoxicity of Asp fl, a major allergen of *A. fumigatus*. Mol Cell Biochem. 1997;175(1-2):21-7.
19. Banerjee B, Greenberger PA, Fink JN, Kurup VP. Immunological Characterization of Asp f 2, a Major Allergen from *Aspergillus fumigatus* Associated with Allergic Bronchopulmonary Aspergillosis. Infection and Immunity. 1998;66(11):5175-5182.
20. Perez-Cuesta U, Aparicio-Fernandez L, Guruceaga X, Martin-Souto L, Abad-Diaz-de-Cerio A, Antoran A, et al. Melanin and pyomelanin in *Aspergillus fumigatus*: From its genetics to host interaction. Int Microbiology (Spanish). 2020;23(1):55-63.
21. Alghamdi NS, Barton R, Wilcox M, Peckham D. Serum IgE and IgG reactivity to *Aspergillus* recombinant antigens in patients with cystic fibrosis. J Med Microbiol. 2019;68(6):924-929.
22. Balloy V, Chignard M. The innate immune response to *Aspergillus fumigatus*. Microbes and Infection. 2009;11(12):919-927.
23. Luther K, Torosantucci A, Brakhage AA, Heesemann J, Ebel F. Phagocytosis of *Aspergillus fumigatus* conidia by murine macrophages involves recognition by the dectin-1 beta-glucan receptor and Toll-like receptor 2. Cell Microbiol. 2007;9(2):368-81.
24. Levitz SM, Diamond RD. Mechanisms of Resistance of *Aspergillus fumigatus* conidia to killing by neutrophils in vitro. J Infect Dis. 1985;152(1):33-42.
25. Maeda K, Caldez MJ, Akira S. Innate immunity in allergy. Allergy. 2018;74(9): 1660-1674.
26. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast Cell: A Multi-Functional Master Cell. Front Immunol. 2016;6(6):620.
27. Rajan TV. The Gell-Coombs classification of hypersensitivity reactions: a re-interpretation. Trends Immunol. 2003; 24(7):376-9.
28. Keir HR, Chalmers JD. Neutrophil extracellular traps in chronic lung disease: implications for pathogenesis and therapy. Eur Resp Rev. 2022;31(163):1-19.
29. 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.
30. Jutel M, Agache I, Zemelka-Wiacek M, Akdis M, Chivato T, Del Giacco S, et al. Nomenclature of allergic diseases and hypersensitivity reactions: Adapted to modern needs: An EAACI position paper. Allergy. 2023;78(11):2851-2874.
31. Kuratsuji T. Studies on leukocyte adherence inhibition test. Part II. Clinical applications of LAI test to detect delayed type hypersensitivity in infants and children. Keio J Med. 1981;30(2):65-9.
32. 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.
33. 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.

34. 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 Innate Non–IgE-mediated Immunoreactivity against *Alternaria alternata*. Asian J Immunol. 2023;6(1):243-251.
35. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non–IgE-mediated Immunoreactivity against *Saccharomyces cerevisiae*. Asian J Immunol 2023;6(1): 234-241.
36. 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 Non–IgE-mediated Immunoreactivity against *Candida albicans* in Patients with Atopic Dermatitis. Asian J Immunol 2023;6; (1):268-276.
37. 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.
38. 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.
39. 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.
Available:http://dx.doi.org/10.1155/2014/860427
40. Olivier CE, Pinto DG, Lima RPS, Silva MD, Santos RAPG, Teixeira APM, 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.
41. Olivier CE, Pinto DG, Santos RAPG, Lima RPS. Dextran's interference over the Leukocyte Adherence Inhibition Test. Academia Letter. 2021; article 3792.
42. 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.
43. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. 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.
44. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. 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.
45. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. 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.
46. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Immunoreactivity against Cobalt. Asian J Immunol. 2023;6(1):174-184.
47. Olivier CE, Pinto DG, Teixeira AP, Santana JL, Santos RA, Lima RP. Contribution of the Leukocyte Adherence Inhibition test to the diagnosis of Non—IgE-Mediated Immunoreactivity against Cochineal Dye. Asian Journal of Immunology. 2023;6(1): 185-95.
48. Burge HA. Airborne Allergenic Fungi: Classification, Nomenclature, and

Distribution. Immunol Allergy Clin North America. 1989;9(2):307-319.
49. Kurup VP, Shen H-D, Banerjee B. Respiratory fungal allergy. Microbes and Infection. 2000;2(9):1101-1110.
50. 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.
51. Rackemann FM. A clinical study of 150 cases of bronchial asthma. Arch Intern Med. 1918;2: 517–22.
52. 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.
53. Cadena J, Thompson GRT, Patterson TF. Aspergillosis: Epidemiology, Diagnosis, and Treatment. Infect Dis Clin North Am. 2021;35(2):415-434.
54. Park SJ, Mehrad B. Innate immunity to *Aspergillus* species. Clin Microbiol Rev. 2009;22(4):535-51.
55. 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. Biomedic J Sci Tech Res. 2021;36(3): 28647-28655.
56. Thomson DMP. Assessment of immune status by the leukocyte adherence inhibition test. Academic Press: New York, 1982;xvii:380.
57. 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.
58. Fink A, Heller L, Eliraz A, Weisman Z, Miskin A, Schlezinger M, et al. Allergen-specific leukocyte adherence inhibition (LAI) assay: sensitivity, specificity and mechanism. Immunol Lett. 1987;16(1):65-70.
59. 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.
60. Sahiner UM, Giovannini M, Escribese MM, Paoletti G, Heffler E, Alvaro-Lozano M, et al. Mechanisms of Allergen Immunotherapy and Potential Biomarkers for Clinical Evaluation. J Person Med. 2023;13(5):845.
61. WMA. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA. 2013;310(20): 2191-4.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/112345