Original Research Article

Endotyping Cellular and Humoral Immunoreactivity against *Allium* spices and Sulfites preservatives in Allergic Patients: A Retrospective Study

ABSTRACT

Background: Spices from the *Allium* genus season food worldwide, and their widespread use may produce allergic reactions by contact or ingestion. However, the Non–IgE-mediated immunoreactivity against these allergens has not yet been adequately investigated.
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 two *Allium* spices (garlic and onion) and a sulfite derivative in patients with Non–IgE-mediated allergic phenotypes.
Methodology: Two cohorts of allergic patients suspected of Non–IgE-mediated hypersensitivity against *Allium* spices and sulfite derivatives were investigated with the help of TTP or LAIT, simultaneously against extracts of garlic, onion, and sodium bisulfite. The results of the semi-quantitative serum TTP against 1 mg/mL garlic extract, 1 mg/mL onion extract, and 1 mg/mL sodium bisulfite were distributed in ranges through cascade distribution charts. The results of the Leukocyte Adherence Inhibition (LAI) percentage promoted by the *ex vivo* challenges against 1 mg/mL garlic extract, 1 mg/mL onion extract, and 1 mg/mL sodium bisulfite were distributed in ranges through cascade distribution charts. The statistical differences inside these cohorts were calculated.
Results: Paired t-test indicated a non-significant difference between garlic TTP and onion TTP (p-value = 0.761); a non-significant difference between sulfites TTP and garlic TTP (p-value = 0.112); a non-significant difference between sulfites TTP and onion TTP (p-value = 0.058); a non-significant difference between garlic TIAL and onion TIAL (p-value = 0.942); a significant difference between sulfites TIAL and garlic TIAL (p-value < 0.001) and a significant difference between sulfites TIAL and onion TIAL (p-value < 0.001).
Conclusion: Results support that the TTP and LAIT performed with sodium bisulfite, garlic, and onion extracts may discriminate diverse degrees of humoral and cellular immunoreactivity in patients suffering from diversified allergic phenotypes.

Keywords: *Allium sativum, Allium cepa; Endotype; Garlic; Hypersensitivity; Leukocyte Adherence Inhibition Test; Non–IgE-mediated Immunoreactivity; Onion; Precipitins, Sulfite derivatives.*

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

1. INTRODUCTION

Allium is a genus of monocotyledonous plants, characterized by their odorous volatile sulfur-containing compounds (most derived from cysteine sulfoxides) with more than eight hundred species, among which quite a few are edible and cultivated as lore for culinary

purposes or therapeutic indications, such as several kinds of garlic (e.g., *Allium sativum* L.), onions (e.g., *Allium cepa* L.), chives (e.g., *Allium schoenoprasum*) and leeks (e.g., *Allium porrum* L.), among others [1]. *Allium* spices are reported to have been used as food and as medicines in the ancient histories of Egypt, China, Japan, India, Greece, and Rome [2].

The first reported clinical case of allergy to an *Allium* species was described in 1950 by Edelstein, who treated a food-pack worker presenting occupational dermatitis after skin contact with garlic and "severe cardiospasm" immediately after ingestion [3]. Shortly after that (1952), the Canadian Medical Association published a report of a cook who was also diagnosed with an occupational allergy to onion and garlic [4]. Finally (1954), the "classic aspects of onion and garlic dermatitis in housewives" was published by the American College of Allergists [5]. Hypersensitivities to *Allium* spices have been reported to produce contact dermatitis, gingivitis, stomatitis, allergic rhinitis, allergic conjunctivitis, and asthma [6, 7]. In Spain, the reported prevalence of documented hypersensitivity against garlic and/or onions in a cohort of allergic patients submitted to routine skin allergic tests was almost 3% [8]. In Saudi Arabia, from a cohort of 108 patients with clinical suspicion of food allergy, 15 (13.8%) had garlic and onion-specific IgE antibodies in their sera [9].

Skin tests with spices gradually became routine to diagnose cooks with hand dermatitis, and the early attempts to identify the sensitizer revealed that it was soluble in water, ethanol, and acetone [10]. In the last decade of the 20th century, doctors became progressively aware of other clinical manifestations that could be caused by hypersensitivity to components of the *Allium* genus: several cases of bronchial asthma, rhinitis, conjunctivitis, and dermatitis were attributed to onion allergy, demonstrated by bronchial provocation tests, nasal provocation tests, and skin tests with onion extracts that cross-reacted with garlic and leek extracts; and in some cases, detection of specific IgE against onion and garlic was possible by Phadezyme® and CAP System RAST FEIA® [11]. Allergic rhinitis elicited by *Allium* allergens was also demonstrated by anterior rhinomanometry [12]. Susceptible patients may also develop urticaria, angioedema, and anaphylactic reactions, especially to young garlic (an unripe and underdeveloped bulb) and raw onions [13, 14]. An experience done with volunteers and patients with gastroesophageal reflux submitted to an esophageal pH probe who had ingested onions did not increase any reflux variables measured in asymptomatic volunteers. However, the ingestion of onions significantly increased reflux episodes in symptomatic subjects compared with asymptomatic subjects after onion ingestion and when compared with meals with no-onion [15].

Even before the reports of *Allium* allergic reactions, the sulfur compounds of *Allium* species were already studied, particularly the diallyl disulfide, an organic molecule with formula $C_6H_{10}S_2$ [16]. Diallyl disulfide is one of the principal components of the distilled oil of garlic and other *Allium* genus plants, produced during the decomposition of allicin released after crushing [17, 18]. Identified as an active component due to its larvicidal activity, the diallyl disulfide was also suspected of producing allergic reactions and was employed to perform diagnostic allergic skin tests (with the help of a 1% solution) [19-21]. Non-IgE-mediated allergic contact dermatitis is one of the most common symptoms of *Allium* allergy, mainly in cooks who handle the spices, despite the use of gloves permeable to diallyl disulfide [22]. Occasionally, when used as a naturopathic remedy by topical application, garlic has also been linked to allergic contact dermatitis [23].

With the help of Immunoblotting, in 2001, Asero *et al*. identified IgE-binding to 15-kDa and 43-kDa onion proteins; after preabsorption of the patient's serum with peach Lipid Transfer Protein (LTP), the IgE-binding to the 15-KDa protein disappeared [24]. Later (2007), Enrico *et al*., by immunoblotting an onion extract with the serum of a patient with onion allergy and anti-IgE, revealed a 12-kDa IgE-binding protein band that was inhibited by onion extract and the peach allergen rPru p 3 (a marker allergen for LTP) [25, 26]. With the help of mass spectrometry, a Taiwan group marked the IgE-binding of a 56-kDa protein as a major allergen for patients with garlic allergy, identified as the alliin lyase (E.C.4.4.1.4), the enzyme responsible for the lysis of alliin into the biologically active allicin molecule upon crushing of a garlic clove [27]. Italian investigators also reported cellular responses, and they identified specific B cell proliferation in onion-allergic patients' plasma incubated with onion extracts (but not in healthy controls) [28]. Cellular techniques using cell sorting, EliSpot, flow cytometry, and confocal microscopy have recently boosted a crescent interest in studying the interaction of LTP allergens with immune cells, particularly with group 2 innate lymphoid antigen-presenting cells, promoting Th2 cell responses involved in allergy pathogenesis [29].

Besides diallyl disulfide, *Allium* species also produce other organic sulfides such as diallyl trisulfide, diallyl sulfide, dipropyl disulfide, dipropyl trisulfide, 1-propenyl propyl disulfide,

allyl methyl disulfide, and dimethyl disulfide [30]. In organic chemistry, "sulfide" or "thioether" refers to the linkage "C–S–C". In inorganic chemistry, "sulfides" are corrosive ionic salts composed of the anion S^{2-}, while "sulfites" (or chiefly British "sulphites") are salts composed of the sulfur dioxide (SO_2^-) which is found in aqueous solution as free SO_2, HSO_3^- and/or SO_3^{2-}. In biological fluids, the sulfite ions are usually combined with carbonyl compounds such as acetaldehydes, proteins, sugars, and others [31]. The solution's free sulfite ions associate and dissociate in equilibria with carbonyl compounds according to the solution's pH at diverse equilibrium constants (pKs) [32].

Allium species' characteristic flavor and pungency depend on the content of "sulfates" (the sulfur tetraoxide SO_4^{-2}) in the soil the plant grows [33]. In Naples, it was reported a particular association of pizza makers' hand dermatitis with positive skin allergy tests done with diallyl disulfide and ammonium persulfate, a flour-strengthening salt with formula $(NH_4)^{2+}(S_2O_8)^{2-}$ [34].

There seems to be some hypersensitivity cross-reactivity among the organic sulfides in *Allium* spices and the inorganic sulfites used as antioxidants and preservatives in industrialized foods, cosmetics, and medicines [35]. Sulfites are the group of inorganic compounds elected by the American Contact Dermatitis Society as the "Allergen of the Year for 2024" [36].

Sulfites were anciently aggregated into the human diet employing *Saccharomyces cerevisiae* fermentation, which reduces sulfate into sulfites and sulfides [37, 38].

Nowadays, sulfites are almost universally added to industrialized foods and beverages to control non-enzymic browning, enzymic browning, oxidation, and microorganisms growing [39]. Several sulfite salts are qualified to be added to industrial food, such as sulfur dioxide (E 220; SO_2), sodium sulfite (E 221; Na_2SO_3), sodium bisulfite (E 222; $NaHSO_3$), sodium metabisulfite (E 223; $Na_2S_2O_5$), potassium metabisulfite (E 224; $K_2S_2O_5$), calcium sulfite (E 226; $CaSO_3$), calcium bisulfite [E 227; $Ca(HSO_3)_2$] and potassium bisulfite (E 228; $KHSO_3$) [40].

Since the seventies, sulfur dioxide has been known as an atmospheric pollutant associated with the rapid development of laryngeal and bronchial spasms, excessive bronchial secretions, and pulmonary edema in sensitive patients, either inhaled or ingested [41]. Anaphylaxis after ingestion of sodium bisulfite was also reported [42]. Besides respiratory symptoms and anaphylaxis, sulfite hypersensitivities are associated with nausea, stomach cramps, diarrhea, urticaria, angioedema, dermatitis, diaphoresis, tingling sensations, flushing, and loss of consciousness [43]. Sulfites are associated with headaches in sensitive individuals [44]. Sulfites are among the main suspects of allergies associated with alcoholic beverages [45]. Several prescription medicines incorporate sulfites as antioxidants, such as topical ophthalmic medications; inhaled bronchodilators (salbutamol, racepinephrine); beta-adrenoceptor agonists (isoprenaline, epinephrine); local anesthetics; injectable corticosteroids; injectable antibiotics; injectable antiarrhythmics; injectable analgesics; antishock agents, including Aramine®, Intropin® and Levophed®; and solutions for parenteral nutrition and dialysis [46-52].

The resources to diagnose sulfite hypersensitivity are *in vivo* and *ex vivo* provocation tests [53]. We routinely employ the Leukocyte Adherence Inhibition Test (LAIT) and the Tube Titration of Precipitins (TTP) in our facilities as a triage to evaluate Non-IgE-mediated immunoreactivity against suspected allergens before the performance of more exhaustive *in vivo* provocation tests [54-60]. To evaluate the potential of the LAIT and TTP to endotyping Non–IgE-mediated cellular and humoral immunoreactivity against garlic, onion, and sodium bisulfite, we retrospectively compiled the electronic medical charts of patients with Non–IgE-mediated allergic rhinitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, allergic sinusitis, allergic migraine, atopic dermatitis, and/or urticaria who were investigated simultaneously for immunoreactivity against these three allergens by one of these assays.

The present study is a proof of concept that hypothesizes that the LAIT and the TTP may differentiate diverse endotypes and degrees of immunoreactivity against *Allium* species and sodium bisulfite among patients suffering from common allergic phenotypes. As the tests were performed simultaneously with the same venous sample with the three allergens, it was possible to calculate a paired t-test to discriminate cross-reactivity between them.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 07/2024), we reviewed the electronic chart of 9,450 outpatients who attended our facility from January 2018 to September 2024.

A cohort of 100 consecutive outside patients (TTP cohort) had been simultaneously submitted to TTP with garlic extract, onion extract, and sodium bisulfite for presenting Non–IgE-mediated allergic rhinitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, allergic sinusitis, allergic migraine, atopic dermatitis, and/or urticaria. This cohort counted 26 males; mean age 39.7 years; SD 16.9 years; range 5 to 79 years; median 39.5 years; modes = 43 (appeared seven times); geometric mean = 35.2 years.

A cohort of 100 consecutive outside patients (LAIT cohort) had been simultaneously submitted to TIAL with garlic extract, onion extract, and sodium bisulfite for presenting Non–IgE-mediated allergic rhinitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, allergic sinusitis, allergic migraine, atopic dermatitis, and/or urticaria. This cohort counted 33 males; mean age 42.9 years; SD 21.3 years; range 2 to 91 years; median 43 years; modes = 43 and 48 years (each appeared four times); geometric mean = 35.1 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 *Allium* spices hypersensitivity who demonstrated a non-reactive or inconclusive skin test against sodium bisulfite, garlic, and onion extracts [61].

2.2 Garlic extract

The peeled garlic was crushed, homogenized, and then left for 48 hours in Coca's solution at 4°C for protein extraction before centrifugation and separation of the water-soluble fraction from solid particles and oily fraction [62]. The protein quantification of the allergen extracts was done according to Bradford's protein-dye binding methodology [63]. The solution was diluted in antigen dilution solution (NaCl 10g; KH_2PO_4 0.72g; Na_3PO_4 2.86g; methylparaben 1g; propylparaben 0.5g; glycerin 400mL; H_2O 600mL) to an estimated protein concentration of 1 mg/mL and stored at 4°C into amber opaque glass vials. The garlic extract solution was used to perform the allergic skin tests, TTP, and LAIT. All relevant and mandatory laboratory health and safety measures have been complied with during the experiments.

2.3 Onion extract

The onion extract solution was prepared using a similar technique employed for the garlic extract.

2.4 Sodium bisulfite solution

The sodium bisulfite powder (IUPAC ID: Sodium hydrogen sulfite; formula $NaHSO_3$) was acquired from Bianquimica™ and diluted with distilled water at 1 mg/mL to perform the allergic skin tests, TTP, and LAIT.

2.5 LAIT: *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

2.5.1 LAIT: Procedure for allergen *ex vivo* challenging

We performed the LAIT as previously described [64-73]. Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with the *Allium* or onion extracts and the unchallenged plasma (added with antigen dilution solution as a control). We collected the plasma with high leukocyte content (buffy coat) from the heparinized tube after one hour of sedimentation at 37 °C. Then, we distributed aliquots of 100 µL into Eppendorf tubes with (or without) the challenging extract and kept them under agitation for 30 minutes (200 rpm at 37 °C).

2.5.2 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 2 hours at 37 °C in the humidified atmosphere of the covered water bath to allow leukocytes to adhere to the glass. Next, we counted the leukocytes, removed the coverslip, and washed the chamber by immersion in a beaker with PBS at 37 °C. Then, we added a drop of PBS to the

hemocytometer's chamber and allocated a clean coverslip over it. The remaining cells were counted in the same squares as previously examined.

2.5.3 LAIT: 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 of the challenged sample divided by LA of unchallenged control plasma multiplied by 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.

2.6 TTP: *In vitro* Investigation: Tube Titration of Precipitins

As previously reported, the semi-quantitative TTP against the aluminum solution was performed in a transparent vitreous tube array [74-76]. 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. Each allergen extract was allocated in sets of eleven glass tubes at progressive duplicated serum dilutions. The progressive dilutions were combined with the 15 µL of the antigen (1 mg/mL) 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 [77].

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 for the garlic extract showed a distribution concentrated on the higher dilutions (Fig 1). There was no negative result. The mean was estimated at 1:247; the median was 1:128; the standard deviation was estimated at 1:180; the mode was 1:512 (appeared nine times).

The TTP for the onion extract showed a distribution concentrated on the higher dilutions (Fig 2). There was no negative result. The mean was estimated at 1:254; the median was 1:256; the standard deviation was estimated at 1:178; the mode was 1:512 (appeared 29 times).

The TTP for the sodium bisulfite solution showed a distribution concentrated on the higher dilutions (Fig 3). There was no negative result. The mean was estimated at 1:207; the median was 1:158; the standard deviation was estimated at 1:176; the mode was 1:128 (appeared 29 times).

The LAIT for the garlic extract showed a wide distribution range of results. Most results were concentrated in the more immunoreactive groups. There were five negative results. The LAI ranged from 0% to 89%. The mean was 51.3%; the median was 57.5%; the standard deviation was 24.0%; the mode was 0% (appeared five times). The cascade distribution demonstrates a wide range of LAI results (Fig. 4). Some patients showed low or moderate immunoreactivity during the ex vivo challenge test. In contrast, others displayed strong immunoreactivity, which could reflect the participation of garlic allergens in a Non–IgE-mediated hypersensitivity condition in these patients.

The LAIT for onion extract showed a wide distribution range of results. Most results were concentrated in the more immunoreactive groups. There were three negative results. The LAI ranged from 0% to 89%. The mean was 52%; the median was 51.2%; the standard deviation was 24.9%; the mode was 62% (appeared seven times). The cascade distribution demonstrates a wide range of LAI results (Fig. 5). Some patients showed low or moderate immunoreactivity during the ex vivo challenge test. In contrast, others displayed strong immunoreactivity, which could reflect the participation of onion allergens in a Non–IgE-mediated hypersensitivity condition.

The LAIT for sodium bisulfite showed a wide distribution range of results. Most results were concentrated in the less immunoreactive groups. There were 27 negative results. The LAI ranged from 0% to 88%. The mean was 27.5%; the median was 25.5%; the standard deviation was 25.1%; the mode was 0% (appeared 27 times). The cascade distribution demonstrates a wide range of LAI results (Fig. 6). Most patients showed low or moderate immunoreactivity during the ex vivo challenge test. At the same time, a few displayed strong immunoreactivity, which could reflect the participation of sulfites in a Non–IgE-mediated hypersensitivity condition in these patients.

The paired t-test indicated a non-significant, minimal difference between garlic TTP and onion TTP; p-value = 0.761.

The paired t-test indicated a non-significant, minimal difference between sulfites TTP and garlic TTP; p-value = 0.112.

The paired t-test indicated a non-significant, minimal difference between sulfites TTP and onion TTP; p-value = 0.058.

The paired t-test results indicated a non-significant minimal difference between garlic TIAL and onion TIAL; p-value = 0.942.

The paired t-test indicated a significantly large difference between sulfites TIAL and garlic TIAL; p-value < 0.001.

The paired t-test results indicated a significantly large difference between sulfites TIAL and onion TIAL; p-value < 0.001.

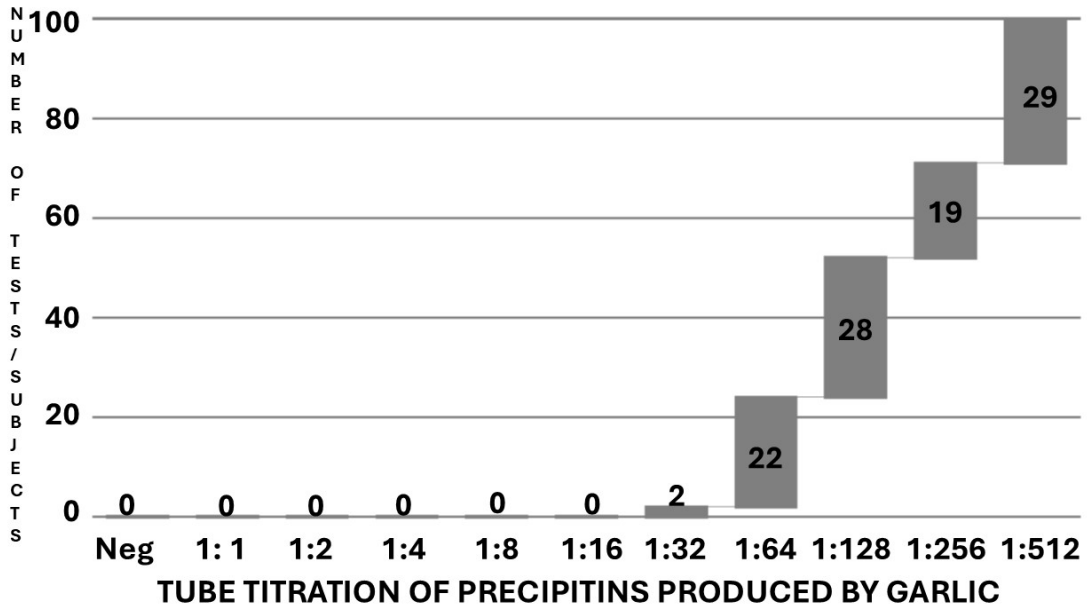

Fig. 1. Cascade distribution chart of the Tube Titration of Precipitins (x-axis %) resulting from the garlic extract against the serum of the TTP cohort of 100 tests/subjects (y-axis).

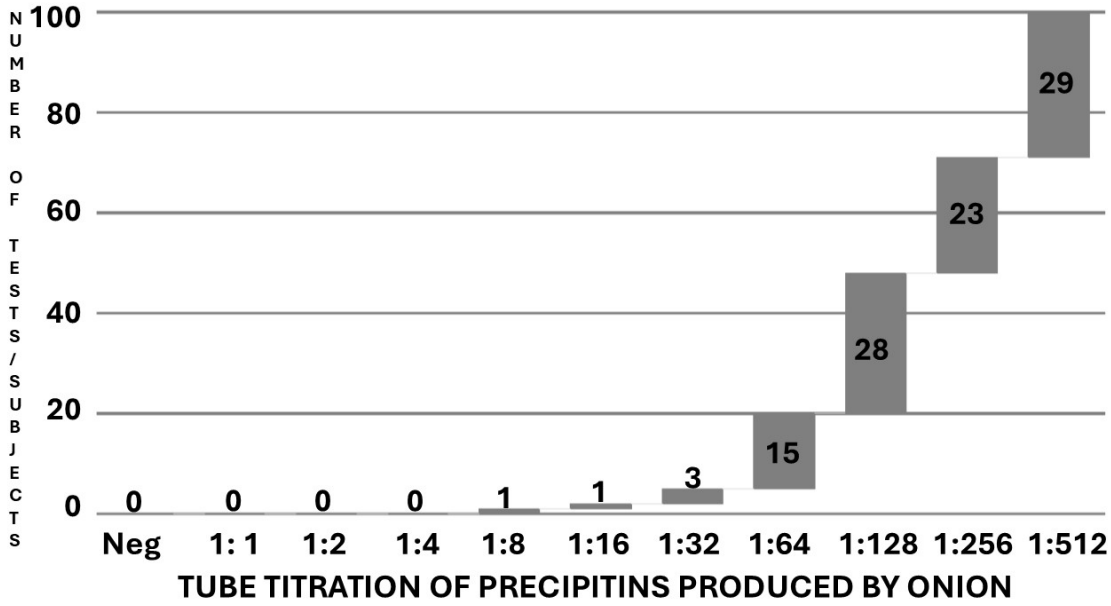

Fig. 2. Cascade distribution chart of the Tube Titration of Precipitins (x-axis %) resulting from the onion extract against the serum of the TTP cohort of 100 tests/subjects (y-axis).

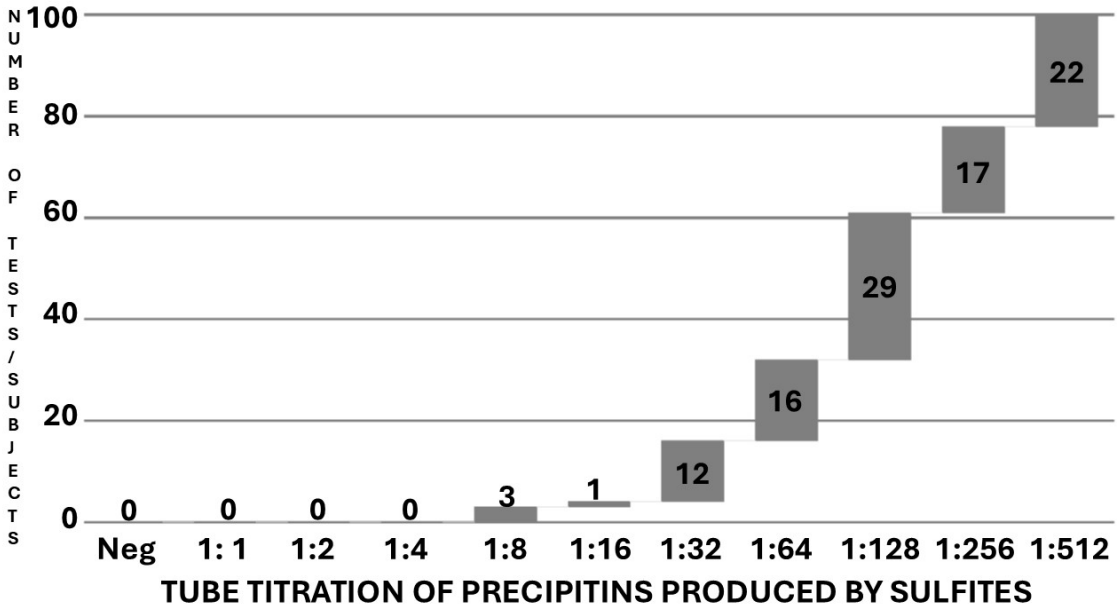

Fig. 3. Cascade distribution chart of the Tube Titration of Precipitins (x-axis %) resulting from the sodium bisulfite extract against the serum of the TTP cohort of 100 tests/subjects (y-axis).

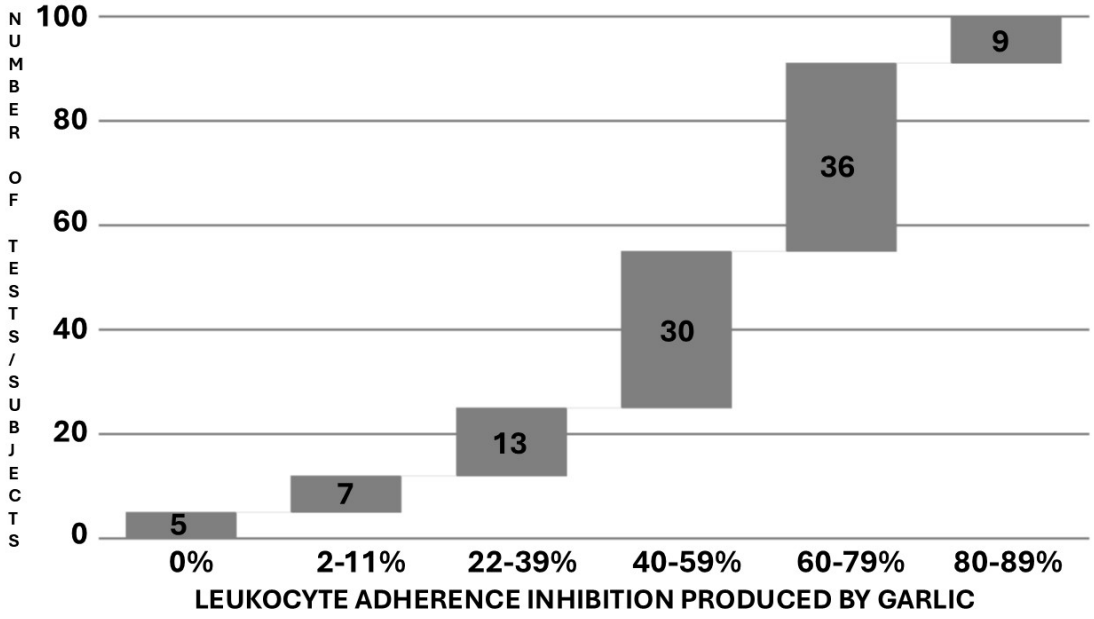

Fig. 4. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the *ex vivo* challenge against garlic extract monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis).

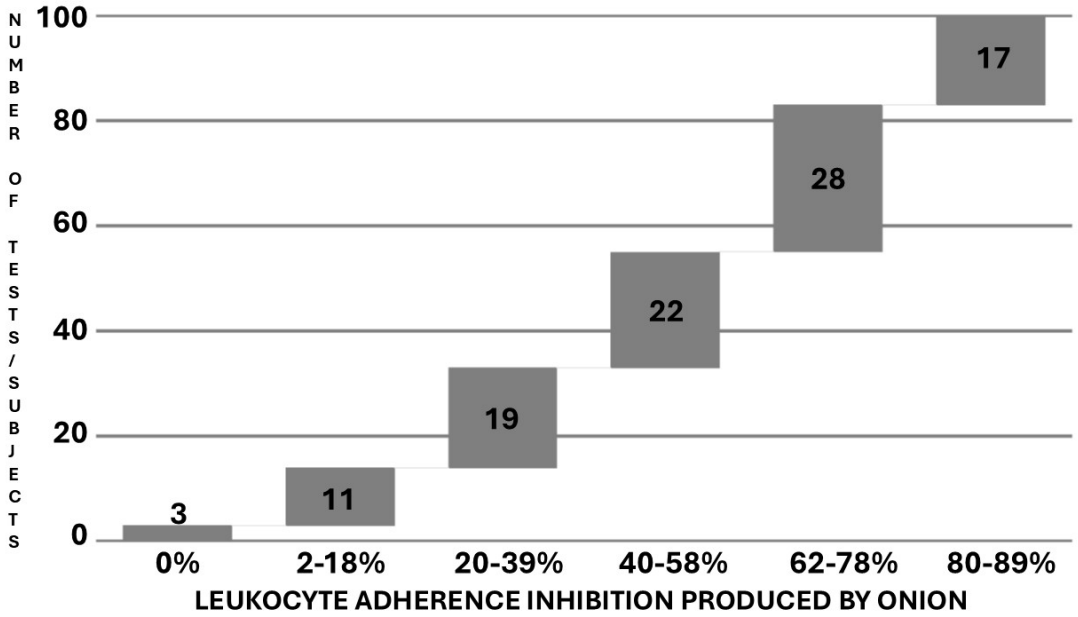

Fig. 5. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo* challenge against onion extract monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis).

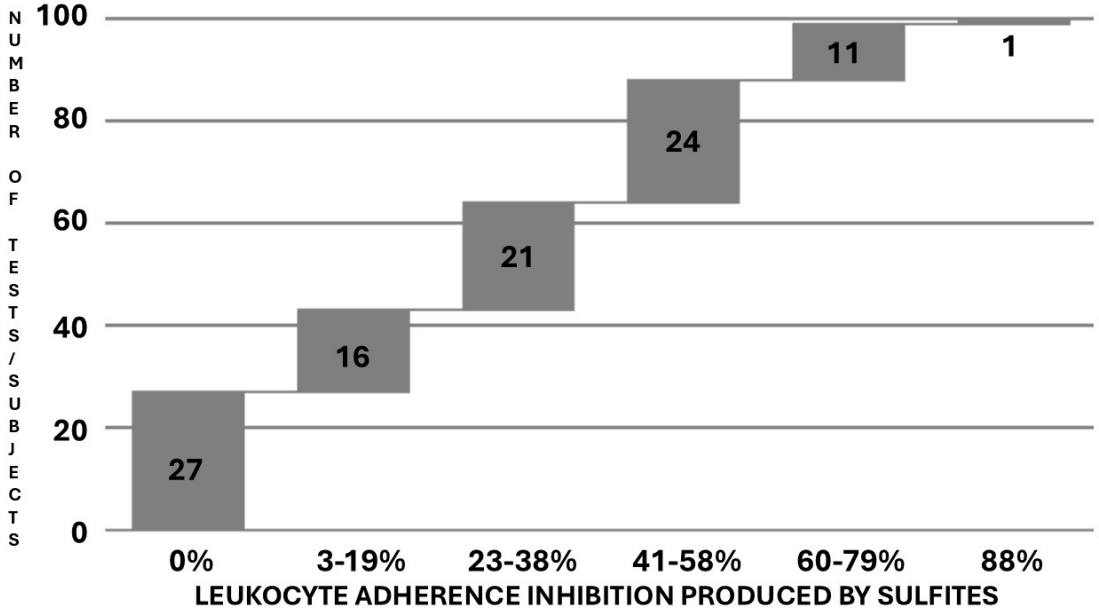

Fig. 6. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo* challenge with sodium bisulfite solution monitored by the Leukocyte Adherence

Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis).

4. DISCUSSION

Soon after the discovery of penicillin (along with the antibiogram) in 1928, the empirical bactericidal properties of *Allium* spices were demonstrated to be "penicillin-like" and proved effective against Gram-positive bacteria, first in animals (1931) and later in humans (1945) [78, 79]. *Allium* extracts also proved effective against fungi, especially their organosulfur derivatives [80, 81].

The early biochemical studies performed with the *Allium* species were motivated by the alliinase enzymatic ability to hydrolyze S-alkyl-substituted cysteine sulfoxide derivatives to the corresponding alkyl alkane thiosulfinates, ammonia, and pyruvic acid [82]. *Allium* species are a natural source of bioactive compounds, including more than 180 active secondary metabolites [83]. Several *Allium* bioactive compounds have been studied for their supposed anticancer, anti-diabetic, anti-inflammatory, antimicrobial, immunomodulatory, and cardioprotective properties [84]. *Allium* spices were studied in several murine models of inflammatory diseases with promisor results [85]. Murine experiments demonstrated that onion bulb extract inhibited the house dust mite-induced phosphorylation of EGFR, ERK1/2, and AKT pathways, inhibiting airway cellular influx, perivascular and peribronchial inflammation, goblet cell hyper/metaplasia, and *ex vivo* eosinophil chemotaxis [86, 87].

Consequently, it is natural to hypothesize that such intense biological activity is probably associated with a great potential to promote immunoreactivity and/or hypersensitization, interfering with metabolic pathways, being proposed even for treating allergic diseases [88]. Therefore, when treating a patient with the Precision Medicine approach, diagnosing or endotyping an eventual allergy, hypersensitivity, or immunoreactivity against any naturopathic therapeutics or nutraceutical food, elective for long-term treatments is remarkably advisable for the prescription of eviction measures and tolerogenic strategies aimed to mitigate diseases symptoms phenotypes [89]. The four classical hypersensitivity mechanisms described by Gell and Coombs have been amplified to seven types with several subtypes, increasing the complexity of the required investigation of the hypersensitivity mechanisms responsible for the disease phenotypes [90, 91]. Endotyping the hypersensitivity mechanisms against sulfites may also help distinguish superimposable phenotypes produced by common pathways such as allergic migraines and sinusitis [92].

The retrospective compilation of our data showed a large distribution of results when we ascertained the results of TTP and TIAL to explore humoral and cellular immunoreactivity against two *Allium* spices and a sulfite derivative. These immunoassays do not identify the exact mechanisms responsible for the clinical condition. Instead, they provide clues about sensitization and immunoreactivity distributed into an extensive spectral range between immune tolerance and symptomatic hypersensitivity.

The semi-quantitative research and titration of precipitins is an essential laboratory exam upon which the fundamentals of Immunology were constructed [93]. Precipitating antibodies suggest a remarkable immune humoral response against the tested antigens [94].

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 allergen [95]. The LAIT did not indicate which pathways produce the resulting final phenomenon (leukocyte adherence inhibition) [96-99].

As indicators of a previous immune response, LAIT and TTP are suitable techniques to quantify an exposome measurement and their correspondent immune response, as proposed by the exposome-wide association study [100].

The comparative results obtained from the TIAL cohort demonstrated a more significative immunoreactivity from the tests performed with garlic extract and onion extract than the results obtained with the sodium bisulfite. This finding states that sulfites are not the only allergen responsible for cellular immunoreactivity against the *Allium* species. On the contrary, several protein allergens in *Allium* spices play a critical role in immunoreactivity against these species. This means that if a patient presents cellular immunoreactivity against *Allium* species, he/she will not necessarily present immunoreactivity against sulfites; however, reciprocation is not probable.

This preliminary retrospective survey demonstrated extensive results from the TTP and the *ex vivo* challenge test monitored by LAIT against two *Allium* species in two cohorts of patients with

various allergic symptoms. 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 with several chemical and biological allergens, demonstrating positive results for some of them. Our results suggest that reactive allergic patients may impair their symptoms by an additional immunoreactivity against *Allium* spices and sulfite derivatives.

5. LIMITATIONS

This study is a retrospective analysis of data collected over six years and nine months. There was no protocol research, 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 point of view of the physician who indicated the exam (CEO) based on a 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 not possible yet. Unfortunately, it was impossible to compare the two procedures because they were taken from different groups of patients.

6. CONCLUSION

Our preliminary results show that the LAIT and TTP may differentiate diverse degrees of immunoreactivity against *Allium* spices in patients clinically diagnosed with Non–IgE-mediated allergies. This methodology may be easily incorporated into specialized centers since the technologies to perform TIAL and TTP are inexpensive and can be performed with minimum laboratory equipment. However, the technique depends on trained biomedical personnel performing artisanal laboratory procedures. As preliminary results, the propaedeutic meaning of these results and the possibility of interferents must be yet established [101]. 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 *Allium* spices and sulfite derivatives [102].

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 [103].

ETHICAL APPROVALS

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

Disclaimer (Artificial intelligence)

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

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Details of the AI usage are given below:

1.

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