Original Research Article

Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non–IgE-mediatedImmunoreactivity against *Saccharomyces cerevisiae*.

ABSTRACT

Aims: To evaluate the potential of the Leukocyte Adherence Inhibition Test (LAIT) to discriminatenon–IgE-mediatedinnate immunoreactivity against *Saccharomyces cerevisiae* in patients with clinical suspicion of allergic reaction to fungal allergens.
Study Design: We retrospectively examined the medical charts of 200 allergic outpatients with urticaria and/or atopic dermatitis associated with the ingestion of fermented food. The individuals were selected by being investigated with an *ex vivo* challenge monitored by LAIT against an extract of *S. cerevisiae*after undetectable specific IgE and not reactiveallergic skin tests to this allergen.
Place and Duration of Study: Instituto Alergoimuno de Americana – São Paulo – Brazil – between January 2018 and August 2023.
Methodology The percentage of Leukocyte Adherence Inhibition (LAI) promoted by the *ex vivo* challenges with *S. cerevisiae* extract was distributed in ranges through a cascade distribution chart to outline the variability of the results.
Results: The meanLAI was 52.5%; SD 24.7%, ranging from 0% to 98%; modes = 0% and 61% (each appeared six times). The cascade distribution chart showed a wide range of LAI results, suggesting that some patients had immunoreactivity against the *S. cerevisiae* allergens while others did not.
Conclusion: Our preliminary results support the conclusion that the*ex vivo* challenges with *S. cerevisiae*monitored by the LAIT can differentiate diverse degrees of non–IgE-mediatedimmunoreactivity against their antigens in allergic patients.

Keywords: Allergy; Saccharomyces cerevisiae; Atopic Dermatitis; Diagnosis; Hypersensitivity; Leukocyte Adherence Inhibition Test; Non–IgE-mediated Immunoreactivity; Urticaria.

1. INTRODUCTION

Saccharomyces cerevisiae (*S. cerevisiae*) is an Ascomycota fungus from the family Saccharomycetaceae from the order Saccharomycetales [1].*S. cerevisiae*is a unicellular budding yeast that evolved for millennia through anthropogenic domestication, purposed to produce fermentative foods, beverages, and fuel[2]. Around the world, several lineages (strains) are adapted to ferment bread, cheese, beer, wine, spirits, and biodiesel [3]. Under adequate conditions, yeast cells can double in numberevery 100 minutes,fermenting the substrate to obtain energy, leaving the residues searched for by humans [4]. In the absence of oxygen, anaerobic fermentation produces ethanol. In the presence of oxygen, aerobic fermentation reduces ethanol to carbon dioxide, which is purposed for raising dough [5]. *S. cerevisiae* is also empirically employed as a nutraceutical and a nutritional supplement due to its content in proteins, vitamins, and minerals [6, 7]. Despite its

allergenicity and invasive capacity, the pharmaceutical industryisbuilding efforts toemploy probiotic strains and prebiotic derivatives of *S. cerevisiae*to treat allergic diseases, dysbiosis, and metabolic syndromes[8-12].

The *S. cerevisiae* cell walls are a composite of glucan and chitin fibers held together by proteinsand mannan[13, 14].*S. cerevisiae* produces more than 20 allergenic proteins that elicit specific IgE production by allergic humans [15]. However, the main immunogenic glycoprotein antigen of *S. cerevisiae*is a 200 kDa glycoprotein (gp200),which elicitsthe production of IgG and IgA anti-*Saccharomyces cerevisiae*specific antibodies [16].Anti-*Saccharomyces cerevisiae* Antibodies (ASCA) are a characteristic feature of diverse inflammatory diseases quantified by various techniques [17]. Strong IgG responses against *S. cerevisiae* and *Candida albicans* are also commonly observable in atopic patients [18].*S. cerevisiae*may elicit the production of specific IgE against protein allergens, such as enolase, invertase, and glucosidase[19, 20]. As a fungus, *S. cerevisiae* also possesses beta-glucans, a Pathogen-Associated Molecular Pattern (PAMP), recognized by innate receptors known as Pattern Recognition Receptors (PRRs) such as the Dectin-1, a primary β-glucan receptor [21]. Through a Dectin-1 agonism, the *S. cerevisiae* glucans stimulates several immune responses, including the production of IgG antibodies [22].Mannan is also aPAMP, acting as aninnate immune target of the *S. cerevisiae* cell wall. Mannan is a polysaccharide composed mainly of D-mannose associated with proteins, glycopeptides, phosphates, and sugars [23, 24].Mannan constitution and antigenicity may vary among strains, eliciting different immune reactions depending onthe hosts' sensitivity[25].The main (but not unique) mannan-interacting surface protein in mammal innate immune cells is the Mannose Receptor (MR), also assigned as the cluster of differentiation number CD206. The mannose receptor is an intricate part of innate immunity and the homeostatic clearance system [26]. Mannan receptors are not specific and may participate in the uptake of unrelated allergens such as Der p 1 and Fel d 1 [27, 28]. Besides innate recognition, mannan may also be recognized by specific IgE antibodies [15]. Chitin, a long-chain polymer of N-acetylglucosamine, is a primary component of cell walls in fungi, arthropods' exoskeletons, mollusks, nematodes, and non-mammalian vertebrates [29, 30]. *S. cerevisiae*chitin is also a PAMP sensed by the human immune system by specific membrane-bound PRRs, inducing trained immunity [31, 32].*Ex vivo* challenges of S. cerevisiae performed with viable Peripheral Blood Mononuclear Cells (PBMC) as well as blood-circulating conventional and plasmacytoid Dendritic Cells demonstrated the production of inflammatory cytokines such as IL-6, IL-10, and IFN-α [33].

While modern scientists are searching for new technologies, they are still helpless to be used in the clinical set and unsuitable for our purposes[34-36].The best option yet is to employ the ancient techniques under the optical of the new knowledge provided by recent scientific advances.

The Leukocyte Adherence Inhibition Test (LAIT) is an immunoassay designed by Halliday to detect antigen-specific immunoreactivity [37-41]. When put in contact with an immunoreactive antigen, leukocytes lose their ability to adhere to glass, exhibiting an easily observable response [39]. Several experimental works associated these responses to clinical and symptomatic non–IgE–mediated hypersensitivity to diverse antigens, including fungi allergens [43-46].

To evaluate the potential of the LAIT to reproduce non-IgE-mediated innate immunoreactivity against the *S. cerevisiae* allergens, we retrospectively examined the medical charts of patients investigated with an *ex vivo* challenge monitored by LAIT against an *S. cerevisiae* extract. These patients, diagnosed with urticaria and/or atopic dermatitis, had clinical suspicion of allergic reactions to fungal allergens, had non-reactive skin tests, and undetectable specific IgE for *S. cerevisiae*.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 05/2023), we proceeded with the electronic chart review of 7,600 allergic patients who attended our outpatient facility from January 2018 to August2023. A cohort of 200 patients had been submitted to an *ex vivo* allergen challenge test with *S. cerevisiae* extract monitored with LAIT. The cohort counted 67 males; mean age 47.8 years; SD 18.5 years; range 18 to 88 years; modes = 34, 43, 52, and 68 years (each appeared seven times). We offer this procedure to patients with urticaria and/or atopic dermatitis associated with the ingestion of fermented food who had an inconclusive investigation performed with allergic skin tests and undetectable specific IgE against *S. cerevisiae* performed with ImmunoCAP® [47].

2.2 Preparation of the *S. cerevisiae* extract

In a beaker were added 50 mg of *S. cerevisiae* (purchased in powder form from a local food merchant) and 25 mL of extracting solution (Propylparaben 0.5g; Methylparaben 1g; Sorbitol 30g; NaCl 5g; $NaHCO_3$ 2.5g; 1,000 mL H_2O). The sample was homogenized and left for 48 hours at four °C for protein extraction. After 48 hours, the supernatant was diluted in 25 mL of antigen dilution solution (NaCl 10g; KH_2PO_4 0.72g; Na_3PO_4 2.86g; methylparaben 1g; propylparaben 0.5g; glycerin 400mL; H_2O 600mL). This solution was used for the execution of the LAIT and the immediate skin tests, done as previously reported.

2.3 *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

We performed the LAIT as previously described [48-58]. Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with *S. cerevisiae* 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 (or without, as used as control) antigen extract (10µL of a solution with 1mg/mL and pH 7.5). 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 groups and the LA from the unchallenged control group: LAR = LA of the challenged sample divided by LA of unchallenged control sample multiplied by 100 (%). To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from 100 (%). We employed the LAI results for the statistics calculations and the cascade distribution chart.

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 meanLAI was 52.5%; SD 24.7%, ranging from 0% to 98%; modes = 0% and 61% (each appeared six times).

There was a wide range of distribution of LAI results, as outlined by the cascade distribution chart in Figure 1. Six patients (3% of the tests) ignored the presence of the allergen on the plasma and presented no inhibition of leukocyte adherence after contact with the *S. cerevisiae* extract. Some patients displayed low or moderate immunoreactivityduring the *ex vivo* challenge test against the *S. cerevisiae* extract. In contrast, others showed a strong immunoreactivity, which possibly would reflect the allergic symptoms after exposure to the allergen.

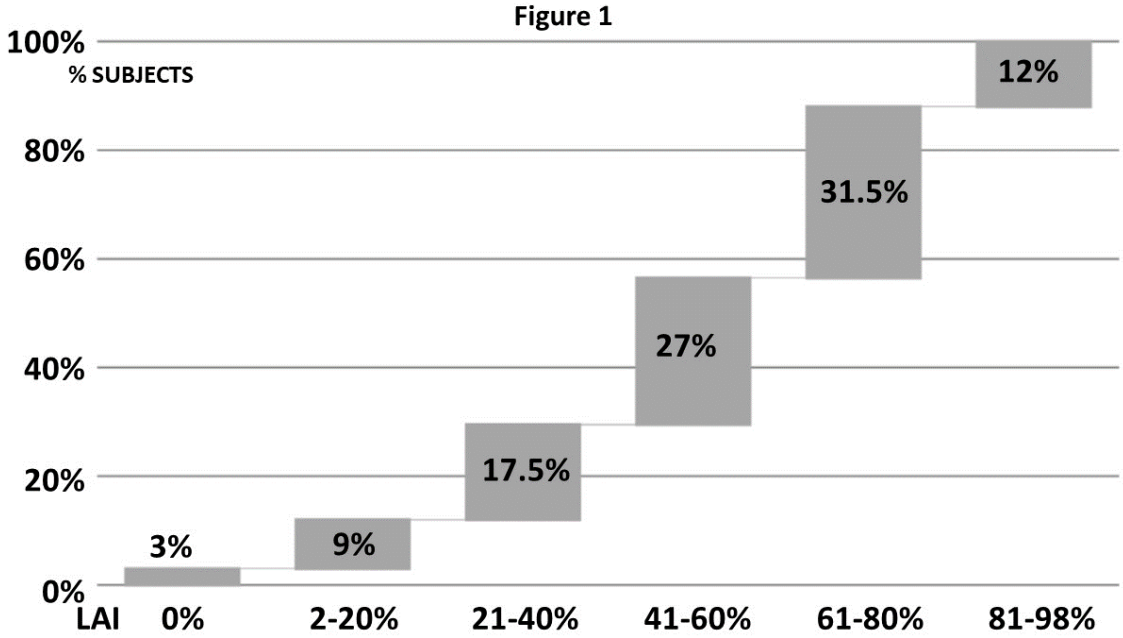

Fig. 1. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivoS. cerevisiae* extract challenges monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective percentage of outcomes over 200 tests (y-axis).

4. DISCUSSION

Hypersensitivity reactions result from the interaction and signal-amplification activities of a group of cytokines initially associated with the adaptive T helper 2 activity [59]. Further studies discovered that Th2 cytokines also influenced a particular group of innate immune cells that respond to their presence, amplifying their influence; these cells were denominated type 2 Innate Lymphoid Cells (ILC2s) [60]. The main stimuli to activate the ILC2s are the alarmins, cytokines activated by microbial activity near the mucosal borders or released by damaged epithelium [61].Type 2 ILCs, as theirs Th2 adaptive counterparts, release cytokines such as IL-4, IL-5, IL-9, and IL-13 [62].

Besides the discovery of Innate Cells with Adaptive Immune Functions, researchers recently reported Adaptive Immune Cells with Innate Immune Functions, which is the case of the Mucosal-Associated Invariant T cells (MAIT) that exhibit rapid effector responsesand can be activated by both T-cellantigen receptor (TCR)-dependent and TCR-independentmechanisms [63]. These MAIT cells are particularly stimulated by gut flora yeasts such as *S.cerevisiae and Candida albicans,* promoting a proinflammatory intestinal and systemic state [64]. This innate recognition turns *S. cerevisiae* into a potential model for studying ex vivo challenges with fresh blood buffy coats or viable PBMC to evaluate non–IgE–mediated innate Th2-like allergic reactions [65, 66]. Nowadays, the standard medical practice is not able to associate these innate pathways to specific clinical conditions since it has not yet incorporated suitable laboratory techniques able to identify and document causal innate mechanisms into its complementary arsenal. At a scientific research level, there are laborious and expensive laboratory techniques, such as the Basophil Activation Test, the Lymphocyte Stimulation Test, or the Leukocyte Migration Inhibition Test, which are not affordable by the traditional clinical lab bench [67-72].

The *ex vivo* challenge test performed with the fresh blood buffy coat allows the interaction of the allergen with all types of peripheral blood immune cells, making possible the participation of reactions orchestrated by adaptive immune cells, innate immune cells, alarmins, cytokines, and adaptive (IgE and Non-IgE) antibody-mediated reactions [73, 74].

The LAIT is an affordable and quick *ex vivo* challenging laboratory technique able to establish a relationship between an allergen and a non–IgE-mediated hypersensitivity immune response

reasonably liable for the symptoms of a particular allergic patient. Since the LAIT solely registers the final resultant phenomenon, the leukocytes' glass-adherence inhibition after contact with tested antigen, it does not explicitly testify for any pathway [75-78].

This preliminary retrospective survey has demonstrated a full range of results from the *ex vivo* challenges against *S. cerevisiae* extract in a group of allergic patients, implying that some patients already had a previous more or less intense immunological experience with their antigens. We employed LAIT as a complementary triage test, mainly when the specific IgE is undetectable, to select worthwhile antigens to proceed with the more laborious *in vivo* provocations. These results suggest that prospective studies with larger double-blind cohorts must be necessary to evaluate the potential contribution of LAIT in the clinical management of patients with *S. cerevisiae* non–IgE-mediated innate hypersensitivity.

5. CONCLUSION

As isolated information, the LAIT positivity does not necessarily prove that the symptoms that motivate the patient to seek medical help happened due to this specific tested antigen. The clinical diagnosis, instead, is better accomplished by the responses to the *in vivo* challenges, the real-world consumption of the agent by the patient, the exclusion of the allergen's source from the patient's diet, and the close observance of the symptoms after its re-introduction. Our preliminary results support that the LAIT may differentiate diverse degrees of *ex vivo*non–IgE-mediated innate immunoreactivity against the *S. cerevisiae* extract, indicating a previous immune experience with this agent. Depending on the intensity of the resulting *ex vivo* interaction, investing in the *in vivo* challenges will be further worthing (or not).

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[79].

ETHICAL APPROVALS

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

REFERENCES

1. Beldal, Ruiz J, Santos A, Van Wyk N, Pretorius IS.*Saccharomyces cerevisiae*. Trends in Genetics. 2019;35(12):956-957.
2. Legras JL, Galeote V, Bigey F, Camarasa C, Marsit S, Nidelet T et al. Adaptation of *S. cerevisiae* to Fermented Food Environments Reveals Remarkable Genome Plasticity and the Footprints of Domestication. Mol Biol Evol. 2018;35(7):1712-1727.
3. Liti G, Carter DM, Moses AM, Warringer J, Parts L, James SA, et al. Population genomics of domestic and wild yeasts. Nature. 2009;458(7236):337-41.
4. Herskowitz I. Life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiol Rev. 1988;52(4):536-53.
5. Pronk JT, Yde-Steensma H, Van-Dijken JP. Pyruvate metabolism in *Saccharomyces cerevisiae*. Yeast. 1996;12(16):1607-33.
6. Moyad MA. Brewer's/baker's yeast (*Saccharomyces cerevisiae*) and preventive medicine: part I. Uro Nurs. 2007;27(6):560-1.
7. Hosseinzadeh P, Javanbakht MH, Mostafavi SA, Djalali M, Derakhshanian H, Hajianfar H, et al. Brewer's Yeast Improves Glycemic Indices in Type 2 Diabetes Mellitus. Int JPrev Med. 2013:4(10): 1131-8.

8. Lew DB, Michael CF, Overbeck T, Robinson WS, Rohman EL, Lehman JM, et al.Beneficial Effects of Prebiotic *Saccharomyces cerevisiae* Mannan on Allergic Asthma Mouse Models. J Immunol Res. 2017;3432701.
9. Sun S, Xu X, Liang L, Wang X, Bai X, Zhu L, et al.Lactic Acid-Producing Probiotic *Saccharomyces cerevisiae* Attenuates Ulcerative Colitis via Suppressing Macrophage Pyroptosis and Modulating Gut Microbiota. Front Immunol. 2021;12:777665.
10. Enache-Angoulvant A, Hennequin C. Invasive *Saccharomyces* infection: a comprehensive review. Clin Infect Dis. 2005;41(11):1559-68.
11. Llopis S, Hernández-Haro C, Monteoliva L, Querol A, Molina M, Fernández-Espinar MT.Pathogenic potential of *Saccharomyces* strains isolated from dietary supplements. PLoS One 2014, 9 (5), e98094.
12. Ramos LS, Mokus L, Frota HF, Santos MV, Oliveira SSC, Oliveira MME, et al. SARS-CoV-2 Post-Infection and Sepsis by *Saccharomyces cerevisiae*: A Fatal Case Report-Focus on Fungal Susceptibility and Potential Virulence Attributes. Trop Med Inf Dis. 2023;8(2).
13. Roelofsen PA. Yeast mannan, a cell wall constituent of baker's yeast. Bioch Bioph Acta 1953;10:477-478.
14. Klis FM, Mol P, Hellingwerf K, Brul S. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 2002;26(3):239-256.
15. Kortekangas-Savolainen O, Kalimo K, Lammintausta K, Savolainen J. IgE-binding components of baker's yeast (*Saccharomyces cerevisiae*) recognized by immunoblotting analysis. Simultaneous IgE binding to mannan and 46-48 kD allergens of *Saccharomyces cerevisiae* and *Candida albicans*. Clin Exp Allergy. 1993:23(3):179-84.
16. Heelan BT, Allan S, Barnes RMR. Identification of a 200-kDa glycoprotein antigen of *Saccharomyces cerevisiae*. Immunol Lett. 1991;28(3):181-185.
17. OlivierCE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) researched by tube precipitins are elevated in patients with dermatologic and gastrointestinal non–IgE–mediated hypersensitivity. Eur J Clin Med. 2023:4(2):25-30.
18. Savolainen J, Kortekangas-Savolainen O, Nermes M, VianderM, Koivikko A, Kalimo K, et al.IgE, IgA, and IgG responses to common yeasts in atopic patients. Allergy. 1998;53(5):506-512.
19. Nittner-Marszalska M, Wójcicka-Kustrzeba I, Bogacka E, Patkowski J, Dobek R. Skin prick test response to enzyme enolase of the baker's yeast (*Saccharomyces cerevisiae*) in diagnosis of respiratory allergy. Med Sci Monit. 2001;7(1):121-4.
20. Horner WE, Armstrong M, El-Dahr J, McCants M, Reese G, Kobernick AK. Prevalence of IgE reactivities in mold-allergic subjects to commercially available fungal enzymes. Allergy Asthma Proc. 2008;29(6):629-35.
21. Kumar H, Kawai T, Akira S. Pathogen Recognition by the Innate Immune System. Int Rev Immunol. 2011;30(1):16-34.
22. Park HY, Yoon HK, Kim JY, Park SR. Heat-Killed *Saccharomyces cerevisiae*, A Dectin-1 Agonist, Selectively Induces IgG4 Production by Human B Cells. Immune Netw 2018;18(6):e46.
23. Mill PJ. Phosphomannans and other components of flocculent and non-flocculent walls of *Saccharomyces cerevisiae*. J Gen Microbiol. 1966;44(3):329-41.
24. Sentandreu R, Northcote DH. The structure of a glycopeptide isolated from the yeast cell wall. Biochem J. 1968;109(3):419-32.
25. Cawley TN, Ballou CE. Identification of two *Saccharomyces cerevisiae* cell wall mannan chemotypes. J Bacteriol. 1972;111(3):690-5.
26. East L, Isacke CM. The mannose receptor family. Bioch Bioph Acta. 2002;1572(2):364-386.

27. Deslée G, Charbonnier AS, Hammad H, Angyalosi G, Tillie-Leblond I, Mantovani A, et al. Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. J Allergy Clin Immunol. 2002;110(5):763-70.
28. Emara M, Royer PJ, Abbas Z, Sewell HF, Mohamed GG, Singh S, et al. Recognition of the major cat allergen Fel d 1 through the cysteine-rich domain of the mannose receptor determines its allergenicity. J Biol Chem. 2011;286(15):13033-40.
29. Yang J, Zhang KQ. Chitin Synthesis and Degradation in Fungi: Biology and Enzymes. Adv Exp Med Biol. 2019;1142:153-167.
30. Tang WJ, Fernandez J, Sohn JJ, Amemiya CT. Chitin is endogenously produced in vertebrates. Curr Biol. 2015;25(7):897-900.
31. Elieh-Ali-Komi D, Sharma L, Dela-Cruz CS. Chitin and Its Effects on Inflammatory and Immune Responses. Clin Rev Allergy Immunol. 2018;54(2):213-223.
32. Rizzetto L, Ifrim DC, Moretti S, Tocci N, Cheng SC, Quintin J, et al. Fungal Chitin Induces Trained Immunity in Human Monocytes during Cross-talk of the Host with *Saccharomyces cerevisiae*. J Biol Chem. 2016;291(15):7961-72.
33. Sabatini A, Guerrera G, Corsetti M, Ruocco G,deBardi M, Renzi S, et al. Human Conventional and Plasmacytoid Dendritic Cells Differ in Their Ability to Respond to *Saccharomyces cerevisiae*. Front Immunol. 2022;13:850404.
34. Stamate S, Chirilov D, Dumitrașcu I, Bogdan Imbrea B, State O. DNA-FET transistors and some applications. Advanced Nano-Bio-Materials and Devices; 2023:7(2):710-723.
35. Ravariu, C. From Enzymatic Dopamine Biosensors to OECT Biosensors of Dopamine. Biosensors, 2023, 13(8):1-21.
36. Hamza M.N, Abdulkarim YI, Saeed SR, Altıntaş O, Mahmud RH, Appasani B et al. Low-Cost Antenna-Array-Based Metamaterials for Non-Invasive Early-Stage Breast Tumor Detection in the Human Body. Biosensors 2022;12:828.
37. Halliday WJ, Miller S. Leukocyte adherence inhibition: a simple test for cell-mediated tumour immunity and serum blocking factors. Int J Cancer. 1972;9(3):477-83.
38. Halliday WJ. Historical Background and Aspects of the Mechanism of Leukocyte Adherence Inhibition. Cancer Res 1979;39(2):558-563.
39. 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.
40. Nogueira-Machado JA, Novato-Silva E, Souza M, Kritz GV. Schistosoma mansoni: cell-mediated immunity evaluated by antigen-induced leukocyte adherence inhibition assay. Immunol Lett.1985;9(1):39-42.
41. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPG, 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.
42. Powell AE, Sloss AM, SmithRN. Leukocyte-Adherence Inhibition: A Specific Assay of Cell-Mediated Immunity Dependent on Lymphokine-Mediated Collaboration between T Lymphocytes. J Immunol.1978;120(6):1957-66.
43. Barrett NA, Maekawa A, Rahman OM, Austen KF, Kanaoka Y. Dectin-2 Recognition of House Dust Mite Triggers Cysteinyl Leukotriene Generation by Dendritic Cells. J Immunol. 2009;182(2):1119-1128.
44. Thomson DM, Grosser N. Immunological mechanisms of tube leukocyte adherence inhibition. Cancer Res.1979;39(2):576-81.
45. 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. Biomed J Scient Tech Res. 2021;36(1):28647-55.

46. Walters BA, Chick JE, Halliday WJ. Cell-mediated immunity and serum blocking factors in patients with chronic dermatophytic infections. Int Arch Allergy Appl Immunol. 1974;46(6):849-57.
47. 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. The Open Allergy J. 2013;6:9-17.
48. 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.
49. 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.
50. Olivier CE, Pinto DG, Lima RPS, SilvaMD, 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.
51. Olivier CE, Pinto DG, Santos RAPG, Lima RPS.Dextran's interference over the Leukocyte Adherence Inhibition Test. Academia Letter 2021; Article number 3792.
52. 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 JClin Med. 2021;2(6):45-50.
53. 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.
54. 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.
55. 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.
56. 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.
57. 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.
58. 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.
59. FortMM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al.IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity. 2001;15(6):985-95.
60. Voehringer D, Reese TA, Huang X, Shinkai K, Locksley RM. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. J Exp Med. 2006;203(6):1435-46.
61. Martinez-Gonzalez I, Mathä L, Steer CA, Ghaedi M, Poon GF, Takei F. Allergen-Experienced Group 2 Innate Lymphoid Cells Acquire Memory-like Properties and Enhance Allergic Lung Inflammation. Immunity. 2016;45(1):198-208.
62. Lloyd CM, Snelgrove RJ. Type 2 immunity: Expanding our view. Sci Immunol. 2018;3:25.

63. Godfrey DI, Koay HF, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. Nat Immunol 2019;20(9):1110-1128.
64. Gargano F, Guerrera G, Piras E, Serafini B, Di Paola M, Rizzetto L, et al.Proinflammatory mucosal-associated invariant CD8+ T cells react to gut flora yeasts and infiltrate multiple sclerosis brain. Front Immunol. 2022;13:890298.
65. Bose N, Wurst LR, Chan AS, DudneyCM, LeRoux ML, Danielson ME,et al. Differential regulation of oxidative burst by distinct β-glucan-binding receptors and signaling pathways in human peripheral blood mononuclear cells. Glycobiol. 2014;24(4):379-91.
66. Mondragão-Rodrigues I, Macedo MF. Buffy Coat Processing Impacts on Monocytes' Capacity to Present Lipid Antigens. Biomed. 2023;11:3.
67. Sugimoto N, Yamaguchi M, Tanaka Y, Nakase Y, Nagase H, AkiyamaH, et al. The basophil activation test identified carminic acid as an allergen inducing anaphylaxis. J. Allergy Clin Immunol In Practice. 2013;1(2):197-199.
68. Chamani S, Mobasheri L, Rostami Z, Zare I, Naghizadeh A, Mostafavi E. Heavy metals in contact dermatitis: A review. J Trace Elem Med Biol. 2023;79:127240.
69. Nordqvist B, Rorsman H. Leucocytic migration in vitro as an indicator of allergy in eczematous contact dermatitis. Trans St Johns Hosp Dermatol Soc. 1967;53(2):154-9.
70. George M, Vaughan JH. In vitro cell migration as a model for delayed hypersensitivity. Proc Soc Exp Biol Med. 1962;111:514-21.
71. Mirza AM, Perera MG, Maccia CA, Dziubynskyj OG, Bernstein IL. Leukocyte Migration Inhibition in Nickel Dermatitis. Int Arch Allergy Applied Immunol. 2009;49(6):782-788.
72. Popple A, Williams J, Maxwell G, Gellatly N, Dearman RJ, Kimber I. The lymphocyte transformation test in allergic contact dermatitis: New opportunities. J Immunotoxicol. 2016;13(1):84-91.
73. Shirakawa T, Kusaka Y, Fujimura N, Goto S, Morimoto K. The existence of specific antibodies to cobalt in hard metal asthma. Clin Exp Allergy. 1988;18(5):451-460.
74. 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 Scient Tech Res. 2021;36(3):28647-55.
75. Thomson DMP. Assessment of immune status by the leukocyte adherence inhibition test. Academic Press: New York. 1982; p xvii, 380 p.
76. 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.
77. Fink A, Heller L, Eliraz A, WeismanZ, Miskin A, Schlezinger M, et al. Allergen-specific leukocyte adherence inhibition (LAI) assay: sensitivity, specificityand mechanism. Immunol Lett. 1987;16(1):65-70.
78. 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. Cellular Immunol 1974;10(3):467-475.
79. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.