ORAL HYPOSENSITIZATION TO NICKEL INDUCES CLINICAL IMPROVEMENT AND A DECREASE IN TH1 AND TH2 CYTOKINES IN PATIENTS WITH SYSTEMIC NICKEL ALLERGY SYNDROME

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Some patients with nickel (Ni) allergic contact dermatitis suffer from systemic (intestinal or cutaneous) symptoms after ingestion of Ni-rich foods and experience symptoms reduction with low-Ni diet, a condition termed "systemic Ni allergy syndrome" (SNAS). We aimed at evaluating whether oral administration of low nickel doses improved clinical conditions and modulated immunological aspects of SNAS, without significant side effects. Thirty-six SNAS patients were enrolled. Treatment started after 1-month of low-Ni diet and consisted in an incremental oral NiOH dose phase (0.3ng to 1.5 μg/week) followed by a 12-months maintenance phase (1.5 μg/week). Randomly, twenty-four patients added Ni therapy to low-Ni diet and 12 remained with diet alone. All patients were allowed rescue medications (antihistamines and topical steroids). After 4 months, Ni-rich foods were gradually reintroduced. *In vitro* allergen-driven IL13, IL5 and IFNγ release by peripheral blood mononuclear cells was evaluated before and after treatment. Twenty-three patients receiving NiOH and the 12 control patients completed the study. Evaluation of SNAS clinical severity (by VAS and drug consumption) showed a significant difference in favor of NiOH-treated patients compared to controls. Twenty of 23 patients in the NiOH group and none in the control group tolerated Ni-rich food reintroduction. Release of all studied cytokines in culture supernatants was significantly lower after NiOH treatment. In conclusion NiOH is effective in reducing symptoms and drug consumption in SNAS and is able to modulate inflammatory parameters.

Nickel (Ni), a ubiquitous metal, is the commonest cause of allergic contact dermatitis (ACD), with a prevalence of about 10% in the adult population (1-4). Ni allergy can cause dermatological lesions not only in skin regions in contact with the metal, but also in other regions, as demonstrated by cases of generalized eczema and urticaria in patients with Ni-containing dental (5-7) or orthopedic (8) prostheses.

Key words: nickel allergy, allergic contact dermatitis, systemic nickel allergy syndrome, orticaria, nickel-rich food, oral hyposensitization

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Ni is also present in many foods, especially vegetables, and it has been observed that ingestion of Ni-rich foods or an oral Ni challenge may elicit eczematous skin reactions in sensitized individuals (9). This phenomenon has been variously termed "systemic Ni contact dermatitis" (10), "systemically-induced eczema", "hematogenous contact eczema" (11) or "systemic nickel allergy syndrome" (SNAS) (9, 12). The last definition, proposed by one of us (MDG), seems more adherent to the clinical picture of the disease. In a wide clinical survey performed in Italy on 1086 patients with Ni-contact allergy, 209 (19.2%) resulted affected not only by systemic cutaneous manifestations (urticaria, edema, eczema, erythema) but also by respiratory and digestive symptoms (nausea, gastric pyrosis, meteorism, abdominal pain, diarrhea and constipation) (13-15). Intestinal biopsies from SNAS patients show, compared to normal histological findings in ACD and healthy subjects, an inflammatory infiltrate of lymphocytes and plasma cells with edema and vasodilatation of the lamina propria (14), furthermore it has been detected an apoptotic decrease of epithelial CD8+ cells and an infiltration of CD4+ cells after an oral challenge with the metal (12). More recently, a significant increase of ICAM-1 levels were found in SNAS patients compared to healthy controls ($p<0.05$), suggesting an inflammatory state induced by ingested Ni (16). Furthermore, an increase in some Th2 cytokines, such as IL13, IL5, and eosinophilic cationic protein was observed in SNAS (17).

At present there are no therapies for SNAS, other than exclusion diet. However, some authors reported an improvement of clinical manifestation of Ni-allergy (ACD or systemic) by administering Ni per os (18-21). In a previous experience, Schiavino and collaborators treated two hundred and ninety patients affected by Ni-related systemic cutaneous and digestive symptoms (female:male ratio 282: 8) with a low-dose (0.1 ng granules) nickel oral hyposensitization (NiOH). Results were excellent, in terms of induction of tolerance to Ni-containing foods, with low incidence of side effects (15). The extremely low dosage of Ni and the absence of laboratory evidence of immunological modulation induced by the treatment, make this study only a preliminary observation, which needs further evaluations. A dose-finding study has been reported by Minelli et al. (National Congress of the Italian Society of Allergy and Clinical Immunology, Rome 4-7 May, 2005), showing that the tolerated dose varies extremely among SNAS subjects, from few nanograms to micrograms. The present study was designed as an open clinical trial to evaluate the tolerability of increasing oral doses of Ni, the safety of a long period treatment with the highest Ni tolerated dose, its clinical efficacy and the potential for oral Ni treatment to modify some immunological characteristics of SNAS. Clinical and immunological end points were evaluated in a group of patients treated by low Ni diet and the administration of NiOH, compared to those obtained in a control group treated by Ni-low diet alone.

MATERIALS AND METHODS

Patients

Thirty-six patients (31F/5M, mean age 39.4±8) with history of SNAS were enrolled in the study. All the patients gave their informed consent. Patients with severe chronic diseases, including active or previous cancers and infections, were excluded.

SNAS diagnosis was based on history, positive Ni patch test, beneficial effects of Ni-free diet and positivity of a double blind placebo-controlled oral Ni challenge. The clinical picture was characterized by an association of gastrointestinal and cutaneous symptoms (dyspepsia, meteorism, diarrhea, eczema, urticaria in regions not in contact with the metal, periorbital angiooedema). Twenty-four patients (66%) were randomly assigned to the active group, treated with NiOH and Ni-low diet (Group A), the remaining 12 to the control group, treated with Ni-low diet alone (Group B). The assignment was established as follows: of every 3 consecutive patients 2 were assigned to the NiOH-treated group and 1 to the control group.

Immunological evaluations

The first 10 NiOH-treated patients and 10 controls underwent immunological evaluations: Ni-driven IL5, IL13 and IFNγ production by cultured peripheral blood mononuclear cells was investigated at the beginning and at the end of the study.

Treatment

Nickel oral hyposensitization (NiOH) was performed with hard gelatin capsules (nickel free, as demonstrated by atomic absorption analysis) containing $NiSO_4 \cdot 6H_2O$ at different dosages (0.1 ng, 1 ng, 10 ng, 0.1 μg, 0.5 μg)

and microcrystalline cellulose as excipient (TIO Nickel, Lofarma SpA, Milan, Italy). Treatment was given 3 times a week increasing progressively the dose from 0.1 ng to 3 μg in 10 weeks with a maintenance phase of 1,5 μg a week for a period of 12 months. All patients were instructed to take rescue medications (antihistamine and/or topical steroids) if necessary.

Diet regimens

In the low-nickel diet the following foods were avoided: avocados, carrots, lettuce, figs, mushrooms, flounder, plaice, herring, tea, apricots, lobster, onions, maize, pears, raisins, asparagus, cabbages, cauliflowers, beans, French beans, yeast, margarine, mussels, oysters, shellfish, potatoes, peas, tomatoes, spinach, prunes, peanuts, oats, cocoa, tomato puree, lentils, almonds, walnuts, hazelnuts. Reintroduction of the Ni-containing diet was gradual, with 2 of the above-listed foods per week.

Clinical assessment

The clinical efficacy of the treatment was evaluated by the recurrence (daily diary) and severity (visual analogical scale –VAS- at each visit) of SNAS symptoms after Ni-rich-food reintroduction, and by use of rescue medications (daily diary). Patients were instructed also to report in the diary any clinical event to evaluate the safety of the treatment.

Visit schedule:

T1 = enrollment: first blood sample drawn for immunological evaluation and assignment to low-Ni diet.

T2 = 1 month after T1, start of NiOH (control group followed the diet only).

T3 = 4 months after T1, start of the reintroduction of the Ni-containing foods.

T4 = 10 months after T1, intermediate evaluation.

T5 = 16 months after T1, final clinical evaluation and second blood sample drawn for immunological study.

VAS for symptoms, rescue medication consumption, possible side effects of the treatment and symptoms induced by Ni-containing food at reintroduction were evaluated at each visit.

Ni oral challenge

Ni oral challenge was carried out in an asymptomatic period (induced by a month of Ni-low diet) administering increasing doses of Ni, from 1.25 to 2.5, 3.75 and 4.5 mg, (capsules made by Lofarma SpA, Milan, Italy) until appearance of SNAS symptoms. Each dose was administered with at weekly intervals.

Patch test

The nickel patch test (Brial Allergen GmbH, Greven, Germany, distributed in Italy by Lofarma SpA, Milan) was applied for 48-72 hours, in accordance with the European Environmental and Contact Dermatitis Research Group guidelines, and was considered positive if an eczematous-vesicular reaction occurred at the contact site.

Peripheral blood mononuclear cell culture and cytokine quantification

Blood samples were collected before and after treatment. Peripheral blood mononuclear cells (PBMC) were isolated and purified from EDTA-treated whole blood by Ficoll-Hypaque (BioSpa Milan, Italy) density gradient centrifugation. Cultures were set up in 1 ml/well (0.8 ml, 1 million cells in complete medium) using 24-wells Costar plastic plates. Cells were stimulated with 0.2 ml of PHA (Sigma Chem. Co., Italy), then incubated at 37°C in humidified atmosphere with 5% CO_2 for seven days. At day 3, 10^{-5} M Ni sulphate was added. Supernatants were stored and evaluated for cytokine quantification. All experiments were performed in triplicate. IL-5, IL-13 and IFNγ were quantified in supernatants using a human cytokine cytometric bead array kit (BD, San Diego, CA, USA).

Statistical analysis

Statistical analysis was performed using the unpaired Mann-Whitney test to evaluate the differences between the two groups and the Chi-square test for categorical variables. A P value <0.05 was considered significant.

RESULTS

Twenty-four patients (20F/4M, mean age 40.5±12.7) were allocated to the NiOH group and 12 to the control group (11F/1M, mean age 38.7±7.9). Twenty-three patients of the NiOH group completed the study. One patient suffered from gastrointestinal symptoms (stomach ache and diarrhea) after 5 maintenance doses of 0.5 μg and refused to continue the study. At the end of the study, after complete reintroduction of Ni-containing foods, 20 out of 23 patients treated with NiOH (87%) remained free from symptoms. Three patients were not able to reintroduce in their diet the last foods of the schedule, corresponding to those with the highest Ni content: spinach, prunes, peanuts, oats, cocoa, tomato purèe, lentils, almonds, walnuts, hazelnuts. None of the control group was able to complete the reintroduction of all Ni-rich foods.

The severity of disease, evaluated by VAS, was similar at the enrollment in the two groups, (VAS=

Table I. *Spontaneous and Ni (10^{-5} M) induced release of studied cytokines from PBMCs of the two group of patients (NiOH-treated patients and controls).*

	Treated group		Diet alone group	
	Spontaneous release	Release in presence of Ni 10^{-5} M	Spontaneous release	Release in presence of Ni 10^{-5} M
IL13	322.5 ± 169.1	479.3 ± 100.3 *	342.4 ± 100.1	480.3 ± 90.8 *
IL5	5.3 ± 2.4	10.5 ± 5.1 **	4.3 ± 1.1	8.9 ± 4.3 **
IFN-γ	7.4 ± 3.5	32.3 ± 16.7 ***	9.2 ± 3.3	44.2 ± 20.3 ***

*Significant changes were found between spontaneous and Ni induced release (*p=0.05; **p=0.03; ***p=0.001) in both groups. No statistical differences were found in cytokine release between the two groups, as in culture without as with Ni.*

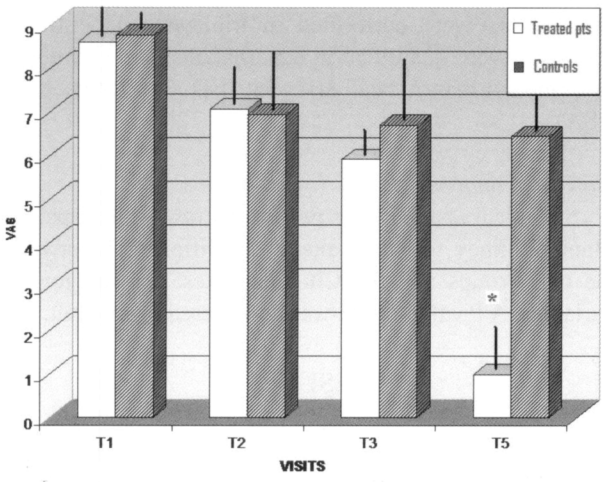

Fig. 1. *Visual analogical scale (VAS) of NiOH-treated patients and controls at each of the four visits. At T5, the values of VAS of treated patients were significantly lower than those observed in the control group. The higher values expresses the most severe clinical pictures. Values are expressed as mean±SD. * = p<0.001 respect T1, T2 and T3 (Chi Square)*

8.62 and 8.78 respectively, p = n.s.). On the contrary, at T5, corresponding to the end of the study, there was a significant difference: 0.99 for the NiOH group and 6.46 for the control patients (p=0.001) (Fig. 1). Rescue drug consumption was significantly lower in the NiOH group in comparison to controls: 56.3% of the NiOH group and 83.0% of the controls at T3, and 4.3% of the NiOH group and 83.0 of the control group at T5 took rescue medications: the Chi-square was highly significant with p <0.001 (Fig. 2).

The safety of treatment was excellent, 21 patients completely tolerated the maximal weekly dose of 1.5 μg during the NiOH maintenance phase. Two patients did not tolerate the highest doses, because of stomachaches, and were treated with lower dosages (600 and 300 ng respectively). No patients showed long-term side effects.

Immunological parameters

No statistical differences were found in spontaneous and Ni-driven cytokine release between the two groups of patients at the beginning of the study (Table I). Before treatment, statistically significant higher levels of IFNγ, IL-5, and IL-13 (p=0.001, 0.03 and 0.05 respectively) were observed in the supernatants of PBMC cultures stimulated with Ni compared to untreated cultures, in all studied patients (Table I, Figs. 3, 4, 5). After NIOH treatment, no significant increase of Ni-induced IFN-γ, IL-5, and IL-13 release by PMBCs was detected, with a significantly lower increase of all cytokines respect the basal conditions and respect to that detected in the group B treated by diet alone (Figs. 3, 4, 5). In particular, Ni-driven IFNγ release decreased by 55.3% ± 1.7 (p<0.01), IL13 release by 31.2% ± 11 (p<0.01) and IL5 release by 58.6% ± 14 (p<0.01), respect to basal values (Fig. 6). On the contrary, no significant changes were observed in patients treated with the elimination diet alone.

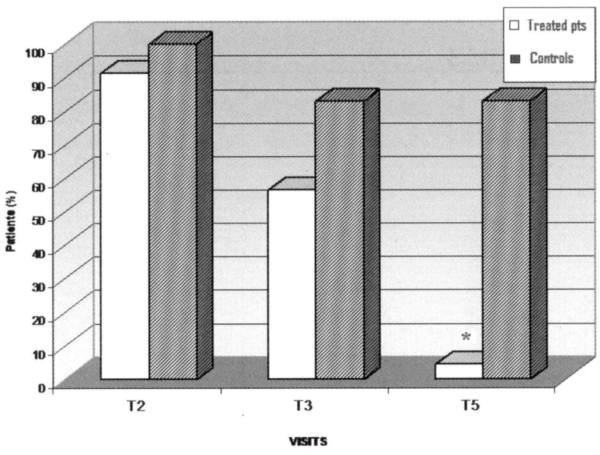

Fig. 2. *Percentages of NiOH-treated patients and controls which necessitated rescue medications. *=p<0.001 respect T2 and T3 (Chi Square)*

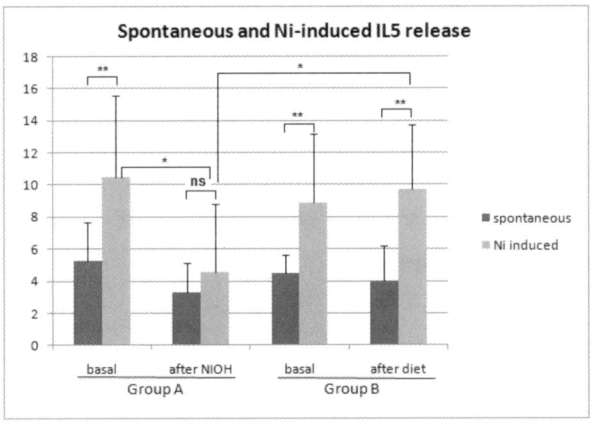

Fig. 3. *Spontaneous and Ni-induced release of IL5 by PBMCs of group A and B patients. IL5 significant increase was found in basal conditions of both groups and in the culture performed at the end of the diet period. No significant increase of IL5 was detected at the end of the NIOH treatment. After treatment, the Ni-induced IL5 release was significantly lower than in basal condition. The Ni-driven IL5 release after NIOH treatment was significantly lower than that detected after diet. (*: p=0.01; **: p=0.03)*

DISCUSSION

This study shows that oral administration of minimal doses of Ni in patients suffering from SNAS results in beneficial effects. In fact, after treatment, the patients were able to reintroduce Ni-rich foods in their diet without the occurrence of systemic symptoms. On the contrary, patients not treated with NiOH, who experienced the disappearance of systemic symptoms during a diet without Ni-rich foods, were unable to completely normalize their diet.

In the recent literature, there are many publications on the systemic effects induced by Ni-containing foods, however, the clinical borders of Ni-induced syndromes are as yet not well defined. In particular, there are no clinical or laboratory data to identify patients suffering from nickel allergic contact dermatitis that will be affected by SNAS, and the immune pathogenesis of SNAS is at present not completely defined. However, all patients affected by SNAS are patch test positives to Ni and the ingestion of Ni-rich foods induces gastrointestinal (abdominal pain, diarrhea, meteorism) and skin symptoms (urticaria, itching, angioedema, diffuse eczematous lesions, flare-up of previous patch test positivity), that disappear with the avoidance of Ni in the diet. The clinical picture of SNAS has been studied by a double blind placebo controlled Ni-challenge, that definitely demonstrated the link between Ni ingestion and symptoms (14). In challenged patients it has been observed histological modifications of the gastrointestinal mucosa characterized by a marked inflammatory infiltrate, characteristically CD4 positive lymphocytes (14). The same histological findings were detected in Ni-allergic patients that accidentally ingested nickel coins (24).

The clinical pictures of the presently studied patients were substantially similar to those of the patients treated in the study by Schiavino et al., who showed excellent results after NiOH, with low incidence of side effects (15, 18). The substantial difference between the two studies is in the Ni dosage: 0.1 ng in the Schiavino et al. study, 1.5 μg (maintenance dosage) in the present study. Other authors performed hyposensitization employing higher dosages, ranging from 0.6 mg (18) to 5 mg (19-20) in patients affected by Ni-ACD. However, SNAS patients do not tolerate such high doses (Minelli et al, communication to the 2006 Congress of the Italian Society of Allergy and Clinical Immunology).

The mechanism of action of NiOH is not

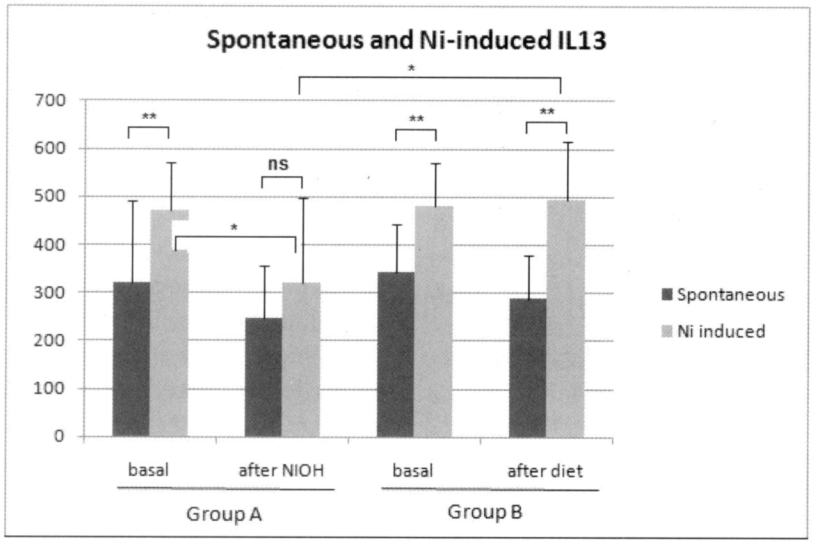

Fig. 4. *Spontaneous and Ni-induced release of IL13 by PBMCs of group A and B patients. IL5 significant increase was found in basal conditions of both groups and in the culture performed at the end of the diet period. No significant increase of IL5 was detected at the end of the NIOH treatment. After treatment, the Ni-induced IL13 release was significantly lower than in basal condition. The Ni-driven IL13 release after NIOH treatment was significantly lower than that detected after diet. (*: p=0.04; **: p=0.05)*

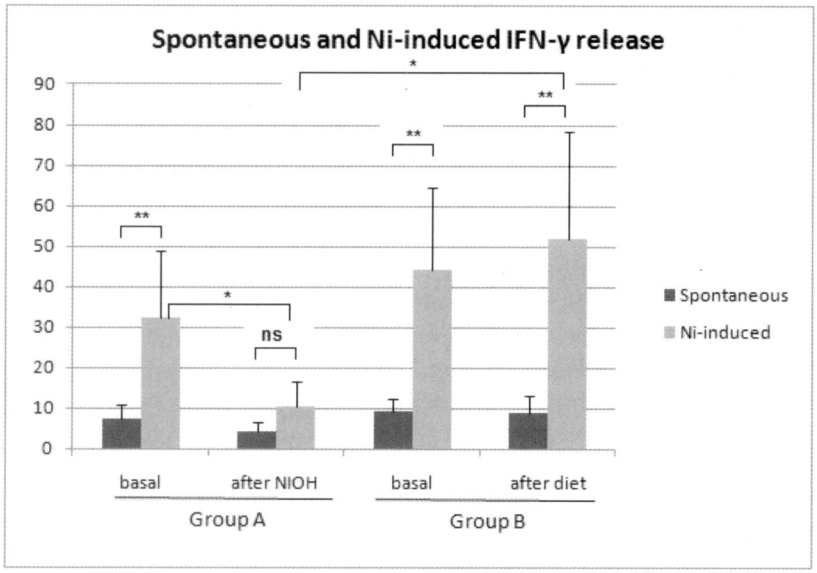

Fig. 5. *Spontaneous and Ni-induced release of IFN-γ by PBMCs of group A and B patients. IFN-γ significant increase was found in basal conditions of both groups and in the culture performed at the end of the diet period. No significant increase of IFN-γ was detected at the end of the NIOH treatment. After treatment, the Ni-induced IFN-γ release was significantly lower than in basal condition. The Ni-driven IFN-γ release after NIOH treatment was significantly lower than that detected after diet. (*: p=0.002; **: p=0,001)*

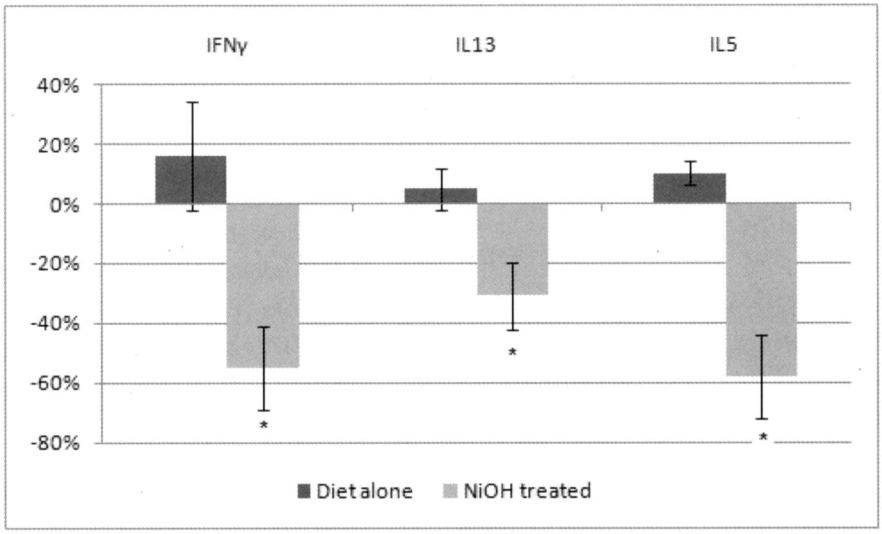

Fig. 6. *Pre/post NiOH treatment changes (%) in in vitro Ni induced IFNγ, IL5 and IL13 release by PBMCs of patients treated by NiOH or by diet alone. NiOH treated patients showed a statistically significant (*: $p < 0.01$) decline in cytokine release respect the values detected before treatment, and respect the release detected in group B patients, treated by diet alone.*

explained, however, it may be related to the possibility of inducing tolerance by oral administration of an antigen. Many studies in animal models demonsttrated induction of tolerance by repeated oral administration of Ni salts. In two studies on guinea pigs by Vreeburg (25) the animals, orally treated with nickel and chromium powder or metallic salts, failed to react to a subsequent immunization, while control animals became clearly hypersensitive. In mice, administration of nickel sulphate ($NiSO_4$) in drinking water for 10 weeks prevented the subsequent sensitization, mediated by CD4-CD8+ T cells (26). Oral administration of Ni as $NiCl_2$ was effective in a mouse model of Ni allergy both for the prevention of subsequent sensitization and for the induction of desensitization in pre-sensitized animals (27). Furthermore, splenic T cells of orally-tolerized donors specifically prevented the subsequent Ni sensitization of the naive recipients (27).

The present study confirmed the immunological findings of SNAS (22, 23), characterized by a mixed increase of Th1 and Th2 cytokines in PBMC cultured in presence of Ni salts. Interestingly, the present study demonstrated a significant reduction of the in vitro Ni-stimulated production of IFNγ, IL5 and IL13 relative to the levels observed before treatment in NiOH-treated patients, whereas no changes were observed in patients that received the elimination diet alone. The reduction of these interleukins, in parallel with the clinically beneficial effects, strengthens the efficacy of the treatment. Clinical efficacy was demonstrated both by the analysis of VASs, that showed significant differences in favor of the NiOH-treated patients relative to controls at T5 (16 months after the start of treatment), and by the use of rescue medications, significantly lower in the NiOH-treated group at T3 and T5. However, the most important and relevant result of the study was that the majority of the NiOH-treated patients (20 out of 23 = 86%) reintroduced all nickel-containing foods without any reactive symptom, compared to none of the control patients.

In conclusion, the results of the present study show that the administration of low doses of Ni in patients suffering from SNAS induces oral tolerance to Ni, without side effects or adverse events. The clinical results are strengthened by the modulation of Th1 (IFNγ) and Th2 (IL5 and IL13) cytokines known to be involved in SNAS. The present is an open study, preliminary to a double-blind placebo-controlled study that the authors already started to completely define the role of oral NiOH

administration in SNAS.

Conflict of interest: The authors Falagiani P, Riva G, Bruno M and Mistrello G are employed in the Scientific Division of Lofarma SpA, Milan, Italy that supplied the drugs used in this study.

REFERENCES

1. Jacob SE, Moennich JN, McKean BA, Zirwas MJ, Taylor JS. Nickel allergy in the United States: A public health issue in need of a "nickel directive". J Am Acad Dermatol 2009; 60:1067-9
2. Greig JE, Carson CF, Stuckey MS, Riley TV. Prevalence of delayed hypersensitivity to the European standard series in a self-selected population. Australas J Dermatol 2000; 41:86-9.
3. Schafer T, Bohler E., Ruhdorfer S, Weigl L, Wessner D, Ilipiak B, Wichmann HE, Ring J. Epidemiology of contact allergy in adults. Allergy 2001; 56:1192-6.
4. Nucera E, Schiavino D, Calandrelli A, et al. Positive patch tests to Euro coins in nickel-sensitized patients. Br J Dermatol 2004; 150:500-4.
5. Levantine AV, Bettley FR. Sensitivity to metal dental plate. Proc R Soc Med 1974; 67:1007.
6. Espana A, Alonso ML, Soria C, Guimaraens D, Ledo A. Chronic urticaria after implantation of 2 nickel-containing dental prostheses in a nickel-allergic patient. Contact Dermatitis 1989; 21:204-5.
7. Fernandez-Redondo V, Gomez-Centeno P, Toribio J. Chronic urticaria from a dental bridge. Contact Dermatitis 1989; 38:178-9.
8. Oleffe J, Wilmet J. Generalized dermatitis from an osteosynthesis screw. Contact Dermatitis 1980; 6: 365.
9. Verna N, Di Claudio F, Balatsinou L, Schiavone C, Caruso R, Renzetti A, Gabriele E, Turi MC, Feliziani A, Di Gioacchino M. Nickel systemic contact dermatitis. Int J Immunopathol Pharmacol 2005; 18(S):11-4.
10. Jensen CS, Menne T, Johansen JD. Systemic contact dermatitis after oral exposure to nickel: a review with a modified meta-analysis. Contact Dermatitis 2006; 54:79-86
11. Erdmann SM, Werfel T. Hematogenous contact eczema induced by foods. Hautarzt 2006; 57:116-20.
12. Di Gioacchino M, Boscolo P, Cavallucci E, et al. Lymphocyte subset changes in blood and gastrointestinal mucosa after oral nickel challenge in nickel-sensitized women. Contact Dermatitis 2000; 43:206-11.
13. Brera S, Nicolini A. Respiratory manifestations due to nickel. Acta Otorhinolaringol Ital 2005; 25:113-5.
14. Di Gioacchino M, Masci S, Cavallucci E, Pavone G, Andreassi M, Gravante M, Pizzicannella G, Boscolo P. Immuno-histopathologic changes in the gastrointestinal mucosa in patients with nickel contact allergy. G Ital Med Lav 1995; 17:33-6.
15. Schiavino D, Nucera E, Alonzi C, et al. A clinical trial of oral hyposensitization in systemic nickel allergy. Int J Immunopathol Pharmacol 2006; 19: 593-600.
16. Minciullo PL, Saija A, Trombetta D, Ricciardi L, Di Pasquale L, Gangemi S. Serum levels of sICAM-1 in subjects affected by systemic nickel allergy syndrome. It J Allergy Clin Immunol 2006; 16:109.
17. Turi MC, Di Claudio F, Schiavone C, Verna N, Perrone A, Cavallucci E, Di Gioacchino M. Systemic nickel allergy syndrome: an update. It J Allergy Clin Immunol 2008; 18:98-102
18. Panzani RC, Schiavino D, Nucera E, Pellegrino S, Fais G, Schinco G, Patriarca G. Oral hyposensitization to nickel allergy: Preliminary clinical results. Int Arch Allergy Immunol 1995; 107:251-4.
19. Morris DL. Intradermal testing and sublingual desensitization for nickel. Cutis 1998; 61:129-32.
20. Sjövall P, Christensen OB, Möller H. Oral hyposensitization in nickel allergy. J Am Acad Dermatol 1997; 17:774-8.
21. Bagot M, Terki N, Bacha S, Moyse D, Suck C, Revuz J. Désensibilation per os dans l'eczéma de contact au nickel: étude clinico-biologique en double insu contre placebo. Ann Dermatol Venereol 1999; 126:502.
22. Boscolo P, Andreassi M, Sabbioni E, Reale M, Conti P, Amerio P, Di Gioacchino M. Systemic effects of ingested nickel on the immune system of nickel sensitised women. Life Sci 1999; 64:1485-91
23. Jensen CS, Lisby S, Larsen JK, Veien NK, Menné T. Characterization of lymphocyte subpopulations and cytokine profiles in peripheral blood of nickel-sensitive individuals with systemic contact dermatitis after oral nickel exposure. Contact Dermatitis 2004; 50:31-8.

24. Mahadi G, Israel DM, Hassall E. Nickel dermatitis and associated gastritis after coin ingestion. J Pediatr Gastroenterol Nutr 1996; 23:74-6.
25. Vreeburg KJ, de Groot K, von Blomberg M, Scheper RJ. Induction of immunological tolerance by oral administration of nickel and chromium. J Dent Res 1984; 63:124-8.
26. Ishii N, Moriguchi N, Nakajima H, Tanaka S, Ameniya F. Nickel sulfate-specific suppressor cells induced by nickel sulfate in drinking water. J Dermatol Sci 1993; 6:159-64.
27. Artik S, Haarhuis K, Begerow J, Gleichmann E. Tolerance to nickel: oral nickel administration induces a high frequency of anergic T cells with persistent suppressor activity. J Immunol 2001; 167: 6794-803.