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*LETTER TO THE EDITOR*

# Modulation of gut microbiota in patients with IBS and systemic nickel allergy after diet and probiotic supplementation: a pilot study

C. Caruso[1*], S. Colantuono[1,2*], G. Celi[3], L. Salerno[4], G. De Martino[5], G. Ianiro[6], C. Casale[4], E. Nucera[1], S. Del Giacco[7] and A. Gasbarrini[8]

*[1]Allergy Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [2]Department of Translational and Precision medicine, Sapienza University, Rome, Italy; [3]Clinica Villa Pia, Rome, Italy; [4]Consultant, Rome, Italy; [5]Consultant, Salerno, Italy; [6]Digestive Disease Center, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [7]Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy; [8]Digestive Disease Center, Fondazione Policlinico Universitario A. Gemelli IRCCS, Catholic University of Sacred Heart, Rome, Italy*

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[*]These authors equally contributed to this paper

To the Editor,

The gastrointestinal tract (GIT) has a very important immune function in developing either effector or tolerant responses to different antigens by balancing the activities of Th1 and Th2 cells as well as regulating Th17 and T regulatory (Treg) cells in the lamina propria (1). Immune dysfunction has been associated with lifestyle changes, such as dietary modifications and increased antibiotic use. These habit changes have profoundly altered the co-evolved relationship between host and microbiota, depleting bacterial populations critical for the maintenance of mucosal homeostasis (2). The gut microbiota can be defined as the trillions of microbes that collectively inhabit the gut lumen, and increasing evidence shows that altered patterns of microbial exposure (dysbiosis) in an early stage of life can lead to the development of adverse food reactions (AFR) by negatively influencing immune system development (3).

The gut microbiota could be considered as a potential target for preventive and therapeutic intervention against AFR. Recent studies have reported the efficacy of intervention in the gut microbiota against AFR. In recent years, strong evidence of a possible relationship between modifications of the gut microbiota composition and development of irritable bowel syndrome (IBS) has been collected (4). The currently reported general population prevalence of nickel (Ni) allergy is 8-18% in the US and Europe; in southern European countries, such as Italy, it is estimated to be 16% (5). In sensitized subjects, ingested Ni may induce gastrointestinal symptoms similar to IBS, in addition to typical systemic cutaneous lesions [systemic nickel allergy syndrome (SNAS)] (6). A low-nickel diet could reduce the systemic manifestations (7).

We report here the results of a pilot observational study to evaluate the effects of probiotic supplementation in patients with IBS and SNAS, that had been previously treated only with dietary intervention without a complete success, in terms of

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*Corresponding Author:*
Dr Cristiano Caruso,
Fondazione Policlinico A. Gemelli,
Largo Gemelli 1,
00168, Rome, Italy,
Tel.: +39 3348079133
e-mail: cristiano.caruso@policlinicogemelli.it



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modulation of faecal microbiota population, reduction of GI symptoms, increase of the patient's quality of life and modification of gut dysbiosis (8).

## MATERIALS AND METHODS

Between March and September 2017 40 consecutive patients, admitted to the Allergy Unit reporting nonspecific gastrointestinal symptoms, were evaluated on the presence of abdominal pain or distension, meteorism, diarrhea and/or constipation (Roma criteria III). Exclusion criteria were: age <18 years; organic gastrointestinal diseases, such as peptic ulcer, inflammatory bowel diseases, celiac disease, gastrointestinal infections, and small intestinal bacterial overgrowth; diabetes mellitus, hepatic, renal or cardiac dysfunction, and thyroid disease or tumor; intake of non-steroidal anti-inflammatory agents and pregnancy, lactation, abuse of alcohol, coffee, tea, and cola intake.

After gastroenterological and allergological evaluation, 14 patients were diagnosed as IBS, 24 as Ni sensitization and SNAS and two patients showed non-specific lipid transfer protein (LTP) sensitization. Age was between 18 and 73 years (median age 40 years). The F/M ratio was 33/7. The median BMI at baseline was 21 (Table I). All patients signed informed consent to participate in the study that was approved by the ethics committee.

All patients had previously been prescribed different diet protocols, without reporting a full improvement of GI symptoms. Eligible participants were adults who i) had a positive Ni-patch test, ii) reported symptoms suggesting SNAS, and iii) improved at least 70% from baseline after one month on a Ni-poor diet (severity of symptoms rated on a visual analog scale - VAS) (6). All patients were evaluated for gut dysbiosis (9) at baseline. DNA extraction method (Next Generation Sequencing) with a commercial kit was performed on stool samples. Direct sequencing of 16S rRNA amplicons is a fast and useful method to determine gut bacterial population (10). Twenty-five out of the 40 patients repeated dysbiosis evaluation also after three months (T1). Quality of life was assessed through IBS QoL (11).

### *Dietary intervention and probiotic supplementation*

IBS patients were arbitrarily divided into two groups, according to a gluten-free diet prescription (11 pts) or a

**Table I.** *Patient characteristics at baseline (T0) and after treatment (T1).*

| | **T0** | **T1** |
|---|---|---|
| n° of patient | 40 | |
| Age in years (median, range) | 40 (18-73) | |
| F/M ratio | 33/7 | |
| IBS (n°) | 14 | |
| Low fodmap diet | 10 | |
| Gluten-free diet | 4 | |
| SNAS (n°) | 24 | |
| LTP sens (n°) | 2 | |
| Dysbiosis (n°/%) | 31/40 (77.5) | 7/25 (28) |
| IBS | 14 | 3 |
| SNAS | 16 | 3 |
| LTP sens | 1 | 1 |
| BMI (median) | 21 | 21.7 |

low Fermentable Oligo-, Di- and Mono-saccharides And Polyols (FODMAP) diet prescription for three months (10 pts). FODMAP are a group of carbohydrates that are poorly absorbed in the small intestine and subsequently fermented in the small or large intestine. Dietary advice on a low FODMAP diet was mostly delivered by a dietitian. Table II is a representative example of common foods that are known to have a high FODMAP content.

Similarly, SNAS patients were prescribed a low-Ni diet (100 μg/kg nickel content during the first four weeks and then up to 200 μg/kg for up to three months). The absolute removal of Ni from the diet is impractical because of its ubiquitous presence in almost all foods; patients were asked to avoid the use of stainless-steel utensils to reduce Ni contamination during cooking.

Two non-specific LTP sensitized patients underwent an

**Table II.** *Foods divided according to the content of FODMAP.*

| Types of sugars | High FODMAP foods | Low FODMAP alternatives |
|---|---|---|
| Oligosaccharides | FOS-Grains: wheat-, rye-, and barley-based products<br>Vegetables: onion, garlic, artichokes, leeks, beetroot, and savoy cabbage | Fruit: banana, most berries (except boysenberries and blackberries), grapes, lemon, lime, mandarin, orange, kiwi fruit, pineapple, passion fruit, and rhubarb |
| Disaccharides | Fruits: watermelon, peaches, persimmon, prunes, nectarines and most dried fruit | Vegetables: capsicum, bok choy, green beans, parsnip, silverbeet, cucumber, carrots, celery, eggplant, lettuce, potatoes, yams, tomatoes, and zucchini |
| Monosaccharides | GOS-Legumes: red kidney beans, baked beans, and soya beans<br>Vegetables: beetroot and peas | Grains: wheat-free grains/flour, gluten-free bread or cereal products, and quinoa |
| polyols | Lactose-Dairy products: cows/goat milk, and yoghurt<br>Fructose (in excess of glucose)<br>Fruits: apples, pears, watermelon, mango, cherries, boysenberries and fruit juice from high-fructose foods<br>Honey-Sweeteners: high-fructose corn syrup<br>Vegetable: asparagus and snap peas<br>Sorbitol fruit: apples, pears, avocado, apricots, blackberries, nectarines, peaches, plums, prunes, and watermelon<br>Mannitol Vegetables: sweet potato, mushrooms, cauliflower, and snow peas | Dairy products: lactose-free, almond or rice-based milk, yoghurt and ice cream, hard cheese, feta and cottage cheese<br>Fruit: banana, grapes, honeydew, melon, kiwifruit, lemon, lime, mandarin, orange, passionfruit, paw paw, and most berries (except boysenberries and blackberries)<br>Sweeteners: maple syrup and golden syrup<br>Sweeteners: Maple syrup, and sugar (sucrose) Fruits: banana, grape, honeydew, melon, kiwifruit, lemon, mandarin, orange, passionfruit, and paw paw |

LTP-free diet; this food allergy is by far the main cause of primary food allergy in Italian adults and generally in the Mediterranean European countries, and is also responsible for the largest number of food-induced anaphylactic reactions (12).

A fixed probiotic supplementation was prescribed along with diet: *Enterococcus faecium*, *Saccharomyces boulardii*, *Lactobacillus acidophilus* (Enterelle©) for the first 12 days, *Lactobacillus salivarius, Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium lactis, Lactobacillus rhamnosus* for up to three months (Acronelle© and Serobioma©).

One study involved two outpatient scheduled visits [baseline (T0) and 3 months after the prescription of diet and probiotic supplementation (T1)] and a phone contact 2 weeks after the conclusion of treatment (T2). Gastrointestinal symptoms were evaluated using VAS before and after treatment. We used VAS-IBS to assess

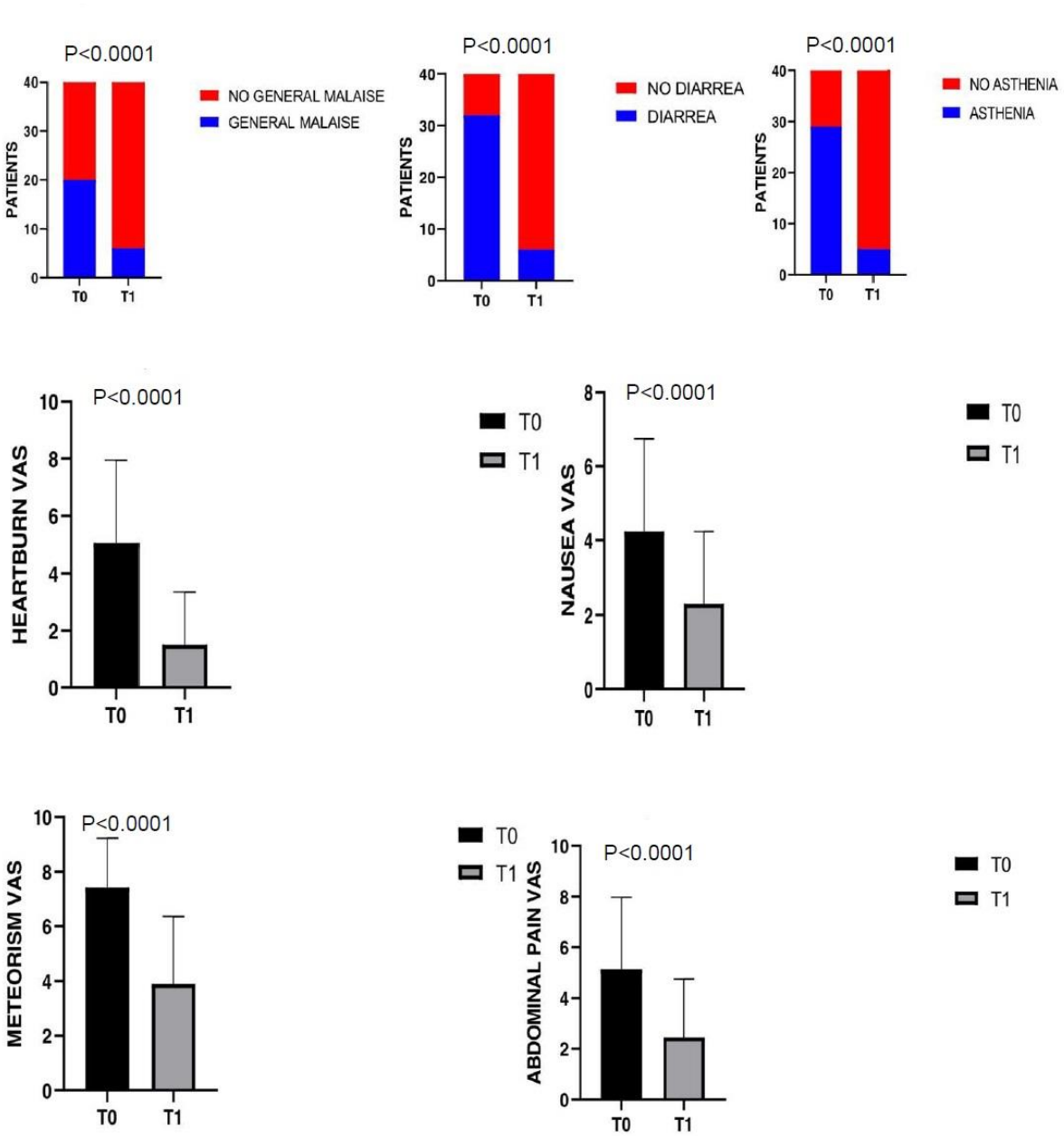


**Fig. 1.** *Improvement of the major items of IBS-VAS at T1 (after diet and probiotic supplementation).*

the presence and severity of symptoms related to IBS and the improvements achieved through dietary and probiotic treatment, assigning a score from 0 to 10 at T0, T1 and T2. The most outstanding physical symptoms identified were divided into six main groups; abdominal pain, diarrhoea, constipation, bloating and flatulence, abnormal bowel passage and vomiting and nausea. all symptoms except vomiting and nausea also support the diagnosis of IBS (13).

In 25 pts that accepted to repeat the test, gut dysbiosis was re-assessed at T1 in order to underline any relevant modification. Two weeks after the conclusion of the prescribed treatment, during a telephone follow-up visit, patients were re-questioned about any variation of clinical symptoms.

*Statistical analysis*

Data analysis was carried out by McNemar's *Chi*-squared test with continuity correction (comparison of proportions) and Paired Sample *t*-test (comparison of means). Probability levels <5% were considered statistically significant.

## RESULTS

At baseline, 31 out of 40 patients had an abnormal dysbiosis index (n.v. 0.070, 0.326). It was not possible to find any significant difference in gut bacteria populations among the three groups of patients, but patients with a higher value of dysbiosis showed a lack of lactate production. Dysbiosis values returned to a normal range after three months of treatment in 18 out of 25 patients that underwent re-assessment at T1.

Comparison of VAS scales means three months after the beginning of the therapy showed significant improvement of gastrointestinal symptoms, in terms of nausea (4.24±2.50 *vs* 2.31±1.93; P<0.0001), heartburn (5.07±2.88 *vs* 1.52±1.84; P<0.0001), meteorism (7.41±1.80 *vs* 3.90±2.47; P<0.0001) and abdominal pain (5.14±2.84 *vs* 2.45±2.31; P<0.0001). After three months there was a significant reduction of patients with diarrhoea (32/40 (80%) *vs* 6/40 (15%); P<0.0001), asthenia (T0 29/40 (72.5%) *vs* 5/40 (12.5%); P<0.0001) and general malaise (20/40 (50%) *vs* 6/40 (15%); P<0.0005) (Fig. 1).

The clinical improvement was confirmed also after the cessation of probiotic supplementation, in fact, VAS score remained unmodified at T2 evaluation, thus indicating the persistence of clinical benefits even after the end of the intervention. No significant difference was found among the groups in terms of improvement of VAS scores: IBS patients (regardless of the diet) as well as Ni-sensitized patients showed similar benefits of clinical symptoms after the intervention. We could speculate that the clinical improvement is mostly due to the probiotic supplementation rather than to different diet protocols, but the size of groups is too small for definitive considerations. Moreover, further studies in a double-blind placebo-controlled setting are needed. No statistical consideration could be made for the two LTP patients. Notably, all patients experienced an improvement of quality of life measured by IBS QoL (11) after the intervention, while no meaningful change of BMI was observed at T1 (Table I).

## DISCUSSION

It has been widely observed that probiotics might be able to exert a beneficial influence on several pathogenetic pathways of IBS, including the restoration of altered gut microbiota, by increasing the number of beneficial bacteria and reducing the number of pathogens because of their reaction to the presence of the coexisting ones, and consequently the decrease of inflammation associated with the proliferation of pathogenic bacteria (9). This way, probiotic supplementation combined with diet could be useful to reduce GI symptoms and improve the quality of life in IBS patients.

In this pilot study, the gut microbial community of SNAS patients was investigated with DNA extraction method on stool samples. Previous findings suggested that the low-Ni diet is useful to improve the symptoms and the quality of life of patients with SNAS (6), however, our preliminary findings hypothesize that its association with probiotics might allow to rapidly decrease the frequency and the severity of symptoms related to meals, likely due to the increase of intestinal gut microbial diversity.

In the present study, for the first time, the effectiveness of probiotic administration coupled to a low-Ni diet in SNAS patients was revealed. Moreover, the

observation that, before the study, all these patients had partially improved with the diet alone may support the idea that probiotic implementation could represent not only a useful but probably a crucial mean for the treatment of these conditions. This data suggests that, although different from an etiopathological point of view, an altered intestinal homeostasis may have a pivotal role in both conditions. However further studies on a larger population are needed to support this hypothesis.

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## REFERENCES

1. Pascal M, Perez-Gordo M, Caballero T, et al. Microbiome and allergic disease. Front Immunol 2018; 9:1584.
2. Plunkett CH, Nagler CR. The influence of the microbiome on allergic sensitization to food. J Immunol 2017; 198(2):581-9.
3. Aitoro R, Paparo L, Amoroso A, et al. Gut microbiota as a target for preventive and therapeutic intervention against food allergy. Nutrients 2017; 9(7):672.
4. Principi N, Cozzali R, Farinelli E, Brusaferro A, Esposito S. Gut dysbiosis and irritable bowel syndrome: the potential role of probiotics. J Infect 2018; 76(2):111-20.
5. Watanabe M, Masieri S, Costantini D, et al. Overweight and obese patients with nickel allergy have a worse metabolic profile compared to weight matched non-allergic individuals. PLoS One 2018; 13(8):e0202683.
6. Di Gioacchino M, Ricciardi L, De Pità O, et al. Nickel oral hyposensitization in patients with systemic nickel allergy syndrome. Ann Med 2014; 46(1):31-37.
7. Rizzi A, Nucera E, Laterza L, et al. Irritable bowel syndrome and nickel allergy: What is the role of the low nickel diet? J Neurogastroenterol Motil 2017; 23(1):101-108.
8. Randazzo CL, Pino A, Ricciardi L, et al. Probiotic supplementation in systemic nickel allergy syndrome patients: study of its effects on lactic acid bacteria population and on clinical symptoms. J Appl Microbiol 2015; 118(1):202-211.
9. Belizário JE, Faintuch J, Garay-Malpartida M. Gut microbiome dysbiosis and immunometabolism: new frontiers for treatment of metabolic diseases. Mediators Inflamm 2018; 2018:2037838.
10. Davidson RM, Epperson LE. Microbiome sequencing methods for studying human diseases. Methods Mol Biol 2018; 1706:77-90.
11. Drossman DA, Patrick DL, Whitehead WE, et al. Further validation of the IBS-QOL: a disease–specific, quality–of–life questionnaire. Am J Gastroenterol 2000; 95(4):999-1007.
12. Asero R, Antonicelli L, Arena A, et al. Causes of food-induced anaphylaxis in Italian adults: a multicentre study. Int Arch Allergy Immunol 2009; 150(3):271-7.
13. Bengtsson M, Ohlsson B, Ulander K. Development and psychometric testing of the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). BMC Gastroenterol 2007; 7:16.