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Contributions of the Leukocyte Adherence Inhibition Test to the Diagnosis of Hypersensitivity Reactions Produced by Nickel

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the potential of the Leukocyte Adherence Inhibition Test (LAIT) as a diagnostic tool for nickel sensitization in patients with clinical suspicion of Nickel Hypersensitivity (NiH).

Study Design: We retrospectively examined the medical charts of a population of 102 patients diagnosed with chronic extensive dermatitis that prevented the employ of the diagnostic Patch Test and were investigated through an *ex vivo* challenge monitored by LAIT against $NiSO_4 (H_2O)_6$.

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Place and Duration of Study: Instituto Alergoimuno de Americana – São Paulo – Brazil – between January 2018 and May 2023.
Methodology: Two groups were separated according to the patient's perception of pruritic reactions after prolonged contact with nickel alloys. Group A was composed of 45 patients denying pruritic reactions to prolonged contact with nickel alloys. Group B was composed of 57 patients that complained of local pruritic reactions produced by prolonged contact with nickel alloys. Cascade distribution charts were mounted with the range groups of Leukocyte Adherence Inhibition (LAI) results for visual comparison between the groups. The difference between the LAI means was studied with the help of the nonparametric Mann-Whitney U Test.
Results: The Cascade distribution charts showed a distinct distribution of LAI when comparing the two groups. This significative difference was supported by the Mann-Whitney U Test under $p < 0.01$.
Conclusion: Our preliminary results support the fact that the LAIT performed with $NiSO_4$ $(H_2O)_6$ has the potential to become a diagnostic tool to help physicians to diagnose patients with NiH.

Keywords: Dermatitis, allergic contact; dermatitis; contact; diagnosis; leukocyte adherence inhibition test; nickel; skin tests.

1. INTRODUCTION

Nickel is a relatively nontoxic corrosion-resistant silver-white chemical element with the symbol Ni and atomic number 28 classified in the periodic table as a transition metal [1]. Nickel is a micronutrient bioavailable from the human diet [2]. Nickel is metabolically active and an essential trace element of the human body [3]. Nickel deficiency results in lower activities of several enzymes, affects carbohydrate metabolism, incorporation of calcium into bones, and iron absorption leading to anemia [4]. However, nickel toxicity may appear at large doses manifested as carcinogenicity, genotoxicity, haematotoxicity, teratogenicity, and immunotoxicity [5]. Used in metallurgy to forge Stainless Steel, nickel may contact human skin through earrings, body piercing, imitation jewelry (*bijou*), clothing accessories (buttons, belt buckles), razors, and occupational hazards [6].

Nickel Hypersensitivity (NiH) is one of the main responsible for Allergic Contact Dermatitis (ACD) in humans [7]. Nickel-related Allergic Contact Dermatitis (Ni-ACD) is a cutaneous hypersensitivity reaction developed after prolonged skin contact with nickel alloys [8]. Haptens are collected from tegument by dendritic cells, which migrate to regional lymph nodes and present the hapten-peptide complexes to specific naïve T cells in a class II MHC-restricted fashion. The innate immune system is suggested to play a crucial role in Nickel sensitization through the Toll-like receptor 4 [9,10]. The sensitized T lymphocytes return to the skin with the guidance of the cutaneous lymphocyte-associated antigen (CLA) [11]. Ni-ACD may be manifest exclusively at the site of the contact or may be systemic, spreading far away from the contact point [12]. The systemic Ni-ACD is a differential diagnosis that overlaps the Intrinsic Atopic Dermatitis phenotype which may also be related to an increased serum nickel level [13].

Besides skin issues, NiH is also responsible for mucosal inflammation, which is named "Allergic Contact Mucositis" (ACM) which promotes oral and gastrointestinal symptoms in humans [14]. Nickel-related Allergic Contact Mucositis (Ni-ACM) produces a low-grade gastrointestinal inflammation manifested as an Irritable Bowel Syndrome-like gastrointestinal disorder, recurrent aphtosis, abdominal bloating, abdominal pain, diarrhea, constipation, nausea, vomiting, and endoscopic findings of chronic gastroduodenitis [15]. Ni-ACM also occurs with nickel contact with oral mucosa through Orthodontic Braces [16]. The systemic liberation of nickel and other metals from orthopedic prostheses is also a great concern [17]. NiH is related to systemic hypersensitivity, defined as the "Systemic Nickel Allergy Syndrome" (SNAS), a condition successfully treated with oral desensitization [18]. The SNAS is related to rhinitis, asthma, urticaria, angioedema, headache, chronic fatigue, post-prandial dyspnea, cystitis, vulvovaginitis, acne, and iron deficiency anemia [19].

Nickel-induced cellular damage may be minimized by nutritional interventions such as a low-nickel / antioxidant-rich / iron-sufficient diet. [20] N-acetylcysteine acts as an antioxidant to reduce oxidative stress, inflammation, and necroptosis caused by nickel exposure [21].

Nickel is present in the soil, drinking water, and in almost all dietary items, but foods usually high in nickel are cocoa, soybeans, oatmeal, nuts, almonds, and legumes; however canned food may have an especially high nickel content liberated from the can alloy [22]. Nickel is not cumulative in the human body since it is easily eliminated by the kidneys. The main blood transport proteins are albumin and nickeloplasmin [23].

Historically, the most used method to clinically investigate the delayed hypersensitivity reactions responsible for ACD is the Patch Test [24]. The Patch Test is nowadays a medical procedure recommended under the guidelines of medical societies working for its standardization [25]. Nickel is one of the main etiologies for the delayed hypersensitivity reactions responsible for ACD as demonstrated by the Patch Test [26]. However, the results of a Nickel Patch Test are not always clinically relevant or reproducible, since the nickel may produce non-specific skin irritant reactions that may mislead the diagnosis of nickel sensitization [27]. The execution of the Patch Test also depends on the existence of an area of skin not affected by the disease, suitable for the placement of an adhesive patch containing the cameras with the studied allergens, which is usually done at the patient's back or the arms. However, sometimes, the patient does not have a sufficient area of uninjured skin to allow this procedure. Additionally, there is also a low risk of sensitization with this procedure [28]. These technical difficulties led some colleagues to search for a laboratory *ex vivo* methodology to diagnose immune sensitization in patients with NiH [29].

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval, from the Instituto Alergoimuno de Americana (Brazil), we proceed with the electronic chart review of a population of 7,200 allergic patients who attended our outpatient facility from January 2018 to June 2023. A group of 102 patients had been submitted to an *ex vivo* allergen challenge test with nickel monitored with LAIT. This procedure was offered to the patient when indicated by the presence of extensive chronic dermatitis preventing the realization of a patch test. This was a very diversified cohort with 17 males; mean age 40.6 years; SD 20.4 years; range 1 to 82 years; modes = 29 years and 42 years (each appeared 6 times); geometric mean = 32.8 years.

This group was divided into 2 groups according to the presence or the absence of the history of the subjects' perception of nickel contact dermatitis (such as cutaneous reactions to metal accessories such as earrings, piercings, metal cloth accessories, razors, bijou, *et cetera*). Group A was defined as the patients that did not perceive any reaction to the prolonged contact with nickel alloys. Group A had 45 participants, with 6 males; mean age 41 years; SD 20.4 years; range 5 to 73 years; mode = 42 years (appeared 4 times); geometric mean = 34.0 years. Group B was defined as the patients who complained of pruritic cutaneous reactions related to prolonged contact with nickel alloys. This a group with 57 participants, with 11 males; mean age 40.2 years; SD 20.3 years; range 1 to 82 years; mode = 29 years (appeared 4 times); geometric mean = 31.9 years.

2.2 Antigen preparation

The [$NiSO_4$ $(H_2O)_6$] was acquired from Labcenter Campinas. The powder was weighted and diluted in a buffer solution [NaCl 10g; KH_2PO_4 0,72g; Na_3PO_4 2,86g; H_2O 600mL], to achieve the final concentration of 1 mg/mL to be employed in the LAIT.

2.3 *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

All patients were submitted to the *ex vivo* challenge tests monitored by the Leukocyte Adherence Inhibition Test (LAIT), against $NiSO_4$ $(H_2O)_6$ to evaluate the cellular response. The LAIT was performed as previously described [30-36]. Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with $NiSO_4$ $(H_2O)_6$ and the unchallenged plasma assay. The plasma with high leukocyte content (buffy coat) was collected from the heparinized tube after one hour of sedimentation at 37 °C and aliquots of 100 µL were distributed into Eppendorf tubes kept under agitation for 30 minutes (200 rpm at 37°C) with (or without, as used as control) antigen extract (10µL of a solution with 1mg/mL and pH 7.5). 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, leukocytes were counted, the coverslip was removed, and the chamber was washed by immersion in a beaker with PBS at 37°C. A drop of PBS was added to the hemocytometer's chamber and a clean coverslip was placed 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 LA from the antigen-specific challenged groups and the LA from the unchallenged control group: LAR = LA of the challenged sample divided by LA of unchallenged control sample; multiplied by 100 (%). To further calculate the Leukocyte Adherence Inhibition (LAI) the LAR was subtracted from 100 (%). The LAI results were further employed for the statistics calculations.

3. RESULTS

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

Group A results: The LAI mean was 34.3%; SD 28.9%; range 0% to 95%; mode = 0% (appeared 14 times).

Group B results: The LAI mean was 49.4%; SD 25.5%; range 0% to 97%; modes = 61% and 77% (each appeared 3 times).

The Cascade distribution charts showed a distinct distribution of LAI when comparing the two groups (Figure 1 and Figure 2). Figure 1 shows the distribution of Group A. Note that there is a great proportion (31%) of negative results (LAI = 0%), which is almost ten times greater than Group B (Figure 2) which was negative in just 3.5% of the results.

The Mann-Whitney U Test Calculator resulted in the value of U as 880.5. The distribution was approximately normal. The Z-Score was 2.70591. The p-value was 0.00672 meaning a significant difference between the two nonparametric groups at $p < 0.01$.

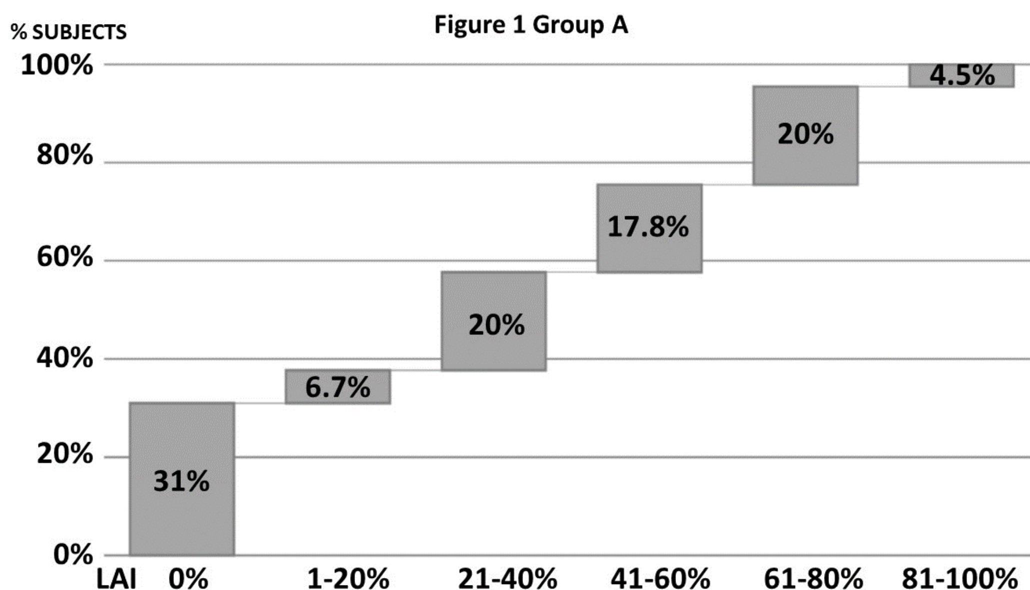

Fig. 1. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition results (x-axis %) of *ex vivo* $NiSO_4$ $(H_2O)_6$ challenges monitored by Leukocyte Adherence Inhibition Tests, according to the respective percentage of results over 45 tests (y-axis) performed on patients of group A (who did not report pruritic reactions to nickel alloys)

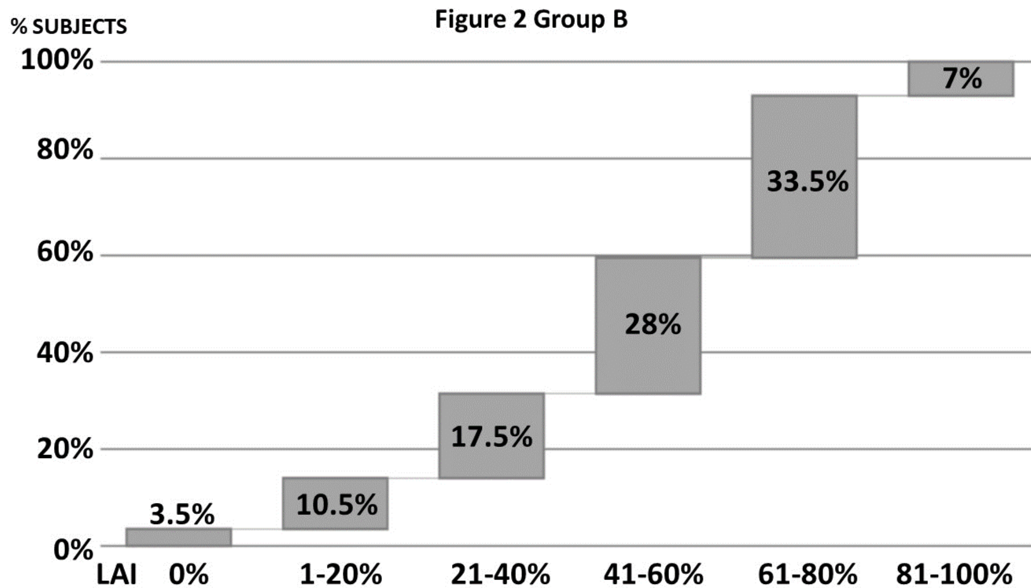

Fig. 2. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition results (x-axis %) of *ex vivo* $NiSO_4$ $(H_2O)_6$ challenges monitored by Leukocyte Adherence Inhibition Tests, according to the respective percentage of results over 45 tests (y-axis) performed on patients of group B (who complained of pruritic reactions to nickel alloys)

4. DISCUSSION

Nickel is the most frequently positive allergen (19%) detected by the standard Patch Test battery of the North American Contact Dermatitis Group [37]. As a public health issue, medical societies are essaying efforts to promote the regulation of nickel use by the population [38]. Most of the knowledge about NiH is derived from murine studies demonstrating activation of $CD8^+$ and $CD4^+$ type 1 T cells which liberate proinflammatory cytokines, responsible for the apoptosis of keratinocytes [39]. NiH is a lymphocyte-mediated Type IV Gell & Coombs delayed hypersensitivity reaction that occurs upon hapten-specific contact in sensitized individuals [40]. Haptens are low-molecular-weight sensitizers, able to be absorbed before to covalently bound to a protein (carrier) [41]. Nickel usually is supposed to bind to proteins before being able to interact with immune cells to become a full antigen allowing antigen presentation and differentiation of T effector cells able to orchestrate hypersensitivity reactions [42]. However, it was already demonstrated that nickel reactivity may not be dependent on protein binding to activate antigen-presenting cells to stimulate the T cell receptor, at least to the HLA-DR–promiscuous VA22/$VB17^+$ TCR human T cell clone SE9, acting in analogy with an idiotypic superantigen [43]. However, as a hapten, anything prevents the possibility of an assemblage of reaginic antibodies against the nickel-protein conjugate to produce humoral (immediate) responses too [44].

The inhibition of the cell migration was the first model for the demonstration of delayed hypersensitivity to an *ex vivo* allergen challenge [45]. This methodology was able to distinguish through *ex vivo* challenges performed with albumin-nickel conjugates patients with NiH from health controls [46]. The *ex vivo* stimulation of T lymphocytes of patients with NiH by nickel sulfate was amplified by the presence of macrophages [47]. Studies had shown that NiH is associated with a broad-spectrum T-cell cytokine response [48,49]. The demonstration of this broad-spectrum cellular response to nickel has created new opportunities to *ex vivo* challenges in the diagnosis of NiH [50]. For 5 years, our Institute has employed *ex vivo* challenge tests monitored by the Leukocyte Adherence Inhibition Test (LAIT) as an alternative to the impossibility to perform a Nickel Patch Test due to the large extension of a patient's skin lesions [44]. The LAIT is a test similar to the Leukocyte Migration Inhibition Test, which exploits similar physiology but is easier to perform and standardize [51].

The LAIT is not a specific research tool to demonstrate any particular immune pathway, since several mechanisms may be involved in the inhibition of leukocyte adherence [52,53]. LAIT is a triage test with a clinical utility to demonstrate the existence of some kind of immunoreactivity against a particular allergen [54]. Several immune mechanisms have been associated with LAIT [55]. Our preliminary retrospective survey has demonstrated that patients with NiH proved by the Patch Test also had LAIT positivity when challenged by $NiSO_4$ $(H_2O)_6$. Our retrospective study does not have information about the follow-up of the patients after the medical orientation to avoid nickel. More studies with prospective larger double-blind cohorts are in need to validate the hypothesis that the LAIT can potentially be used as a diagnostic tool to demonstrate NiH.

We also need to consider that no *in vivo*, *ex vivo,* or *in vitro* positive test makes the diagnosis of "Allergy". Allergy is a disease, clinically diagnosed by the assistant physician. These exams will demonstrate "sensitization" or "immunoreactivity". They are just a "clue". The confirmatory procedure is the *in vivo* Provocation Test. The *in vivo* provocation Test consists of the exclusion of the allergen, with a further re-introduction, which after remission of symptoms and further resurgence will prove causality between the allergen and the disease.

5. CONCLUSION

Our preliminary results support the fact that the LAIT performed with $NiSO_4$ $(H_2O)_6$ after further and more complete studies have the potential to become a diagnostic tool to help physicians to diagnose patients with NiH.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVALS

As per international standards of the Helsinki Declaration the project was submitted and approved by the ethics and research committee of the Institute and the written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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