Contribution of Leukocyte Adherence Inhibition Test in the evaluation of non–IgE-mediated immunoreactivity against peanut proteins in children and adults with Atopic Dermatitis

ABSTRACT

Aim: To evaluate the potential of the Leukocyte Adherence Inhibition Test (LAIT) to evaluate immunoreactivity against peanut proteins in patients with a clinical diagnosis of non–IgE-mediated Atopic Dermatitis (AD).
Study Design: We retrospectively examined the medical charts of a population of 51 children (0 to 17 years) and 275 adults (18 to 93 years) diagnosed with non–IgE-mediated AD who were investigated with an *ex vivo* challenge monitored by LAIT against peanut proteins.
Place and Duration of Study: Instituto Alergoimuno de Americana – São Paulo – Brazil – between January 2018 and February 2024.
Methodology The percentage of Leukocyte Adherence Inhibition (LAI) promoted by the *ex vivo* challenges with peanut proteins was distributed in ranges through a cascade distribution chart to outline the variability of the results.
Results: In the child cohort, the LAI mean was 48.5%; the median was 52%; SD 27.6%; ranging from 0% to 95%; modes = 0% and 63% (each appeared four times). There was a normal wide range of distribution of LAI results, as outlined by the cascade distribution chart. In the adult cohort, the LAI mean was 43.8%; the median was 46%; SD 28.6%; ranging from 0% to 100%; modes = 0% (appeared 45 times). There was a normal wide range of distribution of LAI results, as outlined by the cascade distribution chart.
Conclusion: Our preliminary results support the idea that the LAIT performed with peanut extract may differentiate diverse degrees of ex vivo non–IgE-mediated immunoreactivity against peanut allergens in non–IgE-mediated AD patients, suggesting a potential contribution of LAIT in the stratification of endotypes and the phenotypic classification of non–IgE-mediated AD patients.

Keywords: Adults; Allergy; Atopic Dermatitis; Children; Diagnosis; Endotypes; Food Allergy; Hypersensitivity; Leukocyte Adherence Inhibition Test; Non–IgE-mediated Immunoreactivity; Peanut allergy; Phenotypes, Predictive Biomarker.

Abbreviations:
AD: Atopic Dermatitis
LAI: Leukocyte Adherence Inhibition
LAIT: Leukocyte Adherence Inhibition Test

1. INTRODUCTION

Peanut allergy is considered persistent, affecting 1% to 4.5% of the studied populations; however, fatal anaphylaxis is rare[1].Peanut allergy awarenessis increasing among patientsas diagnostics tools are being improved [2].Peanuts(*Arachis hypogaea*)contain at least 18 proteins

classified among different allergen families (Ara h 1 to 18) [3]. Usually, the allergenicity of the peanut's proteins is studied according to their structure and capacity to elicit antigen-antibody responses [4].“The major peanut allergen, Ara h 2, functions as a trypsin inhibitor, and roasting enhances this function”[5]. “This interference with protein digestion may represent a major determinant of peanut allergenicity. Molecules over 150 Da, at physiologic conditions, do not permeate through enterocytes into the bloodstream”[6]. “However, under inflammatory conditions, a pathological hyperpermeability state allows the permeation of bigger molecules among the damaged enterocytes”[7].

Most published studies about peanut allergy have approached this predicament in the field of IgE-mediated hypersensitivity, while a limited number of them were concerned with the Non–IgE-mediated hypersensitivity [8]. Peanut proteins can elicit the production of IgG and IgE-specific antibodies [9]. Peanut-specific levels of IgG are higher in peanut-allergicthan in non–peanut-allergicchildren[10]. The immune complexes formed among peanut proteins with specific IgG and IgE also interact with activated charcoal, making it a potential therapeutics to treat accidental peanut ingestion by peanut-allergic patients [11].

The dual allergen exposure hypothesis associates peanut allergy, particularly Atopic Dermatitis (AD), with a dual sensitization mechanism through oral ingestion and cutaneous absorption [12]. Unlike the IgE-mediated peanut allergy, which usually manifests itself as an acute reaction, the non–IgE-mediated food allergy may become a chronic condition (if not diagnosed) since the affected subjects may persist indefinitely with the ingestion of the allergenic foods, unaware of the pernicious effect on their immune system [13].

AD is one of the most studied non–IgE-mediated hypersensitivity phenotypes associated with peanut allergy[14]. The tube research of precipitins is a simple and promising methodology to evaluate the presence of antibodies against peanut proteins in the serum of patients with AD [15]. The knowledge of the intrinsic pathways of the immune responses elicited by peanut proteins is essential to define the endotype diagnosis and establish better treatment strategies [16].

At the clinical set, the diagnosis of IgE-mediated peanut allergy is an easy task, accomplished by anamnesis, skin tests, and the laboratory research of specific IgE; however, when employing a multi-omics approach, several clinical phenotypes and endotypes may be differentiated from the primary type I hypersensitivity classification [17]. The main controversy about diagnosing peanut allergy is the gold standard test (*in vivo* oral challenge), which carries a risk of anaphylaxis and requires expensive resources [18]. Although not yet implemented into the routine, the functional immune assays using *ex vivo* challenge with living cells are a promising approach to further understanding the complexities of food allergy endotype-driven allergic reactions [19].

As a proof of concept, to evaluate the potential as a predictive biomarker of the Leukocyte Adherence Inhibition Test (LAIT) to evaluate immunoreactivity against peanut proteins, we retrospectively examined the medical charts of patients with a clinicaldiagnosis of ADandany level of suspicion of non–IgE-mediatedallergy to peanuts. These patients had no reactive (or inconclusive) skin tests to peanut extract or undetectable specific IgE for peanuts and were investigated with an *ex vivo* challenge monitored by LAIT against a peanut extract prepared at our facility.

The present study hypothesizes that the LAIT may differentiate diverse degrees of immunoreactivity against peanut proteins among patients suffering from AD.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 02/2024), we proceeded with the electronic chart review of 8,400 allergic patients who attended our outpatient facility from January 2018 to February 2024. “A cohortof 326 patients with AD was submitted to an *ex vivo* allergen challenge test with peanut extract monitored with LAIT”. [53] This cohort was separated into two groups by age. The child cohort (0 to 17 years) consisted of 51 patients with 27 males; mean age 7.4 years; median agesix years; SD 5.34 years; mode = 6 years (appeared six times). The adult cohort (18 to 93) consisted of 275 patients with 102 males; mean age 50.9 years; median age52 years; SD 19.38 years; mode = 25 years (appeared 12 times). This procedure was offered to patients with atopic

dermatitis, who were submitted to an inconclusive investigation performed with allergic skin tests and undetectable specific IgE against peanuts, performed with ImmunoCAP® [20].

2.2 Antigen preparation

Unroasted, shelled, skinned, and desalted peanuts for the cutaneousand *ex vivo* challenge tests were bought at a local marketplace, extracted, and purified at our laboratory.The peanuts (100g) were crushed with extractor solution (Coca's solution: propylparaben 0.5g, methylparaben 1g, sorbitol 30g, NaCl 5g, $NaHCO_3$ 2.5g, 1,000mL H_2O) [21]. After homogenization, the sample was kept in the refrigerator for 48 hours to extract the proteins. The sample solution was centrifuged (10 min, 4000 rpm) and filtered (80g filter paper) five times. The protein quantification of the allergen extracts was done according to Bradford's protein-dye binding methodology and stored at 4°C [22]. The protein in the sample was diluted in antigen dilution solution (NaCl 10g, KH_2PO_4 0.72g, Na_3PO_4 2.86g, methylparaben 1g, propylparaben 0.5g, glycerin 400mL, H_2O 600mL) to obtain 1mg/mL, concentration used to perform the LAIT and skin tests.

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

The LAIT was performed as previously described [23-31].

3. RESULTS

"As a retrospective survey, there was no research protocol; therefore, we reported the incidental immune investigation as registered in the digital medical charts". [53]

In the child cohort, the LAI mean was 48.5%; the median was 52%; SD 27.6%, ranging from 0% to 95%; modes = 0% and 63% (each appeared four times). There was a normal and wide distribution range of LAI results, as outlined by the cascade distribution chart in Figure 1.

In the adult cohort, the LAI mean was 43.8%; the median was 46%; SD 28.6%; ranging from 0% to 100%; modes = 0% (appeared 45 times). There was a normal and wide distribution range of LAI results, as outlined by the cascade distribution chart in Figure 2.

Four child patients (7.8%) ignored the presence of the allergen on the plasma and presented no inhibition of leukocyte adherence after contact with peanuts. Adults showed a more significant percentage of absolute non-responders (16.3%). Some patients showed low or moderate immunoreactivity during the ex vivo challenge test against peanut proteins, while others showed strong immunoreactivity,possibly explaining the allergic symptoms after exposure to the allergen.

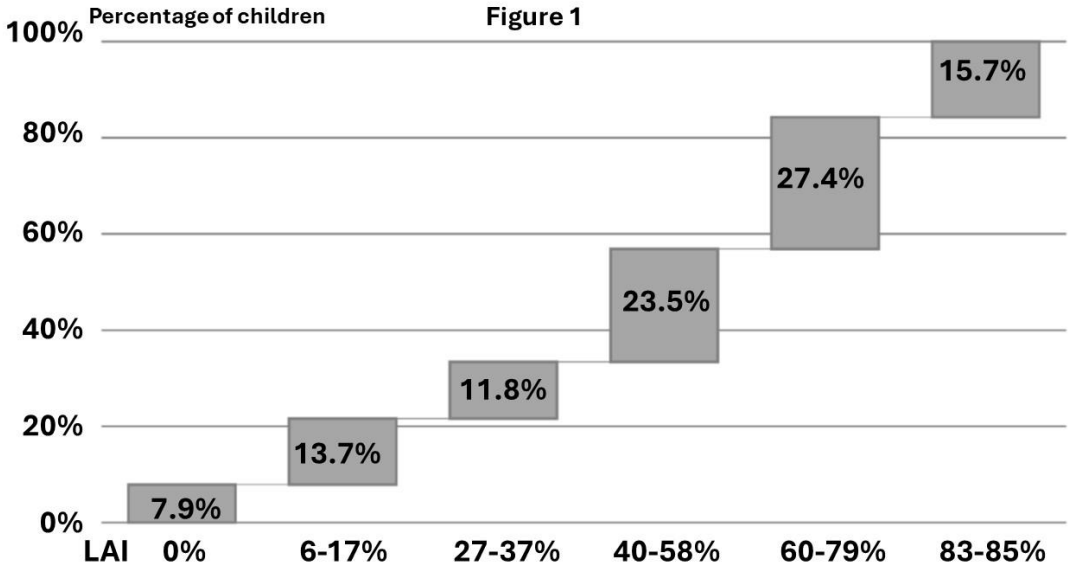

Fig. 1. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo* peanut extract challenges monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over 51Atopic Dermatitis childpatients (0 to 17 years) subjects (y-axis).

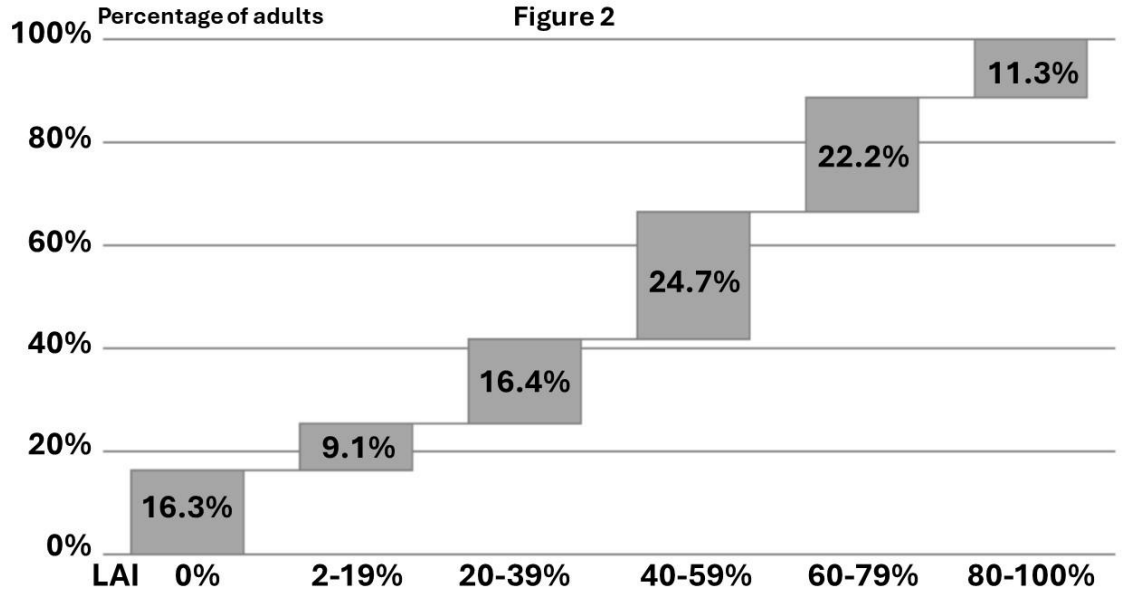

Fig. 2. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo* peanut extract challenges monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over 275 Atopic Dermatitis adult patients (18 to 93 years) subjects (y-axis)

4. DISCUSSION

Diverse AD phenotypes and endotypes associated with several predictive biomarkers are described as initiators of the so-called "atopic march," which usually refers to the co-expression and progression of allergic diseases from childhood to adulthood [32].

The need for predictive biomarkers associated with non–IgE-mediated immunoreactivity against peanuts is increasing with the research of strategies designed to decrease peanuts' immunoreactivity. Several strategies to decrease the allergenicity of proteins have been described, such as thermal treatment, covalent and noncovalent chemical modifications, enzymatic hydrolysis, high-pressure processing, pulsed ultraviolet light, high-intensity ultrasound, irradiation, pulsed electric field, germination, and so on[33-36]. The evaluation of the effect of these treatments is complex and usually depends on the employment of allergic patients to compare the allergenicity or the immunoreactivity of the modified and the unmodified protein. The LAIT is a potential tool to evaluate side-to-side immunoreactivity between the native and the modified protein [37, 38].

"The IgE-mediated peanut allergy is easily investigated at clinical set by cutaneous skin tests or by automatized ImmunoCAP® since most clinical laboratories offer it. However, non–IgE-mediated hypersensitivities are not readily documented by physicians since the laboratory methods designed to diagnose these conditions (such as the Lymphocyte Stimulation Test, the Leukocyte Migration Inhibition Test, or the LAIT) are not universally available by the typical clinical laboratories"[39, 40].

The more effective approach to treating peanut allergy is immunotherapy, a long-term strategy founded on a correct diagnosis, preferentially accomplishing all endotypes responsible for the different phenotypes [41-45]. Sublingual immunotherapy is notably recognized by its sustained effect on peanut desensitization [46].

Until now, immunology societies have recognized at least eight non–IgE-mediated hypersensitivity mechanisms [47].The LAIT observes the adherence inhibition provoked by contact with the tested antigen as a behavior resulting from leukocyte immunoreactivity shared by several immune pathways [48-51]. Using*ex vivo* challenges to investigate immunoreactivity allows a thirty-minute incubation of the allergen with the patient's plasma. This incubation turns the LAIT into a more

sensitive test than the solid-phase research of serum-specific antibodies. The LAIT gives unspecific information that there is some immunoreactivity against peanut allergens. The participation of the immune response against the allergen in the overall clinical picture will be realized in conjunction with the real-world response upon contact with the agent, the exclusion of the agent from the patient's life, and the reappearance after its re-introduction.

This preliminary retrospective survey has demonstrated a great range of results in a group of allergic patients against the *ex vivo* challenge against peanut extract, suggesting several distinct endotypes contribute to the extensive range of phenotypes observed among peanut-allergic patients. The variation of observed results suggests that the LAIT is a potential predictive biomarker to distinguish different endotypes of the AD peanut-allergic patients' physiopathology participants.

5. CONCLUSION

Our preliminary results support that the LAIT performed with peanut extract may differentiate diverse degrees of *ex vivo*non–IgE-mediated immunoreactivity against peanut allergens in Non–IgE-mediated AD patients.More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of*ex vivo* challenges monitored by LAIT in the stratification of endotypes reactions and the phenotypic classification of non–IgE-mediatedfood-allergic patients.

ETHICAL APPROVALS

As a retrospective survey of results recorded *incognito*,approval was given collectively by the institution's ethics committee following the principles of the Declaration of Helsinki[52].

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