



Endotyping Cellular and Humoral Immunoreactivity against Formaldehyde in Patients with Atopic and/or Contact Dermatitis

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Authors' contributions

This work was carried out in collaboration among all authors. Author CEO conceptualized the study, did data curation, formal analysis, literature review, and wrote the original draft. Authors DGP, APMT, CSM, NSR, JLSS and RPSL performed the laboratory procedures. Author RAPGS performed the cutaneous tests. All authors read and approved the final manuscript.

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ABSTRACT

Background: Several publications report that formaldehyde is responsible for hypersensitivity reactions in patients with contact dermatitis, as diagnosed by "in vivo" provocation tests. There is no standardized lab exam that can endotype the mechanisms responsible for these phenotypes, **Aim:** To evaluate the potential of the Tube Titration of Precipitins (TTP) and the Leukocyte Adherence Inhibition Test (LAIT) to discriminate and endotype cellular and humoral immunoreactivity against formaldehyde in patients with contact dermatitis. **Methods:** We retrospectively examined the medical charts of two cohorts (n = 100, each) of patients diagnosed with atopic dermatitis and/or contact dermatitis with clinical suspicion of formaldehyde hypersensitivity, who were investigated with the help of TTP or *ex vivo* challenge tests monitored by LAIT against formaldehyde. The registered results were distributed in ranges through cascade distribution charts. The statistical characteristics of these cohorts were calculated. **Results:** TTP showed a distribution concentrated over the more diluted titrations with no negative result. The mean was estimated at 1:385; the median at 1:512; and the standard deviation at 1:166. The LAIT results demonstrated a wide range. The LAI ranged from 0% to 100%. The mean was 57.5%; the median was 65.5%; the standard deviation was 37.4%. **Conclusion:** Our preliminary results support that the TTP and LAIT performed with formaldehyde solution may discriminate diverse degrees of humoral and cellular immunoreactivity in patients suffering from atopic and/or contact dermatitis. By evaluating the utility of TTP and LAIT as diagnostic tools, the study provides preliminary evidence for endotyping immunoreactivity, which could advance precision medicine in allergy management. The findings may guide clinicians in identifying hidden formaldehyde exposure in products and inform safer therapeutic strategies for sensitized patients. It is worthwhile conducting more in-depth studies to evaluate the usefulness of TTP and LAIT in endotyping non-IgE-mediated hypersensitivity to formaldehyde.

Keywords: Atopic dermatitis; contact dermatitis; endotype; hypersensitivity; formaldehyde; leukocyte adherence inhibition test; precipitins; precision medicine.

ABBREVIATIONS

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

the average value of n (Dankelman & Daemen 1976).

1. INTRODUCTION

The American Contact Dermatitis Society elected Formaldehyde as the "Allergen of the Year 2015" (Pontén & Bruze 2015). Formaldehyde (known as methanal, methylene oxide, oxymethylene, methylaldehyde, and oxomethane) is the simplest aldehyde, with the chemical structure $H_2C=O$ (Gerberich & Seaman 2013). Usually stored as an aqueous solution containing variable amounts of methanol (formalin), it turns on a colorless, pungent, suffocating, flammable gas when liberated into the atmosphere (Commission, 2024). Formaldehyde in aqueous solutions spontaneously hydrates to $H(H_2C=O)OH$ and aggregates to form mixtures of hydrated oligomers: $H(H_2C=O)_n OH$ depending on formaldehyde concentration. Methanol stabilizes aqueous formaldehyde solutions by decreasing

The "plastic age" was inaugurated in 1910 by polymerizing formaldehyde and phenol, producing bakelite (Braun et al., 2013). Nowadays, several industrial uses have been discovered for formaldehyde, turning it into an intermediate chemical tool for the production of adhesives, fabrics, polymers, resins, plastics, paints, lacquers, dyes, explosives, and so forth, stimulating the industrial research of diverse patented productions methods worldwide (Walker, 1964 DIsponible at "Internet Archive": <https://archive.org/details/formaldehyde0000walk>). Formaldehyde may also be formed and liberated in the atmosphere by the incomplete combustion of tobacco, wood, coal, gasoline, diesel, and ethanol in internal combustion engines (Dias et al., 2012). Gaseous formaldehyde at higher concentrations can irritate the eyes and mucous membranes of the respiratory tract, even producing asthma (Bardana & Montanaro 1991; Pougnet et al., 2025; Zhang et al.; 2025).

Initially used as a disinfectant, embalming (anatomy dissection classrooms), and viral inactivator for the production of vaccines, nowadays, formaldehyde is listed as a human carcinogen with restricted uses (Commission, 2021). Formaldehyde is prohibited from use in cosmetic products in most countries. However, the so-called formaldehyde releasers are usually allowed (Commission, 2019). To avoid the inconveniences of formalin, chemists developed the formaldehyde releasers (or formaldehyde donors), reversible linear or cyclic polymers of formaldehyde that slowly release free formaldehyde at levels suppressing microbial growth but (theoretically) sufficiently low not to harm humans (De Groot et al., 2009). These formaldehyde releasers (such as quaternium-15, Diazolidinyl urea, DMDM hydantoin, imidazolidinyl urea, 2-bromo-2-nitropropane-1,3-diol (bronopol), germall-115, preventol, and so forth) are found in cosmetics (creams, lotions, make-up removers, soaps, shampoos, deodorants, toiletries, nail products), cleaning household products (detergents) and industrial chemical products (Flyvholm & Andersen, 1993). Soon, it was realized that formaldehyde releasers were also causes of contact dermatitis in patients sensitized to formaldehyde (Dahlquist & Fregert 1978).

Recently, it was reported that about three-quarters of tested US tattoo inks analyzed by the chromotropic acid method resulted in a positive for formaldehyde releasers (Liou et al., 2021).

Until recently, textile finishes released an elevated level of free formaldehyde (textile-formaldehyde resins), causing frequent textile dermatitis in individuals sensitive to formaldehyde. However, nowadays, clothing finishes release much less free formaldehyde, and allergic contact dermatitis from clothing due to formaldehyde releasers is much less frequent than in the past decades. (Lazarov et al., 2003).

Nowadays, the main question about formaldehyde hypersensitivity is not about the known products with the declared presence of formaldehyde or formaldehyde releasers in their composition but the undeclared presence of formaldehyde in industrial products. In a recent sampling, it was demonstrated by high-performance liquid chromatography (HPLC) that 23 of 130 cosmetic products (18%) (without formaldehyde or formaldehyde releasers on the package ingredient list) were presenting variable

amounts (0.5–507 ppm) of formaldehyde (Søgaard et al., 2024).

The undeclared presence of formaldehyde in industrial products results from the air oxidation of ethoxylated alcohols, such as polyethylene glycols (Bergh et al., 1998). Polyethylene glycols are polymers of ether monomers such as ethylene glycol, ethylene oxide, or oxyethylene, usually available as mixtures of different chain lengths polymers, used as emulsifiers in industrialized food and food supplements (E 1521), medicines (macrogol), cosmetics and housecleaning products (Olivier et al. 2024e). Even corticoid creams may present formaldehyde, mainly when presenting macrogol in their composition (Dahlquist et al., 1980).

The methyl ester of the aspartic acid/phenylalanine dipeptide (aspartame) may also be degraded to formaldehyde and produce systemic allergic dermatitis (Veien & Lomholt 2012, Hill & Belsito 2003, Castanedo-Tardan et al., 2009).

Besides producing their characteristic hypersensitivity conditions (generalized and localized allergic contact dermatitis, airborne symptoms such as rhino-conjunctivitis and asthma, immediate-type allergies such as urticaria and anaphylaxis), hypersensitivity to formaldehyde may also aggravate preexisting dermatoses, producing flares of atopic dermatitis, stasis dermatitis, and rosacea (Goossens & Aerts 2022).

Formaldehyde is unanimous among the diverse batteries recommended for composing diagnostic cutaneous contact test kits (patch tests) (Bruynzeel et al., 1995).

When cutaneous tests are inconclusive, the best way to diagnose formaldehyde hypersensitivity is the exclusion/provocation test, when the patient interrupts the use of the suspected allergen until the symptoms disappear. Then, the allergen is re-introduced to observe reactions. However, this is particularly difficult when polysensitization dominates the clinical picture. In order to shorten the list of suspected allergens, we performed triage tests to elect the allergens that will be emphasized in the exhaustive *in vivo* exclusion/provocation tests.

Cellular immunoreactivity against haptens and hapten-carrier conjugates had been classically

evaluated by the Leukocyte Adherence Inhibition Test (LAIT) (Kuratsuji, 1981).

Humoral immunoreactivity against haptens has been classically evaluated by precipitin research (Rittenberg & Amkraut 1966).

To evaluate cellular immunoreactivity, we employ in our facilities the LAIT (Olivier et al. 2022b, Olivier et al. 2022a, Olivier et al. 2022c, Olivier et al. 2023b, Olivier et al. 2023a).

To evaluate the humoral immunoreactivity, we employ at our facilities the Tube Titration of Precipitins (TTP) (Olivier et al. 2021c, Olivier et al. 2021e, Olivier et al. 2023c, Olivier et al. 2024f, Olivier et al. 2025b).

The present study hypothesizes that the LAIT and the TTP may help differentiate diverse endotypes and degrees of immunoreactivity against formaldehyde among patients suffering from non-IgE-mediated atopic and/or contact dermatitis. To evaluate the potential of the LAIT and the TTP to endotyping non-IgE-mediated immunoreactivity against formaldehyde, we retrospectively compiled the electronic medical charts of patients with these conditions who were investigated with these procedures in our outpatient clinic.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 03/2025), we proceeded with the electronic chart review of 10,270 outpatients who attended our facility from January 2018 to April 2025.

A cohort of 100 outside patients had been submitted to TTP with formaldehyde solution for presenting Atopic and/or Contact Dermatitis. This cohort counted 29 males; mean age 38.8 years; SD 19.6 years; range 3 to 90 years; median 38 years; modes: 7; 9; and 70 years (each appeared 4 times); geometric mean = 32 years.

A cohort of 100 outside patients had been submitted to an *ex vivo* allergen challenge test with formaldehyde solution monitored with LAIT for presenting non-IgE-mediated atopic and/or contact dermatitis. This cohort counted 29 males; mean age 44.7 years; SD 19.1 years; range 9 to 91 years; median 45 years; modes = 35; 48 and

58 years (each appeared three times); geometric mean = 39.5 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 formaldehyde hypersensitivity who demonstrated a non-reactive or inconclusive skin test performed with formaldehyde solution (Olivier et al. 2013).

2.2 Formaldehyde Solution

The formaldehyde solution was prepared with 1.5 mL of a solution of formaldehyde 10% (Perfyl Tech®) diluted to 15 mL with distilled water to finalize a 1 mg/mL solution to perform the allergic skin tests, TTP and LAIT.

2.3 *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

2.3.1 Procedure for allergen *ex vivo* challenging

We performed the LAIT as previously described (Olivier et al. 2012, Olivier et al. 2014, Olivier et al. 2021a, Olivier et al. 2021b, Olivier et al. 2021d). Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with Formaldehyde solution and the unchallenged plasma assay. We collected 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 Formaldehyde solution (10µL) or without Formaldehyde solution (when used as control).

2.3.2 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 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 (phosphate-buffered saline) 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.

2.3.3 Procedure for calculation

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: $LAR = LA \text{ of the challenged sample} / LA \text{ of unchallenged control plasma} \times 100$ (%). To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from 100 (%). We employed the LAI results for the cascade distribution chart and the statistics calculations, both performed with the help of the Microsoft Excel® statistical package.

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

As previously reported, a transparent vitreous tube array performed the semi-quantitative TTP against the Formaldehyde solution (Olivier et al. 2024b, Olivier et al. 2024c, Olivier et al. 2024a, Olivier et al. 2024d, Olivier et al. 2025a). Shortly, the patient's blood was collected in a clot-activator collecting tube. After separation, the serum was centrifugated at 2,000 rpm for 10 minutes. The allergen extracts were allocated in sets of eleven glass tubes at progressive duplicated serum dilutions. The progressive dilutions were combined with the 15 µL of the

antigen solution with 250 µL of the patient's serum, progressively diluted into physiological saline solution (NaCl 0,9%) in the dilution ratios of 1:1; 1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256; and 1:512. One tube was a blank control done with the water and serum to observe occasional spontaneous precipitation (Sia Test). After 24 hours, the tubes were examined, and the titers (the highest dilution factor that yields a positive reading) were recorded (Williams & Chase. 1971).

3. RESULTS

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

The TTP showed a distribution concentrated over the more diluted titrations (Fig 1). There was no negative result. The mean was estimated at 1:385; the median was 1:512; the standard deviation was estimated at 1:166; the mode was 1:512 (appeared 61 times). All Sia tests were negative.

The cascade distribution of LAI results demonstrated a wide range (Fig.2). The LAI ranged from 0% to 100%. The mean was 57.5%; the median was 65.5%; the standard deviation was 37.4%; the mode was 0% (appeared eleven times). About half the patients presented high immunoreactivity during the ex vivo challenge test (LAI > 60%).

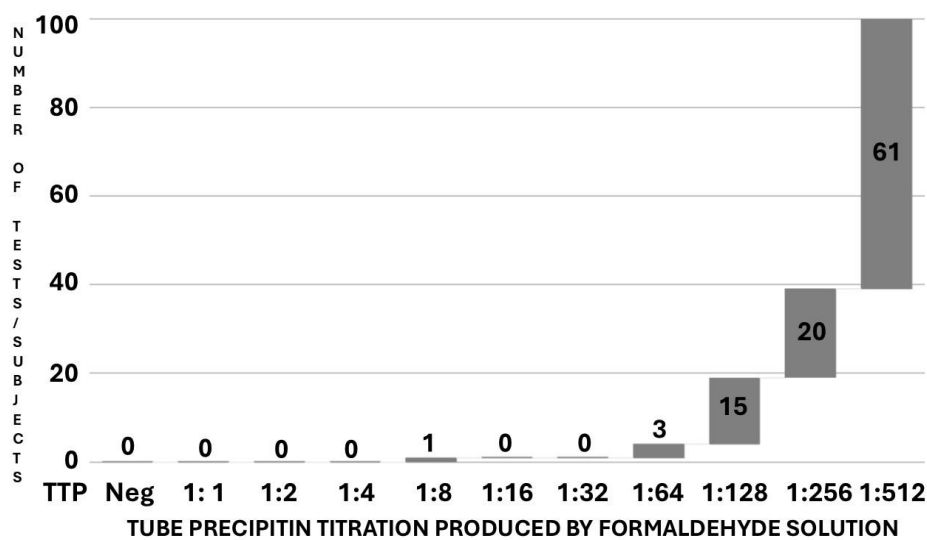


Fig. 1. Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the Formaldehyde solution against the serum of a cohort of 100 tests/subjects (y-axis)

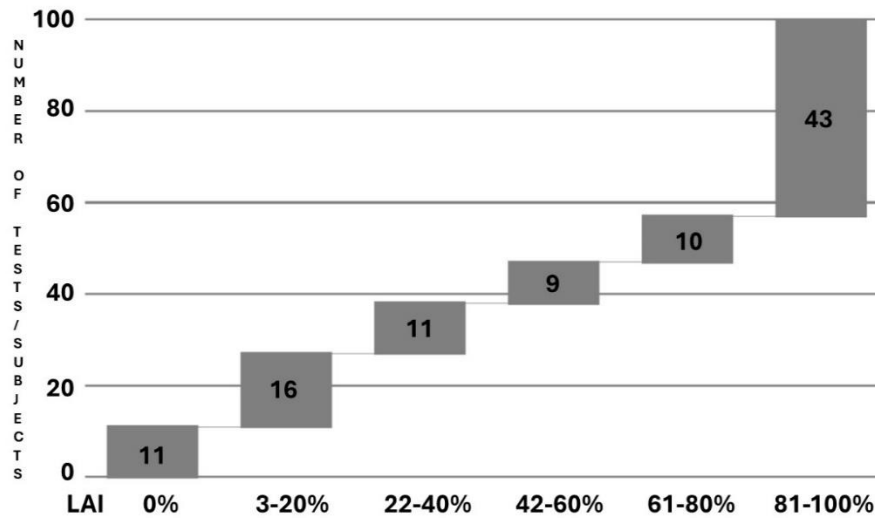


Fig. 2. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo* formaldehyde solution monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over a cohort with 100 tests/subjects (y-axis)

All patients evaluated with TTP demonstrated some degree of humoral immunoreactivity, and most presented positivity by the more diluted titrations. Eleven patients did not present cellular immunoreactivity against Formaldehyde (LAI = zero%), while others presented an extensive range of inhibition of the leukocyte adherence after the *ex vivo* provocation test.

4. DISCUSSION

Despite being universally recognized by its sensitizing properties, little is known about the intrinsic physiopathology of the immunoreactivity (or immunoreactivities) against formaldehyde.

Formaldehyde can establish cross-linking reactions among several amino acids, producing deformation in the proteins' tertiary structure and altering their immunoreactivity (Sompuram et al. 2004, Fraenkel-Conrat et al., 1947; Fraenkel-Conrat & Olcott, 1948b; Fraenkel-Conrat & Olcott 1948a). When absorbed into human blood current, formaldehyde may conjugate with serum proteins, such as the Human Serum Albumin (HAS), forming hapten-carrier complexes able to develop the production of antibodies (IgE, IgM, IgG, and IgA) against their non-self-conjugates (Patterson et al. 1986, Patterson et al. 1989). IgE-mediated sensitization against formaldehyde is not an easy diagnosis but has already been described in children exposed to gaseous formaldehyde (Kramps et al. 1989, Wantke et al.

1996a, Wantke et al. 1996b, Mizuki & Tsuda 2001).

Additionally, to the capacity to produce antibodies against the formaldehyde-HAS conjugated, analysis of individuals occupationally exposed to formaldehyde demonstrated elevated T antigen memory cells and lymphocyte subpopulations of T-helper/suppressor (H/S) ratios ranging from 0.8 to 3.3, suggesting chronic antigenic stimulation by formaldehyde (Thrasher et al., 1988).

There is no standardized lab examination to (unequivocally) diagnose non-IgE-mediated formaldehyde hypersensitivity or to suggest formaldehyde immunoreactivity. However, endotyping the mechanisms responsible for allergic phenotypes is crucial for diagnosing and supervising treatments under personalized medicine and recognizing differential diagnoses among phenotypes (Olivier. 2024). Several phenotypes have been described; however, the endotypes have been poorly explored since the main clinical tools to verify hypersensitivity against formaldehyde are still the cutaneous tests.

As a proof-of-concept, we submitted formaldehyde to an *ex vivo* challenge monitored by the LAIT to demonstrate cellular immunoreactivity. In the same proof-of-concept mentality, we titrated precipitins against formaldehyde to demonstrate humoral

immunoreactivity. Despite non-reactive or inconclusive skin tests, we propose these procedures to patients with atopic dermatitis and/or contact dermatitis with a strong clinical suspicion of hypersensitivity to formaldehyde. The clinical reasoning to indicate these tests is in the assumption that these immunoassays may function as triage tests to reinforce the need for a more exhaustive diagnostic exclusion-provocation test (when the patient excludes the suspected allergen until the symptoms disappear and then re-introduces the allergen to observe the reactions).

As a retrospective cohort analysis, there was no prospective plan. We spreadsheeted a compilation of registered results produced by TTP and LAIT, exploring humoral and cellular immunoreactivity against formaldehyde. These assays provide clues about humoral and cellular immunoreactivity, and the results are distributed in an extensive spectral range, presumably between immune tolerance and symptomatic hypersensitivity. Results provided by LAIT and TTP were interpreted as markers of the immune response after contact with the specific antigen, configuring themselves as techniques to identify exposition to the antigen, immune stimulation, and immunoreactivity, as proposed by the exposome-wide association study (Chung et al. 2024).

At the clinical set, the diagnosis of formaldehyde allergy is accomplished by anamnesis, skin tests, and *in vivo* provocation tests; however, when employing a multi-omics approach, several clinical phenotypes and endotypes may be differentiated (Yoon & Bunyavanich 2025).

This retrospective survey demonstrated that the TTP and the *ex vivo* challenge test monitored by LAIT against formaldehyde can demonstrate significant cellular and humoral immunoreactivity in patients diagnosed with atopic and/or contact dermatitis. However, these immunoassays did not prove *per se* that hypersensitivity to formaldehyde is responsible for these patients' symptoms. This association may only be confirmed by further *in vivo* provocation studies.

None of our patients presented an exclusive reaction to Formaldehyde. We assessed every patient simultaneously with several chemical and biological allergens, demonstrating positive results for some of them, according to clinical suspicions. The most vital suggestion driven by the results is that allergic patients may impair

their symptoms by using creams or cosmetics contaminated with formaldehyde.

In our practice, when preceding *in vivo* cutaneous tests with formaldehyde, we observe immediate cutaneous reactions (obtained by the skin scrape test) and delayed reactions obtained by a forty-eight-hour contact test or a photosensitized ninety-six-hour contact test (patch test). Based on this clinical experience, we can hypothesize that at least three endotypes are associated with formaldehyde hypersensitivity, which may be produced by at least three mechanisms: a predominantly humoral, a predominantly cellular, and a compound of both.

Integrating TTP and LAIT into clinical workflows may guide clinicians in identifying hidden hypersensitivity due to formaldehyde exposure in products and inform safer therapeutic strategies for sensitized patients.

5. CONCLUSION

Our preliminary results show that the LAIT and TTP may differentiate diverse cellular and humoral immunoreactivity degrees against formaldehyde in patients clinically diagnosed with non-IgE-mediated allergies. LAIT and TTP are inexpensive, can be performed with minimum laboratory equipment, and can be incorporated into strategies to address health disparities in respiratory and food allergies (Anagnostou et al., 2025). As a preliminary report, the propaedeutic meaning of the presented results and the possibility of interferents must be yet established (Anouar et al., 2024). More studies focused on the quality-by-design approach with prospective larger double-blind cohorts need to evaluate the potential contribution of LAIT and TTP for endotyping immunoreactivity of patients suspected of symptomatic hypersensitivity against formaldehyde and other similar preservatives (Chiarentin et al., 2023).

6. LIMITATIONS

This study is a retrospective analysis of data collected over six years. There was no protocol research, no control group, and the subject's data were 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 a preliminary study; 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 suggested the exam barely on clinical suspicion led purely by the anamnesis and physical examination. The study lost many of these patients to follow-up, so assuring the relationship between the immunoassays' results and the patient's clinical outcome is impossible.

7. 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 & Mathias 2025).

CONSENT

As a retrospective survey of results recorded *in cognito*, consent was given collectively by the institution's ethics committee following the principles of the Declaration of Helsinki (WMA, 2013).

ETHICAL APPROVALS

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

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) 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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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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