Original Research Article

Endotyping Cellular and Humoral Cross-reactivity against Chicken Meat and Egg Yolk in Non-IgE-mediated Food Protein-Induced Gastrointestinal Allergies.

ABSTRACT

Background: Patients with poultry meat allergy may present several phenotypes regarding clinical presentations and a wide range of symptom severity, suggesting the existence of several endotypes underlying their diseases. The IgE-mediated hypersensitivities are well-established; however, the non-IgE-mediated immunoreactivity against food allergens has not yet been adequately characterized.
Aim: To evaluate the potential of the Tube Titration of Precipitins (TTP) and the Leukocyte Adherence Inhibition Test (LAIT) to discriminate cellular and humoral immunoreactivity against chicken meat extract and chicken egg yolk extract in patients with non–IgE-mediated gastrointestinal food protein-induced allergic phenotypes.
Study Design: We examined retrospectively the medical charts of two cohorts of patients clinically diagnosed with non–IgE-mediated gastrointestinal food protein-induced allergic phenotypes related to consumption of chicken meat and/or chicken egg yolk, who were investigated with the help of TTP or LAIT.
Methodology: The TTP and LAIT's registered results against chicken meat and chicken egg yolk extracts were distributed in ranges through a cascade distribution chart to outline the variability of the results within the cohorts. The Pearson correlation test was used to evaluate the correlation between the results obtained simultaneously with both food allergens.
Results: The LAIT for the chicken meat extract and egg yolk extract showed a wide distribution range of results. The TTP for the chicken meat and egg yolk extracts showed a distribution concentrated on the higher dilutions. The Pearson correlation test showed a non-significant positive correlation between LAIT for egg yolk and LAIT for chicken meat extract; r (٩٨) = ٠,١٥٦, p = ٠,١٢١. The Pearson correlation test showed a non-significant positive correlation between TTP for the egg yolk extract and TTP for the chicken meat extract; r (٩٨) = ٠,١٨١, p = ٠,٠٧٢.
Conclusion: Our preliminary results support that the TTP and LAIT performed with chicken meat and egg yolk extracts may discriminate diverse humoral and cellular immunoreactivity degrees in patients suffering from food protein-induced gastrointestinal allergies. However, there was no statistically significant quantitative correlation between the results.

Keywords: Endotype; Hypersensitivity; Chicken meat; Food Protein-Induced Enterocolitis Syndrome; Leukocyte Adherence Inhibition Test; Non–IgE-mediated Immunoreactivity; Poultry meat; Precipitins.

Abbreviations:
FA: Food Allergy
FPIGA: Food Protein-Induced Gastrointestinal Allergies
LAI: Leukocyte Adherence Inhibition
LAIT: Leukocyte Adherence Inhibition Test
LMIT: Leukocyte Migration Inhibition Test
TTP: Tube Titration of Precipitins

١. INTRODUCTION

Food protein-induced gastrointestinal allergies (FPIGA) and Food Allergies (FA) are yet a conundrum for physicians, gastroenterologists, nutritionists, allergists, but mainly for patients who suffer from these conditions (Olivier ٢٠١٣).

IgE-dependent mechanisms may produce FPIGA; however, most cases are produced by hypersensitivity endotypes not mediated by IgE (Heine ٢٠١٥, Khan ٢٠١٦, Ahmed, *et al.* ٢٠٢١). Non-IgE-mediated FPIGA are associated with a large spectrum of phenotypic conditions, clinically classified by the predominant anatomic localization of the symptoms (such as food protein-induced proctocolitis, food protein-induced enterocolitis, food protein-induced enteropathy, eosinophilic esophagitis, eosinophilic gastroenteritis, food protein-induced gastro-esophageal reflux disease, celiac disease, multiple food protein intolerance of infancy, food protein-induced gastrointestinal motility disorders), as well as some "functional" conditions, such as infantile colic, which food proteins may also induce (Groetch, *et al.* ٢٠٢٥, Huang and White ٢٠٢٥). These so-called food protein-induced allergic syndromes may manifest through exchangeable, heterogeneous, and recurrent symptoms with mild, moderate, or severe presentations, posing diagnostic and therapeutic dilemmas (Heine ٢٠٠٤).

Self-reported allergy to poultry meat is not a very common complaint in clinical practice (Sloan and Powers ١٩٨٦). However, when researched, the incidence of diagnosis of hypersensitivity to chicken meat (*Gallus domesticus*) may be surprising. Sampson reported an incidence of ١٦,٨٪ of positive skin prick tests to chicken meat among children with atopic dermatitis due to FA (Sampson and McCaskill ١٩٨٥).

The first report of chicken meat allergy was described in ١٩٨٢ as a non–IgE-mediated enteropathy in a child who proved to be allergic to chicken meat and cow's milk through provocation tests monitored by jejunal biopsies demonstrating severe villous atrophy after ingestion of chicken meat (Vitoria, *et al.* ١٩٨٢). It took fourteen years for the subsequent description of a non–IgE-mediated Eosinophilic Gastroenteritis to be documented after a chicken meat provocation test monitored by intestinal biopsies (Vandenplas, *et al.* ١٩٩٤). Food protein-induced enterocolitis syndrome is a non-IgE-mediated FPIGA that may present with symptoms such as flatulence, bloating, cramps, postprandial discomfort, vomiting, and diarrhea, or general symptoms, such as failure to thrive, due to chronic exposure to an offending food (Agyemang and Nowak-Wegrzyn ٢٠١٩). Moreover those initially described by pediatricians and attributed to liquid foods, such as cow's milk and liquid infant soy formulas, it was becoming apparent that solid foods (including poultry meat) could also be responsible for food protein-induced enterocolitis syndrome (Nowak-Wegrzyn, *et al.* ٢٠٠٣).

Patients with poultry meat FA may present several phenotypes regarding clinical severity and features, suggesting the existence of several endotypes underlying clinical symptoms (Wanniang, *et al.* ٢٠٢٢). As with any FA, the endotypes behind poultry allergy are primarily classified as IgE-mediated and non–IgE-mediated. Chicken meat allergy has complex sensitization profiles with nine major established allergens and twenty-five proposed candidates (Guiddir, *et al.* ٢٠٢٤). The major chicken meat allergen identified by a proteomics-based approach is a myosin light-chain protein designated as Gal d ٧ (shared by several poultry species), containing the majority of IgE-binding epitopes, characterized by remarkable thermal stability, refolding capacity, and resistance to salivary and gastrointestinal enzymes (Klug, *et al.* ٢٠٢٠). Poultry species develop common allergens, making usual cross-reactivity between chicken meat and turkey meat, as well as with other species (Cahen, *et al.* ١٩٩٨).

Allergic reactions following the administration of hen's egg yolk-based vaccines (such as for yellow-fever and typhus) have been described since the forties (Rubin ١٩٤٦). Allergic symptoms related to ingestion of hen's egg yolk have been reported since the ١٩٥٠s, mainly in children, including regurgitation, eczema, and respiratory symptoms (Todd, *et al.* ١٩٥٧).

Allergy to chicken meat can also develop as a cross-sensitivity against hen's egg proteins (bird-egg syndrome) (Hemmer, *et al.* ٢٠١٦). Bird-egg syndrome is a peculiar IgE-mediated cross-hypersensitivity to egg-yolk alpha-livetin (chicken serum albumin or Gal d ٥), also associated with inhaling birds' feathers and dander (Mandallaz, *et al.* ١٩٨٨, Szépfalusi, *et al.* ١٩٩٤). Three chicken meat allergens; parvalbumin (Gal d ٨), enolase (Gal d ٩), and aldolase (Gal d ١٠) are also present in fish and are responsible for a cross-reactivity hypersensitivity condition called the "fish–chicken syndrome" (Kuehn, *et al.* ٢٠١٦).

Usually, patients present reactions to multiple foods, an issue reinforced by the few laboratory tests which can suggest the possibility of non-IgE-mediated hypersensitivities (Katz

Commented [y١]: Food protein-induced enterocolitis syndrome is a non-IgE-mediated FPIGA that may present with symptoms such as flatulence, bloating, cramps, postprandial discomfort, vomiting, and diarrhea, due to chronic exposure to an offending food, or general symptoms, such as failure to thrive, while avoiding an offending food.

Commented [y٢]: cross-reactive

and Goldberg ٢٠١٤). Some facilities employ the Lymphocyte Stimulation Test for diagnosing hen's egg yolk–induced enterocolitis syndrome (Kajita, *et al.* ٢٠٢٣).

Humoral immunoreactivity against food allergens and aeroallergens had been classically evaluated by Precipitins (Augustin ١٩٥٣, Augustin, *et al.* ١٩٦٠, Cunningham-Rundles, *et al.* ١٩٧٨, Ferguson and Carswell ١٩٧٢, Heiner, *et al.* ١٩٦٢). We also routinely employ the Tube Research (Titration) of Precipitins (TTP) in our facilities as a triage to evaluate humoral non–IgE-mediated immunoreactivity against suspected allergens before performing more exhaustive *in vivo* provocation tests (Olivier, *et al.* ٢٠٢٣b, Olivier, *et al.* ٢٠٢١e, Olivier, *et al.* ٢٠٢١d, Olivier, *et al.* ٢٠٢٤*e, Olivier, et al.* ٢٠٢٤*c).*

The Leukocyte Adherence Inhibition Test (LAIT) and its similar assay, the Leukocyte Migration Inhibition Test (LMIT), have classically been used to differentiate non–IgE-mediated immunoreactivity against microorganisms and aeroallergens (Fink, *et al.* ١٩٨٧, Kallen and Nilsson ١٩٧٩, Kuratsuji ١٩٨١, Thomson ١٩٨٢). The LAIT and the LMIT have also classically been used to differentiate non-IgE-mediated immunoreactivity against food allergens (Allardyce and Shearman ١٩٧٥, George and Vaughan ١٩٦٢, Ashkenazi, *et al.* ١٩٨٠, Butler, *et al.* ١٩٨١, Papageorgiou, *et al.* ١٩٨٣). Non–IgE-mediated cellular immunoreactivity against food allergens has also been reported by Olivier, *et al.* group with the help of the LAIT (Olivier, *et al.* ٢٠٢٢b, Olivier, *et al.* ٢٠٢٢a, Olivier, *et al.* ٢٠٢٢c, Olivier, *et al.* ٢٠٢٣a).

To evaluate the potential of the LAIT and TTP to endotyping non–IgE-mediated cellular and humoral immunoreactivity against egg yolk and chicken meat, we retrospectively compiled the electronic medical charts of patients diagnosed primarily with gastrointestinal food allergies (associated or not with other extra-intestinal allergic phenotypes) related to non–IgE-mediated hypersensitivity, who had been investigated for immunoreactivity by one of two (above-mentioned) assays.

The present study is a proof-of-concept that hypothesizes that LAIT and the TTP may demonstrate a correlation between cellular and/or humoral immunoreactivity between egg yolk and chicken meat proteins in patients suffering from non–IgE-mediated FPIGA.

٢. MATERIALS AND METHODS

٢,١ Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; ٠٥/٢٠٢٥), we reviewed the electronic chart of ١٠,٦٠٠ outpatients who attended our facility from January ٢٠١٨ to July ٢٠٢٥.

A cohort of ١٠٠ consecutive outside patients (TTP cohort) had been submitted to TTP with chicken meat extract and egg yolk extract for presenting non–IgE-mediated FPIGA. This cohort counted ٣٥ males; mean age ٤٣,٦ years; SD ٢٢ years; range ٥ to ٩٤ years; median ٤٢,٥ years; modes = ٤٦ (appeared four times); geometric mean = ٣٦,٥ years.

A cohort of ١٠٠ consecutive outside patients (LAIT cohort) had been simultaneously submitted to LAIT with chicken meat extract and egg yolk extract for presenting non–IgE-mediated FPIGA. This cohort counted ٣٥ males; mean age ٣٤ years; SD ٢٤,٧ years; range ١ to ١٠٠ years; median ٣١ years; modes = ٥ years (appeared six times); geometric mean = ٢٢,٣ years.

This study did not include patients under biological and/or systemic anti-inflammatory therapy. These procedures were offered to patients with clinical suspicion of chicken meat hypersensitivity who demonstrated a non-reactive or inconclusive positive skin test against chicken meat (Olivier, *et al.* ٢٠١٣).

٢,٢. Extracts

٢,٢,١ Chicken meat extraction

Chicken meat (٣٠٠g of breast) acquired from the local market (half cooked and half uncooked) was crushed, homogenized, and then left for ٤٨ hours in a Coca-based extractor solution (propylparaben ٠,٥g, methylparaben ١g, sorbitol ٣٠g, NaCl ٥g, $NaHCO_3$ ٢,٥g, ١,٠٠٠mL H_2O) at ٤°C for protein extraction before centrifugation and separation of the water-soluble fraction(s) from solid particles and oily fraction (Coca ١٩٢٢). The protein quantification of the allergen extracts was done according to Bradford's protein-dye binding methodology (Bradford ١٩٧٦). The solution was diluted in antigen dilution solution (NaCl ١٠g; KH_2PO_4 ٠,٧٢g; Na_2PO_4 ٢,٨٦g; methylparaben ١g; propylparaben ٠,٥g; glycerin ٤٠٠mL; H_2O ٦٠٠mL) to an estimated

protein concentration of ١ mg/mL and stored at ٤°C in amber opaque glass vials. The chicken meat extract solution was used to perform allergic skin tests, TTP, and LAIT. All relevant and mandatory laboratory health and safety measures have been complied with during the experiments.

٢,٢,٢ Egg yolk extract

Three cooked egg yolks and three uncooked egg yolks were prepared similarly with the chicken meat extraction.

٢,٣. LAIT: *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

٢,٣,١ LAIT: Procedure for allergen *ex vivo* challenges

We performed the LAIT as previously described (Olivier, *et al.* ٢٠١٢, Olivier, *et al.* ٢٠١٤, Olivier, *et al.* ٢٠٢١a, Olivier, *et al.* ٢٠٢١b, Olivier, *et al.* ٢٠٢١c).

Shortly, each donor's fresh plasma was divided into two parts and used in parallel *ex vivo* challenging tests with the chicken meat extract (or the egg yolk extract) and the unchallenged plasma (added with antigen dilution solution as a control). We collected plasma with high leukocyte content (buffy coat) from the heparinized tube after one hour of sedimentation at ٣٧°C. Then, we distributed aliquots of ١٠٠ µL into Eppendorf tubes with (or without) the challenging extract and kept them under agitation for ٣٠ minutes (٢٠٠ rpm at ٣٧°C).

٢,٣,٢ LAIT: Procedure for adherence assay

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 ٢ hours at ٣٧°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 phosphate buffer saline (PBS) at ٣٧°C. Then, we added a drop of PBS to the hemocytometer's chamber and placed a clean coverslip over it. The remaining cells were counted in the same squares as previously examined.

٢,٣,٣ LAIT: Procedure for calculations

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 ١٠٠ (%). 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 ١٠٠ (%). To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from ١٠٠ (%). 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.

٢,٤. TTP: *In vitro* Investigation: Tube Titration of Precipitins: TTP

As previously reported, the semi-quantitative TTP against the chicken meat extract (or the egg yolk extract) was performed in a transparent vitreous tube array (Olivier, *et al.* ٢٠٢٤f, Olivier, *et al.* ٢٠٢٤d, Olivier, *et al.* ٢٠٢٤b, Olivier, *et al.* ٢٠٢٤a). Shortly, the patient's blood was collected in a clot-activator collecting tube. After separation, the serum was centrifuged at ٢,٠٠٠ rpm for ١٠ minutes. Each allergen extract was allocated in sets of eleven glass-tubes at progressively duplicated serum dilutions. The progressive dilutions were combined with separated aliquots of ١٥ µL of the allergen extract with ٢٥٠ µL of the patient's serum, progressively diluted into physiological saline solution (NaCl ٠,٩٪) in the dilution ratios of ١:١; ١:٢; ١:٤; ١:٨; ١:١٦; ١:٣٢; ١:٦٤; ١:١٢٨; ١:٢٥٦; and ١:٥١٢. One tube was a blank control done with water and serum to observe occasional spontaneous precipitation (Sia Test). After ٢٤ hours, the tubes were examined, and the titers (the highest dilution factor that yields a positive reading) were recorded (Williams and Chase ١٩٧١).

٣. 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 for the chicken meat extract showed a distribution concentrated on the higher dilutions (Fig. ١). There was no negative result. The mean was estimated at ١:٣٠٧; the median was ١:٢٥٦; the standard deviation was estimated at ١:١٧٥; the mode was ١:٥١٢ (appeared ٣٩ times).

The TTP for the egg yolk extract showed a distribution concentrated on the higher dilutions (Fig. ٢). There were two negative results. The mean was estimated at ١:٣٣٥; the median was ١:٢٥٦; the standard deviation was estimated at ١:١٧٩; the mode was ١:٥١٢ (appeared ٤٨ times).

The Pearson correlation estimated a non-significant, small positive relationship between TTP for the egg yolk extract and TTP for the chicken meat extract; r (٩٨) = ٠,١٨١, p = ٠,٠٧٢. See Fig. ٣.

The LAIT for the chicken meat extract showed a wide distribution range of results. The LAI ranged from ٠٪ to ٩٩٪. The mean was ٤٨,٥٪; the median was ٥٠,٥٪; the standard deviation was ٢٩,٨٪; the mode was ٠٪ (appeared eleven times). The cascade distribution demonstrated a wide range of LAI results (see Fig. ٤). Some patients showed low or moderate immunoreactivities during the *ex vivo* challenge tests. In contrast, others displayed strong immunoreactivities, which could reflect the participation of chicken meat allergens in a non–IgE-mediated hypersensitivity condition in these patients.

The LAIT for the egg yolk extract showed a wide distribution range of results. Most results were concentrated in the more immunoreactive groups. The LAI ranged from ٠٪ to ٩٩٪. The mean was ٥٠,٢٪; the median was ٥١٪; the standard deviation was ٢٧٪; the mode was ٠٪ (appeared eight times). The cascade distribution demonstrated a wide range of LAI results (see Fig. ٥). Some patients showed low or moderate immunoreactivities during the *ex vivo* challenge tests. In contrast, others displayed strong immunoreactivities, which could reflect the participation of egg yolk allergens in a non–IgE-mediated hypersensitivity condition in these patients.

The Pearson correlation estimated a non-significant small positive relationship between LAIT for egg yolk and LAIT for chicken meat extract; r (٩٨) = ٠,١٥٦, p = ٠,١٢١. See Fig. ٦.

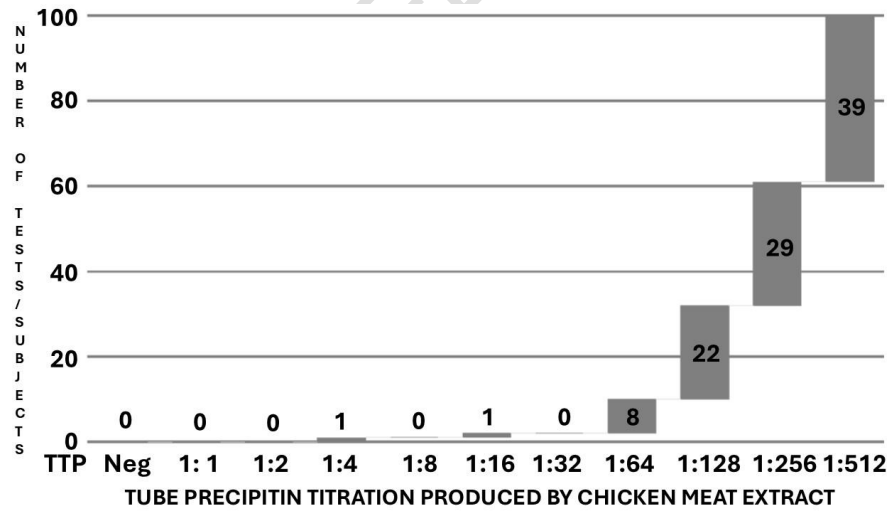

Fig. ١. Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the chicken meat extract against the serums of the TTP cohort of ١٠٠ tests/subjects (y-axis).

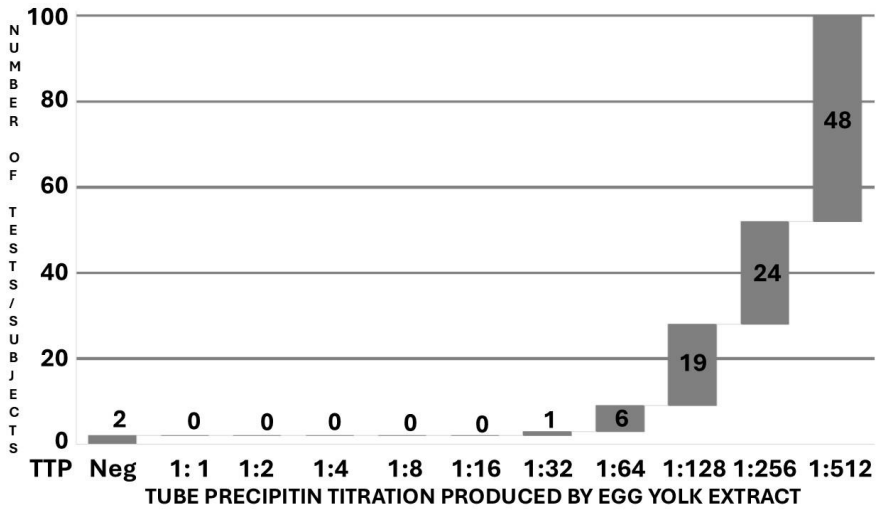

Fig. ٢. Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the hen's egg yolk extract against the serums of the TTP cohort of ١٠٠ tests/subjects (y-axis).

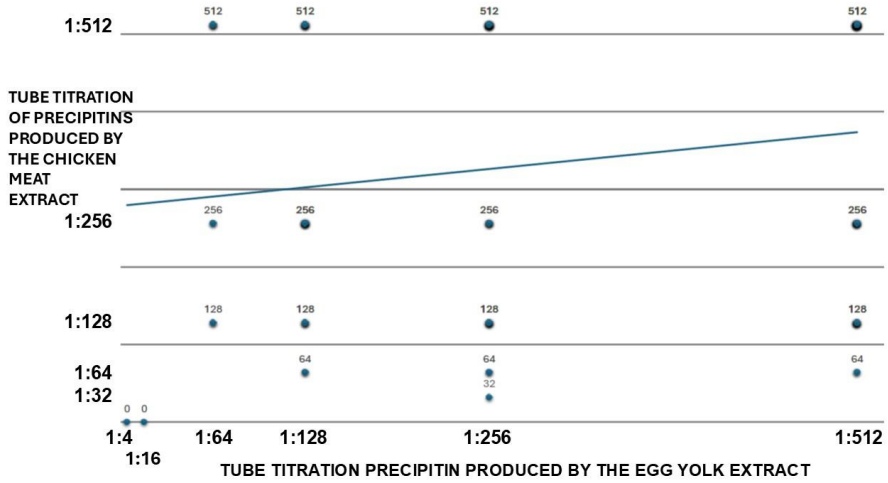

Fig. ٣. Dispersion chart of the Tube Titration of Precipitins (TTP) resulted against hen's egg yolk extract (x-axis), plotted against the TTP results against chicken meat extract (y-axis). The tendency line shows a non-significant, small positive relationship between the assays; r (٩٨) = ٠,١٨١, p = ٠,٠٧٢.

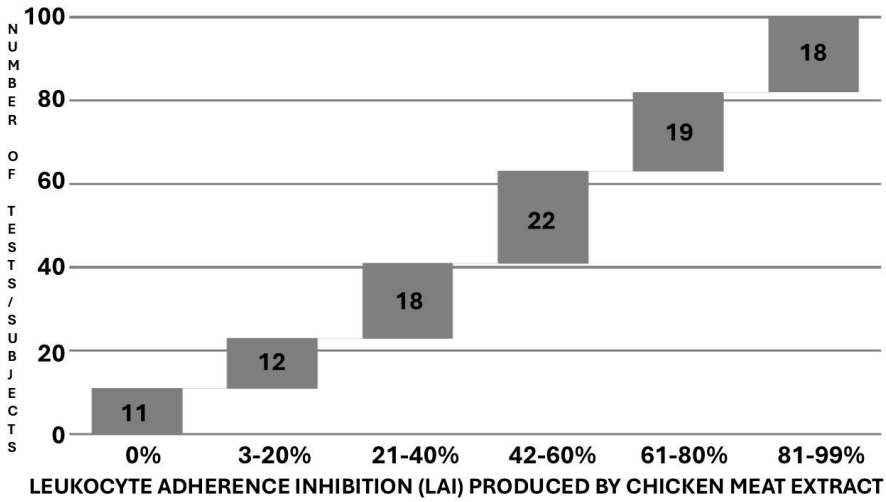

Fig. ٤. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the *ex vivo* challenge tests against chicken meat extract monitored by the Leukocyte Adherence Inhibition Tests (LAITs), according to the respective number of outcomes over the LAIT cohort with ١٠٠ tests/subjects (y-axis).

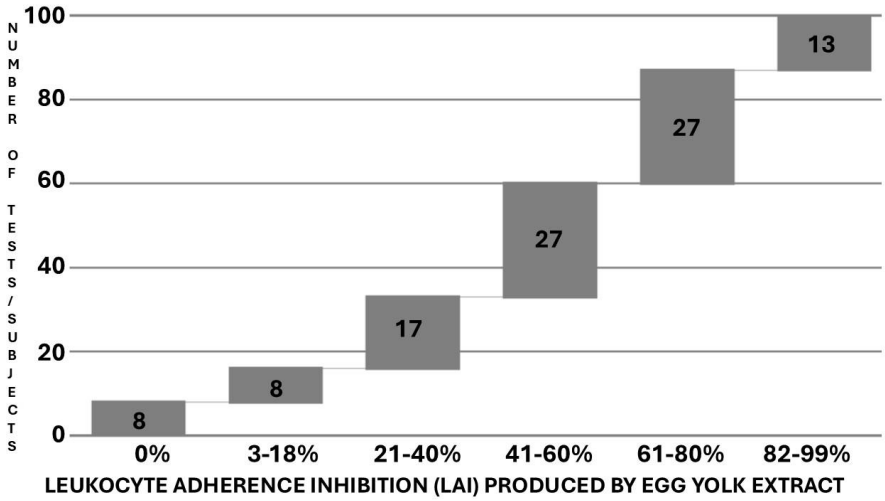

Fig. ٥. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the *ex vivo* challenge tests against hen's egg yolk extract monitored by the Leukocyte Adherence Inhibition Tests (LAITs), according to the respective number of outcomes over the LAIT cohort with ١٠٠ tests/subjects (y-axis).

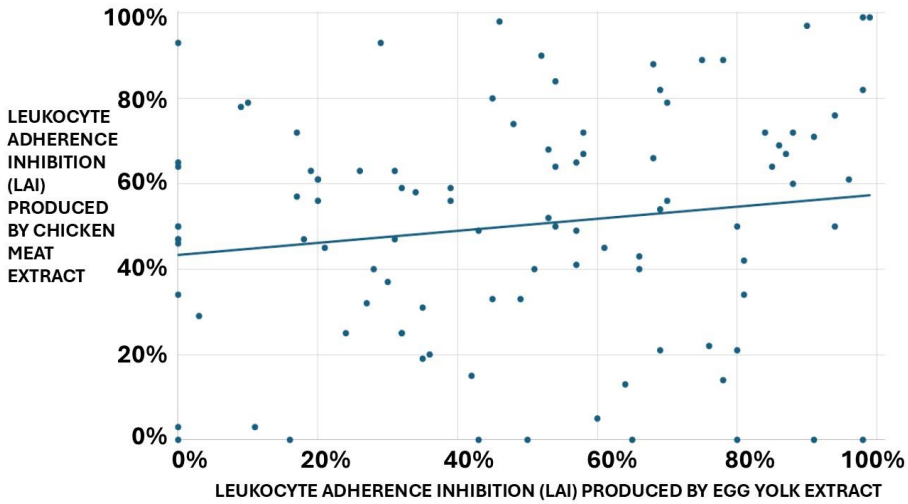

Fig. ٦. Dispersion chart of the Leukocyte Adherence Inhibition (LAI) results of the *ex vivo* challenge tests against hen's egg yolk extract (x-axis %), plotted against the LAI results of the *ex vivo* challenge tests against chicken meat extract (y-axis %). The tendency line shows a non-significant, small positive relationship between the assays; r (٩٨) = ٠,١٥٦, p = ٠,١٢١.

٤. DISCUSSION

The semi-quantitative titration of precipitins is a pioneering technique to evaluate humoral immunoreactivity (Wells ١٩١١, Hunter ١٩٠٥, Olivier, *et al.* ٢٠٢٥b, Olivier, *et al.* ٢٠٢٥a). Precipitating antibodies suggest the presence of a specific humoral immune response against the tested antigens (Gell, *et al.* ١٩٤٦, Ishizaka, *et al.* ١٩٥٩). Before the discovery of IgE, the research of precipitins was the leading way to realize *in vitro* diagnosis of immunoreactivities against antigenic and allergenic agents (Augustin and Hayward ١٩٦٠). The discovery of IgE and its reaginic activity, as well as the concomitant development of the Radio-Allergo Sorbent Test (RAST) to detect specific IgE antibodies, focused the attention of the scientific and medical communities on this particular antibody class, mainly after the incorporation of similar non-radioactive assays into routine clinical assays (Wide ١٩٦٧, Ishizaka and Ishizaka ١٩٦٧).

Although the serum free IgE (as detected by routine immunoassays) is not held responsible for clinical symptoms (as are the tissue-bound IgE antibodies, responsible for the autacoids released after the encountering with the antigen), the research on serum specific-IgE constructed a paradigm in the physician's mentality. The research of tissue-bond IgE, as performed by allergic skin tests, is easily performed; however, demonstration of the tissue-bond IgE antibodies in mucosal sites is a complex task, substituted by the research of eosinophils and T cells in endoscopic biopsies of patients with FA. This technical hindrance did not prevent the creation of the "IgE-mediated Local Allergic Reaction" concept in food hypersensitive patients (Lin, *et al.* ٢٠٠٢). This concept gave further origin to the similar concept of "IgE-mediated Local Allergic Rhinitis", which advocates the mucosal production of IgE

antibodies at insufficient concentrations to be reflected in the blood immunoassays or the skin tests (Rondon, *et al.* ٢٠١٠).

Usually, the correlation and the distribution of simultaneous positive specific-IgE against food allergens and inhalant allergens are weak; however, when properly investigated, polysensitization is more the rule than the exception in FPIGA (Zhang, *et al.* ٢٠٢٥, Čelakovská, *et al.* ٢٠٢٤).

The paradigm of the specific antibody to diagnose the etiology of the allergic symptoms has led to the "IgE culture" and a pressing pursuit to determine the utility of the specific IgG to diagnose the non–IgE-mediated hypersensitivity conditions (Atwah and Koshak ٢٠٢٤). The use of specific IgG against food allergens may be contentious and controversial since the IgG may sometimes act as a hypersensitivity trigger and sometimes as an allergen blocker, depending on its subclass, the antigen-antibody proportion, and the participation of other immune players (Alkhateeb ٢٠٢٠). IgG antibodies can participate in type II (antibody-dependent cell-mediated) and type III (immune complex disease) Gell and Coombs hypersensitivity reactions, which may be theoretically reproduced by the LAIT and the TTP assays, respectively (Olivier, *et al.* ٢٠٢١a). However, there is a lack of effective and practical tools to diagnose gastrointestinal inflammatory reactions due to FA in patients with no evidence of systemic circulatory IgE (Olivier ٢٠٢٢).

The LAIT is an *ex vivo* challenge test performed with a viable leukocyte buffy coat that can theoretically explore most known immune pathways, as it allows the interaction of all immune-circulating participants with the allergens (Olivier, *et al.* ٢٠٢١a). Several immune pathways can produce the final leukocyte adherence inhibition (Tong, *et al.* ١٩٧٩, Halliday, ١٩٧٤).

The present study is a proof-of-concept that hypothesizes that LAIT and the TTP may differentiate diverse degrees of cellular and humoral immunoreactivity against hen's egg yolk and chicken meat allergens among patients suffering from non–IgE-mediated FPIGAs. As the tests were performed simultaneously with the same venous sample with the two allergens, it was possible to calculate a correlation test to distinguish some orders of cross-reactivities between them.

The retrospective compilation of our data showed a large distribution of results when we ascertained the results of TTP and LAIT to explore humoral and cellular immunoreactivity against two chicken food allergens. These immunoassays did not precisely identify the mechanisms responsible for the clinical condition. Instead, they provide evidence about cellular and humoral immunoreactivity distributed into an extensive spectral range that may suggest immune tolerance or hypersensitivity.

This preliminary retrospective survey demonstrated extensive results from the TTP and the *ex vivo* challenge tests monitored by LAIT against hen's egg yolk and chicken meat allergens in two cohorts of non–IgE-mediated food allergic patients. TTP and LAIT are complementary triage tests used at our facilities to select worthwhile antigens to proceed with more laborious *in vivo* provocation tests when the specific IgE is undetectable. None of our patients presented an exclusive reaction to these allergens. Every patient was simultaneously tested for several chemical and biological allergens, demonstrating positive results for some of them. Our results suggest that patients suffering from FPIGA due to hen's egg yolk allergy may experience additional cross-immunoreactivities against chicken meat allergens, and vice-versa.

٥. LIMITATIONS

This study is a retrospective analysis of data collected over seven years. There was no protocol research, and the subjects' data was limited to the essentials available on our electronic sheets. Therefore, we could not establish a cross-comparison between positive and negative controls to validate the results. The number of subjects is appropriate for preliminary analyses; however, future studies must be more comprehensive. The lack of a research protocol implies the possibility of a bias produced by the physician's point of view who indicated the exam (CEO) based on a clinical suspicion led by anamnesis, physical examination, results of skin tests, and the research of specific IgE. The study lost the follow-up of most patients, hampering the registration of the relationship between the immunoassay results and the patient's clinical outcome. Unfortunately, it was also impossible to compare the two procedures with paired tests because they were taken from distinct groups of patients.

Commented [y٣]: research protocol

Commented [y٤]: Chief Executive Officer

٦. CONCLUSION

Our preliminary results show that the LAIT and TTP may differentiate diverse degrees of immunoreactivities against chicken meat and egg yolk extracts in patients clinically diagnosed with non–

IgE-mediated Gastrointestinal Food Allergies. LAIT and TTP are inexpensive, can be performed with minimum laboratory equipment, and can be incorporated into strategies to address respiratory- and food allergy health disparities (Anagnostou, *et al.* ٢٠٢٥). As a preliminary report, the propaedeutic meaning of the presented results and the possibility of interferents must be yet established (Anouar, ٢٠٢٤). More studies focused on the quality-by-design approach, with prospective larger double-blind cohorts needed to evaluate the potential contribution of LAIT and TTP for endotyping cellular and humoral immunoreactivity in patients suspected of hypersensitivity against chicken allergens (Chiarentin, *et al.* ٢٠٢٣).

٧. FUTURE DIRECTIONS AND RECOMMENDATIONS FOR CLINICAL PRACTICE

The primary intended use of *in vitro* or *ex vivo* allergen challenge tests is to spare the patients from being submitted to unnecessary, exhaustive, and dangerous *in vivo* challenge tests. Exploring the humoral and the cellular arms of immune systems, the TTP and LAIT alone or combined may represent, in the near future, a tool for allergists to construct an etiologic diagnosis from their patients, as well as determine the endotypes (mechanisms) of hypersensitivity, in order to choose more convenient and personalized therapies for them. Adding data provided by TTP and LAIT may also contribute to streamlining biomedical research and improving tools such as Large Language Models, usually used by clinicians as a decision support system to enhance diagnostic accuracy (Abers and Mathias, ٢٠٢٥).

CONSENT

As a retrospective survey of results recorded *incognito*, consent was taken collectively by the institution's ethics committee following the principles of the Declaration of Helsinki (WMA, ٢٠١٣).

ETHICAL APPROVALS

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

Disclaimer (artificial intelligence)

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

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