Journal of Applied Health Sciences and Medicine, 6(4):40–55, 2026. DOI: 10.58614/jahsm645

Journal of Applied Health Sciences and Medicine

Journal homepage: https://jpub.org/journal-details.php?journal-id=39

ISSN (E): 2583-1887

Research Article

Endotyping cellular and humoral cross-reactivity among tobacco, tomato, and potato in patients with Allergic Multimorbidity

Celso Eduardo Olivier [1*], Daiana Guedes Pinto [1], Ana Paula Monezzi Teixeira [1], Cibele Silva Miguel [1], Nicole Sartoreto Rocha [2], Alessandra Vieira de Oliveira [2], Luciana Sacilotto Carvalho [2], Raquel Acácia Pereira Gonçalves Santos [2] and Damla Serçe Unat [3]

[1] *Instituto Alergoimuno de Americana, Brazil.*
[2] *Faculdade de Americana, São Paulo, Brazil.*
[3] *Department of Pulmonology, Bakırçay University, Faculty of Medicine, Izmir, Türkiye.*
[*] *Corresponding author: celso@alergoimuno.med.br*

Article Info

Keywords: *Hypersensitivity, Leukocyte Adherence Inhibition Test, Non–IgE-mediated Immunoreactivity, Potato allergy, Precipitins, Solanaceae, Tobacco allergy, Tomato allergy.*

Received: *02.04.2026;*
Accepted: *19.04.2026;*
Published: *24.04.2026*

Abstract

Background: Sensitization to panallergens is associated with allergic multimorbidity and polysensitization. Solanaceae allergens may elicit several allergic endotypes characterized by specific or combined humoral and cellular hypersensitivity mechanisms. Tobacco, potato, and tomato are flowering plants in the Solanaceae family that are known to cause diverse hypersensitivity disease phenotypes. **Study Design:** We examined retrospectively the medical charts of two cohorts of patients clinically diagnosed with non–IgE-mediated multimorbidity allergic phenotypes related to inhalation of tobacco smoke and/or ingestion of tomato and/or potato who were investigated by the Tube Titration of Precipitins (TTP) or the Leukocyte Adherence Inhibition Test (LAIT) simultaneously against the three extracts. **Methodology:** The registered results for the TTP and LAIT against tobacco, potato, and tomato extracts were plotted as ranges using a cascade distribution chart to illustrate the variability within the cohorts. The registered results for Leukocyte Adherence Inhibition (LAI) percentage and precipitin titration was plotted on a cascade distribution chart to illustrate the variability of the results. The correlation between the paired assays was calculated using Pearson's method and is shown in the dispersion graphs. **Results:** The paired t-test indicated no significant difference in LAIT results between tobacco and tomato (p-value = 0.57). Pearson's correlation indicated a significantly moderate positive relationship between tobacco and tomato LAIT results: r (98) = 0.4, p-value <0.001. The paired t-test indicated a significant difference between tobacco and potato LAIT results (p-value = 0.002). Pearson's correlation indicated a significantly moderate positive relationship between tobacco and tomato LAIT results: r (98) = 0.54, p-value <0.001.The paired t-test indicated a significant difference between tomato and potato LAIT results (p-value= 0.005). Pearson's correlation indicated a moderate, significant positive relationship between tobacco and tomato LAIT results (r (98) = 0.216, p = 0.031). The paired t-test indicated that there is a significantly small difference between tobacco TTP results (mean = 242.6; SD = 176.7) and tomato TTP results (mean = 170.1; SD = 154.5), t (99) = 3.1; p = 0.003. Pearson's correlation indicated a non-significant, very small positive relationship between tobacco TTP and tomato TTP: r (98) = 0.00729; p = 0.943. The paired t-test indicated that there is a significantly small difference between tobacco TTP (mean = 242.6; SD = 176.7) and potato TTP (mean = 178.4; SD = 145.2); t (99) = 2.6; p = 0.012. Pearson's correlation indicated that there is a significantly small negative relationship between tobacco TTP and potato TTP: r (98) = 0.214, p = 0.032. The paired t-test indicated that there is a non-significant, very small difference between potato TTP (mean = 178.4; SD = 145.2) and tomato TTP (mean = 170.1; SD = 154.5); t (99) = 0.4; p = 0.725. Pearson's correlation indicated that there is a significantly small negative relationship between potato TTP and tomato TTP: r (98) = 0.238; p = 0.017.**Conclusion:** The preliminary results suggest that the TTP and LAIT may discriminate among diverse levels of humoral and cellular immunoreactivity in patients with tobacco-, tomato-, and potato-related hypersensitivity phenotypes, with a more consistent correlation in the cellular immunoreactivity results than in the humoral assays.

How To Cite: C.E. Olivier, D.G. Pinto, A.P.M. Teixeira, C.S. Miguel, N.S. Rocha, A.V. Oliveira, L.S. Carvalho, R.A.P.G. Santos, D.S. Unat. Endotyping cellular and humoral cross-reactivity among tobacco, tomato, and potato in patients with Allergic Multimorbidity. *Journal of Applied Health Sciences and Medicine*, 6(4):40–55, 2026. https://doi.org/10.58614/jahsm645

1. Introduction

Solanaceae is a family of flowering plants whose most cultivated genera are Nicotiana (to which belong *N. tabacum* or tobacco) and *Solanum* (to which belong *S. tuberosum, the potato, and S. lycopersicum,* the *tomato*) [1, 2].

The Allergen Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) has recognized so far four allergens identified from the *S. tuberosum*:

A. Patatin (Sol t 1 with 43 kDa)
B. Cathepsin D inhibitor (Sol t 2 with 21 kDa)
C. Cysteine protease inhibitor 1 (Sol t 3 with 21 kDa)
D. Serine protease inhibitor 7 (Sol t 4 with 16 kDa) [3].

The Allergen Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) has recognized so far seven allergens identified from the *S. lycopersicum*:

A. Profilin (Sola l 1 with 14 kDa)
B. Beta-fructofuranosidase (Sola l 2 with 50 kDa)
C. Nonspecific lipid transfer protein type 1 (Sola l 3 with 9 kDa)
D. Pathogenesis-related protein (Sola l 4 with 20 kDa)
E. Cyclophilin (Sola l 5 with 19 kDa)
F. Nonspecific lipid transfer protein type 2 (Sola l 6 with 7 kDa)
G. Nonspecific lipid transfer protein type 1 (Sola l 7 with 12.5 kDa) [4].

Over the past two decades, tomato allergen research has focused primarily on identifying new, undescribed putative allergens. Unfortunately, progress in this area appears to be quite slow [5].

The Allergen Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) has not yet published any specific allergen identified from *N. tabacum*.

Contact dermatitis from tobacco leaves has been reported among tobacco harvesters, curers, and cigar makers [6–11]. Allergic skin tests performed with tobacco extracts were first performed in the 1930s when scientists were researching an association among tobacco allergy, respiratory conditions, and cardiovascular diseases [12]. Tests with tobacco extracts, nicotine-free tobaccos, and a 10 percent solution of nicotine salicylate demonstrated that the alkaloid nicotine was not responsible for the positive reactions elicited by the skin tests [13]. In the sixties, several investigators believed that the skin allergic tests performed with tobacco extract would serve as a practical "screening test" determining the importance of tobacco as an etiological factor in several vascular diseases [14–17].

An 18,000 KDa glycoprotein named Tobacco Glycoprotein Purified Antigen (TGP) was considered the primary allergen of tobacco extracts, presenting cross-reactivity with other members of the *Solanacea* family (eggplant, peppers, green pepper, potato, and tomato), which also contain a brown material electrophoretically similar to TGP, cross-reacting with rabbit antibodies to TGP [18]. TGP can be extracted from saline extracts of cured tobacco leaves (TGP-L) and from the cigarette smoke condensate (TGP-CSC), producing immediate cutaneous hypersensitivity when injected intracutaneously in hypersensitive patients [19].

Precipitins against tobacco extracts were first studied in the late seventies, when they were associated with late skin reactions, interpreted as immune-complex reactions to tobacco antigens, and classified as a Gell & Coombs type III hypersensitivity [20–22]. Before the discovery of the reaginic activity of IgE, research on precipitins was the primary method for evaluating humoral immunoreactivity to suspected allergens [23–27]. The Tube Research of Precipitins (TTP) is the most straightforward technique for evaluating the formation of immune complexes after *in vitro* challenges of patients' serum with suspected antigens [28–32]. After the discovery of the reaginic activity of the IgE, investigators reported the presence of IgE-mediated hypersensitivity to antigenic constituents of cigarette smoke [33].

It was also demonstrated that TGP-L and TGP-CSC contain polyphenolic moieties capable of activating factor-XII-dependent pathways, a phenomenon that may underlie the association among cigarette smoking, cardiovascular disease, and chronic obstructive pulmonary disease [34]. Tobacco glycoprotein mimics antibody Fc regions and activates the classical complement pathway, thereby priming immune cells and inducing inflammation [35].

Despite previous studies supporting the existence of immune responses against unburned tobacco, the scientific community remained divided in the late century about the allergic nature of the immunogenic effects of environmental tobacco smoke, mainly because specific IgE against tobacco incineration products could not be demonstrated [36].

Environmental tobacco smoke has been linked with asthma and allergic diseases in children, by skewing the inflammatory profile, aggravating inflammation, promoting infections, tissue damage, and the development of non–IgE-mediated allergic phenotypes by increasing the expression of inflammatory cytokines [37].

Prenatal exposure to Environmental tobacco smoke exacerbated allergen-induced type 2 cytokine production and reduction of IFN-γ production by Natural Killer cells, resulting in an increase in airway eosinophilic inflammation, hyperreactivity, mucus secretion, and cysteinyl leukotriene biosynthesis in the offspring, suggesting a role for innate immunity in the allergic endotypes [38].

Hypersensitivity to tomato may also be responsible for dermatitis induced by contact with the plant and/or the fruit (ripe or unripe) related to heat-stable and/or heat-labile allergens [39]. Pravettoni et al. evaluated the allergenic profile of commercial tomato products using allergic skin tests and specific IgE in tomato-allergic subjects reporting oral allergy syndrome to fresh and cooked tomatoes. Lipid transfer protein (LTP) was the only allergen detected in the peel, pulp, and seeds [40].

To assess the allergens associated with tomato allergy, Asero recruited 97 adults with clinical diagnosis of tomato allergy ascertained by history, positive skin test and specific IgE, reported that the patients were sensitized to patients were sensitized to PR-10 (36%), profilin (8%), both PR-10 (28%) and profilin (18%), LTP alone (8%), LTP plus PR-10 or profilin (1%) [41].

Cross-reactivity among tomato, potato, and latex allergens was evaluated by Reche et al., who demonstrated that the potato allergen inhibited the three allergens (potato, latex, and tomato) with an intensity similar to that observed with the potato allergen alone. SDS-PAGE showed a common band at 44–46 kDa corresponding to patatin, which is implicated in the high cross-reactivity between tomato, latex, and potato observed in the immunoblot and CAP inhibition [42].

Pearson reported that 2.34% of their female adult asthmatic patients complained of sneezing and/or wheezing while scraping uncooked new potatoes, with some presenting with a decrease in FEV after this activity [43]. Swert et al. described seventeen children with potato allergy diagnosed by oral provocation test, proven by *ImmunoCAP*® (Thermo Fisher Scientific, Uppsala, Sweden) and skin allergic tests, presenting eczema (sixteen individuals), gastrointestinal complaints (eight children), urticaria and/or angioedema (five children), wheezing/nasal pruritus (three children), and systemic anaphylaxis (two children) [44]. Quirce et al reported two housewives presenting rhinoconjunctivitis, asthmatic attacks, and contact urticaria after peeling raw potatoes [45]. Jeannet-Peter described a housewife presenting with hives on both hands, itchy, runny eyes and nose, red eyes, sneezing, wheezing, and dyspnea when peeling raw potatoes [46]. Spanish researchers described an 11-year-old girl who presented anaphylactic symptoms after ingestion of potatoes and developed urticaria, angioedema, respiratory, and systemic symptoms on exposure to cooking potatoes or potato pollen. They demonstrated IgE- and non-IgE-mediated antibodies against extracts of potato pulp, peel, and pollen [47]. Potato contact dermatitis was also reported as an occupational hazard [48].

Beyond the canonical IgE-mediated Th2 immunity, pivotal orchestrators of allergic inflammation, such as Th9 cells, have emerged as distinct mechanistic axes underlying atopic dermatitis, allergic rhinitis, and allergic bronchitis [49].

The Leukocyte Adherence Inhibition Test (LAIT) and its similar assay, the Leukocyte Migration Inhibition Test (LMIT), have been used to differentiate non–IgE-mediated immunoreactivity against food allergens [50–54]. The LAIT and the LMIT have also been used to differentiate Non–IgE-mediated immunoreactivity against microorganisms and airborne allergens [55–58].

Non–IgE-mediated cellular immunoreactivity to food allergens has also been reported by our group using the LAIT [59–62]. Non–IgE-mediated cellular immunoreactivity against aeroallergens and microorganisms had also been reported by our group employing the LAIT [63–67]. The primary use of TTP is as a triage to evaluate non–IgE-mediated immunoreactivity against suspected allergens before performing more exhaustive *in vivo* provocation tests [68–71].

To evaluate the potential of the LAIT and TTP to endotype cross-reactive cellular and humoral non–IgE–mediated immunoreactivity against tobacco, tomato and potato allergens, we retrospectively compiled the electronic medical charts of patients diagnosed with non–IgE–mediated allergic multimorbidity involving respiratory and dermatological symptoms (such as allergic rhinitis, allergic conjunctivitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, atopic dermatitis, and/or urticaria) who were investigated for immunoreactivity against these three extracts simultaneously using one of these assays.

The present study was designed as a proof-of-concept study, hypothesizing that LAIT and the TTP may correlate with cellular and/or humoral immunoreactivity against tobacco, tomato, and potato extracts in patients with non–IgE–mediated Allergic Multimorbidity.

2. Materials and Methods

2.1. Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 01/2026), we reviewed the electronic chart of 11.500 outpatients who attended our facility from January 2018 to March 2026, selecting those diagnosed with allergic multimorbidity who were evaluated simultaneously with LAIT or TTP against tobacco, potato, and tomato extracts.

A cohort of 100 consecutive outside patients (TTP cohort) had been submitted to TTP with tobacco, potato and tomato extracts for presenting non–IgE-mediated Allergic Multimorbidity as defined by the concomitant or consecutive presence of at least two allergic phenotypes such as allergic rhinitis, allergic conjunctivitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, atopic dermatitis, and/or popular urticaria. This cohort included 32 males; mean age 34.3 years; SD 19.8 years; range 2 to 75 years; median 34.5 years; mode 44 years (appeared 5 times); geometric mean 26.2 years.

A cohort of 100 consecutive outside patients (LAIT cohort) had been submitted to LAIT with tobacco, potato and tomato extracts for presenting non–IgE-mediated Allergic Multimorbidity as defined by the concomitant or consecutive presence of at least two allergic phenotypes such as allergic rhinitis, allergic conjunctivitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, atopic dermatitis, and/or popular urticaria. This cohort counted 25 males; mean age 39.2 years; SD 18,8 years; range 5 to 87 years; median 39 years; modes = 8, 11, 23, 34, 39, 43, 44, each appeared 4 times; geometric mean = 33.4 years.

This study excluded patients receiving biological and/or systemic anti-inflammatory therapy. These procedures were offered to patients with clinical suspicion of hypersensitivity to tobacco, potato, and tomato allergens who had non-reactive or inconclusive skin test results to these allergens [72, 73].

2.2. Preparation of the allergen's extracts

Preparation of the Tobacco extract

Cured tobacco leaves (200g) of Virginia Bright (rope-rolled air desiccated leaves), acquired from a small local producer of Socorro, São Paulo, were macerated, homogenized and left for 48 hours in a extractor solution (propylparaben 0.5g, methylparaben 1g, sorbitol 30g, NaCl 5g, $NaHCO_3$ 2.5g, 1,000 mL H_2O) at 4°*C* for protein extraction before, centrifugation (for 10 minutes at 4,000 rpm) and separation of the water-soluble fraction from solid particles by filtration (filter paper 80g/m_2) [74]. Protein quantification of the allergen extracts was performed using the Bradford protein-dye binding method [75]. The solution was diluted in an antigen dilution solution (NaCl 10g; KH_2PO_4 0.72g; Na_3PO_4 2.86g; methylparaben 1g; propylparaben 0.5g; glycerin 400 mL; H_2O 600 mL) to an estimated protein concentration of 1 mg/mL and stored at 4°*C* in amber opaque glass vials. The 1 mg/mL extracted solution was used for allergic skin tests, TTP, and LAIT. All relevant and mandatory laboratory health and safety measures have been complied with during the experiments.

Preparation of the tomato extract

Three tomatoes (without seeds) were peeled, macerated, homogenized, and treated in the same manner as the tobacco extract.

Preparation of the potato extract

Four potatoes (two cooked and two raw) were peeled, macerated, homogenized, and treated similarly to the tobacco extract.

2.3. LAIT: *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

We performed the LAIT as previously described [76–80]. Shortly thereafter, each donor's fresh plasma was divided into two portions and used in parallel *ex vivo* challenge tests with the allergen extracts and unchallenged plasma (with the antigen dilution solution added as a control). 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 with (or without) the challenging extract and kept them under agitation for 30 minutes (200 rpm at $37°C$).

After incubation, the challenged plasma was transferred to a standard Neubauer hemocytometer counting chamber with a plain, non-metallic glass surface and left to stand for 2 hours at $37°C$ in a humidified atmosphere within the covered water bath, allowing leukocytes to adhere to the glass. Next, we counted the leukocytes, removed the coverslip, and washed the chamber by immersing it in a beaker containing phosphate-buffered saline (PBS) at $37°C$. 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.

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 Leukocyte Adherence (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 the unchallenged control plasma, multiplied by 100%. To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from 100%. We used the LAI results for the cascade distribution chart and for the statistical calculations, both performed in Microsoft Excel 2022 (Microsoft Corp., Redmond, WA, USA).

2.4. TTP: *In vitro* Investigation: Tube Titration of Precipitins

As previously reported, the semi-quantitative TTP was performed in a transparent vitreous tube array [81]. Shortly thereafter, the patient's blood was collected in a clot-activator tube. After separation, the serum was centrifuged at 2,000 rpm for 10 minutes. Each allergen extract was allocated to 11 glass tubes at progressively diluted serum concentrations. The progressive dilutions were combined with separated aliquots of 15 μL of the allergen extract 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 containing 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 [82]. We used the titration results for the cascade distribution chart and for the statistical calculations, both performed in Microsoft Excel 2022 (Microsoft Corp., Redmond, WA, USA).

3. Results

In this retrospective survey, we report on the incidental immune investigation recorded in the digital medical charts.

The TTP cascade graph for the tobacco extract showed a distribution concentrated toward higher dilutions Figure 1. No negative results were observed. The lower titration was 1:32; the mean was estimated at 1:242; the median was 1:256; the standard deviation was estimated at 1:176; the modes were 1:256 and 1:512 (each appeared 26 times); the geometric mean was estimated at 1:175.

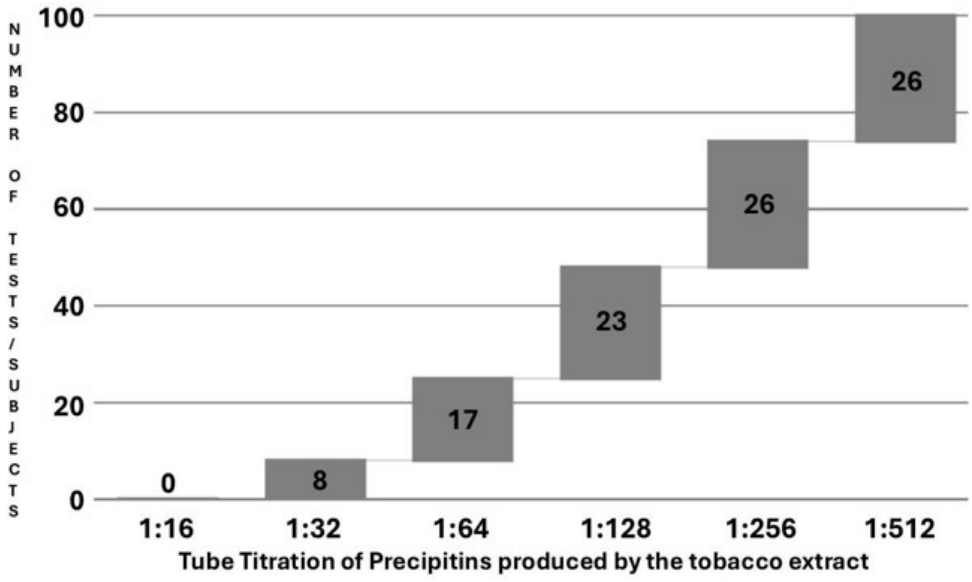

Figure 1: Cascade distribution chart of the Tube Titration of Precipitins (TTP on x-axis %) resulting from the tobacco extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

The TTP cascade graph for the tomato extract showed a distribution concentrated on the intermediate dilutions Figure 2. No negative results were observed. The lower titration was 1:16; the mean was estimated at 1:170; the median was 1:128; the standard deviation was estimated at 1:154; the mode was 64 (appeared 33 times); the geometric mean was estimated at 1:119.

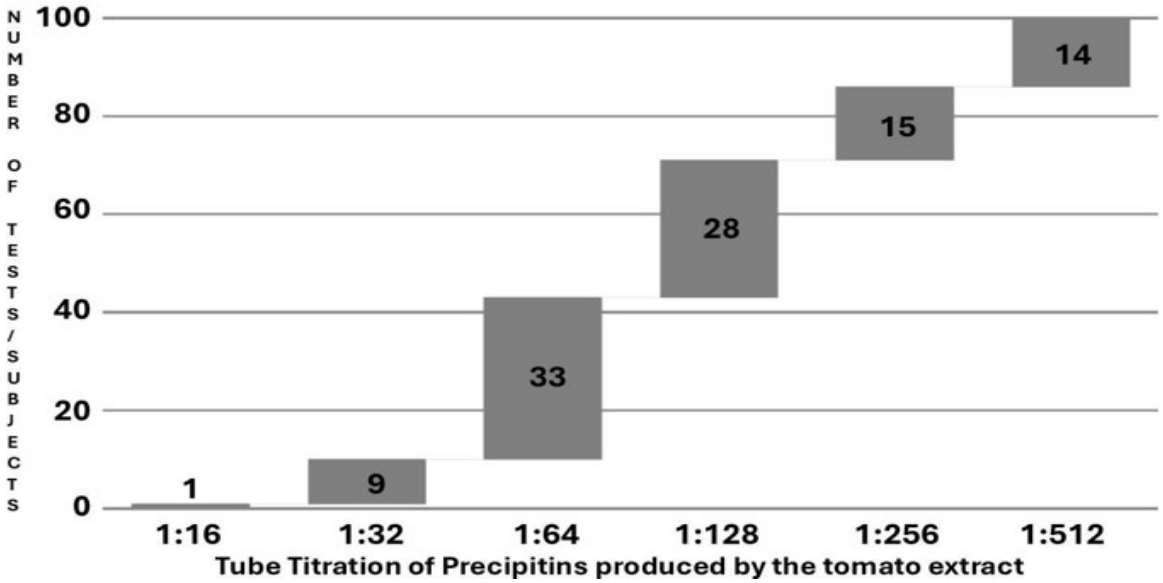

Figure 2: Cascade distribution chart of the Tube Titration of Precipitins (TTP on x-axis %) resulting from the tomato extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

The TTP cascade graph for the potato extract showed a distribution concentrated on the intermediate dilutions Figure 3. No negative results were observed. The lower titration was 1:16; the mean was estimated at 1:178; the median was 1:128; the standard deviation was estimated at 1:144; the mode was 128 (appeared 31 times); the geometric mean was estimated at 1:130.

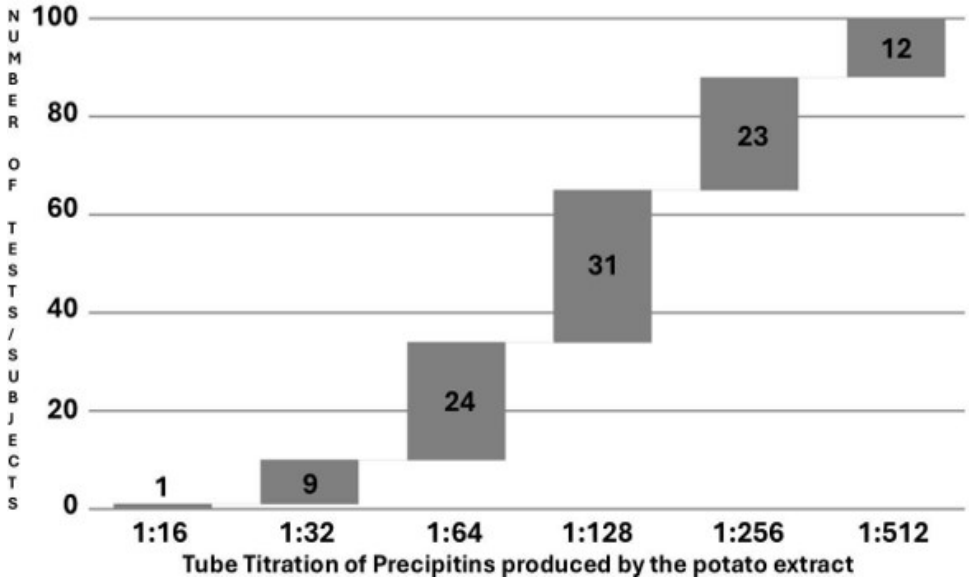

Figure 3: Cascade distribution chart of the Tube Titration of Precipitins (TTP on x-axis %) resulting from the potato extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

The paired t-test indicated that there is a significantly small difference between tobacco TTP results (mean = 242.6; SD = 176.7) and tomato TTP results (mean = 170.1; SD = 154.5), t (99) = 3.1 ; p = 0.003. Pearson's correlation indicated a non-significant, very small positive relationship between tobacco TTP and tomato TTP: r (98) = 0.00729; p = 0.943 see Figure 4.

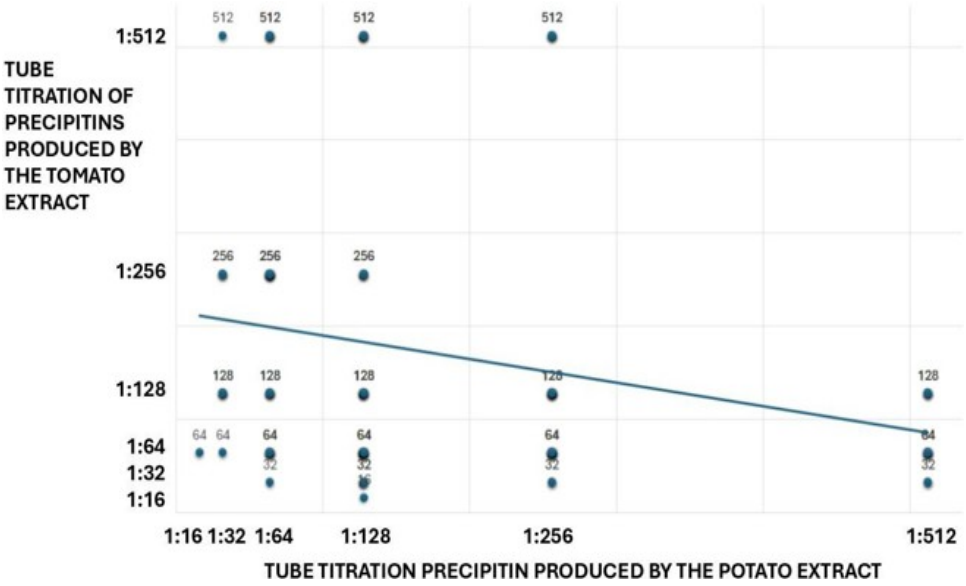

Figure 4: Dispersion chart of the Tube Titration of Precipitins results of the *in vitro* serum challenge against potato extract (x-axis %), plotted against the *in vitro* serum challenge against tomato extract (y-axis %)

The paired t-test indicated that there is a significantly small difference between tobacco TTP (mean = 242.6; SD = 176.7) and potato TTP (mean = 178.4; SD = 145.2); t (99) = 2.6; p = 0.012. Pearson's correlation indicated that there is a significantly small negative relationship between tobacco TTP and potato TTP: r (98) = 0.214, p = 0.032 see Figure 5.

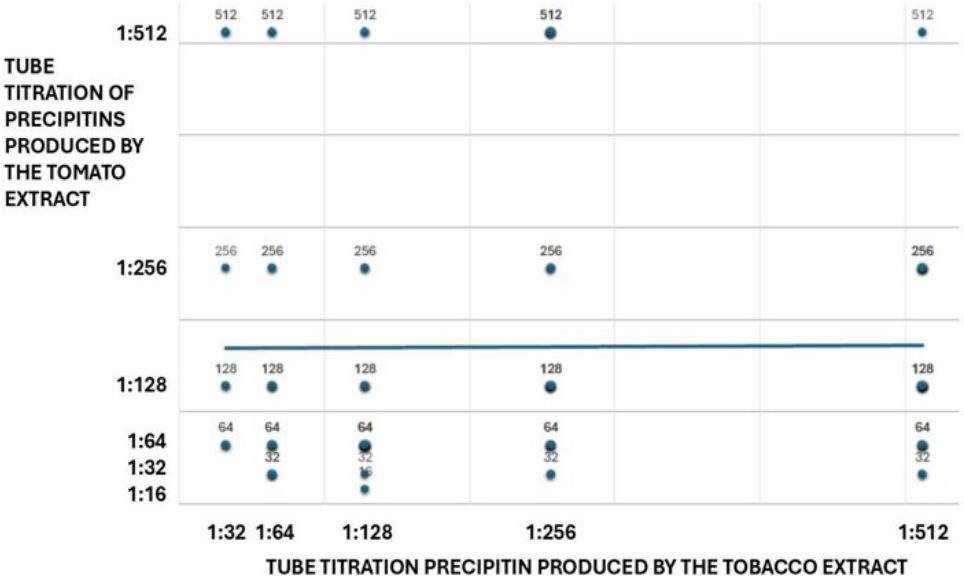

Figure 5: Dispersion chart of the Tube Titration of Precipitins results of the *in vitro* serum challenge against tobacco extract (x-axis %), plotted against the *in vitro* serum challenge against tomato extract (y-axis %)

The paired t-test indicated that there is a non-significant, very small difference between potato TTP (mean = 178.4; SD = 145.2) and tomato TTP (mean = 170.1; SD = 154.5); t (99) = 0.4; p = 0.725. Pearson's correlation indicated that there is a significantly small negative relationship between potato TTP and tomato TTP: r (98) = 0.238; p = 0.017 see Figure 6.

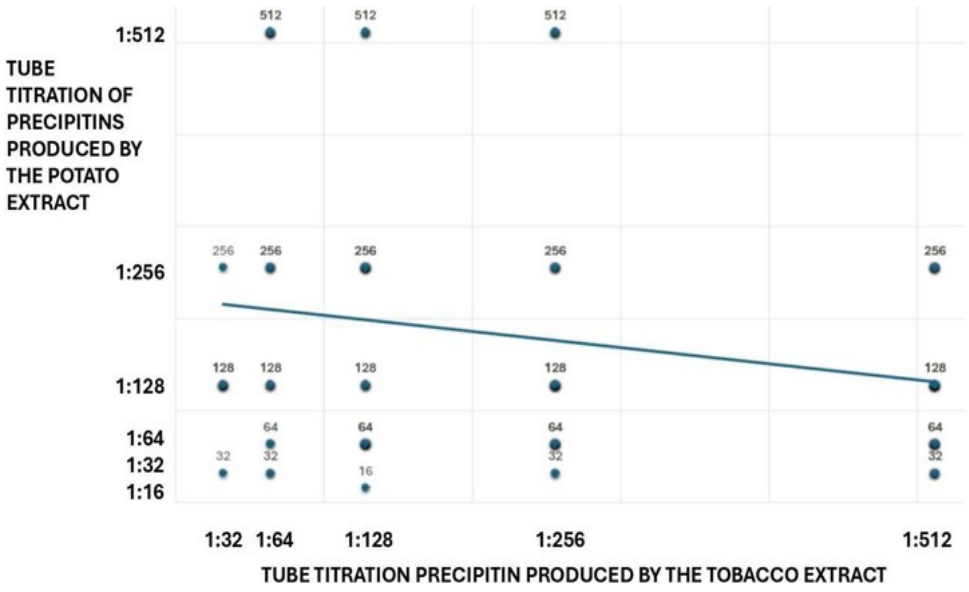

Figure 6: Dispersion chart of the Tube Titration of Precipitins results of the *in vitro* serum challenge against tobacco extract (x-axis %), plotted against the *in vitro* serum challenge against potato extract (y-axis %)

The LAIT cascade graph for the tobacco extract showed a wide distribution range of results. The LAI ranged from 0% to 99% see Figure 7. The mean was 64%; the median was 71%; the standard deviation was 28.2%; the mode was 0% (appeared seven times). The cascade distribution demonstrates a wide range of LAI results. The LAIT cascade graph for the tomato extract showed a wide distribution range of results see Figure 8. The LAI ranged from 0% to 99%. The mean was 62.2%; the median was 72%; the standard deviation was 29.9%; the mode was 0% (appeared nine times). The cascade distribution demonstrates a wide range of LAI results. The LAIT cascade graph for the potato extract showed a wide distribution range of results. The LAI ranged from 0% to 99% see Figure 9. The mean was 71.9%; the median was 77.5%; the standard deviation was 23.4%; the modes were 60, 98, and 99, each appearing 5 times. The cascade distribution demonstrates a wide range of LAI results. The paired t-test indicated no significant difference between tobacco and tomato LAIT results (p-value = 0.57). Pearson's correlation indicated a significantly moderate positive relationship between tobacco and tomato LAIT results: r (98) = 0.4, p-value <0.001 see Figure 10.

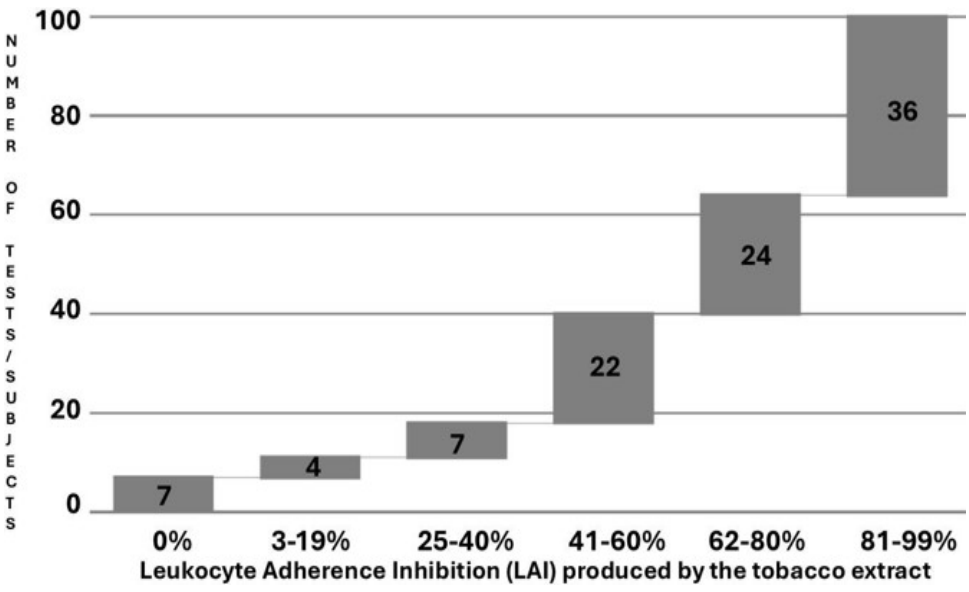

Figure 7: Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the *ex vivo* challenge test against tobacco extract monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

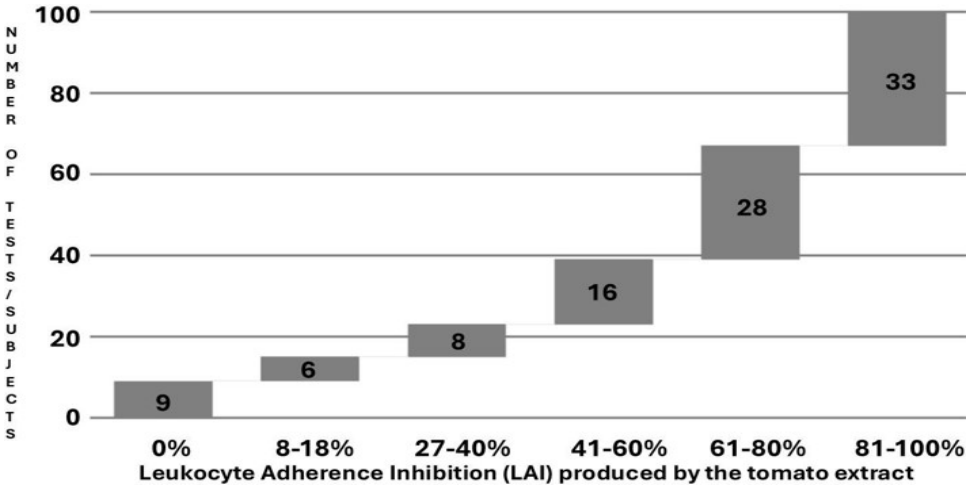

Figure 8: Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the *ex vivo* challenge test against tomato extract monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

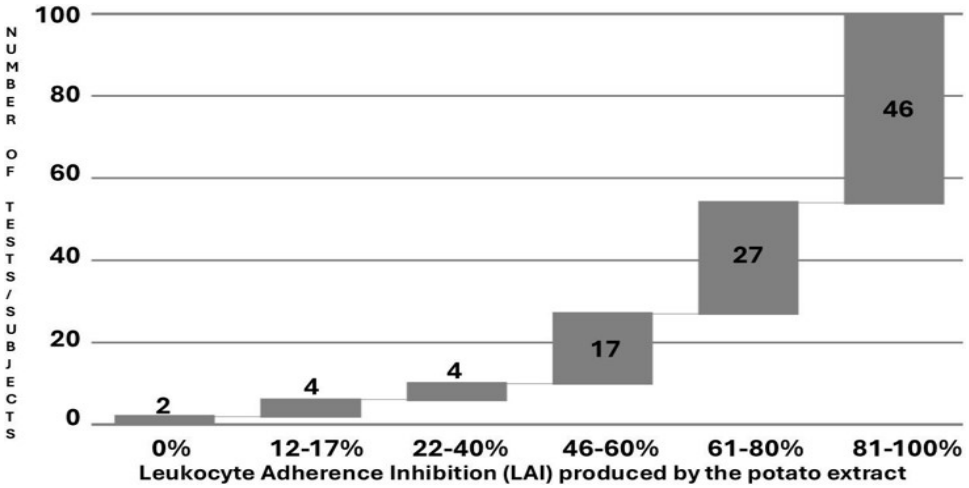

Figure 9: Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the ex vivo challenge test against potato extract monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

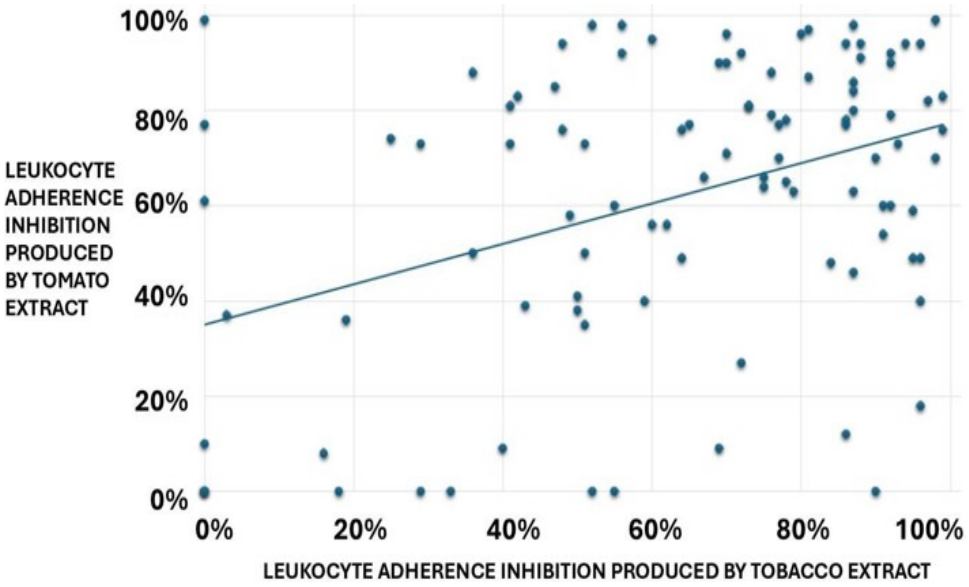

Figure 10: Dispersion chart of the Leukocyte Adherence Inhibition (LAI) results of the *ex vivo* challenge test against tobacco extract (x-axis %), plotted against the LAI results of the *ex vivo* challenge test against tomato (y-axis %)

The paired t-test indicated a significant difference between tobacco and potato LAIT results (p-value = 0.002). Pearson's correlation indicated a significantly moderate positive relationship between tobacco and tomato LAIT results: r (98) = 0.54, p-value <0.001 Figure 11.

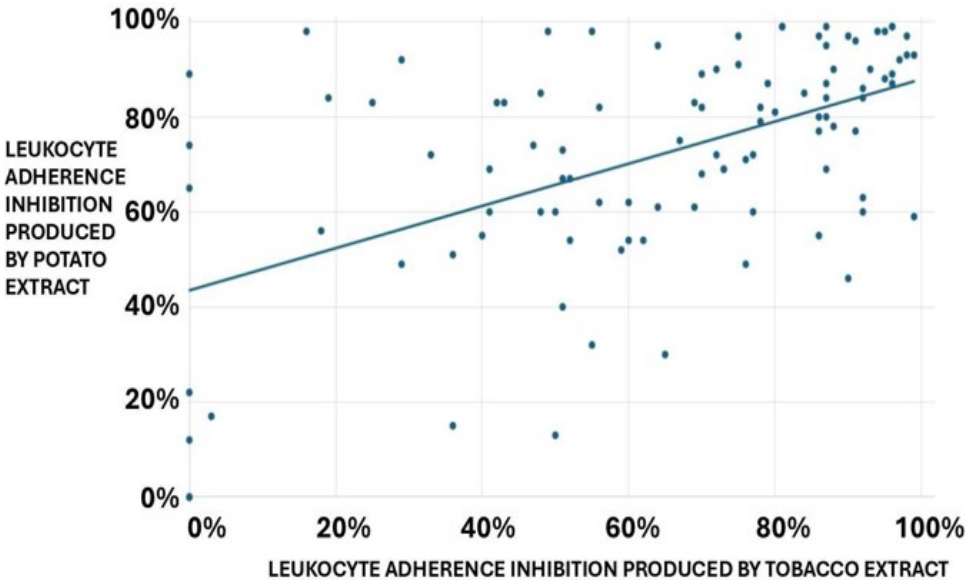

Figure 11: Dispersion chart of the Leukocyte Adherence Inhibition (LAI) results of the *ex vivo* challenge test against tobacco extract (x-axis %), plotted against the LAI results of the *ex vivo* challenge test against potato (y-axis %)

The paired t-test indicated a significant difference between tomato and potato LAIT results (p-value = 0.005). Pearson's correlation indicated a significantly moderate positive relationship between tobacco and tomato LAIT results r (98) = 0.216, p = 0.031 see Figure 12.

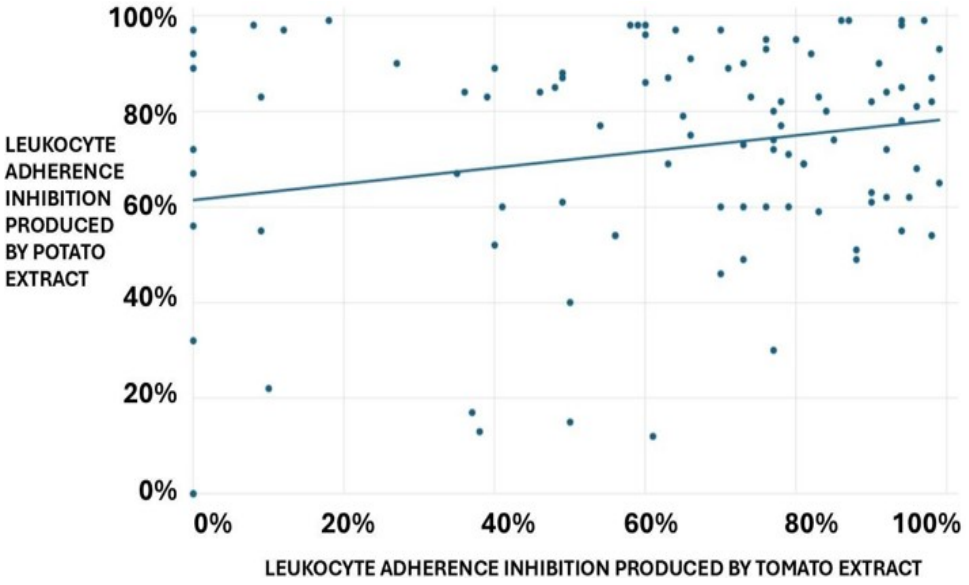

Figure 12: Dispersion chart of the Leukocyte Adherence Inhibition (LAI) results of the *ex vivo* challenge test against tomato extract (x-axis %), plotted against the LAI results of the *ex vivo* challenge test against potato (y-axis %)

4. Discussion

"Tobacco blindness" was a caricatured term used by concerned medical scientists to refer to their colleagues who failed to report and measure tobacco use (and environmental tobacco inhalation) when evaluating the factors influencing the health and diseases of their subjects in medical publications [83].

Allergic diseases have a complex pathogenesis involving multiple immune cells, antibodies, cytokines, and inflammatory mediators that trigger immunoreactivity to otherwise harmless proteins, leading to allergic multimorbidity [84, 85]. Despite the current trend toward clinically diagnosing the etiology of allergic diseases, predominantly through molecular diagnosis of specific IgE against major allergens, non-IgE-mediated mechanisms of hypersensitivity are increasingly recognized as contributors to respiratory allergies and as targets for diversified therapeutic strategies [86, 87].

Active (voluntary or mainstream) and passive (involuntary or environmental) tobacco smoke exposures skew immune responses, causing cellular and humoral immunosuppression and/or immunostimulation, contributing to inflammatory and allergic conditions [88–90]. The innate immune system is particularly prone to mounting inflammatory responses after exposure to tobacco smoke, primarily in epithelial cells

and macrophages [91, 92]. In an inflammatory environment, macrophages exhibit polarization and differentiation into distinct phenotypes, producing cytokines and chemokines that participate in allergic reactions [93]. Cigarette smoke also stimulates the release of proinflammatory cytokines from innate sources, such as natural killer cells [94]. Dendritic cells, however, are the link between innate inflammation induced by tobacco and adaptive immune sensitization of T and B cells [95, 96]. Tobacco smoke skews adaptive immune responses by impairing Th1 responses and augmenting Th2 responses, thereby impairing immune tolerance [97]. Chronic exposure to tobacco smoke inhibits the leukocyte adherence observed by LAIT in mice exposed to environmental tobacco [98]. Allergen challenge reduces catalase activity and downregulates the Nuclear factor erythroid 2-related factor 2 (Nrf2) expression. Deficiency in Nrf2 is associated with diminished antioxidant gene expression, elevated type 2 cytokine levels, and impaired Treg cell differentiation, thereby increasing vulnerability to tobacco smoke-induced lung injury, airway inflammation, and asthma [99].

The genome sequencing showed low genetic divergence of *N. tobacco* from its ancestors and synteny (conservation of gene order on chromosomes) with *S. lycopersicum* and *S. tuberosum* [100].

Nonspecific cellular inflammatory markers can exhibit cross-reactivity among allergens of distinct origins, eliciting different allergic phenotypes [101–104]. Cross-reactivity among tobacco and latex allergens, as well as polysensitization may act with clinical significance to synergistically impair respiratory hypersensitivity symptoms [105]. The diagnosis of polysensitization and cross-reactivity syndromes may be difficult to establish, as excluding a single allergen may not alleviate symptoms, thereby complicating the identification of the culprits [106]. Knowledge of cross-reactivity facilitates clinical suspicion of correlated allergens, enabling a more precise diagnosis, as in the case of Solanacea cross-reactivity [107]. Diagnosing cross-sensitization is relevant for planning diets and group-specific multiallergen desensitization immunotherapy for polysensitized patients [108–110].

The retrospective compilation of our data showed a wide distribution of results when we assessed TTP and LAIT to evaluate humoral and cellular immunoreactivity to the allergens studied.

Most patients showed strong immunoreactivity to tobacco, potato, and tomato extracts, which could reflect the involvement of these allergens in a Non–IgE-mediated hypersensitivity condition.

These immunoassays did not precisely identify the mechanisms responsible for the clinical condition. Instead, they provide evidence of cellular and humoral immunoreactivity distributed across an extensive spectral range, which may suggest immune tolerance or hypersensitivity. As the tests were performed simultaneously using the same venous sample for the three allergens, it was possible to calculate correlations to quantify cross-reactivity among them.

LAIT and TTP are diagnostic approaches, not intended to provide a molecular diagnosis of the factor responsible for the allergic phenotype, but rather to provide an overall assessment of the intensity of the patient's cellular and humoral immunoreactivity against the allergen extract.

This preliminary survey demonstrated extensive results from the TTP, and the *ex vivo* challenge test, monitored by LAIT, was conducted against tobacco, tomato, and potato allergens in two cohorts of patients with allergic multimorbidity. TTP and LAIT are complementary triage tests used at our facilities to select worthwhile antigens for more labor-intensive *in vivo* provocation testing when specific IgE is undetectable. None of our patients presented an exclusive reaction to these allergens. Every patient was simultaneously evaluated for several known allergens, with some positive results.

Our results suggest that when exposed simultaneously to both allergens, allergic patients may experience a synergistic worsening of symptoms via cross-reactive hypersensitivity mechanisms.

5. Limitations

This study is a retrospective analysis of data collected over seven years since our facility began employing laboratory immune assays. There was no protocol research, and the subject's 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 studies; however, future studies must be more comprehensive. The lack of a research protocol raises the possibility of bias stemming from the physician who ordered the exam (CEO), driven by clinical suspicion based on the anamnesis, physical examination, cutaneous allergy test results, and IgE-specific testing. The study lost many of these patients to follow-up, so it is not yet possible to assess the relationship between the immunoassay results and patients' clinical outcomes. Unfortunately, it was not possible to compare the two procedures using paired t-tests because they were obtained from distinct patient groups.

6. Conclusion

The preliminary results suggest that the TTP and LAIT performed with tobacco, tomato, and potato extracts may discriminate for diverse humoral and cellular levels of immunoreactivity in patients with Allergic Multimorbidity. There is a significant association between allergen immunoreactivities. LAIT and TTP are inexpensive, can be performed with minimal laboratory equipment, and can be incorporated into strategies to address health disparities in multimorbidity and allergies [111]. As a preliminary report, the propaedeutic meaning of the presented results and the possibility of interferents must be established [112]. More studies using a quality-by-design approach, with larger, prospective, double-blind cohorts, are needed to evaluate the potential contribution of LAIT and TTP to endotyping cellular and humoral immunoreactivity in patients suspected of hypersensitivity to tobacco, tomato, and potato extracts [113].

7. Future Directions and Recommendations for Clinical Practice

The primary intended use of *in vitro* or *ex vivo* allergen challenge tests is to spare patients from unnecessary, exhaustive, and dangerous *in vivo* challenge tests that are required to prescribe desensitization treatments [114]. Exploring the humoral and cellular arms of the immune system, the TTP and LAIT, alone or combined, may soon serve as tools for allergists to construct etiologic diagnoses in their patients and determine the endotypes (mechanisms) of hypersensitivity, in order to choose more convenient and personalized therapies for them. Adding data from TTP and LAIT may also help streamline biomedical research and improve tools such as Large Language Models, which clinicians often use as decision-support systems to enhance diagnostic accuracy [115].

Article Information

Acknowledgements: The Instituto Alergoimuno de Americana funded this work.

Author Contributions: Celso Eduardo Olivier - Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing; Damla Serçe Unat - Supervision; Daiana Guedes Pinto, Ana Paula Monezzi Teixeira, Cibele Silva Miguel, Nicole Sartoreto Rocha, Alessandra Vieira de Oliveira, Luciana Sacilotto Carvalho, Raquel Acácia Pereira Gonçalves Santos - Methodology.

Funding / Financial Support: This study was supported by the Instituto Alergoimuno de Americana.

Competing Interests: The authors have declared that no competing interests exist.

Ethical Approval: The study was approved by the Institutional Review Board of the Instituto Alergoimuno de Americana (Brazil; January/02/2026).

Informed Consent: As a retrospective survey of results recorded in Cognito, consent was obtained collectively by the institution's ethics committee in accordance with the principles of the Declaration of Helsinki. Cross-sectional and retrospective digital medical records were collected, and file data were saved anonymously.

Data Availability Statement: Data available on reasonable request.

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