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EDITORIAL

Foreword: Supplement on immune manipulation of patients with upper respiratory infections

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The present Supplement includes topics that are very common in daily clinical practice. Each issue will be presented and discussed, showing innovative solutions and approaches.

The first theme concerns the immune manipulation of patients with upper respiratory infections and treatment with antibiotics. Antibiotics are presently overused with harmful consequences, such as adverse events, systemic dysbiosis, and increased antibiotic resistance. As a result, immune manipulation using natural components modulating the immune response is advantageous (1). In this regard, a series of clinical experiences performed in otolaryngologic settings investigated two food supplements. The first product (Abincol@) is a consolidated probiotic mixture containing *Lactobacillus plantarum* LP01 (formerly *Lactobacillus plantarum*) (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million of living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 million of living cells). The other product is a new multi-component food supplement (Abincol Immuno®) that contains a probiotic mixture (*Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus*), zinc, inulin, and vitamin D. Many patients with upper respiratory infections took these food supplements and experienced fewer symptoms.

The second issue regards a widespread challenge in daily practice, such as gastric reflux (2). Two primary diseases may occur, such as gastroesophageal reflux disease (GERD) and laryngopharyngeal reflux (LPR). These diseases may affect any age: from infancy to ageing. Managing these digestive diseases is challenging and mainly involves paediatricians, general practitioners, gastroenterologists, and otolaryngologists. In addition, patients with gastric reflux complaints frequently ask for advice from pharmacists. An educational program involved these professional positions. Updating the knowledge on this argument significantly affected the prescriptive attitude. Proton pump inhibitors and alginates are the most common medications. Among these products, Marial(R) and esomeprazole achieved deep interest. Namely, international guidelines recommend the add-on use of omeprazole for patients with gastric reflux disorders (3). Consistently, the outcomes provided by the current survey confirmed the therapeutic synergy acquired by add-on PPI to alginates.

The third matter concerns a common issue in clinical practice represented by intestinal diverticula (4). An Italian survey recruited a large sample of patients with symptomatic diverticulosis and diverticulitis treated with a new multi-component food supplement (Divercol®). This product contains seeds of *Plantago psyllium*, dry extract of *Perilla*

Keywords: immune manipulation, Abincol®, Abincol Immuno®, gastric reflux, Marial®, diverticular disease, Divercol®, urological disorders, Broser®, rhinitis, Rinalt®, wound healing

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frutescens, Citrus paradisi seeds dry extract, a mixture of tyndallized probiotics (*Lactobacillus rhamnosus, L reuteri, L acidophilus*), and riboflavin.

The fourth issue concerns the outcomes reported by a national survey conducted in patients with medical and postsurgical urological problems (5). The patients were treated with a food supplement (Broser®) containing bromelain, escin, and selenium.

The fifth topic regards a new medical device that has innovative characteristics (Rinalt@). This product contains a phyto-polymer (Mucotannil@ complex), including hydroalcoholic extract of Icelandic moss (*Cetraria islandica*) and a high-polysaccharide extract from the inner bark of Larch (*Larix decidua*) and Sea Buckthorn (*Hippophae rhamnoides*) leaf/bud extract. The medical device also contains seawater, dexpanthenol, and essential balsamic oils. This multi-component product is indicated for the adjunctive treatment of respiratory disorders characterized by mucus hyperproduction, including infectious rhinitis and allergic rhinitis (6,7).

Another issue concerns the use of probiotic mixture as supportive care in oncologic patients. The last topic regards a demanding medical problem, such as the management of wounds, mainly concerning the difficult to treat injuries (8). Regenerative Medicine is a new health branch that deals with wound healing. An innovative Regeneration Medicine protocol represents an ideal approach to manage patients with difficult-to-treat wounds. This protocol consists of a multidisciplinary approach and uses different integrated treatments.

Conflict of Interest

All authors state that there is no conflict of interest.

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EDITORIAL

Immune manipulation in managing patients with upper airways infections: a new strategy

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The human body harbours about 100 trillion colonies of microorganisms inhabiting various body parts, including the respiratory tract and the digestive tube (1). These microbes are defined microbiota and populate the various organs in different proportions and with diverse functions. The microbiota aims to promote and maintain a healthy immune system and impede colonization by pathogens (2). During childhood, the composition of commensal microbiota modify over time, and adequate microbial exposure allows the physiological maturation of the immune-competent cells resident in the airways, intestine, and brain (3). The impaired composition of commensal microbiota is defined as dysbiosis. Dysbiosis can cause impairment of the immune system and amplify the inflammatory response.

There is growing thought that upper respiratory infections are increasing worldwide (4). Consequently, acute and chronic respiratory infections contribute to burden the health service (5). Furthermore, a high-fat, low-fibre diet and broad antibiotic use are the leading causes of dysbiosis. Consequently, restoring dysbiosis using probiotics could be a profitable strategy for reinstating physiologic microbiota, such as eubiosis (6).

The inhibition of pathogenic bacteria proliferation is the most relevant mechanism of the action exerted by probiotics (7). Other mechanisms include: i) the antagonism of adhesion and co-aggregation ability by pathogens, through competition for binding sites and nutritional sources, ii) the secretion of antimicrobial substances, iii) the enhancement of intestinal barrier function by regulation of tight junctions and mucins expression, and iv) the immunomodulation by interaction to receptors of microorganism-associated molecular patterns (8,9). In addition, probiotics produce short-chain fatty acids, such as butyrate, propionate, and acetate, that exert relevant immunoregulatory functions, mainly by modulating extracellular and intracellular signaling molecules. These fatty acids bind to cell surface G protein-coupled receptors, modulate the immune function indirectly, inhibit histone deacetylases, the cell interior, and regulate gene transcription in the immune response. Probiotics also promote B-cell differentiation and antibody production (10). Microbiota metabolites also include essential amino acids, mostly tryptophan, which dampens TNF-α-induced activation of NF-κB and reduce expression of the pro-inflammatory cytokines. Also, gut bacteria synthesize vitamins from complex B, which play a vital role in regulating the immune system (11). The anti-inflammatory effects of probiotics entail a significant increase in anti-inflammatory IL-10 production and a decrease of pro-inflammatory cytokines, such as IL-1β, TNFα, and IL-6 (12). Moreover, probiotics can decrease the type 3 immune response, favouring the differentiation of anti-inflammatory Treg/type 1 regulatory intestinal T cells (13). In particular, Lactobacilli activate human dendritic cells and lead to a type 1 immune response,

Keywords: Probiotics, zinc, inulin, vitamin D3, upper airways, infection, inflammation, otolaryngologist, clinical experience

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characterized by T-helper 1 polarization, which increases IFN-γ and IL-12 production, cytokines devoted to fighting infections (14).

However, the gut–lung axis, such as the cross-talk between the intestinal tract and the airways, sustains a bidirectional communication through the blood system and lymphatic drainage. Thus, the gut-airways axis modulates the local immunity of both the gut and the respiratory tract, as well as their respective microbial patterns and composition (15). This issue represents the basis for understanding how an oral probiotic can influence upper airways and help manage respiratory infections (16).

The probiotic approach

Based on this background, a probiotic mixture (Abincol®) was launched some years ago. Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (formerly *Lactobacillus plantarum*) (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 million of living cells). The rationale for using this probiotic mixture is that these probiotics are living microorganisms that confer a health benefit to the host when administered in adequate amounts. These probiotics produce microbial transformation in the intestinal microbiota and exert several health-promoting properties, including maintaining the gut barrier function and modulation of the host immune system (17). Moreover, the effects of this probiotic mixture may be complementary (also referred to as additive) and synergistic (18). These probiotic strains produce growth factors that strengthen the gut epithelium and antimicrobial-anti-inflammatory mediators (short-chain fatty acids, bacteriocins, hydroperoxides, bile acids, and lactic acids), killing harmful microorganisms (19).

Consequently, their cellular components are released in the gut environment, activating immune responses by modulating pro-inflammatory cytokine production and immunoglobulin synthesis, besides improving macrophage and lymphocyte activity (20). Also, non-immunological benefits associated with these probiotics include the digestion and absorption processes, competition with potential pathogens for nutrients and intestinal adhesion sites, pH alterations, agglutination of pathogenic microorganisms, and sequestration of metabolic toxins (21). Furthermore, animal models and *in vitro* assays demonstrated that these probiotics also decrease apoptosis, increase the mucus synthesis, tissue repair, redistribution, and production of tight junctions in gut epithelial cells, thus reducing the intestinal permeability and enhancing the barrier protection and function (22). However, it has to underline that the underlying mechanisms of probiotics are dependent on the specific microbial strain, and the effectiveness is also disease-specific. Thus, the probiotic choice should be carefully oriented to a specific strain in a particular disease.

Abincol® has been fruitfully used in patients with chronic intestinal diseases (23), in patients undergoing bowel preparation (24), and in post-surgical intestinal dysbiosis (25). More recently, this food supplement has been tested in an experience conducted by a panel of Italian otolaryngologists (26). This experience considered both the evaluation of doctors' points of view in managing patients with upper respiratory diseases and patients' point of view with rhinosinusitis, pharyngotonsillitis, otitis media, and laryngotracheitis (27-31). The obtained outcomes had clinical relevance as they were obtained in real-world settings. Therefore, this probiotic mixture represented a reliable therapeutic option in clinical practice.

New perspectives

The next advancement was the fulfilment of a new multi-component food supplement containing a probiotic mixture (*Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus*), zinc, inulin, and vitamin D.

Vitamin D (VD) is an essential hormone for humans as it exerts pleiotropic effects, including anti-inflammatory activity (32). Throughout the body, many cells express the VD receptor (VDR) and the enzyme 1α-hydroxylase (33). A relationship between VD status and the incidence and the severity of RI in children has been found in many observational studies; mainly, the link between severe deficiency and susceptibility to RI is prototypically represented by the high respiratory morbidity in children with rickets

(34). Low VD status (< 50 nmol/L) is an independent risk factor for treatment failure and delayed recovery from severe lower RI in children (35). VD supports the innate and adaptive immune response and plays a role in fighting pathogens, suggesting the need to guarantee an adequate status, particularly for patients with acute or chronic infections with profound VD deficiency. The benefit is notably higher in those receiving daily or weekly VD without additional bolus doses (36).

Zinc is necessary for mounting an efficient and balanced immune response, mainly against pathogens (37). However, zinc also affects hematopoiesis, including differentiation and maturation into immune cell subtypes.

Inulin is a fructan, such as a fructose polymer. Inulin is an unavailable carbohydrate (i.e., not digestible by enzymes produced by the human body) present in various foods of plant origin. It is generally extracted from chicory or artichoke; arriving in the intestine without being digested, inulin is used by the bacteria of the intestinal microbiota that ferment it for nourishment (38, 39). Through this mechanism, inulin promotes the growth of intestinal bacteria that are allies of health; inulin promotes, for example, good intestinal function. Furthermore, inulin helps reduce the amount of cholesterol present in food. Therefore, inulin, as a substratum for probiotics, is a prebiotic.

Lactobacillus plantarum, Lactobacillus rhamnosus, and *Lactobacillus delbrueckii subsp. Bulgaricus* are well-known probiotics widely used as a food supplement to maintain optimal eubiosis (40-43). Therefore, these components exert a relevant influence on the immune system. Therefore, this new product has been tested in patients with respiratory infections. Promising results emerged from the clinical experience.

Conclusive remarks

As the immune response is significantly affected during infections and antibiotics alter microbiota balance, immune manipulation could represent an attractive strategy for managing respiratory infections. Namely, probiotics, overall if a mixture, and active components could reinforce the immune system providing a short resolution of symptoms.

Conflict of interest

All authors state that there is no conflict of interest.

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CLINICAL TRIAL

A multi-component food supplement with a probiotic mixture, zinc, vitamin D, and inulin for upper respiratory infections

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Upper respiratory infections represent a common problem in clinical practice. Doctors very frequently use antibiotics in managing infections. However, antibiotics alter the physiological microbiota, causing dysbiosis. Dysbiosis worsens respiratory symptoms, changes gut function, amplifies inflammation, and impairs the immune response. Probiotics significantly restore respiratory and gut microbiota, expediting recovery and preventing relapse. In this regard, a probiotic mixture (Abincol®), containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii* LDD01, is fruitfully used in common practice. Recently, a new multi-component food supplement (Abincol Immuno®) has been launched.

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This product contains an innovative probiotic mixture (*Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus*), zinc, inulin, and vitamin D. This product has been tested in a practical experience by a panel of Italian otorhinolaryngologists who visited a large number of patients with upper airways infections. All patients were treated with antibiotics and Abincol@ for two weeks, then a subgroup (ratio 1:1) took a one-month course of Abincol Immuno® (Group A), the other patients served as control (Group B). Patients were evaluated at baseline (T0), at the end of antibiotic treatment (T1), at the end of the Abincol Immuno® course (T2), and at the end of 4-month follow-up (T3). Globally, 3.819 outpatients (mean age 45 years) were enrolled: 1.936 (50.7%) in Group A and 1.883 (49.3%) in Group B. This food supplement significantly reduced the rate of patients with cough (p=0.03), fatigue (p<0.001), headache (p<0.01), and malaise (p=0.01). Both food supplements were well tolerated, and no significant adverse reactions were observed. In conclusion, the current clinical experience suggested that immune manipulation with this multi-component product may be considered an effective and safe therapeutic option in managing patients with an upper respiratory infection and treated with antibiotics.

Keywords: upper respiratory infection, mucosal microbiota, antibiotics, probiotics, inulin, vitamin D, zinc, clinical experience

The human body is not sterile as it contains many microbes, which are widely distributed in all organs exposed to the external environment. These microorganisms are mainly bacteria and constitute a unique, differentiated, and dense ecosystem named microbiota (1). There is growing interest in clarifying microbiota's exact composition and function (2). The microbiota is characterized by a diversity directed to contrast pathogens optimally. Altered microbiota is named dysbiosis. Dysbiosis fosters respiratory infections, and *vice versa* respiratory infections amplify dysbiosis (3). In this regard, antibiotics significantly change the intestinal and respiratory microbiota, further worsening dysbiosis (4).

Upper respiratory infections represent a daily challenge in otorhinolaryngological (ORL) practice. These infections may be acute or chronic. Otorhinolaryngologists frequently prescribe antibiotics in managing patients with respiratory infections. However, antibiotic overuse promotes antibiotic resistance and mucosal dysbiosis (5). As a result, it is common to use probiotics in respiratory infections and treat them with antibiotics to restore the microbiota environment (6).

Abincol@ is a food supplement containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 million living cells). This food supplement has been tested in an experience conducted by a panel of Italian otorhinolaryngologists (7). This experience considered the evaluation of doctors' point of view in managing patients with upper respiratory diseases and the point of view of patients with rhinosinusitis, pharyngotonsillitis, otitis media, and laryngotracheitis (8-12). The obtained outcomes had clinical relevance as they were obtained in real-life settings. Therefore, this probiotic mixture represented a reliable therapeutic option in clinical practice.

Further, a new multi-component food supplement (Abincol Immuno®), containing a probiotic mixture (*Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus*), zinc, inulin, and vitamin D has been launched.

Vitamin D (VD) is an essential hormone for humans as it exerts pleiotropic effects, including anti-inflammatory activity and immune-stimulating effects (13,14). Zinc is necessary for mounting an efficient and balanced immune response, mainly against pathogens, and promotes the maturation of immunocompetent cells (15). Inulin is a fructan, such as a fructose polymer; it is a prebiotic that serves as a nutrient for probiotics (16,17). *Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus* are well-known probiotics widely used as a food supplement (18-21).

Based on this background, a panel of Italian otorhinolaryngologists tested this new food supplement in patients with upper respiratory infections managed in real-life settings.

MATERIALS AND METHODS

The present experience was conducted by a panel of Italian otorhinolaryngologists, distributed across Italy, assuring extensive and complete national coverage during the fall-winter 2020-2021. These specialists recruited all consecutive outpatients visited for an upper respiratory infection.

The inclusion criteria were: diagnosis of upper airways infection, both genders, adulthood, and antibiotic prescription. In addition, exclusion criteria were to have comorbidities and concomitant medications able to interfere with the evaluation of the outcomes.

All patients signed informed consent. The procedures were conducted in a real-world setting, such as an ORL clinic. The patients were subdivided into two groups: Group A was treated with an oral antibiotic for 7-10 days associated with a 2-week course of Abincol®, then patients took a four-month course of Abincol Immuno®. Group B was treated with antibiotics for 7-10 days and a 2-week course of Abincol® alone. Voluntarily, some patients could take further courses of Abincol Immuno®.

Each stick of Abincol Immuno® contains inulin 372.4 mg, zinc 10 mg (corresponding to 100% of the daily reference nutritional value), vitamin D 5 μg (corresponding to 100% of the daily reference nutritional value), *Lactobacillus plantarum* LP01 ($1x10^9$ CFU), *Lactobacillus rhamnosus* CRL 1505 ($1x10^9$ CFU), and *Lactobacillus delbrueckii subsp. Bulgaricus* LB2 ($2x10^8$ CFU). The food supplements were taken following the specific indications, such as one stick/daily.

Patients were visited at baseline (T0), at 14 days (T1), at the end of the Abincol® course, at six weeks (T2), at the end of the Abincol Immuno® course in Group A, and at 4-months (T3). Clinical examination was performed in all patients at each visit. The evaluation parameters were the presence and severity of some symptoms, including fever, fatigue, malaise, headache, pain, cough, nausea, meteorism, diarrhoea, and constipation.

Patients used a visual analogue scale (VAS) to assess the perception of the symptom severity. The VAS scale ranged from 0 (no symptom) to 10 (very intense symptom). Safety was measured by reporting the occurrence of adverse events. All clinical data were inserted in an internet platform that guaranteed the patients' anonymity and the findings' recording accuracy.

Statistical analysis of the data was conducted to evaluate the intragroup and intergroup comparisons. Intragroup analysis was performed using the paired Wilcoxon test to compare severities and the McNemar test to compare proportions. Intergroup analysis was performed using the Wilcoxon test to compare severities and Chi-square test to compare frequencies. The statistical software was STATA 15.1, College Station, Texas 77845 USA.

RESULTS

Globally, 3.819 outpatients (mean age 45 years) were enrolled: 1.936 (50.7%) in Group A and 1.883 (49.3%) in Group B. The baseline data were superimposable in the two groups, so the groups were well-matched. At T1, there was no significant difference between groups concerning the frequency and severity of symptoms.

At T2, Group A patients had a lower frequency of cough ($p=0.032$), fatigue ($p<0.001$), headache ($p<0.01$), and malaise ($p=0.01$), as reported in Fig. 1 and Fig. 2. However, there was no difference in the symptom severity between groups, even if Group A patients experienced fewer symptoms than controls. At T3, there was no intergroup difference for both symptom frequency and severity.

The intragroup analysis showed that both frequency and severity of symptoms progressively diminished ($p<0.001$ for all times) in the two groups, respectively. In addition, the nutraceutical products were well-tolerated in all subjects.

DISCUSSION

Since ancient times, popular medicine has employed natural remedies, including the fermented-milk derivatives rich in bacteria, mostly *Lactobacilli*. Presently, there is growing scientific interest in developing food supplements to prevent and/or soothe human diseases, sparing pharmacological medications. In this context, probiotics continue to have a central role in the field of nutraceuticals. There is extensive evidence that they effectively restore gut and respiratory microbiota (22).

Antibiotics are frequently used in patients with an upper respiratory infection, but not always antibiotics

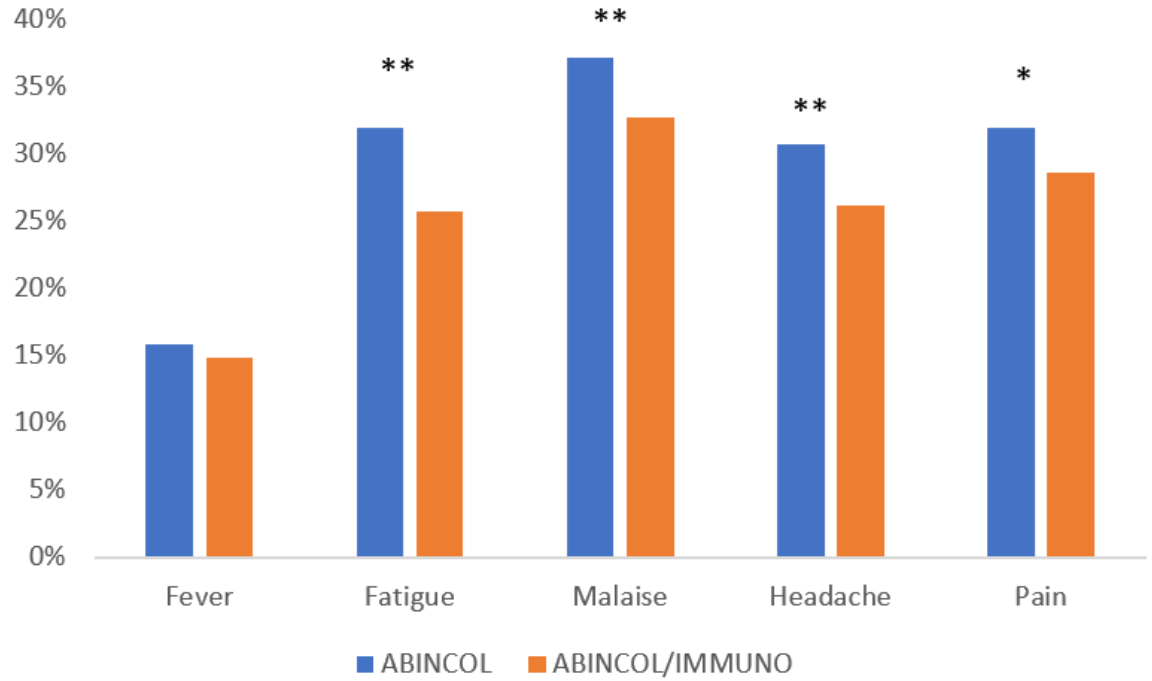

Fig. 1. *Percentages of patients with symptoms at T2 in the two groups. *p<0.010; **p<0.005*

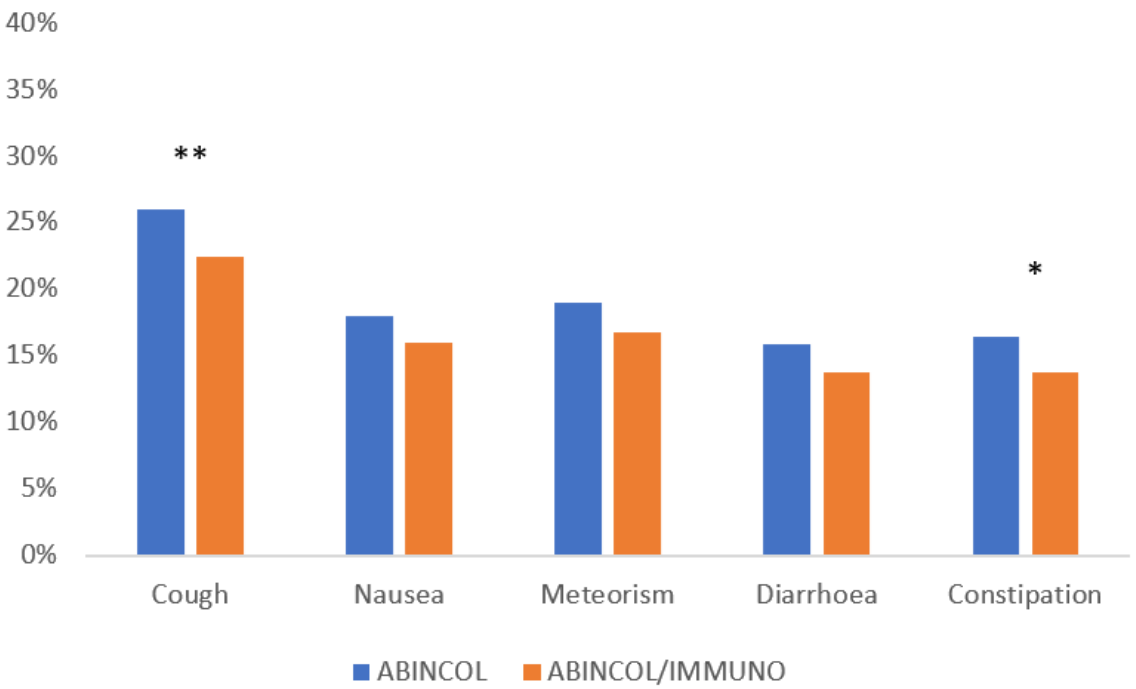

Fig. 2. *Percentages of patients with symptoms at T2 in the two groups. *p<0.010; **p<0.005*

are correctly prescribed. Overuse/abuse of antibiotics entails intestinal and respiratory dysbiosis. Moreover, infections *per se* promote dysbiosis; to interrupt this vicious circle, probiotics may represent a fruitful choice (23, 24). However, it could be more favourable to associate other components able to potentiate the immunomodulatory activity of probiotics. In this regard, the new food supplement Abincol Immuno® combines a probiotic mixture with three immune-stimulant components, including vitamin D, zinc, and prebiotic inulin. This association allows achieving a relevant immune manipulation as reported by the current experience. Indeed, the combined courses of Abincol® and Abincol Immuno® significantly reduced the persistence of some symptoms. In particular, malaise, headache, and fatigue are constitutional symptoms associated with respiratory infections. They represent the active involvement of the immune response and the associated inflammation that involve the whole body. Therefore, the significant frequency reduction means that the multi-component product can manipulate the immune system dampening the hyperactivation consequent to the infectious stimulation. In addition, the reduction of patients with cough depended on the direct activity on respiratory microbiota.

It is well known that the single components exert a relevant effect on the immune functions (25, 26). In particular, zinc is necessary for the physiological maturation and functioning of the immune system. Vitamin D plays a crucial role in modulating the immune response, and its defect significantly impairs the defensive mechanisms against pathogens. The prebiotic inulin is fruitful nourishment for probiotics, so it exerts a synergistic effect together with them. Therefore, the present experience demonstrated that the multi-component nutraceutical Abincol Immuno® combined with a previous Abincol® course exerted systemic and local effects, expression of an efficient immune manipulation in patients treated with antibiotics for respiratory infections.

It has to be noted that the present survey cannot be considered a formal investigative study. Consequently, further studies should be conducted with a rigorous methodology, designed according to randomized-controlled criteria. However, the strength of this survey is the considerable number of enrolled patients and the real-world setting. The outcomes could, therefore, mirror the facts observable in clinical practice. Moreover, it has to be noted that there was an interesting diminishing trend for all considered symptoms in patients treated with both food supplements. It suggests that active and sustained immune system manipulation hastens the recovery from upper respiratory infections.

In conclusion, the current clinical experience suggested that immune manipulation with this multi-component product may be considered an effective and safe therapeutic option in managing patients with an upper respiratory infection and treated with antibiotics.

Conflict of interest

All authors state that they have no conflict of interest.

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CLINICAL TRIAL

Probiotics, zinc, inulin, and vitamin D as an ancillary treatment for patients with acute rhinosinusitis

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Acute rhinosinusitis (ARS) affects the nose and paranasal sinuses together. Acute rhinosinusitis is usually suspected on the clinical ground, but the diagnosis is confirmed by endoscopy. Antibiotic therapy is used for ARS patients in almost all patients. However, antibiotics induce dysbiosis, such as a disturbed balance of physiological microbiota. Dysbiosis impairs the immune system and slows down the resolution of inflammatory events consequent to the infection. The current clinical experience was conducted in patients with ARS visited in an otolaryngologic setting. All patients were treated with antibiotics and Abincol@ for two weeks, then a subgroup (ratio 1:1) took a one-month course of Abincol Immuno® (Group A), the other patients served as control (Group

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B). Patients were evaluated at baseline (T0), at the end of antibiotic treatment (T1), at the end of the Abincol Immuno® course (T2), and at the end of 4-month follow-up (T3). Globally, 601 outpatients (mean age 46 years) were enrolled: 305 (50.7%) in Group A and 296 (49.3%) in Group B. The baseline data were superimposable in the two groups, so the groups were well-matched. At T2, Group A patients had a lower frequency of cough (p=0.05), fatigue (p=0.04), and nausea (p=0.02). However, there was no difference in the symptom severity between groups, even if Group A patients experienced fewer symptoms than controls. In conclusion, the present clinical experience demonstrated that immune manipulation with this multi-component product might be considered an effective and safe therapeutic option in managing patients with acute rhinosinusitis and treated with antibiotics.

Keywords: acute rhinosinusitis, mucosal microbiota, antibiotics, probiotics, inulin, vitamin D, zinc, clinical experience

Acute rhinosinusitis (ARS) is an infectious disease involving the nose and paranasal sinuses (1). The ARS duration is, by definition, less than one month (2). In the United States, ARS accounts for approximately 30 million primary care visits and a cost of $11 billion annually (3). It is also a common reason for antibiotic prescriptions worldwide. According to the guidelines concerning antibiotic stewardship, it should be recommended to diagnose bacterial ARS correctly (4,5). However, it is challenging to establish bacterial causes in clinical practice (6). Thus, antibiotics are empirically prescribed by doctors for patients with ARS. However, antibiotic treatment is commonly associated with microbiota perturbation, such as dysbiosis. Therefore, probiotics are commonly prescribed to restore the physiological balance of microbiota in clinical practice. Probiotics could improve the intestinal and respiratory microbiota equilibrium, reduce respiratory symptoms, and induce general wellbeing (7). In this regard, Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (formerly *Lactobacillus plantarum*) (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 million living cells). An Italian experience showed that Abincol® was effective as an add-on treatment in patients with upper respiratory infections, including rhinosinusitis, pharyngotonsillitis, otitis media and laryngotracheitis (8-12). A new multi-component food supplement (Abincol Immuno®) contains a probiotic mixture (*Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus*), zinc, inulin, and vitamin D. It has been launched very recently.

Vitamin D (VD) is an essential hormone for humans as it exerts pleiotropic effects, including anti-inflammatory activity and immune-stimulating effects (13,14). Zinc is necessary for mounting an efficient and balanced immune response, mainly against pathogens, and promotes the maturation of immunocompetent cells (15). Inulin is a fructan, such as a fructose polymer; it is a prebiotic nutrient for probiotics (16,17). *Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus* are well-known probiotics widely used as a food supplement (18-21).

A panel of Italian otorhinolaryngologists tested this new food supplement in a large number of patients with ARS and taking antibiotics, followed-up in real-life settings.

MATERIALS AND METHODS

This experience was conducted in Italian otorhinolaryngology centres across Italy, assuring extensive and complete national coverage during the fall-winter 2020-2021. These specialists recruited all consecutive outpatients who visited for ARS. The inclusion criteria were: diagnosis of acute rhinosinusitis, both genders, adulthood, and antibiotic treatment. In addition, exclusion criteria were to have comorbidities and concomitant medications able to interfere with the evaluation of the outcomes. All patients signed informed consent. The procedures were conducted in a real-world setting, such as an ORL clinic.

The patients were subdivided into two groups: Group A was treated with an oral antibiotic for 7-10 days associated with a 2-week course of Abincol®, then patients took a four-month course of Abincol Immuno®, and Group B was treated with antibiotics and a 2-week course of Abincol®

alone. Voluntarily, some patients could take further courses of Abincol Immuno®. Each stick of Abincol Immuno® contains inulin 372.4 mg, zinc 10 mg (corresponding to 100% of the daily reference nutritional value), vitamin D 5 mg (corresponding to 100% of the daily reference nutritional value), *Lactobacillus plantarum* LP01 ($1x10^9$ CFU), *Lactobacillus rhamnosus* CRL 1505 ($1x10^9$ CFU), and *Lactobacillus delbrueckii subsp. Bulgaricus* LB2 ($2x10^8$ CFU). The food supplements were taken following the specific indications, such as one stick/daily.

Patients were visited at baseline (T0), after 14 days (T1), such as at the end of the Abincol® course, after six weeks (T2), such as at the end of the Abincol Immuno® course in Group A, and after a 4-month follow-up (T3). Clinical examination was performed in all patients at each visit. The evaluation parameters were the presence and severity of some symptoms, including fever, fatigue, malaise, headache, pain, cough, nausea, meteorism, diarrhoea, and constipation. Patients used a visual analog scale (VAS) to assess the perception of the symptom severity. The VAS scale ranged from 0 (no symptom) to 10 (very intense symptom). Safety was measured by reporting the occurrence of adverse events.

All clinical data were inserted in an internet platform that guaranteed the patients' anonymity and the findings' recording accuracy. Statistical analysis of the data was conducted to evaluate the intragroup and intergroup comparisons. Intragroup analysis was performed using the paired Wilcoxon test to compare severities and the McNemar test to compare proportions. Intergroup analysis was performed using the Wilcoxon test to compare severities and the Chi-square test to compare frequencies. The statistical software was STATA 15.1, College Station, Texas 77845 USA.

RESULTS

Globally, 601 outpatients (mean age 46 years) were enrolled: 305 (50.7%) in Group A and 296 (49.3%) in Group B. The baseline data were superimposable in the two groups, so the groups were well-matched. At

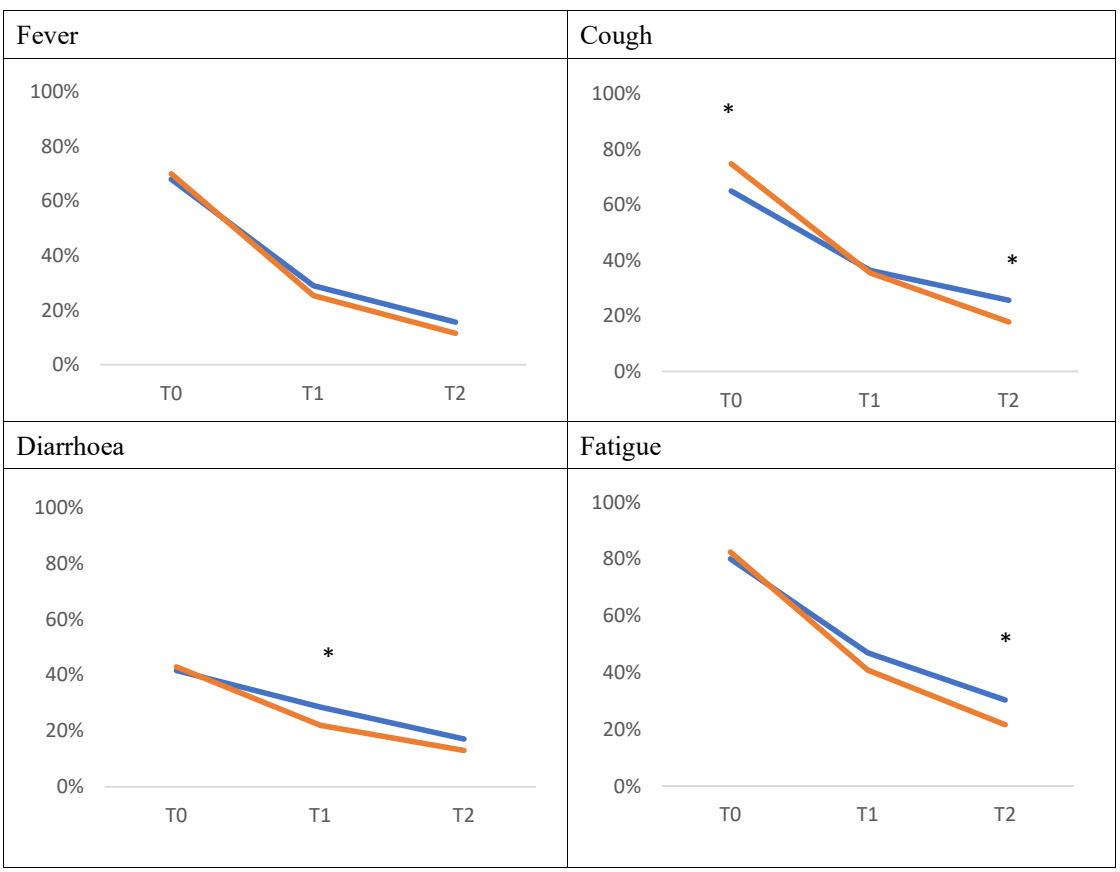

Fig. 1. *Percentages of patients with symptoms at T2 in the two groups*

T1, there was no significant difference between groups concerning the frequency and severity of symptoms.

At T2, Group A patients had a lower frequency of cough (p=0.05), fatigue (p=0.04), and nausea (p=0.02), as reported in Fig. 1, 2, and 3. However, there was no difference in the symptom severity between groups, even if Group A patients experienced fewer symptoms than controls.

At T3, there was no intergroup difference for both symptom frequency and severity. The intragroup analysis showed that both frequency and severity of symptoms progressively diminished ($p<0.001$ for all times) in each group. The nutraceuticals were well-tolerated in all subjects.

DISCUSSION

Acute rhinosinusitis is a widespread disease in clinical practice. Acute rhinosinusitis is usually associated with a respiratory infection, mainly caused by viral or bacterial pathogens (22). Acute rhinosinusitis usually follows an acute upper respiratory infection, such as the common cold. Predisposing factors include anatomic defects, allergy, tobacco smoking, and immune suppression. The ARS diagnosis is based on clinical features and confirmed by endoscopic outcomes (23). When ARS is suspected, clinicians empirically prescribe antibiotic treatment. However, antibiotics may frequently cause dysbiosis, both in intestinal and respiratory levels. Consequently, probiotics have become a common preventive practice in antibiotic-treated patients (24).

Among the available probiotics, Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion living cells), *Lactobacillus lactis, subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii subspecies delbrueckii* LDD01 (200 million living cells). Some clinical studies showed that Abincol® improved symptom severity in patients with intestinal

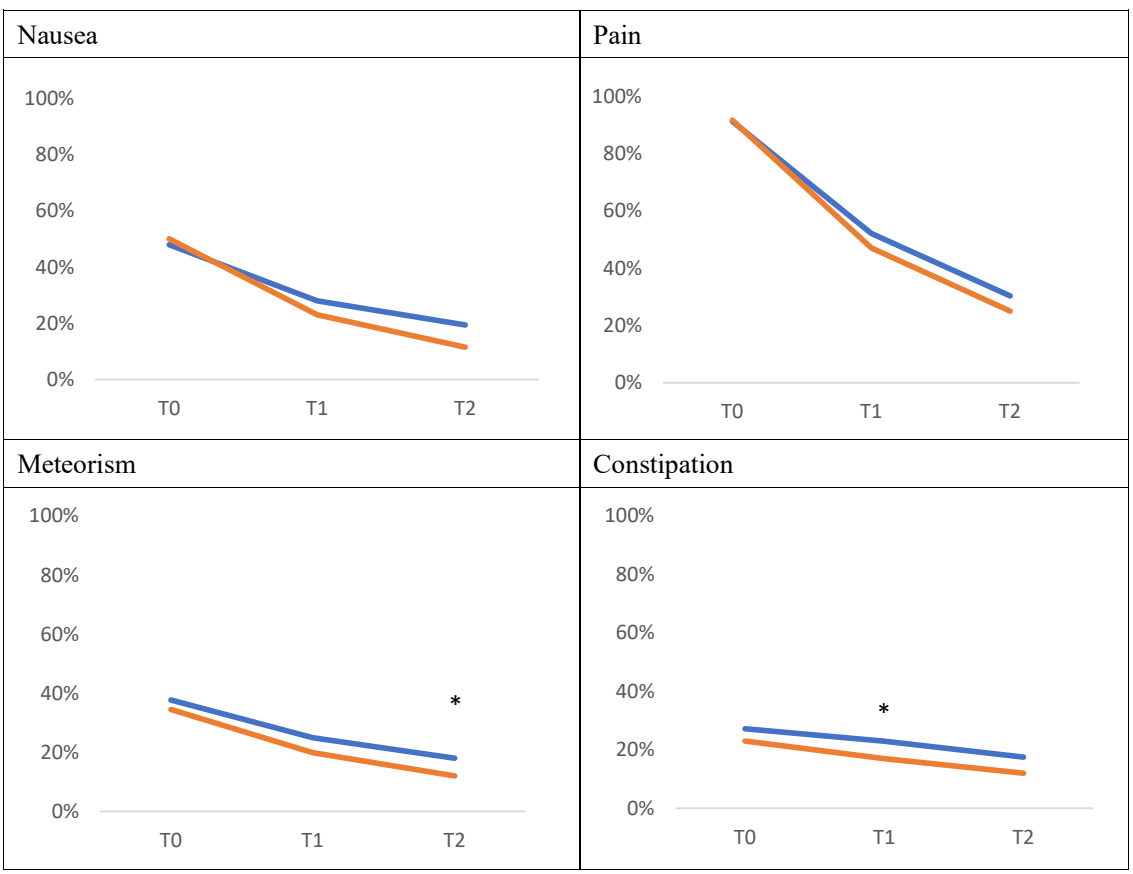

Fig. 2. *Percentages of patients with symptoms at T2 in the two groups*

problems (25-28). Moreover, Abincol® has been fruitfully used in patients with respiratory infections (8-12). A new food supplement Abincol Immuno®, containing a probiotic mixture, vitamin D, zinc, and the prebiotic inulin, has been launched. The rationale is the synergistic potential of these components to ensure a more robust immune manipulation. Therefore, the current experience tested the combination of two courses, including Abincol® and Abincol Immuno®, in half of the patients, for demonstrating an improved immune manipulation in patients treated with antibiotics for ARS.

This association allowed to achieve relevant outcomes as reported by the current experience. Indeed, the combined courses of Abincol® and Abincol Immuno® significantly reduced the persistence of some symptoms. In particular, malaise is a constitutional symptom associated with respiratory infections. Nausea is the expression of gastrointestinal involvement in ARS and potentially consequent to antibiotic treatment. They represent the active involvement of the immune response and the associated inflammation that involve the whole body. Therefore, the significant frequency reduction means that the multi-component product can manipulate the immune system dampening the hyperactivation consequent to the infectious stimulation. In addition, the reduction of patients with cough depended on the direct activity on respiratory microbiota. However, it has to be noted that also the frequency of the other symptoms diminished in patients who took Abincol Immuno®.

The present survey cannot be considered a formal investigative study. Consequently, further studies should be conducted with a rigorous methodology, designed according to randomized-controlled criteria. However, the strength of this survey is the considerable number of enrolled patients and the real-world setting. The outcomes could, therefore, mirror the facts observable in clinical practice. Moreover, it has to be noted that there was an interesting diminishing trend for all considered symptoms in patients treated with both food supplements. It suggests that active and sustained immune system manipulation hastens the recovery from upper respiratory infections.

In conclusion, the present clinical experience demonstrated that immune manipulation with this multi-component product might be considered an effective and safe therapeutic option in managing patients with acute rhinosinusitis and treated with antibiotics.

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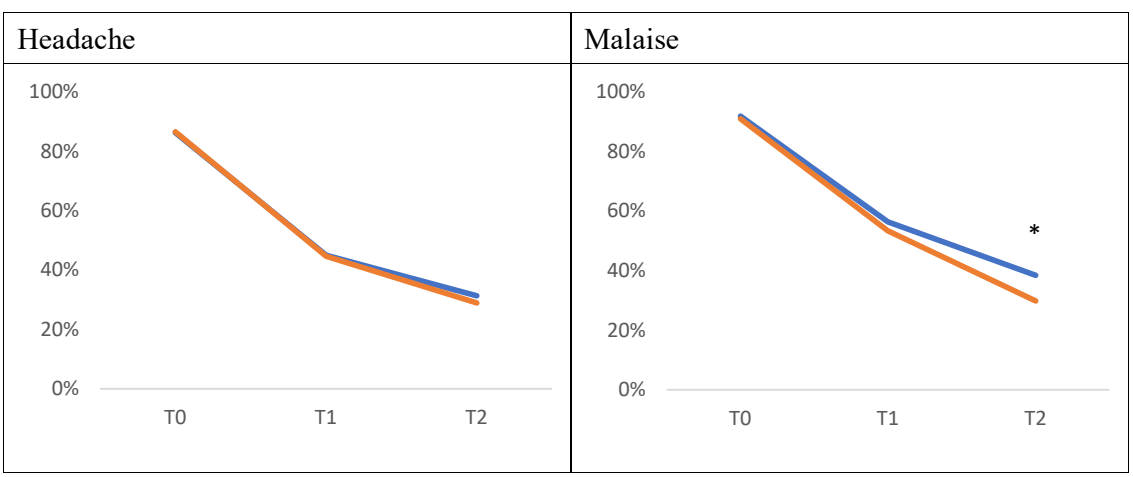

Fig. 3. *Percentages of patients with symptoms at T2 in the two groups*

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CLINICAL TRIAL

Probiotics, zinc, inulin, and vitamin D as an ancillary treatment for patients with acute pharyngotonsillitis

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Acute pharyngotonsillitis is a common disease, mainly characterized by a sore throat. The diagnosis is usually performed on the clinical ground, and antibiotic therapy is frequently used in clinical practice on an empirical basis. Antibiotics frequently induce intestinal and respiratory dysbiosis associated with slow recovery. Therefore, probiotics are commonly prescribed in patients treated with antibiotics to restore the physiological microbiota. The current clinical experience was conducted in patients with acute pharyngotonsillitis and treated with antibiotics. All patients were treated with antibiotics and Abincol@ for two weeks, then a subgroup (ratio 1:1) took a one-month course of Abincol Immuno® (Group A), the other patients served as control (Group B). Patients were evaluated at baseline (T0), at the end of antibiotic

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treatment (T1), at the end of the Abincol Immuno® course (T2), and at the end of 4-month follow-up (T3). Globally, 922 outpatients (mean age 46 years) were enrolled: 452 (49%) in Group A and 470 (51%) in Group B. At T2, Group A patients had a lower frequency of all symptoms. Both nutraceuticals were well tolerated. In conclusion, the present clinical experience demonstrated that immune manipulation with this innovative multi-component product might be considered an effective and safe therapeutic option in managing patients with acute pharyngotonsillitis and treated with antibiotics.

Keywords: acute pharyngotonsillitis, antibiotic therapy, dysbiosis, probiotics, zinc, inulin, vitamin D

Pharyngitis is clinically typified by sore throat sustained by the inflammation of the posterior oropharynx. Bacterial infection is a common cause of acute pharyngitis and requires adequate different treatment (1). Namely, identifying the pathogen should be clinically relevant to treat the infection properly (2). The palatine tonsils are an anatomic structure positioned in the pharynx and are constituted of lymphatic tissue. The palatine tonsils are a component of the Waldeyer's ring and the adenoids (nasopharyngeal tonsil), tubal, and lingual tonsil. The Waldeyer's ring represents the first defence against inhaled and/or ingested pathogens and provides the first immunological response. Acute tonsillitis usually results from a viral or bacterial infection and, when uncomplicated, presents a sore throat and fever (3). However, differentiation between bacterial and viral infection can be difficult in clinical practice. Consequently, antibiotics are frequently prescribed for tonsillitis in a primary care setting.

As pharyngitis is often associated with tonsillitis, the term pharyngotonsillitis is used to define this respiratory infection (4, 5). Acute pharyngotonsillitis frequently accounts for bacterial pathogens, including the group A beta-hemolytic *Streptococcus* (6). Therefore, patients with acute pharyngotonsillitis frequently visit medical offices, emergency departments, and urgent care centres (7). Acute pharyngotonsillitis may be associated with complications, so antibiotics are widely prescribed to prevent them (8). However, antibiotic treatment provokes a microbiota perturbation, such as dysbiosis. Therefore, probiotics are usually prescribed along with antibiotics in clinical practice (9). In this regard, Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 million living cells). It was placed on the market some year ago. This product has been previously tested in patients with gastroenterological disorders (10-12).

More recently, a new multi-component food supplement (Abincol Immuno®), containing a probiotic mixture (*Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus*), zinc, inulin, and vitamin D has been launched.

Vitamin D (VD) is an essential hormone for humans as it exerts pleiotropic effects, including anti-inflammatory activity and immune-stimulating effects (13,14). Zinc is necessary for mounting an efficient and balanced immune response, mainly against pathogens, and promotes the maturation of immunocompetent cells (15). Inulin is a fructan, such as a fructose polymer; it is a prebiotic that serves as a nutrient for probiotics (16,17). *Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus* are well-known probiotics widely used as a food supplement (18-21).

Therefore, the present clinical experience evaluated the potential role of the add-on treatment with this new product in patients with acute pharyngotonsillitis and under antibiotic treatment.

MATERIALS AND METHODS

The recent experience was conducted in Italian otorhinolaryngology centres, distributed across Italy, assuring extensive and complete national coverage during fall-winter 2020-2021. These specialists recruited all consecutive outpatients visited for acute pharyngotonsillitis. The inclusion criteria were: diagnosis of acute pharyngotonsillitis, both genders, adulthood, and

antibiotic treatment. In addition, exclusion criteria were to have comorbidities and concomitant medications able to interfere with the evaluation of the outcomes. All patients signed informed consent. The procedures were conducted in a real-world setting, such as an ORL clinic.

The patients were subdivided into two groups: Group A was treated with an oral antibiotic for 7-10 days associated with a 2-week course of Abincol®, then patients took a four-month course of Abincol Immuno®, and Group B was treated with antibiotics and a 2-week course of Abincol® alone. Voluntarily, some patients could take further courses of Abincol Immuno®. Each stick of Abincol Immuno® contains inulin 372.4 mg, zinc 10 mg (corresponding to 100% of the daily reference nutritional value), vitamin D 5 mg (corresponding to 100% of the daily reference nutritional value), *Lactobacillus plantarum* LP01 ($1x10^9$ CFU), *Lactobacillus rhamnosus* CRL 1505 ($1x10^9$ CFU), and *Lactobacillus delbrueckii subsp. Bulgaricus* LB2 ($2x10^8$ CFU). The food supplements were taken following the specific indications, such as one stick/daily.

Patients were visited at baseline (T0), after 14 days (T1), such as at the end of the Abincol® course, after six weeks (T2), such as at the end of the Abincol Immuno® course in Group A, and after a 4-month follow-up (T3). Clinical examination was performed in all patients at each visit. The evaluation parameters were the presence and severity of some symptoms, including fever, fatigue, malaise, headache, pain, cough, nausea, meteorism, diarrhoea, and constipation. Patients used a visual analog scale (VAS) to assess the perception of the symptom severity. The VAS scale ranged from 0 (no symptom) to 10 (very intense symptom). Safety was measured by reporting the occurrence of adverse events.

All clinical data were inserted in an internet platform that guaranteed the patients' anonymity and the findings' recording accuracy. Statistical analysis of the data was conducted to evaluate the intragroup and intergroup comparisons. Intragroup analysis was performed using the paired Wilcoxon test to compare severities and the McNemar test to compare proportions. Intergroup analysis was performed using the Wilcoxon test to compare severities and the Chi-square test to compare frequencies. The statistical software was STATA 15.1, College Station, Texas 77845 USA.

RESULTS

Globally, 922 outpatients (mean age 46 years) were enrolled: 452 (49%) in Group A and 470 (51%) in Group B. The baseline data were superimposable in the two groups, so the groups were well-matched. At T1, there was no significant difference between groups concerning the frequency and severity of symptoms.

At T2, Group A patients had a lower frequency of all symptoms. However, there was no significant difference in the symptom severity between

Table I. *Frequencies of symptoms referred by patients at T2*

	ABINCOL	ABINCOL+ ABINCOL IMMUNO
fever	15.9%	14.7%
cough	32.5%	29.7%
diarrhoea	17.6%	16.7%
fatigue	35.4%	30.0%
nausea	17.9%	17.0%
pain	33.1%	29.1%
meteorism	19.9%	16.4%
constipation	14.9%	13.9%
headache	31.5%	24.8%
malaise	39.1%	36.5%

groups, even if Group A patients experienced fewer symptoms than controls (Table I, Fig. 1-2-3).

At T3, there was no intergroup difference for both symptom frequency and severity in the groups. The intragroup analysis showed that both frequency and severity of symptoms progressively diminished ($p<0.001$ for all times). The nutraceutical was well-tolerated in all subjects.

DISCUSSION

The sore throat is the pivotal symptom referred by patients with acute pharyngotonsillitis. However, many other symptoms may be present in these patients. In addition, some symptoms are constitutional, such as malaise, fatigue, fever, and headache, or depend on gastrointestinal impairment due to the antibiotic treatment, including nausea, diarrhea, constipation, meteorism. Furthermore, antibiotic treatment may frequently cause dysbiosis, both at intestinal and respiratory levels. Consequently, the use of probiotics to restore physiological microbiota has become a widespread practice.

In this context, Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii subspecies delbrueckii* LDD01 (200 million of living cells). In addition, some clinical studies showed that Abincol® was effective as an add-on treatment in patients with upper respiratory infections, including rhinosinusitis, pharyngotonsillitis, and otitis media and laryngotracheitis (22-26).

A new food supplement Abincol Immuno®, containing a probiotic mixture, vitamin D, zinc, and the prebiotic inulin, has been launched. The rationale is the synergistic potential of these components to ensure a more robust immune manipulation. Therefore, the current experience tested the combination of two courses, including Abincol® and Abincol Immuno®, in half of the enrolled patients to demonstrate improved

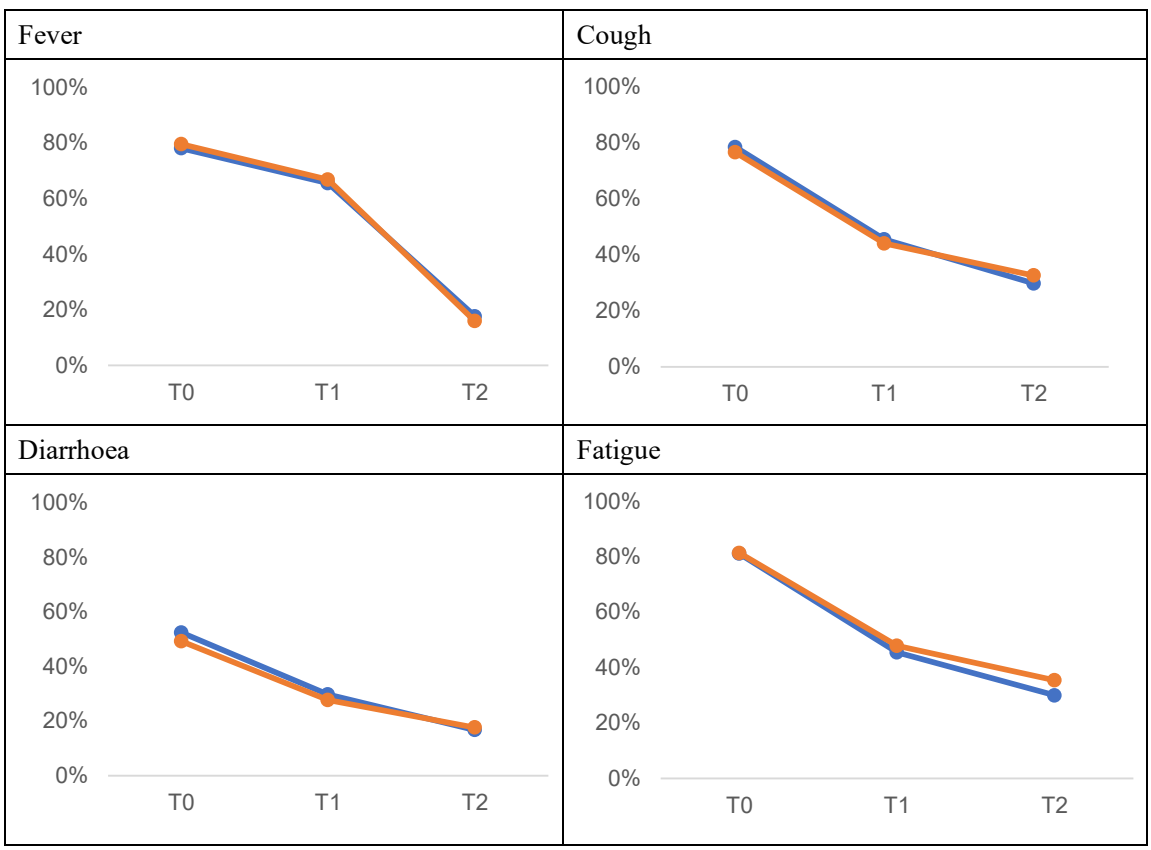

Fig. 1. *Percentages of patients with symptoms at T2 in the two groups*

immune manipulation in patients treated with antibiotics for acute pharyngotonsillitis.

The current experience of a large group of patients with acute pharyngotonsillitis showed that this association achieved relevant outcomes. Indeed, the combined courses of Abincol® and Abincol Immuno® reduced the persistence of all symptoms. Namely, it has to be noted that the frequency of the symptoms diminished in patients who took Abincol Immuno®. Therefore, immune manipulation might represent a relevant strategy for managing acute pharyngotonsillitis patients (27,28).

The present survey cannot be considered a formal investigative study. Consequently, further studies should be conducted with a rigorous methodology, designed according to randomized-controlled criteria. However, the strength of this survey is the considerable number of enrolled patients and the real-world setting. The outcomes could, therefore, mirror the facts observable in clinical practice.

In conclusion, the present clinical experience demonstrated that immune manipulation with this innovative multi-component product might be considered an effective and safe therapeutic option in managing patients with acute pharyngotonsillitis and treated with antibiotics.

Conflict of interest:

All authors state that there is no conflict of interest.

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Probiotics, zinc, inulin, and vitamin D as an ancillary treatment for patients with acute otitis

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Acute otitis media (AOM) affects the middle ear and is clinically characterized by earache as the main symptom. Otitis media may be clinically suspected, but the otoscopy usually confirms the diagnosis. Antibiotic therapy is used in clinical practice, primarily in patients with severe symptoms. However, antibiotics often induce intestinal and respiratory dysbiosis associated with some clinical problems. Dysbiosis impairs the immune system and slows down the resolution of inflammatory events consequent to the infection. The current clinical experience was conducted in patients with AOM and taking antibiotics. All patients were treated with antibiotics and Abincol® for two weeks, then a subgroup (ratio 1:1) took a one-month course of Abincol Immuno® (Group A), the remaining patients served as control (Group B). Patients were evaluated at baseline (T0), at the end of antibiotic treatment (T1), at the end of the Abincol

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Immuno® course (T2), and at the end of 4-month follow-up (T3). Globally, 821 outpatients (mean age 46 years) were enrolled: 389 (47.4%) in Group A and 432 (52.6%) in Group B. At T2, Group A patients had a lower frequency of fatigue (p=0.02) and pain (p=0.01). However, there was no difference in the symptom severity between groups, even if Group A patients experienced more minor symptoms than controls. In conclusion, the present clinical experience demonstrated that immune manipulation with this new multi-component product might be considered an effective and safe therapeutic option in managing patients with acute otitis media and treated with antibiotics.

Keywords: otitis media, acute, antibiotic therapy, dysbiosis, probiotics, zinc, inulin, vitamin D

Otitis media (OM) is an inflammation of the middle ear (1). Acute otitis media (AOM) usually lasts a few weeks. Acute OM is frequently associated with effusion and follows an acute upper respiratory infection, typically the common cold (2-4). Even if AOM is one of the most common diseases in young children (5, 6), AOM can also affect adults, mostly the youngest. Acute OM has a relevant burden for society, because of frequent medical consultation, large antibiotic prescriptions, and sometimes surgery, mainly in young people (7-10). It also negatively impacts the patient's quality of life and has a pharmacoeconomic burden.

Bacteria may infect the middle ear primitively or successively to a viral infection. Acute OM can be a severe condition that should be promptly treated. Therefore, antibiotics are commonly used in daily practice to treat AOM when a bacterial cause is suspected (11). Unfortunately, antibiotic treatment is frequently associated with microbiota perturbation, such as dysbiosis involving the digestive tract and airways, including the Eustachio tube. Therefore, probiotics are usually prescribed along with antibiotics in clinical practice to restore the physiological balance of microbiota.

Probiotics could improve intestinal and respiratory microbiota equilibrium, reduce respiratory symptoms, and promote general wellbeing (12). In this regard, Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 million living cells). It has been recently placed on the market. This product has been previously tested in patients with gastroenterological disorders (13-15). More recently, an innovative multi-component food supplement (Abincol Immuno®), containing a probiotic mixture (*Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus*), zinc, inulin, and vitamin D has been launched.

Vitamin D (VD) is an essential hormone for humans as it exerts pleiotropic effects, including anti-inflammatory activity and immune-stimulating effects (16, 17). Zinc is necessary for mounting an efficient and balanced immune response, mainly against pathogens, and promotes the maturation of immunocompetent cells (18). Inulin is a fructan, such as a fructose polymer, a prebiotic that serves as a nutrient for probiotics (19, 20). *Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus delbrueckii subsp. Bulgaricus* are well-known probiotics widely used as a food supplement (21-24).

Therefore, the present clinical experience evaluated the potential role of the add-on treatment with this new product in patients with acute otitis media and under antibiotic treatment.

MATERIALS AND METHODS

The recent experience was conducted in Italian otorhinolaryngology centres across Italy, assuring extensive and complete national coverage during fall-winter 2020-2021. In addition, these specialists recruited all consecutive outpatients visited for acute otitis media.

The inclusion criteria were: diagnosis of acute otitis, both genders, adulthood, and antibiotic treatment. In addition, exclusion criteria were to have comorbidities and concomitant medications able to interfere with the evaluation of the outcomes. All patients signed informed consent. The procedures were conducted in a real-world setting, such as an ORL clinic.

The patients were subdivided into two groups: Group A was treated with an oral antibiotic for 7-10 days associated with a 2-week course of Abincol®, then patients took a four-month course of Abincol Immuno®, and Group B was treated with antibiotics and a 2-week course of Abincol® alone. In addition, some patients could voluntarily take further courses of Abincol Immuno®.

Each stick of Abincol Immuno® contains inulin 372.4 mg, zinc 10 mg (corresponding to 100% of the daily reference nutritional value), vitamin D 5 mg (corresponding to 100% of the daily reference nutritional value), *Lactobacillus plantarum* LP01 ($1x10^9$ CFU), *Lactobacillus rhamnosus* CRL 1505 ($1x10^9$ CFU), and *Lactobacillus delbrueckii subsp. Bulgaricus* LB2 ($2x10^8$ CFU). The food supplements were taken following the specific indications, such as one stick/daily.

Patients were visited at baseline (T0), after 14 days (T1), such as at the end of the Abincol® course, after six weeks (T2), such as at the end of the Abincol Immuno® course in Group A, and after a 4-month follow-up (T3). Clinical examination was performed in all patients at each visit. The evaluation parameters were the presence and severity of some symptoms, including fever, fatigue, malaise, headache, pain, cough, nausea, meteorism, diarrhoea, and constipation. Patients used a visual analogue scale (VAS) to assess the perception of the symptom severity. The VAS scale ranged from 0 (no symptom) to 10 (very intense symptom). Safety was measured by reporting the occurrence of adverse events.

All clinical data were inserted in an internet platform that guaranteed the patients' anonymity and the findings' recording accuracy. Statistical analysis of the data was conducted to evaluate the intragroup and intergroup comparisons. Intragroup analysis was performed using the paired Wilcoxon test to compare severities and the McNemar test to compare proportions. Intergroup analysis was performed using the Wilcoxon test to compare severities and the Chi-square test to compare frequencies. The statistical software was STATA 15.1, College Station, Texas 77845 USA.

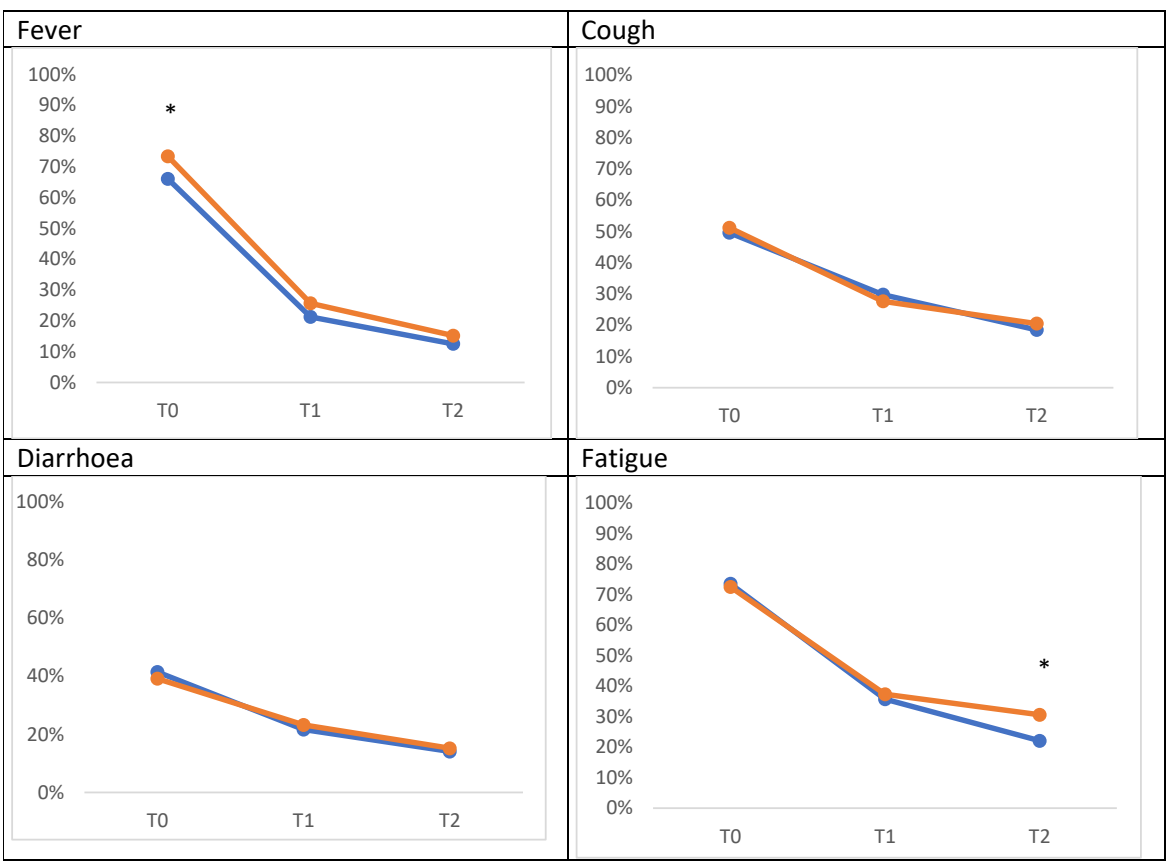

Fig. 1. *Percentages of patients with symptoms (fever, cough, diarrhea and fatigue) at T2 in the two groups*

RESULTS

Globally, 821 outpatients (mean age 46 years) were enrolled: 389 (47.4%) in Group A and 432 (52.6%) in Group B. The baseline data were superimposable in the two groups, so the groups were well-matched. At T1, there was no significant difference between groups concerning the frequency and severity of symptoms.

At T2, Group A patients had a lower frequency of fatigue (p=0.02) and pain (p=0.01), as reported in Fig. 1, 2, and 3. However, there was no difference in the symptom severity between groups, even if Group A patients experienced fewer symptoms than controls. At T3, there was no intergroup difference for both symptom frequency and severity in both groups.

The intragroup analysis showed that both frequency and severity of symptoms progressively diminished (p<0.001 for all times). In addition, the nutraceutical was well-tolerated in all subjects.

DISCUSSION

Albeit acute otitis media is a typical pediatric infection, it also affects adults. Acute OM usually follows an acute respiratory infection, mainly caused by viral or bacterial pathogens (25). As a result, AOM places a significant burden on health care systems worldwide. Although symptomatic relief could be enough for some patients, other cases require antibiotics, especially if suspected complications or symptoms are severe (26). However, antibiotics cause dysbiosis, both at the intestinal and respiratory levels.

Consequently, probiotics have become a common practice to contrast dysbiosis. In this context, Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii subspecies*

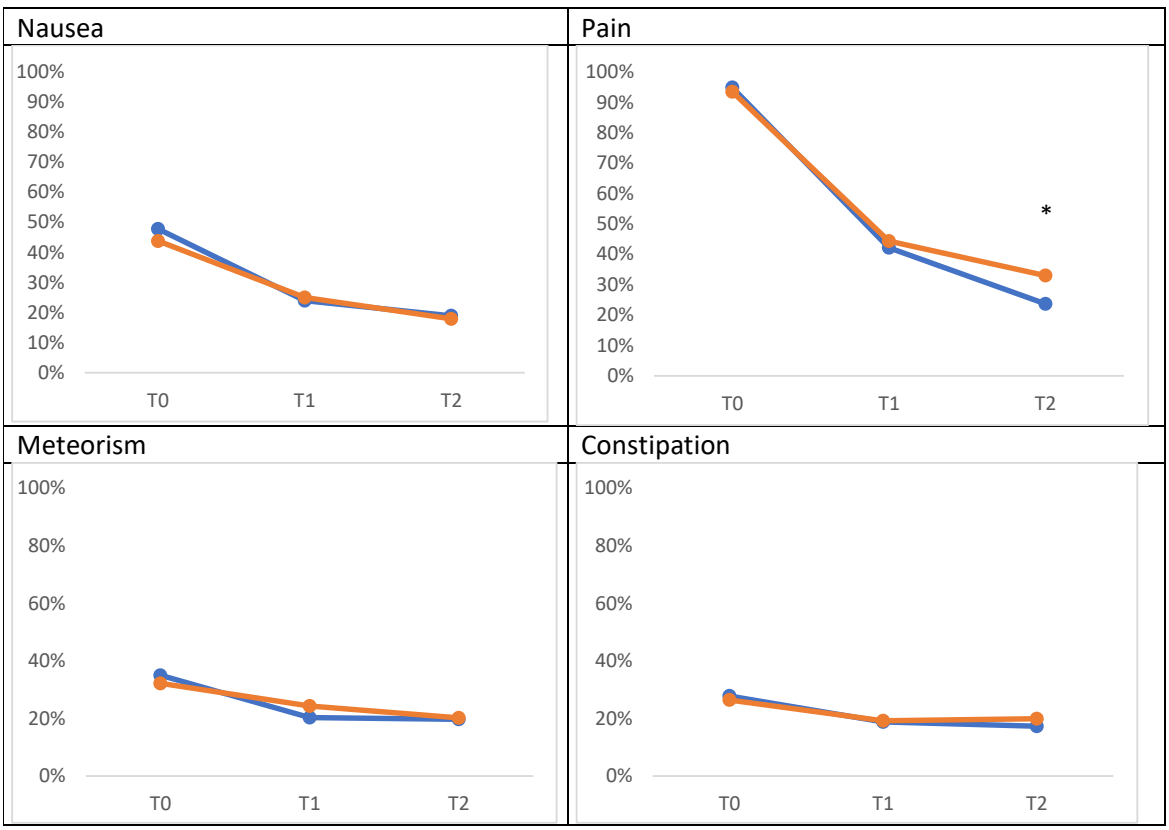

Fig. 2. *Percentages of patients with symptoms (nausea, pain, meteorism and constipation) at T2 in the two groups*

delbrueckii LDD01 (200 million of living cells). In addition, some clinical studies showed that Abincol® was effective as an add-on treatment in patients with upper respiratory infections, including rhinosinusitis, pharyngotonsillitis, otitis media, and laryngotracheitis (27-31).

A new food supplement Abincol Immuno®, containing a probiotic mixture, vitamin D, zinc, and the prebiotic inulin, has been launched. The rationale is the synergistic potential of these components to ensure a more robust immune manipulation. Therefore, the current experience tested the combination of two courses, including Abincol® and Abincol Immuno®, in half of the enrolled patients to demonstrate improved immune manipulation in patients treated with antibiotics for acute otitis media.

The current experience, conducted on a large group of patients with acute OM, showed that this association achieved relevant outcomes. Indeed, the combined courses of Abincol® and Abincol Immuno® significantly reduced the persistence of some symptoms. In particular, fatigue is a constitutional symptom frequently associated with respiratory infections and consequent to antibiotic treatment. Therefore, the significant reduction of frequency means that the multi-component product can manipulate the immune system dampening the hyperactivation consequent to the infectious stimulation and the detrimental effects of antibiotics. In addition, the reduction of patients with pain depended on the direct activity on local microbiota. However, it has to be noted that also the frequency of the other symptoms diminished in patients who took Abincol Immuno®.

The present survey cannot be considered a formal investigative study. Consequently, further studies should be conducted with a rigorous methodology, designed according to randomized-controlled criteria. However, the strength of this survey is the considerable number of enrolled patients and the real-world setting. The outcomes could, therefore, mirror the facts observable in clinical practice.

In conclusion, the present clinical experience demonstrated that immune manipulation with this new multi-component product might be considered an effective and safe therapeutic option in managing patients with acute otitis media and treated with antibiotics.

Conflict of interest:

All the authors declared no conflict of interest.

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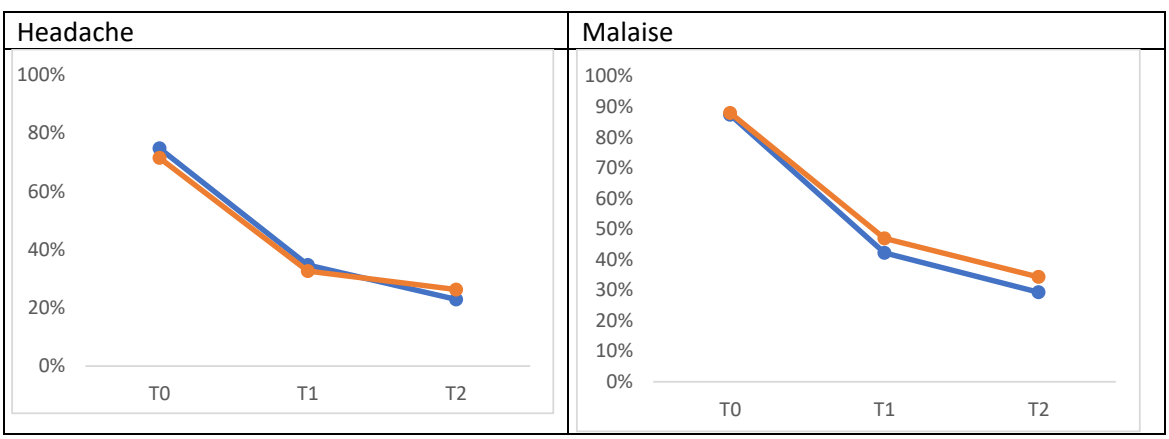

Fig. 3. *Percentages of patients with symptoms (headache and malaise) at T2 in the two groups*

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The gastric reflux: a challenge in clinical practice

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Gastric reflux, such as the spill of gastric contents outside the stomach, may become symptomatic when the refluxate damages the esophageal mucosa, such as the gastroesophageal reflux disease (GERD). The worldwide prevalence of GERD is continuously increasing in the last few years. The guidelines for diagnosing and treating GERD are well consolidated and timely updated. In addition, gastric content, even only gaseous, may spill out the esophagus, damaging other organs, mainly airways. As a result, the extra-esophageal manifestations of reflux have been considered from a multidisciplinary point of view; the symptoms of laryngopharyngeal reflux (LPR) are precisely defined as autonomous disorders. The otolaryngologists have codified guidelines for the LPR diagnosis, but the treatment is still poorly specified. Consequently, many healthcare professionals, including general practitioners, pediatricians, gastroenterologists, otolaryngologists, other medical specialists, and pharmacists, manage GERD and LPR patients. Many therapeutic options are available. The most common medications include proton pump inhibitors, alginates, and antacids.

Gastric reflux is a physiological phenomenon that occurs during and after meals. On the contrary, gastroesophageal reflux disease (GERD) is defined as a "disease that develops when the reflux of stomach contents induces troublesome symptoms and/or complications" (1). This umbrella definition was devised to encompass the broad spectrum of GERD manifestations, including endoscopically evident diseases (esophagitis, stricture, Barrett's metaplasia, and adenocarcinoma), troublesome esophageal symptoms (heartburn, regurgitation, and chest pain), without endoscopically evident disease, and potential extra-esophageal manifestations, including asthma, cough, and laryngopharyngeal reflux (LPR). The GERD definition, widely referred to as the Montreal definition, is adequate in identifying the potential manifestations of GERD by the common element stemming from the reflux of gastric content into the esophagus. However, it also implies some clinically relevant practical problems. In particular, the appropriate diagnostic work-up, the use of proton pump inhibitors (PPIs), and the approach to alternative therapeutic strategies need adequate consideration.

GERD is classified into "erosive GERD" with esophageal mucosal break and "non-erosive GERD" (NERD) with symptoms alone. In addition, GER is classified into "acidic-GER" and "non-acidic (weakly acidic, alkaline) GER" (1).

PRACTICAL MANAGEMENT

From a diagnostic point of view, if GERD is suspected,

Keywords: gastric reflux, gastroesophageal reflux disease, laryngopharyngeal reflux, proton pump inhibitor, alginate, antacids

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the patient should be clinically evaluated by specific questionnaires and the PPI test. The typical symptoms of GERD are heartburn and regurgitation, but patients may not necessarily accurately perceive their symptoms (2). Thus, in addition to asking for typical symptoms of GERD, a careful interview concerning other expressions may be helpful. GER may cause typical symptoms and extraesophageal symptoms, including chronic cough, bronchial asthma, the discomfort of the pharynx and larynx, sore throat, and non-cardiac chest pain (2). Since extraesophageal symptoms may be the only symptoms of GER, it is essential to take them in the differential diagnosis. In particular, laryngopharyngeal reflux is a disorder involving upper airways due to the contact of the gastric refluxate with these tissues (3).

In clinical practice, if harmful gastric reflux is highly suspected, two types of algorithms are proposed: i) the administration of standard-dose PPI, which is the first-line treatment option of GERD, is started first without endoscopy (therapy at clinics without endoscopy equipment), such as an empiric trial based on the *ex juvantibus* criteria; and ii) upper digestive endoscopy is performed first before PPI therapy (4,5). If a symptomatic resolution was obtained with the PPI test before endoscopy, they are considered transient symptoms, and treatment should be discontinued. If the symptoms persist or relapse, endoscopy needs to be performed. However, if the PPI test is started before endoscopy, erosive GERD and non-erosive-GERD cannot be differentiated by the endoscopy, even if done afterwards. Other diagnostic tests include pH-manometry, imaging, pepsin assay, and pH measurement with 24 h monitoring.

The PPI test may be inconclusive in a prominent number of patients and may not be reasonable, mainly concerning the reimbursement; an alternative diagnostic strategy could be based on administering the patients of validated questionnaires. The most commonly used are reflux symptoms index (RSI) and gastroesophageal reflux disease impact scale (GIS); both are quick, self-completed, validated, and reliable (3,4).

In addition, it has to be underlined that noxious gastric reflux may affect any age. However, consistently, gastric reflux complaints are the most common medical problem in infancy. Moreover, LPR is undoubtedly much more common than imagined. So, particular attention should be paid to "irritative" symptoms reported by patients, including sore throat, dysphagia, dysphonia, and raclage.

Therapeutic options

From a therapeutic point of view, many options have been proposed; they include neutralization of gastric acid content using PPI, antihistamines (H2-receptor antagonists), antacid chemical compounds, and antireflux barrier (using alginates), prokinetics, inhibitors of gastric sphincters, protection of mucosal tissue, neuromodulators, nociceptor antagonists, lifestyle modification, dietary regimens, and (in very selected cases) surgery (6). It is to note that many medications are over the counter products (OTC), and self-medication is the overall approach by the patients themselves. In addition, the pharmacist usually advises an OTC for gastric reflux symptoms.

Medical prescriptions usually include PPI, alginates, and antacids. Indeed, PPI class is very effective and has changed the therapeutic strategy. In particular, esomeprazole could be the ideal PPI reference as it is effective and safe in most patients.

However, an increasing new way of thinking is reluctant on an enthusiastic use of PPI, mainly concerning potential side effects (5-7). Therefore, new interest has been directed to alginates, as they offer different positive mechanisms of action and are very safe (8-10). As a result, alginates may represent a fruitful therapeutic option in treating both GERD and LPR, also as monotherapy as suggested by the NICE guidelines (11, 12).

In this regard, the medical device Marial[@], manufactured by Aurora, Milan, Italy, is a multi-component product containing magnesium alginate and E-Gastryal[®]. E-Gastryal[®] contains hyaluronic acid (HA), hydrolyzed keratin, tara gum, and xanthan gum. The rationale of this combination concerns the knowledge of the inflammatory events caused by physical and chemical stimuli, the symptom polymorphism of the patients with reflux, and the pathogenic role of some molecules involved in the healing process.

The endogenous HA promotes the hydration of the damaged tissues (by hygroscopic capacity) and, at the same time, determines the modulation of the reconstitution of the extra-cellular matrix (ECM) by plastic capacity (13). Namely, molecules with high hygroscopic activity, such as HA and mucopolysaccharides, create intense water replacement and eliminate toxic substances from the matrix, rebooting the gel phase of ECM. Consequently, recovery of tissue alkalinity occurs, and the reparative process begins induced by fibroblasts.

Magnesium alginate combined with E-Gastryal® has been proven to effectively relieve the discomfort caused by gastric reflux, preventing and alleviating the injury to mucous membranes. In addition, this compound can actively regenerate the damaged tissue by repairing and regenerating mucous membranes and by mucoadhesive and film-forming characteristics that prolong the contact time with the mucous membranes and consequently improve the effectiveness (14).

DISCUSSION

In these last years, considerable experience has accumulated about its effectiveness and safety in managing patients with GERD and LPR by otolaryngologists and gastroenterologists (15-21). However, there was an interest to intercept the actual management of GERD and LPR patients in different settings. Therefore, an extensive survey included pharmacists, pediatricians, and primary care doctors to investigate the observed behaviour in managing patients with gastric reflux. This initiative also provided an educational phase. So, the effect of updating on prescriptive attitude was also investigated.

The outcomes underlined the pivotal role exerted by PPI and alginates as the primary therapeutic option in managing gastric reflux problems. In particular, updating the knowledge about pathophysiology, diagnosis, and treatment of gastric reflux disorders significantly affected the prescriptive attitude. Thus, there is a shared opinion that the best options are using PPI as an add-on to alginates or alginates alone for patients with GERD or LPR. The following papers will report in detail the findings of this educational program.

Therefore, the practical management of patients with GERD and LPR relies on combined consolidated compounds, such as alginates and PPI. In particular, the best choice may include a multi-component device containing alginate and cytoprotective molecules integrated with a proton pump inhibitor.

As there is scarce evidence about the attitude to manage GERD and LPR by primary care doctors and pediatricians and the advice provided in pharmacy, it is vital to promote updating initiatives aiming at learning the practical management of GERD and LPR. These projects can provide relevant outcomes, fruitful in daily practice. Indeed, active participation significantly changed the prescriptive attitude.

Conflict of interest:

All authors state that there is no conflict of interest.

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Gastric reflux management in pharmacy

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Gastroesophageal reflux disease (GERD) is a widespread disease, as about a quarter of the Western population has GERD symptoms at least weekly, and GERD is the most frequent reason for outpatient gastroenterology consultation. Laryngopharyngeal reflux (LPR) is the extra-oesophageal counterpart of GERD, as the upper airways are the target of the extraesophageal spill out of gastric contents. GERD and LPR treatment are commonly based on proton pump inhibitor (PPI) use, but PPI may be ineffective in some patients and potentially unsafe if administered for a very long time. As a result, other therapeutic options include alginates and antacids. Most of the compounds used for managing gastric reflux are available as over the counter products or medical devices. Thus, pharmacist plays an active role in advising these products. The current experience resulted from the involvement of a group of Italian pharmacists who participated in an educational project on managing gastric reflux problems. This experience, involving 372 Italian pharmacies, provided some interesting findings. First, 63% of subjects visiting a pharmacy required treatment for GERD, and 38% had LPR.

Moreover, more than 40% of subjects did not follow a correct diet or adequate gastric reflux lifestyle. In this regard, the pharmacist advised a diet and lifestyle change in 43% of subjects as a first-line option. PPI was the most common medication, both as monotherapy or polytherapy, about past or concomitant treatment for gastric reflux symptoms. Alginates were the second choice as both alone or add-on. Very notably, the updating of the knowledge about gastric reflux significantly affected the attitude of pharmacists to advise OTC products. As a result, there was a reduction of PPI suggestions and increased alginates. In particular, the combined strategy, such as the PPI add-on to alginates, was the most common option.

Gastroesophageal reflux disease (GERD) is a gastrointestinal motility disorder depending on the reflux of stomach contents into the esophagus and oropharynx (1). The reflux of stomach contents, causing troublesome symptoms and/or complications, identifies GERD (2). GERD is characterized by two cardinal esophageal symptoms: heartburn and regurgitation. In addition, extra-esophageal symptoms involving upper airways define the laryngopharyngeal reflux (LPR) syndrome (3). GERD and LPR are also commonly associated with lower quality of life, poor sleeping, and decreased work productivity. Complications of GERD include reflux esophagitis (most common), esophageal stricture, Barrett's esophagus, and esophageal adenocarcinoma. Complications of LPR may include chronic inflammation, recurrent infections, and cancer (4).

Safe and effective treatments for GERD and LPR include correct diet, lifestyle adjustment, medications, and, in some cases, surgery. In addition, many

Keywords: gastric reflux, gastroesophageal reflux disease, laryngopharyngeal reflux, proton pump inhibitor, alginate, antacid, pharmacy

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medications can be accessed as over-the-counter (OTC) or medical devices for short-term management or by doctor prescription for long-term management. In this regard, the pharmacist can advise patients on appropriate OTC medications and/or medical devices and help address their adverse reactions and drug interactions.

From an epidemiological point of view, GERD is a very prevalent disease; indeed, about 25% of the Western population has GERD symptoms at least weekly (5). In addition, GERD is a frequent reason for outpatient consultation (6).

The pathophysiology of gastric reflux is complex, and many pathogenic mechanisms concur with the development of the disease. Many risk factors have been identified, including hiatus hernia, obesity, high-fat diet, tobacco smoking, alcohol consumption, Helicobacter pylori infection, pregnancy, genetics, medications, and consumption of foods able to increase the acid output (7-10).

Fletcher first described the postprandial gastric acid pocket in 2001 (11). Evidence from pH studies indicated that the pH of the region just below the lower esophageal sphincter was lower than that of the stomach, occasionally after a meal, despite the average buffering effect of food. Thus, it was identified that a postprandial gastric acid pocket containing unbuffered, extremely low pH, such as about 2 contents, accumulates at the top of the stomach contents (normal pH 4–5), 10–15 minutes after eating. It occurs when gastric juices do not mix properly with the meal so that it floats at the top of the stomach contents to form a layer that can persist for up to 2 hours. Compared to healthy controls, people with GERD tend to produce a larger acid pocket, which floats higher into the gastroesophageal junction (allowing for more reflux occurrences), has a lower pH, and lasts for longer 11,12). The acid pocket contributes more to symptoms if the patient lies down after eating and in people with hiatus hernia.

Laryngopharyngeal reflux shares the exact pathophysiological mechanisms of GERD but differs in the upper airway involvement. In addition, inflammation and immune response actively exert a relevant pathogenic mechanism causing symptom occurrence.

The pharmacist plays a relevant role in managing patients with GERD and LPR (3). The pharmacist should consider some issues, such as confirming the GERD diagnosis based on the patient's symptomatic history, ruling out any reason for referral to a doctor (alarming symptoms), and focusing on the nature, severity, and frequency of symptoms when determining treatment (13). Moreover, the pharmacist advises patients about OTC treatment and/or medical devices for intermittent/mild-moderate symptoms of gastric reflux. The main recommendations include a series of points (13). First, to take acid-suppressive therapy consistently at the same time each day, when symptoms are worse (i.e., morning or evening). Second, take PPIs 1 hour before the most important meal (this allows the short half-life drug maximum time to inhibit the active proton pump). The PPIs may take up to 3 days to reach their desired effect, with a maximum effect after five days. The PPIs may not be effective in all people with GERD symptoms. Fourth, to take over-the-counter PPIs for a maximum of 14 days. Antacids and alginates can be used to treat breakthrough symptoms. The PPIs are generally well tolerated (common (1% – 10%) adverse reactions include diarrhea, headache, nausea, abdominal pain, and constipation). Third, to advise on diet and lifestyle adjustment. Consult with a doctor if PPI therapy fails to improve symptoms, symptoms worsen or concerning signs or symptoms occur.

Based on this background, an experience has been conducted on a large group of Italian pharmacists to explore the practical attitude to manage patients with GERD and LPR in pharmacy. The pharmacists participated in an educational program on this issue that included a re-assessment of gastric reflux management after the updating.

MATERIALS AND METHODS

The current experience was conducted in 372 pharmacies, distributed in the whole of Italy, so assuring a comprehensive and complete national coverage. The pharmacists were asked to recruit all consecutive patients seen because of gastric reflux complaints.

Pharmacists collected detailed history and administered the RSI questionnaire. Medical history concerned the past

and concomitant treatment for gastric reflux. Moreover, the pharmacist advised the patients about dietary and lifestyle changes and/or pharmacological medications.

Reflux Symptom Index (RSI) is a self-administered nine-item questionnaire developed by Belafsky for assessing symptoms in patients with reflux disease (14). It is so simple that it can be completed in less than 1min. Each item's scale ranges from 0 (no problem) to 5 (severe problems), with a maximum score of 45. It has been concluded that RSI has high reproducibility and validity for the diagnosis of reflux if an RSI score >13 is defined as abnormal (14). Therefore, RSI may be considered a practical tool in the approach of patients with suspected LPR (15).

Demographic and clinical characteristics were described using means, absolute numbers, or percentages. The analysis was performed using GraphPad Prism software, GraphPad Software Inc, CA, USA.

RESULTS

Globally, 8,155 patients [4,116 (50.5%) males, 4,038 (49,5%) females, mean age 49.33 years] were interviewed. In detail, 63% went to the pharmacy to buy something for symptoms attributable to gastric reflux; 45.2% of them had a medical prescription. A positive RSI score, such as ≥ 13, was achieved by 3,207 (38%) subjects. About 43.8% of subjects did not adopt correct dietary rules; consistently, about 41.7% did not follow a correct lifestyle.

About the previous/concomitant treatments for gastric reflux, 2,934 (35.4%) subjects took medications. Fig. 1 shows the detailed data: 1,914 (67%) subjects took one product alone, whereas 942 took a polytherapy. In particular, 751 took a combination of PPI with other products, primarily alginates and antacids. Nine-hundred-and-thirty subjects chose monotherapy with PPI, alginates were preferred by 359 subjects, and antacids monotherapy by 411 subjects. Pharmacists advised 3,618 (43%) patients to change diet and lifestyle as the first step.

About the treatment advised by pharmacists, monotherapy was proposed to 4,566 (66.3%) subjects,

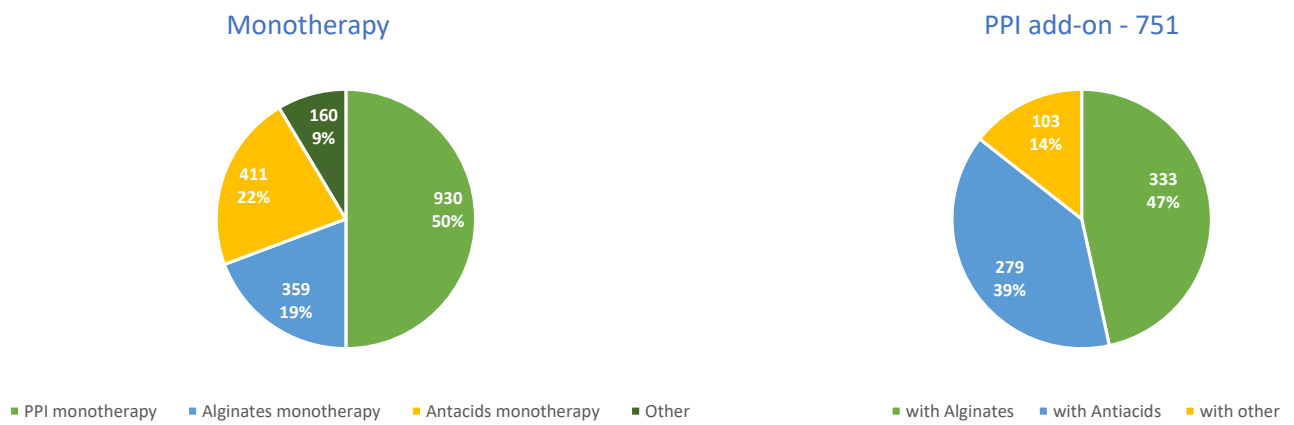

Fig. 1. *Past and current treatments for gastric reflux in pharmacy*

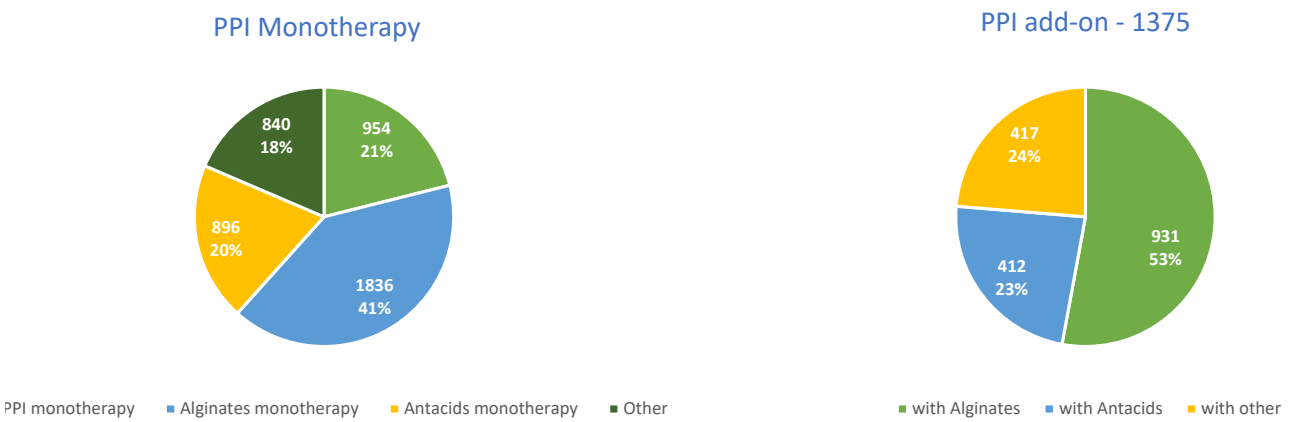

Fig. 2. *Treatments advised by pharmacists after the educational program.*

whereas 2,320 (33.7%) had polytherapy suggestions. Fig. 2 shows the details about the advised treatments by pharmacists. The most common product in monotherapy was alginate, whereas the combination PPI with alginates was the most frequent polytherapy.

Notably, there was a shift concerning the PPI: the rate of PPI add-on in respect to the prescribed global PPI passed from 44.7% to 58%.

DISCUSSION

The pharmacists may play an essential role in managing subjects with gastric reflux complaints by helping them to verify their diagnosis, referring them to a doctor for the assessment of alarming symptoms, and recommending treatment, including diet, lifestyle, and medications (3). Pharmacists can advise patients on the safe and effective use of PPIs, help cease inappropriate PPIs, and address their adverse reactions and interactions with other medications.

Avoiding caffeine, chocolate, spicy foods, and mint may lead to improvement in gastric reflux symptoms. Thus, counselling should be done on a case-by-case basis, and overweight patients should be encouraged to do so to lose weight. Smoking and alcohol lower low esophageal sphincter pressure, so patients should be counselled to stop smoking and/or stop/limit drinking alcoholic beverages when needed. It is recommended that patients, who present with nocturnal GERD symptoms, have to be instructed to elevate the head of the bed. However, as lifestyle change cannot be sufficient, a medical treatment is necessary for most patients with GERD or LPR. The first-line treatment of gastroesophageal reflux disease continues to be the anti-secretory drugs, most commonly proton pump inhibitors (PPIs). Of course, dietary and lifestyle modifications, including avoidance of acidic foods, carbonated beverages, alcohol, spicy food, and tobacco, in any case, should be recommended. In patients who are partially or non-responsive to medical therapy, it is hypothesized that it may be due to medication non-compliance, inappropriate dosing or timing, weakly acidic or alkaline reflux, increased volume of reflux leading to esophageal distension, or esophageal hypersensitivity (16-18). So, as suggested by guidelines, other medical options should be considered. In this regard, PPI add-on, alginates, and antacids are the most common alternative strategies. In this regard, recent guidance provided helpful information about the use of over-the-counter PPI in pharmacy (13). In particular, a short PPI course may represent the first step, followed by a re-assessment if ineffective. A recent meta-analysis showed that alginates had higher odds (OR = 4.42) of resolving gastric reflux symptoms than placebo or antacids (19). Alginates may offer some advantage in respect to PPI because of may create a raft that blocks the refluxate. In addition, alginates display an optimal safety profile. So, alginates can be taken for very long-standing periods.

The current experience in pharmacy provided some interesting findings. First, 63% of subjects visiting a pharmacy with gastric reflux complaints required treatment for GERD. Moreover, 38% of them had LPR. Interestingly, more than 40% of subjects did not follow a correct diet or an adequate lifestyle. In this regard, the pharmacist advised a diet and lifestyle change in 43% of subjects as a first-line option.

The PPI was the most common medication, both as monotherapy or polytherapy, about past or concomitant treatment for gastric reflux symptoms. Alginates were the second choice as both alone or add-on. Very notably, the updating of the knowledge about gastric reflux significantly affected the attitude of pharmacists to advise patients. As a result, there was a reduction in PPI use and an increase in alginates. In particular, the combined strategy, such as the PPI add-on to alginates, was the most common option.

A balanced and equilibrate association between over the counter PPI and alginates could, indeed, represent the ideal approach in managing patients at the pharmacy level. Of course, a short re-assessment is required to achieve the best control of gastric reflux.

In conclusion, the current experience demonstrated that the management of gastric reflux in pharmacy represents an important step for patients. Moreover, the pharmacist may positively contribute to the control of gastric reflux issues. Education programs are useful to improve the knowledge of gastric reflux and implement the advice skill. Alginates and PPIs are the most advantageous options for gastric reflux.

Conflict of interest:

All authors state that there is no conflict of interest.

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Gastric reflux: the pediatrician's point of view

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Gastroesophageal reflux (GER) in infants is the most common causes for physician consultation worldwide. Moreover, GER disease (GERD) also affects children. In addition, laryngopharyngeal reflux (LPR) may also affect children. As a result, the pediatrician has to often manage these diseases in daily practice. An educational program concerning updating knowledge about this issue was performed in a panel of 246 Italian pediatricians. The prescriptive attitude was assessed before and after the educational course. History, mainly regarding treatments, Infant Gastroesophageal Reflux Questionnaire (I-GERQ), GERD-Q, and reflux symptom index (RSI) were administered. The group of infants included 1,800 infants. The group of children included 1,706 subjects.

There was a shift for monot herapy in infants' management from 86.3% to 96%. In particular, the quote of alginate monotherapy increased from 79% to 94%. On the contrary, proton pump inhibitor (PPI) monotherapy decreased from 17.5% to 5%. Polytherapy substantially did not change. There was a shift for monotherapy from 74% to 88.4% in children's management. In particular, the quote of alginate monotherapy increased from 34% to 65.3%.

On the contrary, PPI monotherapy decreased from 30% to 10.7%. Polytherapy substantially did not change. In conclusion, the current experience demonstrated that GERD is a widespread medical problem in infants and children, and LPR significantly affects children. Updating the knowledge on gastric reflux significantly changed the prescriptive attitude of pediatricians. Alginates were the most common substance used in managing infants and children with gastric reflux. Therefore, PPI is used mostly combined with alginates.

Gastroesophageal reflux (GER) in infants is the most common causes for physician consultation worldwide (1). GER is defined as the natural movement of gastric contents from the stomach into the esophagus or mouth, which may be swallowed or regurgitated (2, 3). Regurgitation may occur after feedings more than six times per day in some infants (4). Many infants experience several episodes of GER during 24 hours without any adverse effects over the first few months of life (2). GER is present in over three-fourths of the infant population and appears in males approximately two times more often than females (5). Infants may begin having GER before two months of age (5). The most common clinical feature of GER in infants is post-prandial regurgitation, which will often go away around 12 months of age without any treatment (2, 3). The reflux episodes will often decrease as the infant approaches one year of age (4). Usually, the infant will have no apparent symptoms other than uncomplicated spitting up, appearing comfortable, and showing good weight gain (6). However, a thorough evaluation is

Keywords: gastric reflux, gastroesophageal reflux disease, laryngopharyngeal reflux, proton pump, inhibitor, alginate, antacid, pediatricians

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needed if poor weight gain, persistent irritability, and respiratory problems persist (2, 3).

The management of gastric reflux in infants relies on dietary recommendations and medications. Food thickeners and changes in feed volumes or frequency are suggested. Common medications used for treating GERD in infants are antacids and acid suppressants (3). Studies have shown acid suppressants to be more successful in treating GERD, which has led to increased use of proton pump inhibitors (PPIs) in the last years (7).

Even if relatively rare, gastroesophageal reflux may also affect children (8-11). The prevalence of gastroesophageal reflux disease symptoms in children aged 3 to <18 years ranges from 1.8% to 22% (12). The pathophysiology of GERD in children is similar to that in adults. However, children may present gastroesophageal and extraesophageal symptoms distinct from classic heartburn. In addition to a growing awareness of the disorder's high prevalence, increasing evidence supports GERD being a lifelong condition in some individuals that begins in childhood. Although the diagnostic workup in children compared with adults may differ, studies suggest that the early detection and treatment of GERD in childhood may result in better adult disease outcomes, improved quality of life, and decreased overall healthcare burden (13).

The GER workup includes history, physical examination, and specific questionnaires to parents and oldest children (14-16).

Treatments for pediatric GERD are based on the clinical indications of the specific child with GERD and range from conservative measures (positioning, formula changes) and pharmacotherapy to surgery. However, antisecretory agents, particularly the PPIs, are quite safe, effective, and due to their superiority in resolving acid-related disease, they could be appropriate interventions in GERD occurring in childhood to prevent long-term sequelae (17-19) successfully. Moreover, alginates and antacids are also widely used in clinical practice. In particular, alginates provide the best safety profile.

In addition, the output of gastric contents outside the esophagus may impact on upper airways. As a result, laryngopharyngeal reflux (LPR) occurs. Notably, LPR is different from GER as the symptoms are different (20). However, the management of LPR is similar to GERD.

The current experience aimed to evaluate the practical attitude of a group of Italian pediatricians in managing infants and children with gastric reflux before and after an educational program on this topic.

MATERIALS AND METHODS

The current experience included 246 Italian pediatricians distributed across the country, assuring a wide and complete national coverage. In addition, pediatricians participated in an educational program to update the knowledge concerning gastric reflux management.

The experience included two groups of subjects, such as infants and children. The inclusion criteria were to suspect gastric reflux and both genders. Exclusion criteria were to have comorbidities able to interfere with the evaluation of outcomes. As this survey was based on real-world practice, the doctors had the complete liberty of choosing the preferred medications based on the best practice.

The visit included a detailed history of symptoms and current treatments for gastric reflux, clinical examination, and administration of specific questionnaires.

The Infant Gastroesophageal Reflux Questionnaire (I-GERQ) had been developed and validated to assess GERD-related symptoms in infants (14). The I-GERQ-R used in the study was a 14-item caregiver-completed symptom assessment scale. All items were based on a 1-week recall period. Response choices ranged from 2–5 categories; higher scores indicated a more significant symptom burden. The cutoff of 16 serves to define normal results.

The GERD questionnaire (GERD-Q) was self-administered to children. It consists of six questions about the frequency of GERD- related symptoms during the past seven days (9). Questions numbered 1, 2, 5, and 6 are scaled 0 for "0 days", 1 for "1 day", 2 for "2–3 days", and 3 for "4–7 days." Questions numbered 3 and 4 are scaled 3 for "0 days", 2 for "1 day", 1 for "2–3 days", and 0 for "4–7 days," resulting in a GERD-Q score ranging from 0 to 18. A score of ≥8 was considered to define positivity.

Reflux Symptom Index (RSI) is a self-administered nine-item questionnaire developed by Belafsky for assessing symptoms in patients with reflux disease (21). It is so simple that it can be completed in less than 1min. Each item's scale ranges from 0 (no problem) to 5 (severe problems), with a

maximum score of 45. It has been concluded that RSI has high reproducibility and validity for the diagnosis of reflux if an RSI score >13 is defined as abnormal (21). Therefore, RSI may be considered a practical tool in the approach of patients with suspected LPR (22).

Analyses were performed using GraphPad Prism software, GraphPad Software Inc, CA, USA.

RESULTS

Infant Group

The group of infants included 1,800 infants, 956 (53.1%) males, and 844 (46.9%) females; the mean age was 7.44 months.

I-GERQ was positive (score>16) in 47% of infants.

Globally, 219 (12%) infants were currently treated: 189 (86.3%) with monotherapy and 30 (13.7%) with polytherapy. About monotherapy, 149 (79%) subjects took alginates, 33 (17.5%) PPI, and 7 (3.5%) ranitidine. About polytherapy, 28 subjects took alginates and PPI add-on, and 2 ranitidine plus PPI.

The prescribed treatments included dietetic recommendations for 608 (33.8%) infants. Medications were prescribed in 915 infants. Monotherapy was prescribed in 879 (96%) infants, including alginates in 826 (94%), PPI in 43 (5%), and ranitidine in 10 (1%). Polytherapy was prescribed in 36 infants using the association between PPI and alginates.

Therefore, there was a shift for monotherapy from 86.3% to 96%. In particular, the quote of alginate monotherapy increased from 79% to 94%. On the contrary, PPI monotherapy decreased from 17.5% to 5%. Polytherapy substantially did not change.

Children Group

The group of children included 1,706 subjects, 908 (53%) males and 798 (47%) females; the mean age was 8.28 years. Globally, 711 (41.7%) children had a GERD-Q score>8; 471 (66.2%) harmed the quality of life. Moreover, 653 (38%) had an RSI score>13.

Only 228 (13.3%) children were ongoingly treated; 169 (74%) took a monotherapy, 51 (30%) with PPI, 58 (34%) with alginates, and 47 (28%) with antacids. Polytherapy (with PPI as an add-on) was prescribed to 59 children; 22 (37%) took alginates plus PPI, and 23 (39%) antacids associated with PPI (Fig. 1).

About the diagnosis, 82% of children had a confirmed diagnosis, such as 711 (42%) children had GER and 526 (38%) had LPR.

Treatment was prescribed in 1,205 (70.6%) children; 1,065 (88.4%) were treated with monotherapy, including alginates in 696 (65.3%), 163 (15.3%) with antacids, and 114 (10.7%) with PPI (Fig. 2). Polytherapy was prescribed in 140 (11.6%) children, PPI was an add-on with alginates in 80 children (57%), and with antacids in 37 (26.4%).

Therefore, there was a shift for monotherapy from 74% to 88.4%. In particular, the quote of alginate monotherapy increased from 34% to 65.3%. On the contrary, PPI monotherapy decreased from 30% to 10.7%. Polytherapy substantially did not change.

Fig. 1. *Current treatment for GER in children before educational course*

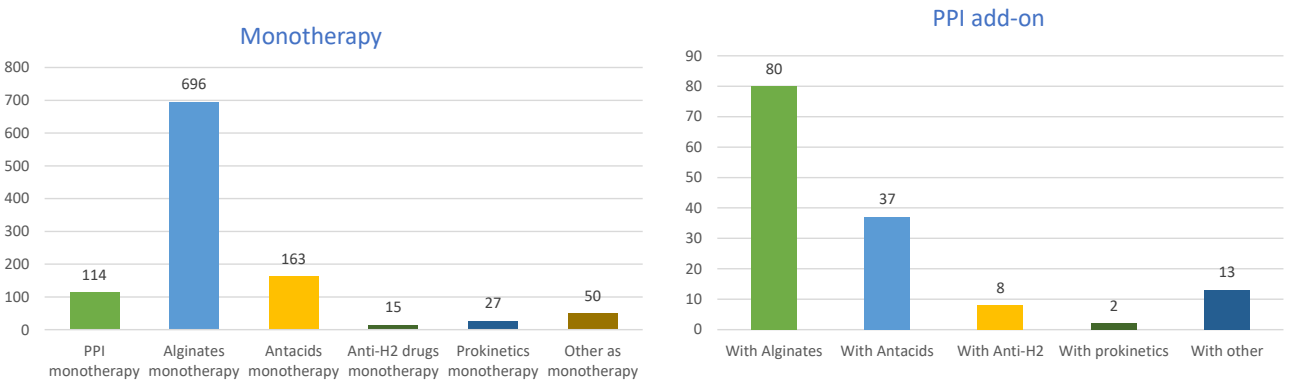

Fig. 2. *Prescribed treatments for GER in children.*

DISCUSSION

Gastric reflux is the most common medical problem in infants, and it is also common in children. The GER diagnosis is difficult in infants and also in children. Suggestive history and validated questionnaires are reliable to suspect GERD. Specific questionnaires may also support the suspect of LPR.

The current experience provided interesting outcomes. In particular, the findings highlighted the clinical relevance of GERD and LPR in infants and children. Even though the present experience is not methodologically rigorous, the obtained information mirrors the daily practice. As a result, the outcomes provide valuable data to understand the pediatricians' attitude in managing infants and children with gastric reflux. In addition, the updated knowledge about this topic, learned by an educational program, significantly affected the prescriptive practice.

Gastric reflux in infants is a relevant challenge for pediatricians as the current experience reported a prevalence of about 50% in the selected population. However, a specific treatment was prescribed in a limited quote of infants: only 12% of infants received active treatment for gastric reflux. In this context, alginates were the most common product used by pediatricians. Updating the knowledge on gastric reflux provided a significant increase in prescription: from 12% to 33.8%, such as almost the triple. Moreover, the updating also affected the quote of alginates prescription, mainly concerning the monotherapy, as the percentage of prescription increased from 79% to 94%.

The GERD was also a common problem in children, as more than 40% of them had a GERD-Q score positive, such as>8, and 2/3 had impaired quality of life. Consistently, LPR was also a common issue in children, as nearly 40% had LPR. These data underline the clinical relevance of gastric reflux also in childhood. These findings could depend on wrong dietary regimens and lifestyles that significantly increase the prevalence of gastric reflux symptoms in childhood. However, only 13% of children received specific treatment for gastric reflux. Alginates, antacids, and PPI were the most common medications prescribed, mainly as monotherapy. Also, the updated knowledge on gastric reflux significantly changes the pediatricians' attitude to treat children for children. Namely, 62% of children had a prescription. Alginates were the most commonly used medication, and the quote increased from 1/3 to 2/3 of all products.

The current experience demonstrated that alginates are very popular in pediatric practice probably for two main reasons: they are effective and safe. Moreover, their use significantly increased after adequate updating of the knowledge on this topic.

The present experience has relevant methodological biases but provided exciting outcomes based on a large sample of infants and children collected by a representative panel of Italian pediatricians.

In conclusion, this experience demonstrated that

GERD is a prevalent medical problem in infants and children, and LPR significantly affects children. Updating the knowledge on gastric reflux significantly changed the prescriptive attitude of pediatricians. Alginates are the most common substance used in managing infants and children with gastric reflux. Therefore, educational programs are welcome to improve the clinical practice.

Conflict of interest:

All authors state that there is no conflict of interest.

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Gastric reflux management at primary care level

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Gastroesophageal Reflux (GER) is a common disease; if associated with oesophageal damage is named GER disease (GERD) or if it damages upper airways as laryngopharyngeal Reflux (LPR). Although gastric reflux is common, its management remains challenging at the primary care level. The current experience evaluated the impact of an educational project about gastric reflux on the prescriptive attitude of primary care doctors. A large group of patients (20,778) with gastric reflux symptoms was evaluated before and after the educational program, involving 708 Italian physicians working at the primary care level. Before the update, only one-quarter of patients were actively treated for gastric reflux problems. The most common medications were PPI (72%), alginates (40%), and antacids (33%). A monotherapy was more frequently prescribed than polytherapy. After the educational program, 71% of patients had GERD diagnosis, and 29% LPR. In addition, 84% of patients had a medical prescription for harmful gastric reflux. Polytherapy was prescribed in 9,422 patients, mainly using PPI as an add-on associated with alginates, prokinetics, or antacids. Monotherapy was used in 8,069 patients, alginates were prescribed in 52% of patients, PPI in 28.6%, and antacids in 9.4%. In conclusion, the current experience confirmed the worth of continuing medical education and the importance of correctly managing patients with gastric reflux concerns. GERD and LPR should be adequately treated using appropriate medications, mainly concerning alginates and PPI.

Gastric Reflux is a para-physiologic event, but when the refluxate damages the extra-gastric organs and provokes symptoms, it becomes a disease, such as gastroesophageal disease (GERD). Moreover, the extra-oesophageal involvement may affect upper airways, so making the laryngopharyngeal Reflux (LPR) may happen.

Both GERD and LPR are very common in clinical practice. Both disorders have a relevant burden on society. Population-based studies reported that 19.8% of North Americans complain of typical symptoms of GERD (heartburn and regurgitation) at least weekly (1). Also, GERD accounted for $9.3 to $12.1 billion in direct annual healthcare costs in the United States, higher than any other digestive disease. The prevalence of GERD in the primary care setting becomes even more evident; namely, GERD is the most frequently first-listed gastrointestinal diagnosis (2, 3)

Expenditures for extra-oesophageal manifestations of reflux could surpass $50 billion, 86% of which could be attributable to pharmaceutical costs (2, 3). Therefore, noxious gastric reflux represents a significant medical issue that deserves adequate attention (4).

From a pathophysiological point of view, gastric reflux includes different mechanisms. The transient lower oesophageal sphincter relaxation, hiatus hernia, acid pocket, visceral hypersensitivity, and obesity represent the most important causes of gastric reflux. In

Keywords: gastric Reflux, GERD, laryngopharyngeal Reflux, PPI, alginates, primary care

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addition, impaired oesophageal and extra-oesophageal mucosal integrity, poor oesophageal clearance, and delayed gastric emptying are associated with GERD development. In addition, another pathogenic factor is a neural reflex sustained by acid exposure: the so-called Reflex-Reflux (5).

Laryngopharyngeal reflux is most commonly presented as laryngeal symptoms such as coughing, hoarseness, dysphagia, *globus*, and sore throat, but there can also be nose and sinus ear signs and eye involvement (6). Epidemiological studies have shown that the prevalence of this LPR may be extremely high, that it has specific characteristics of an outbreak, and that it is one of the most common causes of patient visits to their family medicine physicians, but also to otolaryngologists, gastroenterologists, paediatricians, pulmonologists, allergists, and psychiatrists (7, 8). In addition, LPR is a multifactorial syndrome with a vast clinical representation during the disease and with complications, so it requires and deserves a multidisciplinary approach.

The management, including workup and treatment, of patients with GERD and/or LPR is complex and challenging. Recent papers extensively discussed this topic (9-11). Nevertheless, the approach in managing patients with gastric reflux problems remains valuable in the primary care setting. Probably, this uncertain attitude depends on insufficient knowledge of the topic. As a result, an educational program on this issue has been promoted. In order to assess the consequence of this project on clinical practice, the current experience evaluated possible management changes after updating the knowledge on this topic in the primary care setting.

MATERIALS AND METHODS

The current experience included 708 Italian primary care doctors, distributed across the country, assuring wide and complete national coverage. In addition, the doctors recruited consecutive patients who visited in their offices.

The inclusion criteria were to have both genders with symptoms evocative of gastric reflux. Exclusion criteria were to have comorbidities able to interfere with the evaluation of outcomes. As this survey was based on real-world practice, the doctors had the liberty to choose the preferred medications based on the best practice.

The visit included a detailed history of symptoms and current treatments for gastric reflux, clinical examination, and administration of specific questionnaires.

The gastroesophageal reflux disease impact scale (GIS) comprises eight questions about the frequency of the following occurrences in the past week (12). The questions are acid-related symptoms, chest pain, extra-esophageal symptoms, the impact of symptoms on sleep, work, meals, and social occasions, and the use of additional non-prescription medication. Four response options are provided to describe frequency over the previous two weeks: ‘none of the time’ (score 1), ‘a little of the time’ (score 2), ‘some of the time’ (score 3), and ‘all of the time’ (score 4).

The reflux symptom index (RSI) is a self-administered nine-item questionnaire developed by Belafsky for assessing symptoms in patients with reflux disease (13). It is so simple that it can be completed in less than 1min. Each item’s scale ranges from 0 (no problem) to 5 (severe problems), with a maximum score of 45. It has been concluded that RSI has high reproducibility and validity for the diagnosis of reflux if an RSI score >13 is defined as abnormal (13). Therefore, RSI may be considered a practical tool in the approach of patients with suspected LPR (14).

Analyses were performed using GraphPad Prism software, GraphPad Software Inc, CA, USA.

RESULTS

The experience concerned 20,778 patients, 10,523 (50.6%) males and 10,255 (49.4%) females, the mean age was 50.4 years. A gastroscopy was recently performed in 3,532 (17%) patients; hiatus hernia was diagnosed in 2,184 (62%) subjects. About the GIS questionnaire results, 7,100 (34.2%) reported GER symptoms sometimes, and 1,717 (8.3%) daily. About RSI questionnaire results, 8,701 (41.9%) patients scored ≥13.

Concerning the dietary regime, 7,629 (36.7%) patients reported wrong diet attitudes, including feast (53%), excessing water drinking (33%), plentiful dinner (41%), and eating hastily (48%). Concerning the lifestyle norms, 7,642 (36.8%) patients followed improper rules, including dining late (51%), laying down after meals (56%), keeping the head down

(44%), wearing tight clothes (17%), and physical activity on a full stomach (10%).

Globally, 5,545 (26.7%) patients were currently treated for gastric reflux; the most common medications included PPI (72%), alginates (40%), and antacids (33%). Fig. 1 reports the detailed findings. Near 3,000 (54%) patients took a monotherapy; the most common medication was PPI (68%), followed by alginates (16%) and antacids (12%). Polytherapy with PPI was the most common as taken by 1,965 patients; the most frequent associations were with alginates (58%), antacids (32%), and prokinetics (20%). Globally, PPI was currently taken by 3,998 patients; monotherapy was taken by 2,033 (50.9%) patients and PPI as an add-on in 1,965 (49.1%); thus, the ratio was about 1/1.

After updating the knowledge about gastric reflux, the prescriptive attitude changed. Globally, 14,777 (71%) patients had GERD diagnosis, and 6,063 (29%) LPR one.

Medications were prescribed in 17,491 patients, such as in 84% of the visited patients; treatments were monotherapy in 8,069 (46%) patients and polytherapy in 9,422 (54%) patients reported in Fig. 2. There was a shift: monotherapy decreased from 54% to 46%.

Concerning monotherapy, alginates were the most common treatment as prescribed in 4,196 (52%) patients, followed by PPI in 2,308 (28.6%) and antacids in 762 (9.4%). Concerning polytherapy, PPI was used as an add-on in 9,422 patients; the most common associations were: with alginates in 7,241 (76.9%) patients, prokinetics in 1,758 (18.6%), and antacids in 1,700 (18%). In particular, PPI was prescribed in 11,730 patients, 2,308 (19.7%) patients as monotherapy, and 9,422 (80.3%) as polytherapy.

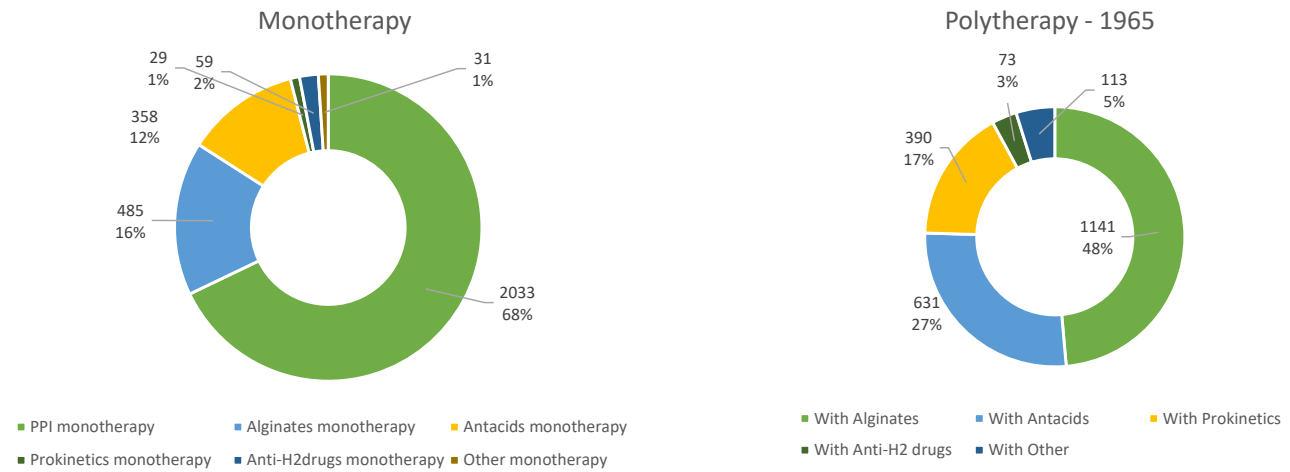

Fig. 1. *Current treatments for gastric reflux before educational course.*

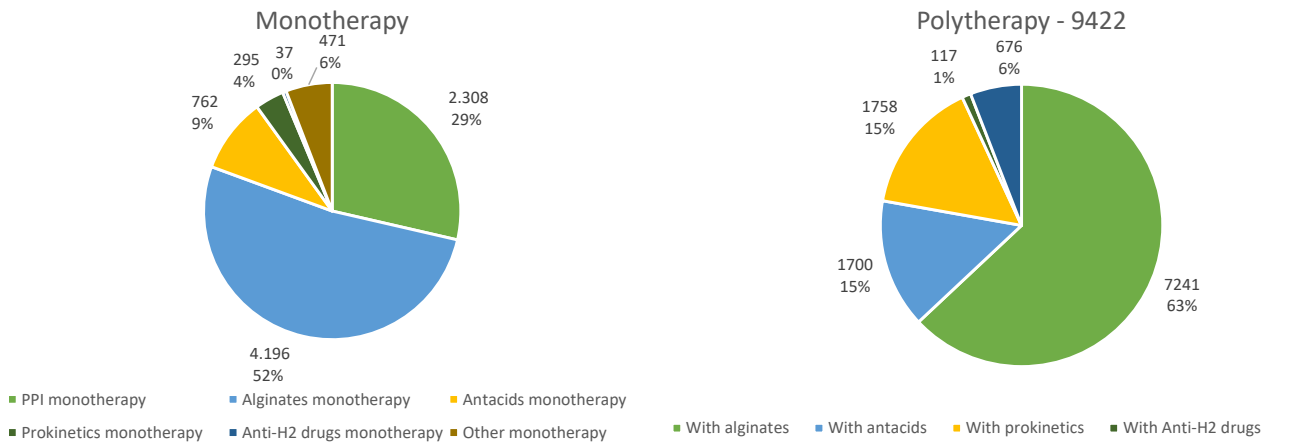

Fig. 2. *Prescribed treatments in patients with GERD and/or LPR.*

DISCUSSION

Gastric Reflux is a common medical problem that significantly involves primary care doctors. However, there is still concern about its management, mainly depending on inadequate knowledge of the topic. For this reason, the current experience aimed to evaluate the practical attitude in managing patients with gastric reflux at primary care before and after an update program on this matter. An unrestricted grant promoted the educational program.

The obtained outcomes provided exciting suggestions. First, the experience included over 20,000 outpatients with gastric reflux symptoms. Second, a so much sample provided a sustained clinical relevance to the findings as, rarely, surveys included such large populations.

GERD diagnosis was made in about 70% of patients, LPR diagnosis in 29%. This outcome confirms the importance of considering LPR as a common consequence of gastric reflux.

Inappropriate diet and lifestyle were common as about 40% of patients had wrong behaviours. This fact underlines the importance of adequately considering these behavioural aspects in clinical practice.

Some interesting results were obtained concerning the therapeutic strategy before updating the knowledge on this topic. First, few patients were treated for gastric reflux as only one-quarter took medications for gastric reflux. In this context, monotherapy, such as a single compound prescription, was the preferred option as 54% of patients took one medication alone. PPI was the most commonly prescribed drug in this context, as about ¾ of patients take this medication class. Moreover, alginates and antacids were commonly used, in 40% and 33%, respectively. Antacids are very popular, but they are often self-prescribed. However, PPI was also an add-on, primarily associated with alginates (58%) and antacids (32%). Therefore, the ratio between PPI monotherapy and polytherapy was about 1:1.

The educational project significantly affected the prescriptive attitude of primary care doctors. Thorough history documented a recent upper digestive endoscopy which demonstrated hiatus hernia in about two-thirds of subjects. The administration of specific questionnaires, such as GIS and RSI, identified 70% of patients with GERD and 30% with LPR. This finding confirmed the relevance of gastric reflux in daily practice. Consequently, primary care doctors prescribed treatment in more than 80% of patients. Polytherapy was more frequently advised with an inversion rate from 46% to 54%. Alginates were the most common compound prescribed as monotherapy, as used in more than 50% of patients, followed by PPI (nearly 30%) and antacids (nearly 10%). On the contrary, polytherapy with PPI was the most frequent prescription, more often associated with alginates (77%) or prokinetics or antacids (18.6 and 18%, respectively).

These results, therefore, reaffirmed the importance of educational programs, mainly for primary care doctors. As a result, the clinical practice significantly changed as a more thorough workup, and more appropriate prescription occurred after updating the knowledge about gastric reflux. Two primary outcomes emerged: more frequent use of PPI, primarily as an add-on, and widespread prescription of alginates, both alone or associated with PPI. The awareness of the relevance of gastric reflux convinced primary care doctors to change the prescriptive attitude, moving toward a frequent use of medications. In addition, the alginates' role was reconsidered. In this regard, alginate may be considered a fruitful and relevant option in many patients with reflux disease. In particular, the knowledge about the utility of alginates derives from an exciting research area investigating the pathogenic role of the so-called "acid pocket."

The acid pocket is a short zone of unbuffered highly acidic gastric juice that accumulates in the proximal stomach after meals; as the acid pocket is the source of acid reflux, it increases the propensity for it through conventional mechanisms, such as hiatus hernia, and has been considered a significant cause of gastric reflux. Alginate is an anionic polysaccharide occurring naturally in brown algae and has a unique property in the treatment of gastric reflux by eliminating the acid pocket. Thus, alginate formulation can reduce postprandial symptoms by neutralizing the acidity of gastric contents. In addition to neutralizing the gastric acidity, more importantly, alginates form a foamy gel that is like a raft floating on the surface of gastric contents after interacting with gastric acid, and this barrier-like gel displaces the acid pocket from the oesophageal-gastric junction and protects both the oesophageal and

the upper respiratory mucosa from the acid and non-acid Reflux by gel coating. Therefore, alginate protects the oesophageal and upper respiratory mucosa from acid and non-acid Reflux and displacement of the acid pocket away from the esophagus. In addition, an old concept could be revised for GERD and LPR therapy: the "cytoprotection" of mucosal tissues (15-20).

In conclusion, the current experience confirmed the relevance of continuing medical education and the importance of correctly managing patients with gastric reflux. In particular, GERD and LPR should be adequately treated using appropriate medications, mainly concerning alginates and PPI.

Conflict of interest:

All authors state that there is no conflict of interest.

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Diverticular disease: a new therapeutic opportunity

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Diverticular disease is a common medical problem, mainly affecting older people. Different phenotypes have been described, including symptomatic diverticular disease, diverticulitis, symptomatic uncomplicated diverticular disease (SUDD), and segmental colitis associated with diverticulosis (SCAD). The management of diverticular disease consists of antibiotics and anti-inflammatory drugs that should be used for very long times. However, side effects commonly occur. Therefore, a new multi-component food supplement (Divercol®) has been recently launched to be employed in these patients. This product contains seeds of *Plantago psyllium*, dry extract of *Perilla frutescens, Citrus paradisi* seeds dry extract, a mixture of tyndallized probiotics (*Lactobacillus rhamnosus, L reuteri, L acidophilus*), and riboflavin.

The recent experience evaluated its preventive and adjunctive activity in 388 patients with SUDD, symptomatic diverticular disease, and diverticular disease and treated with multiple antibiotic courses. Patients took a one-week antibiotic course, then a two-week Divercol® course (one stick/day). The assessed symptoms included: intestinal movement alteration, meteorism/flatulence, and abdominal pain. The visual analogue scale measured patients' perception of symptoms. All patients perceived a significant ($p<0.001$ at all times) improvement of all measured parameters. In addition, the treatment was safely tolerated.

In conclusion, the current clinical experience suggested that the multi-component product Divercol® may be an effective and safe adjunctive therapeutic option in managing patients with diverticular disease.

The diverticulosis of the colon is an anatomic modification of the colonic wall characterized by pockets, such as the *diverticula* occurring after a hernia of the colonic mucosa and sub-mucosa consequent to a defect of the muscle layer (1). The term diverticulum derives from the Latin verb *divertere,* meaning to deviate. Diverticulosis is defined as the presence of colonic diverticula, which may be symptomatic or complicated. Diverticular disease is defined when symptoms occur (2). Symptomatic, uncomplicated diverticular disease (SUDD) is a subtype of diverticular disease, characterized by persistent symptoms without macroscopic colitis or diverticulitis. However, diverticulitis identifies the macroscopic inflammation of diverticula associated with acute or chronic complications. Complicated diverticular disease includes abscess, peritonitis, obstruction, fistulas, or hemorrhage. Segmental colitis

Keywords: diverticular disease, management, probiotics, psyllium, Perilla, grapefruit, riboflavin

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is a localised inflammatory disorder associated with diverticulosis (SCAD) (3).

From a clinical point of view, most subjects with colonic diverticula remain symptomless, such as having diverticulosis. However, about 20% of subjects with diverticulosis develop symptoms, including recurrent abdominal pain or discomfort, bloating, and changing bowel habits, such as a symptomatic diverticular disease. In addition, a minimal quote (about 4%) of patients develop acute diverticulitis (4).

The prevalence of the disease is up to 10% of the general population, higher in elderly patients over 65 years old (up to 65% of people aged over 70 years); recent studies showed an increment also in youngers over 40 years old (5).

Diverticulosis may depend on complex interactions among genetic factors, alteration of colonic motility, lifestyle conditions, including tobacco smoking, obesity, alcohol consumption, low fibre, and meat intake with diet (6). Moreover, a role of unbalanced microbiota (dysbiosis) has been recently hypothesized (7). Preliminary data suggested that colonic symptoms were associated with the reduced representation of taxa with a possible anti-inflammatory effect, including *Clostridium* cluster IV and overgrowth of Enterobacteriaceae, Bifidobacteria, and *Akkermansia* (8). As a result, the use of probiotics has been hypothesized suitable for diverticulosis. Probiotics may modify the gut microbial balance leading to health benefits due to their anti-inflammatory effects and capability to enhance anti-infection defences by maintaining adequate bacterial colonization in the gastrointestinal tract and inhibiting colonic bacterial overgrowth and metabolism of pathogens (9). Therefore, probiotics have been tested in several trials. A recent systematic review evaluated 11 studies on this topic (10). However, the inclusion/exclusion criteria, type of probiotics, and duration of treatments were very variable. Thus the available outcomes did not support sweeping conclusions.

The management of diverticular disease depends on the precise diagnosis and includes antibiotics, anti-inflammatory drugs, and surgery in severe cases (11). In addition, dietary and lifestyle changes, fibre-rich meals, and counselling may be helpful (12). Also, biophenol-rich nutraceutical supplementation may be used as adjuvant therapy as it is safe and exerts anti-inflammatory activity (13).

As SUDD is a prevalent problem among patients with diverticulosis, affecting up to 20% of them, and has a typical natural history characterized by a long-term duration and acute relapses (14), it requires particular attention and appropriate therapy, including topical medications (15, 16).

In the context of non-pharmacological remedies for diverticular disorders, a new multi-component food supplement (Divercol®) has been recently launched. This product contains seeds of *Plantago psyllium*, dry extract of *Perilla frutescens, Citrus paradisi* seeds dry extract, a mixture of tyndallized probiotics (*Lactobacillus rhamnosus, L reuteri, L acidophilus*), and riboflavin. Therefore, the current experience aimed to evaluate the effectiveness of this food supplement in preventive or adjunctive therapy for patients with diverticular disease, diverticulitis, or SUDD.

MATERIALS AND METHODS

The current experience involved a panel of Italian gastroenterologists, distributed across Italy, assuring complete national coverage. The experience was performed in early 2021. The patients, who had a diagnosis of diverticular disease, were stratified into three groups. Group A included patients with SUDD; Group B patients with symptomatic diverticular disease; Group C patients with diverticular disease and treated with antibiotic courses.

Patients were visited at baseline (T0), then a one-week antibiotic course was prescribed. At the end of this preventive antibiotic course, patients were re-visited (T1); a two-week course with the food supplements started. Finally, after 21 days (T2), patients were re-evaluated.

The food supplement was prescribed as a stick daily for 14 consecutive days. A stick of Divercol® contains *Plantago psyllium* seed powder 3 g, dry extract of *Perilla frutescens* 100 mg (of which 2.5 mg of polyphenols), dry extract of *Citrus paradisi* seeds 100 mg (of which 50 mg of bioflavonoids), a mixture of tyndallized probiotics, including *Lactobacillus rhamnosus* $1x10^9$ CFU, tyndallized *Lactobacillus reuteri* $1x10^9$ CFU, and *Lactobacillus acidophilus* $1x10^9$ CFU, and vitamin B2 (riboflavin) 1.4 mg (100% of the recommended daily dose).

Each patient measured the perception of symptom severity

using a visual analogue scale, where 0 means no symptoms and 10 is the worst symptom. The assessed symptoms included: intestinal movement alteration, meteorism/flatulence, and abdominal pain. In addition, safety was evaluated, collecting adverse events.

RESULTS

The current experience included 388 outpatients, 206 males and 182 females, with a mean age of 62.9 years. Group A consisted of 107 (31.7%) patients, Group B 146 (43.2%), and Group C 85 (25.1%).

Group A

The perception of intestinal movement alteration significantly improved after the antibiotic treatment ($p<0.001$), consistently the severity diminished at T2 ($p<0.001$), as reported in Fig. 1. Furthermore, the severity of meteorism/flatulence significantly diminished at T1 ($p<0.001$) and T2 ($p<0.001$). In addition, the intensity of abdominal pain significantly decreased at T1 ($p<0.001$) and T2 ($p<0.001$).

Group B

The perception of intestinal movement alteration significantly improved after the antibiotic treatment ($p<0.001$), consistently the severity diminished at T2 ($p<0.001$), as reported in Fig. 2. Furthermore, the severity of meteorism/flatulence significantly diminished at T1 ($p<0.001$) and T2 ($p<0.001$). In addition, the intensity of abdominal pain significantly decreased at T1 ($p<0.001$) and T2 ($p<0.001$).

Group C

The perception of intestinal movement alteration significantly improved after the antibiotic treatment

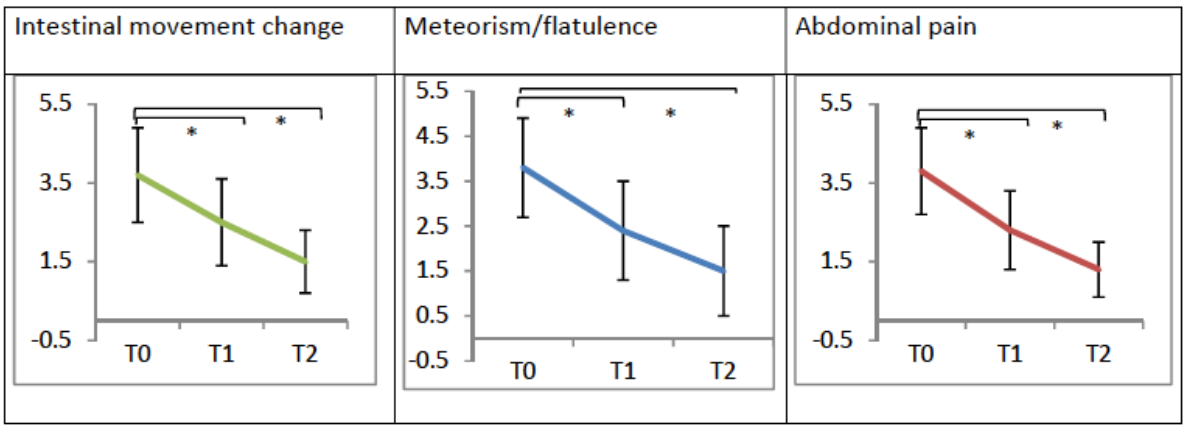

Fig. 1. *Mean (± standard deviation) of symptom severity in Group A patients at the three observation times (T0, T1, and T2).*

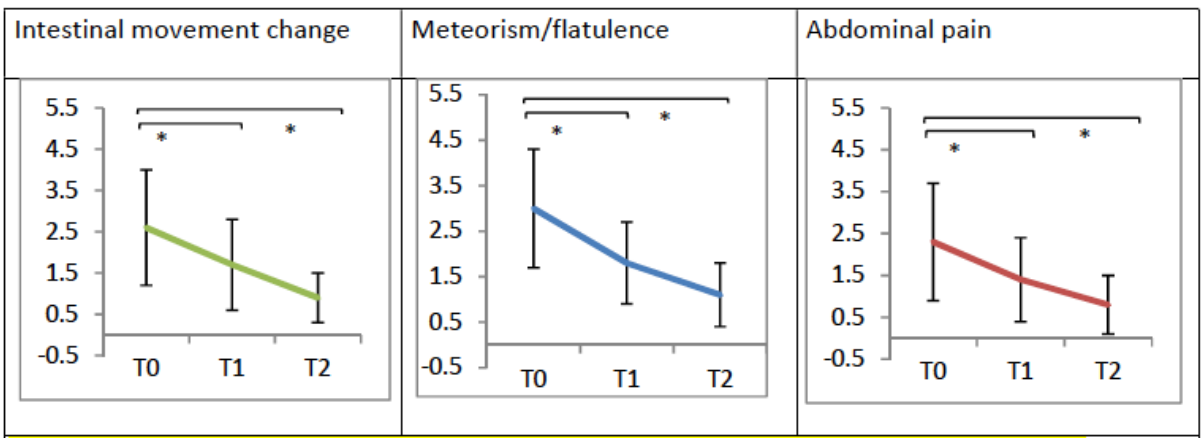

Fig. 2. *Mean (± standard deviation) of symptom severity in Group B patients at the three observation times (T0, T1, and T2).*

($p<0.001$), consistently the severity diminished at T2 ($p<0.001$), as reported in Fig. 3. Furthermore, the severity of meteorism/flatulence significantly diminished at T1 ($p<0.001$) and T2 ($p<0.001$). In addition, the intensity of abdominal pain significantly decreased at T1 ($p<0.001$) and T2 ($p<0.001$). The food supplement was well tolerated, and no relevant adverse events were reported.

DISCUSSION

Diverticular disease represents a common medical problem, mainly concerning older people while also affecting younger subjects. Diverticular disease is a chronic inflammatory disorder that significantly affects patients' quality of life and significantly burdens health services.

Treatment of diverticular disease generally is not resolutive as the pathogenesis is multifactorial. In addition, dysbiosis and impaired intestinal transit are involved in the disease. Therefore, it is important to address the treatment toward different targets. Antibiotics and anti-inflammatory drugs, including mesalazine, are widely used but have relevant side effects, including dysbiosis and increased resistance. As a result, there is a growing interest in multi-component food supplements that could restore eubiosis, regulate intestinal movements, and exert anti-inflammatory, antioxidant, and antimicrobial activities. In this regard, the multi-component Divercol® may represent an interesting option in diverticular disease management. Namely, the single components exert fruitful actions.

Psyllium is a widely used remedy for constipation. It traps water in the intestine increasing stool water, easing defecation, and altering the colonic environment (17). Moreover, psyllium restores gut microbiota and reduces inflammatory events.

Perilla frutescens (L.) is an annual herbal medicinal, aromatic, functional food, and ornamental plant that belongs to the mint family, Lamiaceae (18). Perilla has traditionally been prescribed to treat depression-related disease, anxiety, asthma, chest stuffiness, vomiting, coughs, colds, flu, phlegm, tumors, allergies, intoxication, fever, headache, stuffy nose, constipation, abdominal pain, and indigestion, and acts as an analgesic, anti-abortive agent, and a sedative. In addition, 271 natural molecules have been identified in perilla organs, including phenolic acids, flavonoids, essential oils, triterpenes, carotenoids, phytosterols, and fatty acids tocopherols, and policosanols. In addition to solvent extracts, the individual compounds include rosmarinic acid, perillaldehyde, luteolin, apigenin, tormentic acid, and isoegomaketone. In addition, perilla showed various biological activities such as antioxidant, antimicrobial, anti-allergic, antidepressant, anti-inflammatory, anticancer, and neuroprotection effects.

Grapefruit (*Citrus paradisi* Mcfad) is a perennifolium tree 5-6 m high with fruit of about

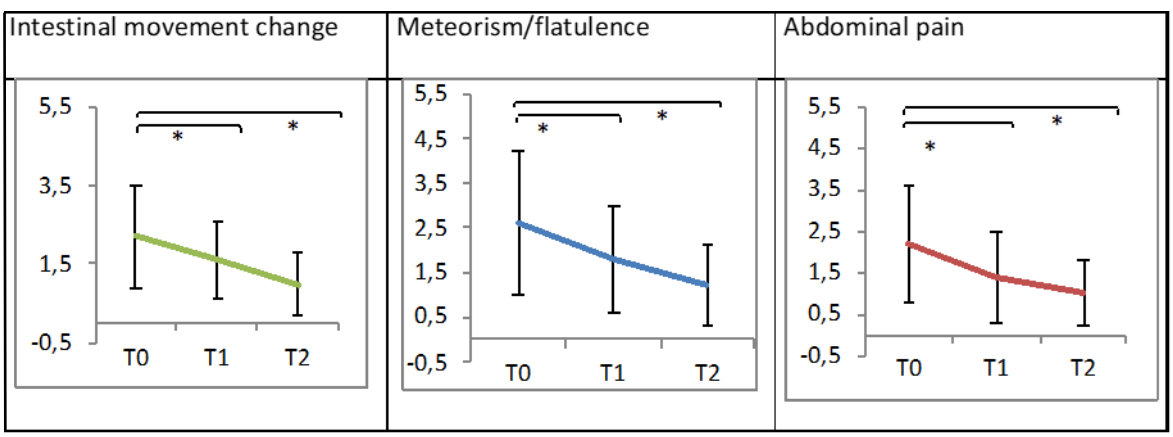

Fig. 3. *Mean (± standard deviation) of symptom severity in Group C patients at the three observation times (T0, T1, and T2).*

15 cm in diameter, protected by peel. We can find about 11-14 segments (carpels), each surrounded by a membrane containing the juice sacs and the seeds (19). The fruit is made up of numerous compounds and has nutritive value because of various vitamins and minerals, among other chemicals. In addition, the seeds display a variety of biomedical, antigenotoxic, and chemopreventive effects, undoubtedly related to the presence of the numerous chemicals that have been determined to constitute the fruit.

Lactobacillus rhamnosus is one of the most widely used probiotic strains (20). Various health effects are well documented, including preventing and treating gastrointestinal infections and diarrhea and stimulating immune responses that promote vaccination or even prevent specific allergic symptoms.

L. acidophilus is a homofermentative, microaerophilic, short-chain Gram-positive microorganism with rod morphology having its bacteriocins belonging to class II a (21). Several bacteriocins of L. acidophilus have been isolated and characterized. It exerts strong inhibitory actions against food spoilage, and pathogenic bacteria make them an important class of bio preservatives. L. acidophilus can be added as an adjunct in many food fermentation processes contributing to unique taste, flavor, and texture. It also preserves the products by producing lactic acid and bacteriocins.

Lactobacillus reuteri is a well-studied probiotic bacterium that can colonize many mammals. In humans, *L. reuteri* is found in different body sites, including the gastrointestinal tract, urinary tract, skin, and breast milk (22). First, *L. reuteri* can produce antimicrobial molecules, such as organic acids, ethanol, and reuterin. Due to its antimicrobial activity, *L. reuteri* can inhibit the colonization of pathogenic microbes and remodel the commensal microbiota composition in the host. Second, *L. reuteri* can benefit the host immune system. For instance, some *L. reuteri* strains can reduce the production of pro-inflammatory cytokines while promoting regulatory T cell development and function. Third, bearing the ability to strengthen the intestinal barrier, the colonization of *L. reuteri* may decrease the microbial translocation from the gut lumen to the tissues. Microbial translocation across the intestinal epithelium has been hypothesized as an initiator of inflammation. Therefore, inflammatory diseases, including those located in the gut and remote tissues, may be ameliorated by increasing the colonization of *L. reuteri*.

Riboflavin is vitamin B2 (23). The role of riboflavin has also been dealt with in preventing a wide array of health diseases like migraine, anemia, cancer, hyperglycemia, hypertension, diabetes mellitus, and oxidative stress directly or indirectly. The riboflavin deficiency has a profound effect on iron absorption, metabolism of tryptophan, mitochondrial dysfunction, gastrointestinal tract, brain dysfunction, and metabolism of other vitamins and is associated with skin disorders. In addition, riboflavin is an antioxidant nutrient that may prevent lipid peroxidation and reperfusion oxidative injury.

Based on this background, the current experience demonstrated that the multi-component food supplement Divercol® might be an effective adjunctive therapy in managing patients with diverticular disease. In particular, this product significantly regulated intestinal movements, reduced symptoms associated with dysbiosis, such as meteorism/flatulence, and relieved the abdominal pain due to gas overproduction. Moreover, Divercol® was able to exert preventive activity in patients treated with antibiotics for an extended period. Antibiotics significantly affect the gut microbiota, promoting dysbiosis. This experience showed that this supplement food significantly improved symptoms associated with antibiotic overuse. In fact, the beneficial effects were more evident after antibiotic discontinuation, confirming the positive activity exerted by this multi-component food supplement.

It has to be noted that the present survey cannot be considered a formal investigative study. Consequently, further studies should be conducted with a rigorous methodology, designed according to randomized-controlled criteria. However, the strength of this survey is the considerable number of enrolled patients and the real-world setting. The outcomes could, therefore, mirror the facts observable in clinical practice.

In conclusion, the current clinical experience suggested that the multi-component product Divercol® may be an effective and safe adjunctive

therapeutic option in managing patients with diverticular disease.

Conflict of interest:

All authors state that there is no conflict of interest.

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Rinalt®: a new adjunctive therapeutic option for rhinitis

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Rhinitis is an inflammatory disease of the nose. The rhinitis classification considers different phenotypes characterized by the etiopathogenic mechanisms. The most common types include infectious rhinitis, allergic rhinitis, non-allergic rhinitis, vasomotor rhinitis, atrophic rhinitis, medicamentosa (iatrogenic) rhinitis, and hormonal rhinitis. Natural remedies are even more esteemed and requested by the patients. In this regard, Rinalt® is a Medical Device Class II CE formulated as a nasal spray. Rinalt® contains Mucotannil® complex, seawater, dexpanthenol, and essential balsamic oils. Rinalt® is an adjunctive treatment for respiratory disorders characterized by mucus hyperproduction, including infectious rhinitis and allergic rhinitis. This formulation results in a protective barrier against pathogens, allergens, irritants, and noxious substances. These effects are obtained thanks to the active components. Its components exert many activities, including antimicrobial, anti-inflammatory, antioxidant, decongestant, and mucolytic properties. Therefore, this product may be fruitfully used in clinical practice.

Rhinitis is a common heterogeneous chronic disorder in both children and adults. It is usually characterized by inflammation of the nasal mucosa and is clinically defined by the presence of one or more of the following symptoms: nasal congestion, pruritus, sneezing, rhinorrhea, and posterior nasal drainage. (A)

The rhinitis classification considers different phenotypes based on the etiopathogenic mechanisms. The most common types include infectious rhinitis, allergic rhinitis, non-allergic rhinitis, vasomotor rhinitis, atrophic rhinitis, medicamentosa (iatrogenic) rhinitis, and hormonal rhinitis, as reported in Table I. Infectious rhinitis is one of the most frequent types of rhinitis, and it is caused by viral or bacterial pathogens, even if the viruses are most common (1).

Acute viral rhinitis, such as the common cold, is undoubtedly the most frequent infectious disease. The common cold is characterized by congestion of the nasal mucosa and occlusion of the sinus ostia. It is usually a self-limited illness confined to the upper respiratory tract, but in some patients, viral rhinitis can spread to adjacent organs or predispose to bacterial complications. In fact, bacterial rhinitis usually follows viral rhinitis (superinfection). The common cold symptoms include fever, headache, sneezing, rhinorrhea, nasal congestion, sore throat, and cough. These symptoms, as a rule, last less than ten days, except for the cough that persists for weeks. In this regard, the "ten-day mark," such as a symptom duration exceeding ten days, should suggest the suspect of acute rhinosinusitis (2). The treatment of the common cold is based on symptom relief, as there is no causal therapy. Many therapeutic options are available, but the patient preference is addressed to natural remedies (3).

Keywords: rhinitis, medical device, natural remedies, nasal spray, Mucotannil® complex, seawater, dexpanthenol, essential balsamic oils

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Allergic rhinitis (AR) is the most common immune-mediated disease as it may affect up to 40% of the general population, with approximately 80% of those diagnosed with allergic rhinitis developing symptoms before age 20 years. Boys tend to have an increased incidence of AR in childhood, but women are more commonly affected in adulthood. (B) The pathogenic mechanisms consist of an allergen-specific functional defect of T regulatory cells and T helper 2 polarization (4). Consequently, there is the production of allergen-specific IgE (sensitization) and eosinophilic infiltrate of the nasal mucosa (5). The typical symptoms of allergic rhinitis immediately appear after exposure to the causal allergen. They include nasal itching, sneezing, watery rhinorrhea, and nasal obstruction, and the last express the allergic inflammation (6). The pharmacological treatment of allergic rhinitis rests on the use of antihistamines and intranasal corticosteroids (7). Antihistamines are the first-line medication for allergic rhinitis as they are quickly effective and safe. Interestingly, some of them, including cetirizine and fexofenadine, also reduce nasal congestion (8,9). However, this effect depends on the modulation of allergic inflammation (10).

Non-allergic rhinitis (NAR) affects over 22 million people in the United States and is present more commonly in women, with an age of onset usually > 40 years. A non-IgE-mediated mechanism characterizes non-allergic rhinitis. This group, in fact, consists of patients with symptoms of rhinitis but without any identifiable allergic triggers. NAR is a diagnosis of exclusion in patients negative for systemic IgE when the many other causes of rhinitis have been ruled out. (B, C)

The most common phenotype is the non-allergic rhinitis with eosinophil infiltrate (NARES); other types include mast cell phenotype (NARMA), mast cell, eosinophil (NARME), and neutrophil (NARNE). Therefore, nasal cytology is mandatory to diagnose these diseases (11). The treatment of non-allergic rhinitis is based on the relief of symptoms and includes the same pharmacological classes used for allergic rhinitis (12).

Atrophic rhinitis is a chronic disease condition characterized by atrophy of the nasal mucosa, formation of nasal crusts, and a typical foul odour. It shows a female predominance (5:1) and usually affects elderly subjects or complicates allergic rhinitis. (D)

Non-pharmacological remedies

There is also a growing interest in non-pharmacological remedies for managing rhinitis. Many people prefer to use natural remedies instead of drugs. The common thought believes that traditional medicine is equally effective and overall safer than medications. In this regard, many food supplements and medical devices are available. The market is continuously increasing, and the patient's preference is even more oriented toward non-chemical products, mainly for children (3,13-16).

Consequently, more and more people prefer to use complementary medicine, for example, herbal medications, saline solutions, probiotics, oligo-elements, and vitamins (17). In this regard, there is a growing interest in nutraceuticals. Nutraceuticals are just substances of natural origin that can have a positive effect on the state of health. At present, nutraceuticals, with proven efficacy, are popularly associated with conventional therapy to speed up recovery, make it long-lasting, and avoid aggressive therapeutic regimens, including systemic corticosteroids, or at least limit their duration if they are needed (18,19). Therefore, their use is widespread, and new products are continuously developed.

Rinalt®: a new adjunctive therapeutic option for rhinitis.

Rinalt® is a Medical Device Class II CE formulated as a nasal spray. Rinalt® contains Mucotannil® complex, seawater, dexpanthenol, and essential balsamic oils. Rinalt® is an adjunctive treatment for respiratory disorders characterized by mucus hyperproduction, including infectious rhinitis and allergic rhinitis. This formulation results in a protective barrier against pathogens, allergens, irritants, and noxious substances. These effects are obtained thanks to the active components.

Mucotannil® complex is based on polysaccharides from larch inner bark and Icelandic moss and tannins from sea buckthorn extract. These combined substances exert a triple action: i) inactivation of pathogens, typically an antiviral activity provided by tannins, ii) decongestion by seawater, and iii) protection, polysaccharides and tannins form a protective barrier

on the respiratory mucosa; Mucotannil® complex has a high content of mucoadhesive mucilages, tannins, and flavonoids that form a double barrier by the complexation between tannins and mucus proteins, and the direct muco-adhesivity by polysaccharides. In particular, Mucotannil® complex contains hydroalcoholic extract of Icelandic moss (*Cetraria islandica*) and a high-polysaccharide extract from the inner bark of Larch (*Larix decidua*) and Sea Buckthorn (*Hippophae rhamnoides*) leaf/bud extract, with a robust protective action towards influenza and other seasonal viral affections, thanks to its high content of tannins which create a "barrier" upon the tissues which prevents viral attacks and spread. In addition, it also contains essential oil of peppermint and small amounts of aromatic substances with potent balsamic action.

Mucotannil® complex: an innovative product

The use of mucoadhesive spray systems, as with the Mucotannil® complex, promises several advantages concerning more conventional routes of administration, including i) more easily localization at the target site, ii) more prolonged residence time, and iii) increased topical concentration gradient.

In addition, the Mucotannil® complex presents a series of favourable characteristics:

- the mucilaginous polysaccharide components have a relevant mechanical interaction with mucins, forming adhesive "gels" that create a thin and tenacious protective barrier on mucosal surfaces and prevent them from coming in contact with irritants, allergens, or pathogens.

- the mucilaginous polysaccharide hold large amounts of water within their molecular network and release it slowly. This fact assures adequate hydration to the epithelial tissues, displaying lenitive and emollient effects. The effective lenitive effect against irritation reduces mucus secretion. Moreover, hydration favours the fluidization and elimination of mucus in excess.

- the sea buckthorn may prevent viral infections, shorten the infection duration, and relieve respiratory symptoms.

- the muco-adhesiveness reduces the washout caused by the mucociliary clearance; thus, the stay in contact with the mucosa is much more prolonged.

The single components include:

- Icelandic moss (*Cetraria islandica*) contains mucoadhesive polysaccharides (branched galactomannans) that improve the "barrier effect" of the formulation, as well as a high content (about 70%) of (1-3),(1-4)-α-D-glucans (lichenin and isolichenin) with biological activity (20). Icelandic moss also contains other secondary metabolites, including the α-lactonic acids protolichesterinic and fumarprotocetraric, fumaric acid, tannins, and peculiar terpenic substances, such as cetraric acid, which have antibiofilm and antioxidant activities (21).

- Larch (*Larix decidua*) contains neutral polysaccharides (arabinogalactans) having a structure of highly branched 6-*β*-*D*-Galactans, characterized by muco-adhesivity (22,23). The protective, lenitive, and emollient activities provided by the mucilaginous

Table I. *Classification of rhinitis*

Infectious rhinitis (acute, chronic)
Allergic rhinitis (seasonal, perennial)
Non-allergic rhinitis (with eosinophils NARES, with mast cells, with mast cells and eosinophils, with neutrophils)
Vasomotor rhinitis
Atrophic rhinitis
Medicamentosa rhinitis
Hormonal rhinitis

polysaccharides depend on a mechanical pathway, including the hydration of the mucus layer promoting mucus discharge. Moreover, various components (phenols, polyphenols, saponins, carotenoids, and lipids) contrast the bacterial attachment and exert an antioxidant effect.

-Sea buckthorn (*Hippophaë rhamnoides*) is a plant growing in Europe and Russia. In traditional medicine, the leaves are used as astringent (24). Young leaves and buds of the plant are very rich in flavonoids, tannins, and triterpenes: the total phenolics in the fresh leaves expressed as gallic acid equivalents are about 1.3-1.6% w/w. Tannins exert antiviral activity. The leaves also contain flavonoids, inhibiting the pathogen's virulence, mitigating symptoms, and accelerating the healing. Tannins exert astringent activity and interact with the viral capsid proteins, with the formation of insoluble complexes and precipitation. Consequently, tannins may prevent viral infections and block the exit of the virus after replication (25,26). In addition to antiviral effects, tannins may disrupt or prevent the formation of bacterial biofilms (27). Furthermore, flavonoid glycosides and flavanols exert a significant antioxidant effect, hastening the mucosal recovery in damaged tissues (28).

Other components

Seawater has decongestant, anti-inflammatory, and antimicrobial activities; it also promotes epithelial repair (29).

Panthenol (also called pantothenol) is the alcohol analogue of pantothenic acid (vitamin B_5) and is a provitamin of B_5. In organisms, it is quickly oxidized to pantothenic acid. It is a viscous, transparent liquid at room temperature. Panthenol is used as a moisturizer to improve wound healing in pharmaceutical and cosmetic products (30). It improves hydration, reduces inflammation, and accelerates mucosal wounds' rate of healing (31). Panthenol readily penetrates the mucous membranes (including the intestinal mucosa), quickly oxidized to pantothenic acid. It is also used in the biosynthesis of coenzyme A, which controls a wide range of enzymatic reactions.

Peppermint essential oil and pure menthol, and anethole have been traditional remedies against respiratory ailments for a long time. Laboratory and clinical studies have shown that spray formulations containing aromatic essential oils, already at low doses, have a balsamic effect which in a short time can induce great improvements in symptom severity (32).

Conclusive remarks

Rhinitis is an umbrella term including many upper airway disorders. Common cold, allergic rhinitis and vasomotor rhinitis indeed are the most prevalent types of rhinitis. As a result, there is increasing attention to natural products that are effective and safe. In this context, Rinalt® is a multi-component medical device indicated for the adjunctive treatment of rhinitis. Its components exert many activities, including antimicrobial, anti-inflammatory, antioxidant, decongestant, and mucolytic properties. Therefore, this product may be fruitfully used in clinical practice.

Conflict of interest:

All authors state that there is no conflict of interest.

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A multicomponent food supplement with bromelain, escin, and selenium in patients with urological medical disorders

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Urological disorders are very common and affect any age. The most common medical conditions include cystitis, urolithiasis, renal colic, and prostatitis. Broser® is a food supplement containing bromelain, escin, and selenium. Its efficacy has been positively tested in patients with otorhinolaryngological disorders. Therefore, the present clinical experience investigated the efficacy and safety of this product in patients with medical urological disorders and treated with standard therapy. The add-on Broser® course lasted 2 weeks. The oral nutraceutical was taken following the specific indications, such as two tablets/daily. Patients were visited at baseline (T0) and after the treatment (T1). Patients in Group A had to take Broser® one tablet b.i.d. per 20 days. Evaluation parameters were the following signs and symptoms: pain, functional powerlessness, fever, oedema, malaise, dysuria, and infection severity. Globally, 1,204 patients participated in this clinical experience. The median age was 52 years. The food supplement significantly reduced the symptom severity and the incidence of symptoms. In conclusion, the present clinical experience demonstrated that the add-on therapy with Broser® significantly reduced the severity and incidence of urological complaints. Therefore, this food supplement could be fruitfully used in clinical practice.

Urological disorders represent a common condition in men (1). Different diseases may affect men, but prostatitis is particularly frequent as up to 50% of men report being affected by symptoms suggesting prostatitis during their lifetime (2). Prostatitis is a group of clinical syndromes which has been categorized by the United States National Institutes of Health (NIH) into several distinct entities as follows: acute bacterial prostatitis (ABP; category I), chronic bacterial prostatitis (CBP; category II), chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), inflammatory type (category IIIA) and noninflammatory type (category IIIB), and asymptomatic inflammatory prostatitis (category IV), as recently pointed out (3, 4).

Also, urolithiasis is a common condition that requires a timely workup as symptoms are usually very intense, and complications may occur (5). In addition,

Keywords: medical urological disorders, bromelain, escin, selenium, real-life study

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urinary tract infections, mainly concerning cystitis, are extremely frequently, mostly in women (6). Also, an infection may significantly worsen up and become life-threatening (7). As a result, adequate management needs prompt symptom relief and resolution of infectious events, including antibiotics (8).

As an inflammatory reaction is associated with these urological disorders, the use of nutraceuticals, provided with anti-inflammatory activity, has been proposed since long ago. In this regard, a multicomponent food supplement (Broser®) has been recently marketed.

Broser® tablet currently contains bromelain 100 mg, escin 30 mg, and selenium 42.5 mcg. This nutraceutical exerts anti-inflammatory, anti-oedema, antioxidant, and draining activities thanks to the active components.

Bromelain is a complex mixture of protease extracted from the fruit or stem of the pineapple plant (9). Bromelain gained universal acceptability as a phytotherapeutic agent due to its history of safe use and lack of side effects. The beneficial effects are due to multiple factors. Bromelain increases bioavailability and reduces the side effects associated with various antibiotics. Furthermore, bromelain acts as an immunomodulator and has anti-oedematous, anti-thrombotic, and anti-inflammatory activity. Bromelain has the *in vitro* ability to modulate surface adhesion molecules on T cells, macrophages, and natural killer cells and may also induce the secretion of IL-1β, IL-6, and tumour necrosis factor α (TNFα) by peripheral blood mononuclear cells (10). Furthermore, Bromelain influences blood coagulation by increasing the serum fibrinolytic ability and inhibiting the synthesis of fibrin, a protein involved in blood clotting (11). In addition, bromelain can reduce the average number of days for the complete disappearance of pain and post-surgery inflammation.

Escin is the major active principle from *Aesculus hippocastanum* (Hippocastanaceae), such as the horse chestnut tree (12). Escin is a natural mixture of triterpene saponins. Escin effectively prevents the formation of oedema in models of inflammation that reproduce the initial exudative phase. The mechanism of the anti-oedematous effect, in addition to the previously mentioned sensitization to Ca ions, that results in a 'sealing effect' on small vessels permeable to water, has also been related to reduced hypoxia-induced activation of human endothelial cells. Escin can well antagonize the reduction in ATP content and increased phospholipase A2 responsible for releasing precursors of inflammatory mediators. There is, furthermore, a reduced neutrophil adherence/activation, all resulting in the protection of veins and reduced oedema. Escin also exerts antiviral and antiallergic activity (13).

Selenium is an essential trace element that exerts many functions, including anti-inflammatory activity (14). Epidemiological data suggest a positive association between selenium deficiency and chronic inflammation. Selenium supplementation of patients with chronic disorders has improved their health status and quality of life. In septic shock patients with acute inflammation accompanied by severe pathology, selenium supplementation at high concentrations lessened mortality and improved health status (14). Adequate concentrations of selenium are essential for initiating immunity and regulating excessive immune responses and chronic inflammation. Selenium-dependent effects are mediated via the downregulation of the redox-sensitive transcription factor NF-kB and the epigenetic control of gene expression regulation (9). The selenium-metabolites modulate cell signalling, DNA methylation (directly or through one-carbon metabolism), histone acetylation, and finally, gene expression (14).

Based on this background, an Italian survey explored the effectiveness of Broser® effectiveness in patients with upper airway diseases (15). The outcomes were exciting so that this food supplement is commonly used in otolaryngological practice. Furthermore, based on this background, a nationwide survey investigated the Broser® use in patients with urological disorders.

MATERIALS AND METHODS

The current survey was conducted in urological Italian ORL clinics, distributed in the whole of Italy, so assuring a wide and complete national coverage during the fall-winter 2020-2021. Furthermore, these Italian urologists were asked to recruit all consecutive patients visited because of common urological problems, mainly prostatitis, urolithiasis, and cystitis.

Patients were consecutively recruited during the specialist visit. The inclusion criteria were: to have a diagnosis of the inflammatory urological disorder, both genders, and adulthood. Exclusion criteria were to have comorbidities able to interfere with the evaluation of outcomes. As this survey was based on real-world practice, the doctors had the liberty to choose the preferred medications based on the best practice. The patients were subdivided into Group A taking Broser® and Group B without supplementation that served as a control group. The group assignment was random on a 1:1 ratio.

All patients signed informed consent. All the procedures were conducted in a real-world setting. The treatment course lasted 20 days. The oral nutraceutical was taken following the specific indications, such as two tablets/ daily. Patients were visited at baseline (T0) and after the treatment (T1). Patients in Group A had to take Broser® one tablet b.i.d. per 20 days.

Evaluation parameters were the following signs and symptoms: pain, functional powerlessness, fever, oedema, malaise, dysuria, and infection severity. Their severity was measured by a six-point scale, where 0=no symptom,

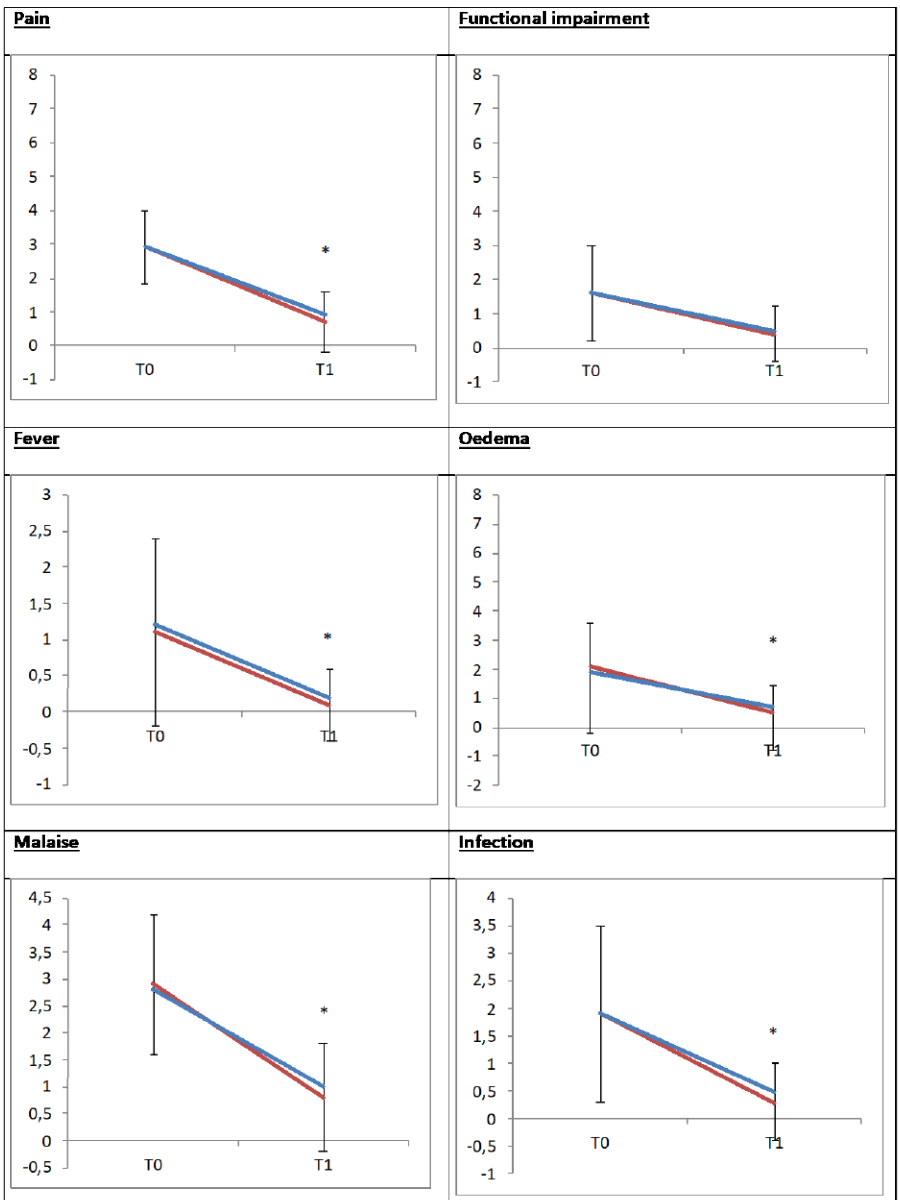

Fig. 1. *Visual analogic scale scores before (T0) and after add-on therapy (T1) with Broser® in Group A (in red) and Group B*

1=mild, 2=medium; 3=moderate; 4=more than moderate; and 5=severe.

Also, urologists evaluated the effectiveness of the treatment as resolved, improved, unchanged. Compliance was also assessed as good or bad.

Safety was measured by reporting the occurrence of adverse events.

RESULTS

Globally, 1,204 patients participated in this clinical experience. The median age was 52 years (Interquartile range: 39-65). Patients were stratified into Group A, including 617 (51.3%) patients and Group 586 (48.7%). In Group A, the main diagnoses were: urolithiasis (57 subjects, 9.2%), cystitis (87, 14.1%), deferential cystitis (73, 11.8%), renal colic (91, 14.8%), and prostatitis (284, 46%). In Group B, 318 (patients were treated with antibiotics, urolithiasis (56, 9.6%), cystitis (92, 15,7%), deferential cystitis (65, 11.1%), renal colic (92, 15.7%), and prostatitis (258, 44%).

About the medical treatment in Group A, 318 (51.5%) were treated with antibiotics, 160 (25.9%) with NSAID, and 57 (9.2%) with antipyretics. In Group B, 327 (55.8%) patients took antibiotics, 303 (51.7%) NSAID, and 52 (8.9%) antipyretics.

Regarding the intragroup analysis, all symptoms significantly decreased in both groups at T1 ($p<0.001$ for all).

The intergroup analysis showed that patients in Group A experienced less severe symptoms at T1 than controls, as reported in Fig. 1. Consistently, patients in Group A had significantly less frequent symptoms than controls, as shown in Fig. 2. In addition, the product was well-tolerated, and no clinically relevant adverse events were reported.

DISCUSSION

Medical urological disorders frequently affect the global population at any age. Consequently, these problems require adequate attention and should be timely treated as they sometimes could be serious.

The current clinical experience explored the effectiveness and safety of add-on therapy with a multicomponent food supplement (Broser®).

The outcomes were promising as the perception of symptom severity of all complaints but functional impairment significantly decreased after the add-on course. Consistently, the percentage of patients with symptoms, but functional impairment, significantly diminished after the complementary therapy.

In particular, patients taking this multicomponent product experience less severe pain, malaise, and dysuria. In addition, fever, oedema, and infectious severity were significantly less than patients treated with standard therapy alone. Moreover, about 20-30% fewer subjects had urological symptoms.

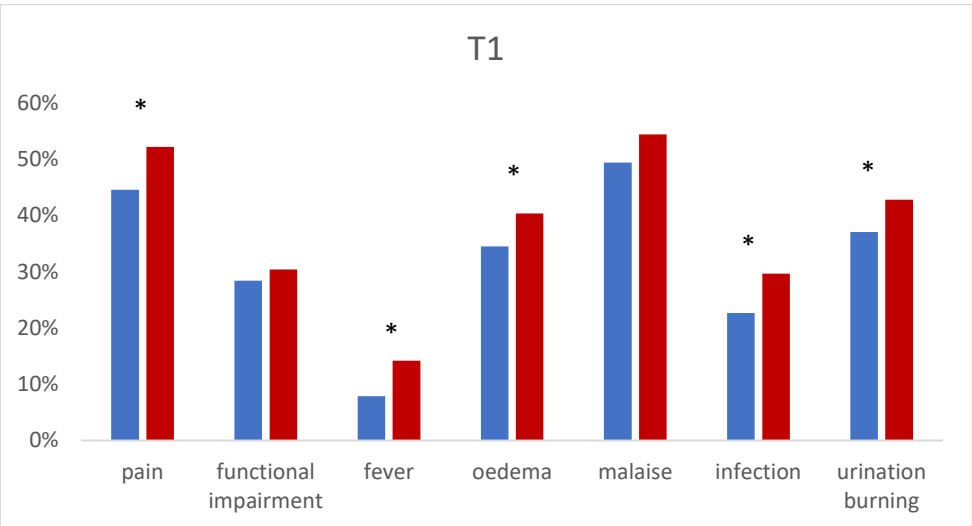

Fig. 2. *Prevalence of urological complaints after add-on therapy (T1) with Broser® in Group A (in blue) and Group B*

These relevant results depend on the active component contained in the product. In particular, bromelain, escin, and selenium exert relevant anti-inflammatory activities that improve clinical features.

Notably, the add-on treatment did not cause clinically relevant adverse events and was well tolerated.

Of course, the present clinical experience was conducted practically without rigorous methodology. As a result, further studies should be conducted to confirm these preliminary findings. However, a large number of enrolled patients and the real-life setting assure a practical relevance of the results as they mirror what happens in clinical practice.

In conclusion, the present clinical experience demonstrated that the add-on therapy with Broser® significantly reduced the severity and incidence of urological complaints. Therefore, this food supplement could be fruitfully used in clinical practice.

Conflict of interest:

All authors state that there is no conflict of interest.

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A multicomponent food supplement with bromelain, escin, and selenium in patients with urological post-surgical complaints

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Urological surgery is common at any age. The most common surgical procedures include urological surgery, endoscopy, genitals surgery, and cystoscopic procedures. Broser® is a food supplement containing bromelain, escin, and selenium. Its efficacy has been positively tested in patients with otorhinolaryngological disorders. Therefore, the present clinical experience investigated the efficacy and safety of this product in patients undergoing urological surgery and treated with standard therapy. The add-on Broser® course lasted 2 weeks. The oral nutraceutical was taken following the specific indications, such as two tablets/daily. Patients were visited at baseline (T0) and after the treatment (T1). Patients in Group A had to take Broser® one tablet b.i.d. per 20 days. Evaluation parameters were the following signs and symptoms: pain, functional powerlessness, fever, oedema, malaise, dysuria, and infection severity. Globally, 697 patients participated in this clinical experience. The median age was 58 years. The food supplement significantly reduced the symptom severity and the incidence of symptoms. In conclusion, the present clinical experience demonstrated that the add-on therapy with Broser® significantly reduced the severity and incidence of symptoms consequent to urological surgery. Therefore, this food supplement could be fruitfully used in clinical practice.

Urological surgery is frequent enough in men and commonly includes surgery for benign prostatic hypertrophy, prostate cancer, bladder cancer, prostate adenoma, urolithiasis and kidney cancer (1, 2). Urological units are widespread, and many men undergo surgery. As a result, post-surgical problems are very common. Pain, urinary symptoms, fever, malaise frequently affect patients, and other complaints may occur (3, 4). Also, endoscopic procedures, including catheterizing, frequently cause inflammation and potentially infections of the urinary tract (5-9).

Therefore, inflammation is the main pathophysiologic event associated with urological surgery. Therefore, the use of nutraceuticals, provided with anti-inflammatory activity, is common practice.

Keywords: urological surgery, bromelain, escin, selenium, real-life study

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In this regard, a multicomponent food supplement (Broser®) has been recently marketed.

Broser® tablet currently contains bromelain 100 mg, escin 30 mg, and selenium 42.5 mcg. This nutraceutical exerts anti-inflammatory, anti-oedema, antioxidant, and draining activities thanks to the active components.

Bromelain is a complex mixture of protease extracted from the fruit or stem of the pineapple plant (10). Bromelain gained universal acceptability as a phytotherapeutic agent due to its history of safe use and lack of side effects. The beneficial effects are due to multiple factors. Bromelain increases bioavailability and reduces the side effects associated with various antibiotics. Furthermore, bromelain acts as an immunomodulator and has anti-oedematous, anti-thrombotic, and anti-inflammatory activity. Bromelain has the *in vitro* ability to modulate surface adhesion molecules on T cells, macrophages, and natural killer cells and may induce the secretion of IL-1β, IL-6, and tumour necrosis factor α (TNFα) by peripheral blood mononuclear cells (11). Furthermore, Bromelain influences blood coagulation by increasing the serum fibrinolytic ability and inhibiting fibrin synthesis, a protein involved in blood clotting (11). In addition, bromelain can reduce the average number of days for the complete disappearance of pain and post-surgery inflammation.

Escin is the major active principle from *Aesculus hippocastanum* (Hippocastanaceae), such as the horse chestnut tree (12). Escin is a natural mixture of triterpene saponins. Escin has been shown to be effective in preventing the formation of oedema in models of inflammation that reproduce the initial exudative phase. The mechanism of the anti-oedematous effect, along with the sensitization to Ca ions resulting in a 'sealing effect' on small vessels permeable to water, has also been related to reduced hypoxia-induced activation of human endothelial cells. Escin can well antagonize the reduction in ATP content and increased phospholipase A2 responsible for releasing precursors of inflammatory mediators. There is, furthermore, a reduced neutrophil adherence/activation, all resulting in the protection of veins and reduced oedema. Escin also exerts antiviral and antiallergic activity (13).

Selenium is an essential trace element that exerts many functions, including anti-inflammatory activity (14). Epidemiological data suggest a positive association between selenium deficiency and chronic inflammation. Selenium supplementation of patients with chronic disorders has improved their health status and quality of life. In septic shock patients with acute inflammation accompanied by severe pathology, selenium supplementation at high concentrations lessened mortality and improved health status (14). Adequate concentrations of selenium are essential for initiating immunity and regulating excessive immune responses and chronic inflammation. Selenium-dependent effects are mediated via the downregulation of the redox-sensitive transcription factor NF-kB and the epigenetic control of gene expression regulation (15). The selenium-metabolites modulate cell signalling, DNA methylation (directly or through one-carbon metabolism), histone acetylation, and gene expression (15).

Recently, an Italian survey explored the effectiveness of Broser® in patients with otorhinolaryngological diseases, including post-surgical conditions (16). The survey provided exciting outcomes. In addition, bromelain is currently used in oral surgery, mainly concerning the third molar surgery (17-19). The rationale of its use is based on anti-inflammatory and fibrinolytic activity. Recently, a clinical study explored the activity of a non-pharmacological compound in patients undergoing urological surgery (20). Therefore, based on this background, a nationwide survey investigated the Broser® use in patients with post-surgical urological problems.

MATERIALS AND METHODS

The current survey was conducted in urological Italian ORL clinics, distributed in the whole of Italy, so assuring a wide and complete national coverage during the fall-winter 2020-2021. These Italian urologists were asked to recruit all consecutive patients undergoing a urological procedure, mainly including urological surgery, endoscopic surgery, cystoscopy, and cystoscopic procedures.

Patients were consecutively recruited during the specialist visit. The inclusion criteria were: to have a

diagnosis of an inflammatory urological disorder, both genders, and adulthood. Exclusion criteria were to have comorbidities able to interfere with the evaluation of outcomes. As this survey was based on real-world practice, the doctors had the liberty to choose the preferred medications based on the best practice. The patients were subdivided into Group A taking Broser® and Group B without supplementation that served as control group. The group assignment was random on a 1:1 ratio.

All patients signed informed consent. All the procedures were conducted in a real-world setting. The treatment course lasted 2 weeks. The oral nutraceutical was taken following the specific indications, such as two tablets/daily. Patients were visited at baseline (T0) and after the treatment (T1). Patients in Group A had to take Broser® one tablet b.i.d. per 20 days.

Evaluation parameters were the following signs and symptoms: pain, functional powerlessness, fever, oedema, malaise, dysuria, and infection signs. Their severity was measured by a six-point scale, where 0=no symptom, 1=mild, 2=medium; 3=moderate; 4=more than moderate; and 5=severe. Also, urologists evaluated the effectiveness of the treatment as resolved, improved, unchanged. Compliance was also assessed as good or bad. Safety was measured by reporting the occurrence of adverse events.

The statistical analysis was performed using the non-parametric Mann-Whitney test. The statistical program was STATA, v 15.1, distributed by StataCorp, College Station, Texas, 77845, USA.

RESULTS

Globally, 697 patients participated in this clinical experience. The median age was 58 years

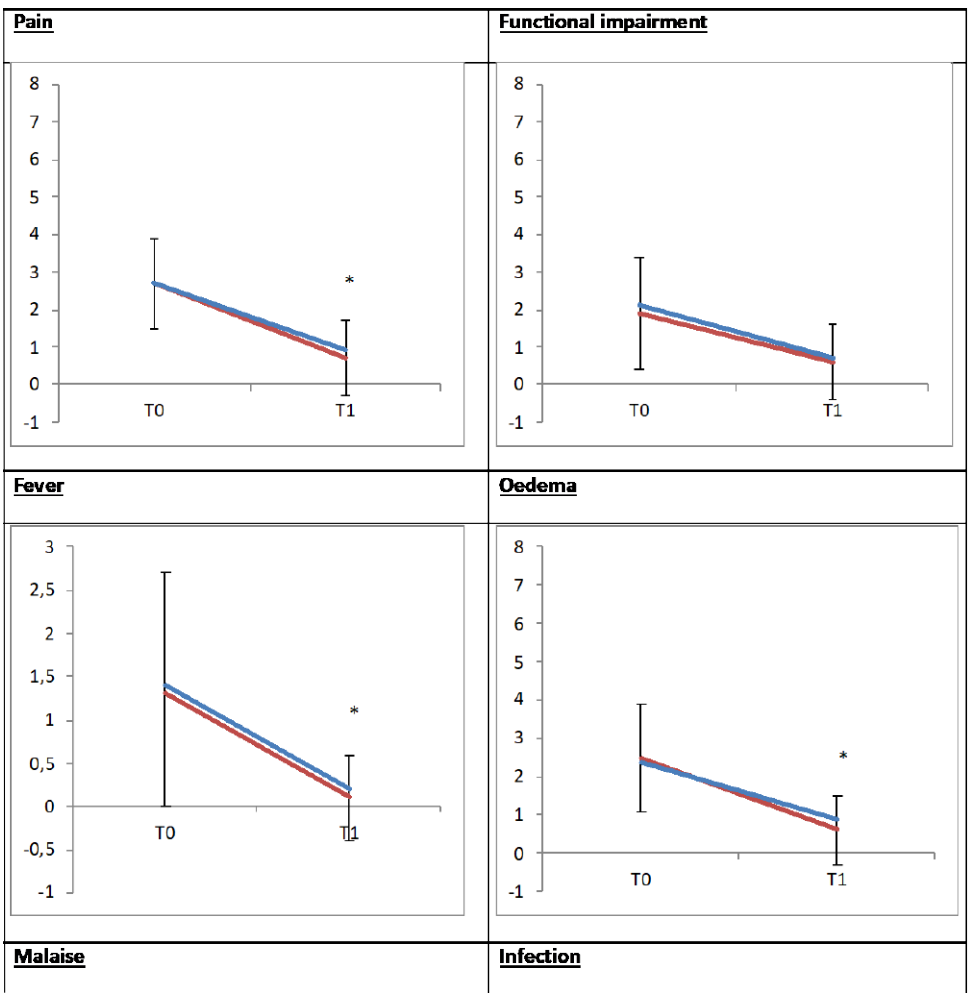

Fig. 1. *Visual analogic scale scores before (T0) and after add-on therapy (T1) with Broser® in Group A (in red) and Group B*

(Interquartile range: 45-71). Patients were stratified into Group A, including 356 (51.1%) patients and Group B, 341 (48.9%). In Group A, the main operations were: urological surgery or endoscopy (114 subjects, 32%), genitals surgery (141, 39.6%), and cystoscopic procedures (71, 19.9%). In Group B, 113 (33.1) patients had urological surgery or endoscopy, 122 (35.8%) genitals surgery, and 84 (24.6%) cystoscopic procedures.

About the medical treatment in Group A, 250 (70.2%) were treated with antibiotics, 73 (20.5%) with NSAID, and 38 (10.7%) with antipyretics. In Group B, 252 (73.9%) patients took antibiotics, 163 (47.8%) NSAID, and 43 (12.6%) antipyretics.

Regarding the intragroup analysis, all symptoms significantly decreased in both groups at T1 ($p<0.001$ for all).

The intergroup analysis showed that patients in Group A experienced less severe symptoms at T1 than controls, as reported in Fig. 1. Consistently, patients in Group A had significantly less frequent symptoms than controls, as shown in Fig. 2. In addition, the product was well-tolerated, and no clinically relevant adverse events were reported.

DISCUSSION

Urological surgery procedures are common in the global population at any age. Consequently, these conditions require adequate attention and should be timely treated as they sometimes could be serious.

The current clinical experience explored the effectiveness and safety of add-on therapy with a multicomponent food supplement (Broser®).

The outcomes were very promising as the perception of symptom severity of all complaints but functional impairment significantly decreased after the add-on course. Consistently, the percentage of patients with symptoms, but functional impairment, significantly diminished after the complementary therapy. In particular, patients taking this multicomponent product experience less severe pain, malaise, and dysuria. In addition, fever, oedema, and infectious severity were significantly less than patients treated with standard therapy alone. Moreover, about 15-30% fewer subjects had urological symptoms.

These relevant results depend on the active component contained in the product. In particular,

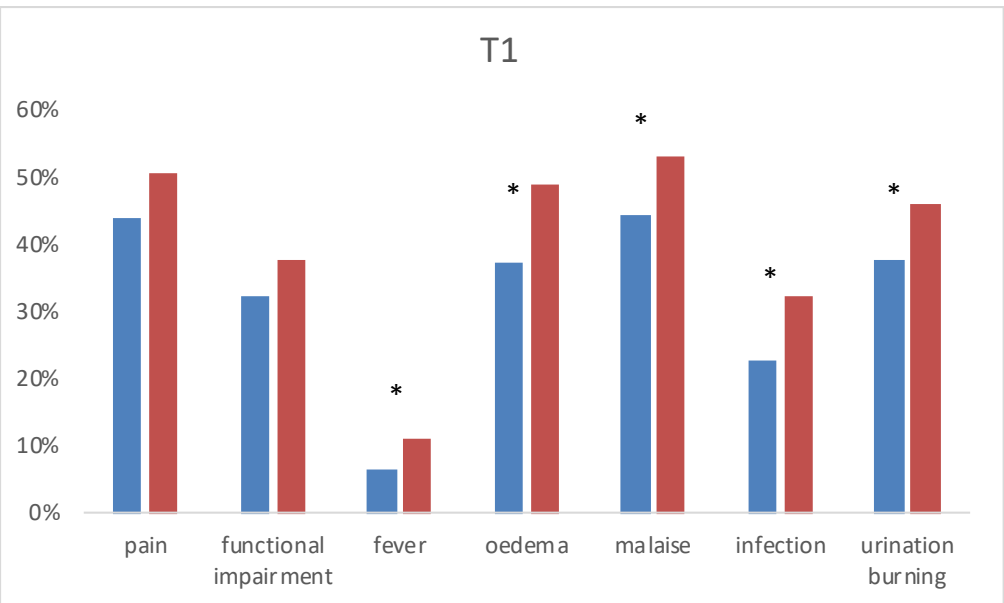

Fig. 2. *Prevalence of urological complaints after add-on therapy (T1) with Broser® in Group A (in blue) and Group B.*

bromelain, escin, and selenium exert relevant anti-inflammatory activities that improve clinical features. Notably, the add-on treatment did not cause clinically relevant adverse events and was well tolerated.

Of course, the present clinical experience was conducted practically without rigorous methodology. As a result, further studies should be conducted to confirm these preliminary findings. However, the large number of enrolled patients and the real-life setting assured a practical relevance of the results as they mirror what happens in clinical practice.

In conclusion, the present clinical experience demonstrated that the add-on therapy with Broser® significantly reduced the severity and incidence of symptoms consequent to urological surgery. Therefore, this food supplement could be fruitfully used in clinical practice.

Conflicts of interest statement:

All authors state that there is no conflict of interest.

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Wound healing: a new management strategy

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Wound healing is a complex process whose understanding is constantly evolving. Until recently, the wound healing mechanism was imagined as a fibroproliferative response for producing a scaring, such as a repairing process. Recently, great attention has been addressed to biomaterials, stem cells, and bioengineered tissues. In this context, a new healthcare branch has developed: Regenerative Medicine. The healing process consists of three phases: the inflammatory reaction, cell proliferation (excessive or impaired), and extracellular matrix (ECM) remodelling. However, wound repair is usually characterised by the incomplete regeneration of the original tissue with hyperproduction of organised collagen, which can lead to new tissue production, with an 80% similarity to the original tissue. Also, impaired reparation leads to an abnormal fibroproliferative response, causing hypertrophic or keloid scars. Regenerative Medicine has promoted the study of tissue regeneration, mainly concerning the use of stem cells and electromagnetic applications.

Consequently, the research developed *in vitro* and *in vivo* cellular models to investigate the tissue repair and regeneration process. Particular interest has been pointed to the role of humoral factors (cytokines and growth factors), genetic factors (genes expressed in the various phases of the process), and cellular factors. In addition, attention has been paid to fibroblasts, collagen, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs), agents fundamental for the extracellular matrix (ECM) remodelling. Thus, the new perspective shifted from inflammation to regeneration. This idea concerns treating the patient with skin and mucosal wounds, using local anti-inflammatory compounds targeting endothelial cells, ECM, anti-inflammatory cytokines, and MMPs. The regeneration pathway starts from the platelet activation leading to the recruitment of neutrophils (natural "debrider"), the release of TGF-β1, PDGF, TNF-α, and IL-1, and regulation of adhesion molecules expression. Macrophages are further recruited to ensure sustained debridement, release proinflammatory cytokines, and amplify the fibroproliferative response in the context of chronic inflammation. Macrophages include the M1 subpopulation, which is devoted to eliminating invading microorganisms and promoting the early inflammatory response. The M2 subpopulation aims to eliminate damaged cells and tissues and promote neo-angiogenesis and tissue remodelling. In addition, ECM may amplify some mechanisms of the healing process. In conclusion, Regeneration Medicine represents the keystone for a new holistic approach to wound management.

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The healing process consists of three phases: the inflammatory reaction, cell proliferation (excessive or impaired), and extracellular matrix (ECM) remodelling. However, wound repair is characterised

Keywords: wound, healing, regenerative medicine

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by the incomplete regeneration of the original tissue with hyperproduction of organised collagen, which can lead to new tissue production, with an 80% similarity to the original tissue. Impaired reparation leads to an abnormal fibroproliferative response, causing hypertrophic or keloid scars.

Regenerative Medicine has promoted the study of tissue regeneration, mainly concerning the use of stem cells and electromagnetic applications. Consequently, the research developed *in vitro* and *in vivo* cellular models to investigate the tissue repair and regeneration process. Particular interest has been pointed to the role of humoral factors (cytokines and growth factors), genetic factors (genes expressed in the various phases of the process), and cellular factors. In addition, attention has been paid to fibroblasts, collagen, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs), agents fundamental for the extracellular matrix (ECM) remodelling.

The wound repair aims to control excessive inflammation, including proliferation, differentiation, and function of inflammatory cells. The new perspective shifted from inflammation to regeneration. This idea concerns the treatment of the patient with skin and mucosal wounds, using local anti-inflammatory compounds targeting endothelial cells, ECM, anti-inflammatory cytokines, and MMPs.

The regeneration pathway starts from the platelet activation leading to the recruitment of neutrophils (natural "debrider"), the release of TGF-β 1, PDGF, TNF-α,andIL-1,andtheregulationofadhesionmolecules expression. Further, macrophages are recruited to ensure sustained debridement, release proinflammatory cytokines, and amplify the fibroproliferative response in the context of chronic inflammation. Macrophages include the M1 subpopulation devoted to eliminating invading microorganisms and promoting the early inflammatory response. The M2 subpopulation aims to eliminate damaged cells and tissues and promote neo-angiogenesis and tissue remodelling. In addition, ECM may amplify some mechanisms of the healing process.

Regenerative Medicine: a new approach

The clinical approach to the patients with wounds should first consider the underlying conditions, comorbidities, nutritional status, and concomitant medical treatments. Consistently, the repair, regeneration, and healing process are highly individual. In addition, the response singularity depends on specific immunopathological pathways (6).

The innate immune response lasts from 2 hours to 5 days and marks the inflammatory phase. Cell migration and inter-cellular and cell-cell matrix adhesion occur. After one week, the cellular proliferation phase begins. It involves granulation of the newly formed tissue and change of the cell populations, such as the transition from M1 to M2, blockage of monocytes, increase in fibroblasts, and deposition of type III and VII collagen. Wound retraction and re-epithelialisation characterise this phase. The remodelling phase increases collagen deposition and tensile strength, substituting collagen type III by type I. These biological processes may overlap.

A physiological repair may be defective because of different causes. A defect incurs chronic non-responding skin lesions in ECM remodelling. Abnormal collagen deposition blocks the action of fibroblasts, and re-epithelialisation is halted, resulting in an inflammatory process that becomes chronic. Any disruption of the combined and synergistic action between cytokines and M1 and M2 macrophages supports non-healing. In chronic lesions, proteases alter the granulation tissue, stopping cell migration for scarring.

The role of M1 macrophages in the inflammatory process and M2 macrophages in the repair and regeneration process is still debated, especially about the disrupted M1/M2 ratio associated with chronicization. Understanding the M1/M2 switching is vital to genotype and type the receptors of the two subpopulations. The M2 population is divided into four subclasses, M2a, M2b, M2c, and M2d. The type of polarisation depends on two transcription regulators, the interferon regulatory factors IRF5 and IRF4. A correlation has been demonstrated between IFR5, high levels of M1, and inflammation, and, also, between IRF4 and M2.

The Anti-Inflammatory Regenerative Medicine (AIMED) Protocol

The care and treatment of non-healing wounds is the main challenge for Regenerative Medicine (7,8). The non-healing wounds have a significant burden on healthcare expenditure, including hospital and social

spending and management complexity. In addition, it is difficult to treat wounds to have a profound impact on the costs, well-being, and quality of life of both patients and their families.

The skin is a significant barrier, protecting from numerous agents that may damage the body. In addition, the skin system is a defence mechanism to maintain a balance by involving various molecular, cellular, immune, endocrine, and neurological mechanisms. Understanding these mechanisms has led to the development of numerous new drugs and medical devices for skin diseases.

The wound repair and regeneration require a complex network that often may stall, so impairing healing. A Regenerative Medicine Center (RMC) cares for patients with a clinically relevant wound. A chronic skin wound outpatient clinic requires a multidisciplinary approach to treat vascular lesions, pressure lesions, diabetic foot lesions, autoimmune and rheumatic lesions, post-surgical skin lesions, burns, and scars.

The skin regeneration damaged by multiple *noxae* is possible by interacting with the outside environment and constantly renewing. Namely, the skin can self-repair and react to lesions by epidermal stem cells located in the dermis and epidermis. In patients with recalcitrant skin lesions, comorbidities, such as chronic disease, diabetes, vascular insufficiency, peripheral oedema secondary to heart failure, malnutrition, bedsores, and infections can affect the responsiveness to treatments.

These different stages of repair are always present in all types of lesions, concerning their timescales, interaction, genetic, humoral, cellular, and ultrastructural mechanisms. In addition, a key role is played by fibrin deposition and hydration of the matrix by hyaluronic acid, stimulating the production of fibroblasts. In addition, however, other cell types (granulocytes, monocytes, M1 macrophages) and cytokines play a role in shutting the inflammatory, proliferative phase (endothelial cells, fibroblasts, and keratinocytes) the ECM remodelling phase.

From repair to regeneration: Regeneration 3.0 (the Prometheus Project)

The RMC aimed to offer an innovative solution to the current difficulties in managing non-healing skin lesions. In the research progress, we defined Regeneration 1.0, meaning a dressing process involving advanced dressings. This type of dressing is required to maintain an adequate wound moisture level, be partly or totally occlusive, and passively absorb the exudate, with a function determined by the patient's metabolism and biological "performance."

Regeneration 2.0, in contrast, involves the use of bioactive dressings with a biological action on the wound (hyaluronic acid, collagen, silver, etc.). From this perspective, the RMC investigated a sterile gauze dressing with bioactive substances (hyaluronic acid, carnosine). Namely, persistent inflammation exerts a relevant role in non-healing wounds. In particular, nitric oxide contrasts the harm caused by ROS that, through systemic or topical treatment with antioxidants (carnosine), can be turned around in non-responding lesions. Moreover, bovine colostrum, at pH 6.8, acts against the tissue acidosis found in damaged tissues.

Regeneration 3.0 prioritises combining the anti-inflammatory activity of the nine proteins acting as growth factors in the bovine colostrum, the homeostatic, angiogenic, and reorganisational activities of the matrix, the modulation of collagen synthesis, and the remodelling of the epithelium. The choice of bovine colostrum relies on some properties: barrier action, anti-inflammatory action, pain relief, reduction and absorption of exudates, contrasting bacterial and fungal proliferation, antioxidant action, hydration, and protection against skin diseases and dermatosis.

The RMC treats patients with chronic wounds of various aetiologies using the AIMED model. This method assures: i) optimal wound management with great awareness of dressing protocols and the use of medications existing on the market, integrated into the AIMED model; ii) a rapid healing, iii) a prompt pain relief, iv) reduced complications, v) reduced healthcare expenditure, vi) a better adherence and patient's and family's satisfaction, and vii) a reduced rate of recurrence.

DISCUSSION

The study of biological molecules has enabled a glimpse of a possible new key for interpreting

biological phenomena linked to the management of the inflammatory process, and some such molecules could be prototypes for others still to come. The greater availability of water molecules around more hydrophilic molecules and the better organisation of the body's water seem to produce a more significant and better biological response. The study of the hydrodynamic behaviour of numerous molecules always comes back to the endothelium, ECM, and resident and inflammatory cells. In addition, the role of the cell membrane seems to be worthy of attention, as its structure enables substances to travel or be transported into the cell. The cytoskeleton is a system of canals (microtubules) that transport substances and information is transmitted; it occurs as the molecules, including water (up to 80% of the cytoskeleton), have a dipole moment, such as an electrically charged spatial distribution involving a positively charged pole and a negatively charged pole. All macromolecules become biologically active only if immersed in an aqueous matrix. Thus, it demonstrates the predominant role of water in living beings.

Stem cells deriving from adipose tissue have a migratory capacity that enables them to reach the target site through the blood, enabling rapid access to the entire body. The target organs then capture them through complex interactions with endothelial cells to leave the circulation for tissue regeneration. Thus, stem cells could offer an opportunity for the regenerative treatment of skin lesions but within the scope of holistic principles. However, stem cells only work after transplantation if they can communicate with the stem cells already resident in the tissue, acting as a starter and stimulating the existing cells through the cell membrane. The limitation of this technique is the low efficiency of the differentiation process and the oncogenic risk caused by the use of viral vectors.

Moreover, it has to be underlined that the wound healing process is a complex process intertwined with the biological mechanisms causing individuals to become ill. Systemic and local factors combine to cause the process to become chronic and perpetuate itself in the skin wound, an expression of it all. Bacterial infections, which are difficult to combat due to antibiotic resistance, greatly complicate the roles of our innate immune system and lymphocytes.

In conclusion, we know the key players in the wound healing process, and we have new molecules available to act on them (9,10). However, the future must necessarily lie in the transfer of molecules and information between the endothelium, ECM, and cell membrane, which can be directed towards tissue regeneration if the resident stem cells have the chance of communicating and interacting with new therapeutic models; all this without forgetting the human being, at the centre of research and scientific evolution.

Conflict of interest:

All authors state that there is no conflict of interest.

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LumiHeal® and Anti-inflammatory Regenerative Medicine (AIMED): a new management strategy for shortening wound healing and effective outcomes, from Repair 1.0 to Wound Regeneration 4.0

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Wound healing may require a long-standing period. As a result, patients, including their families and society, must sustain a relevant burden. Moreover, patients have to remain disabled also for a long time. Therefore, the healthcare service has to expend applicable costs. In the past, the Regeneration Medicine 3.0 represented the keystone for a holistic approach to wound management. However, there was also the need to consider the socio-economic impact on hospital costs and regional management spending review. Consequently, it is demanding to revise the care strategies for patients with skin wounds integrated into hospital-territory stewardship. In this regard, an innovative approach (Wound Regeneration 4.0) permitted to obtain exciting outcomes. Namely, an integrated protocol, combining anti-inflammatory, regenerative medicine, shortened the duration of wound healing, improved the quality of life of patients and family and significantly diminished the costs. In this context, the LumiHeal® gel associated with multi-LED light exerts a series of activities that actively promote wound healing. Moreover, LumiHeal® gel is active in all phases of wound healing and is effective on both chronic and acute wounds. The current outcomes suggest a multidisciplinary approach based on clinical, pharmacological, and economic issues that represent a point of departure for a new phase, such as the Wound Regeneration 4.0.

The Regenerative Medicine: a new approach

Patients with wounds require adequate attention and a thorough workup, mainly if they have complex treating lesions (1). Consequently, the repair, regeneration, and healing process are highly individual. In addition, the response singularity depends on specific immunopathological pathways. Therefore, regenerative Medicine is appropriate for managing wounds, mainly in complicated patients (2). Anyway, Regenerative Medicine relies on well-defined standards and protocols. We would report the personal experience in managing patients with wounds applying an innovative approach.

The patient with a difficult-to-treat lesion, such as a lesion that does not heal and progress throughout the normal phases of recovery (inflammation, proliferation, and remodelling), constitutes the paradigm of the chronic patient (no responders). The chronic patient is in the position of having to tackle and coexist with one or more diseases over time. This situation should be adequately managed to ensure an acceptable quality of life. In addition, chronic illness entails health economic aspects which are scarcely measurable, both considering the expensive materials and wound chronicity over time.

Notably, the care of chronic wounds accounts near

Keywords: wound, healing, regenerative medicine, anti-inflammatory, NPWT, Regenerative medicine, LumiHeal®, wound regeneration 4.0.

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4% of the total costs of the national healthcare service. The costs for wounds and lesions account for 15-20% for the dressing, 30-35% for personnel costs, and 50% for hospitalization. Expenditure is increasing seeing that to care for 2 million Italians with pressure wound affects public health costs for almost 1 billion euros a year.

The management of difficult-to-treat wounds in the outpatient setting requires close collaboration between the hospital and territory to guarantee clinical care continuity, even in light of the new LEA (essential levels of assistance) proposed by each Region in the new regional care pathways for chronic and sub-acute patients.

Clinical care continuity should be guaranteed everywhere. A possible solution could be to create *"integrated hospital-territory diagnostic-therapeutic pathways"* for treating all chronic diseases, in which a multidisciplinary network of professionals can make a difference in the management, evolution, and outcome of the injury.

The personal experience

The personal experience concerned an innovative model of care based on an original therapeutic approach to skin lesions, called AIMED (anti-inflammatory, regenerative medicine). This protocol is based on the circularity of the different phases.

The regenerative medicine outpatient clinic (RMC Regenerative Medicine Center) for recalcitrant lesions was established in the summer of 2015. This clinical project initially brought together professionals with different skills with a single mission: globally care for difficult wounds, involving patients and their families. Personal motivation was the main characteristic common to all project members. Each professional built a new organizational model and a close-knit network of activities with their essential personal expertise. Each member has embraced the philosophy of caregiving, associating respect for the clinical priorities of the patients and their families, adopting national and international methods and guidelines for wound management.

The clinical management includes assessment of the lesion, global evaluation of the patient, management and removal of the cause leading to the formation of the lesion, application of validated and shared protocols, prevention and management of complications, ultrasound treatment of skin lesions to remove necrotic and fibrinous tissue, acting as a bactericide and stimulating tissue regeneration, surgical procedures for biopsies, surgical debridement, and removal of lesions with reconstruction and/or skin grafts, documentation of the procedures through shared records, the definition of protocols based on technological innovations and biomarkers, consultancy and cooperation with other units (hospital, hospice, and primary care), and promotion of research and teaching on this matter.

According to international guidelines, the lesions are classified by type and stage, and the various types of advanced treatment are assessed and selected concerning the type of lesion and the operating protocol, with attention to pharmacoeconomic issues.

From repair to regeneration: Regeneration 3.0 phase

The RMC aimed to offer an innovative solution to the current difficulties in managing non-healing skin lesions. In the research progress, we defined *Repair 1.0*, meaning a dressing process involving advanced dressings. This type of dressing is required to maintain an adequate wound moisture level, be partly or fully occlusive, and passively absorb the exudate, with a function determined by the patient's metabolism and biological "performance."

Repair 2.0, in contrast, involves the use of bioactive dressings with a biological action on the wound (hyaluronic acid, collagen, silver, etc.). From this perspective, the RMC investigated a sterile gauze dressing with bioactive substances (hyaluronic acid, carnosine). Namely, persistent inflammation exerts a relevant role in non-healing wounds. In particular, nitric oxide contrasts the harm caused by ROS that, through systemic or topical treatment with antioxidants (carnosine), can be turned around in non-responding lesions (3). Moreover, bovine colostrum, at pH 6.8, acts against the tissue acidosis found in damaged tissues.

Regeneration 3.0 prioritizes combining the anti-inflammatory activity of the nine proteins acting as growth factors in the bovine colostrum, the homeostatic, angiogenic, and organizational activities of the matrix, the modulation of collagen synthesis,

and the remodelling of the epithelium. The choice of bovine colostrum relies on some properties: barrier action, anti-inflammatory action, pain relief, reduction and absorption of exudates, contrasting bacterial and fungal proliferation, antioxidant activity, hydration, and protection against skin diseases and dermatosis.

The RMC treats patients with chronic wounds of various aetiologies using the AIMED model. This method assures: i) optimal wound management with great awareness of dressing protocols and the use of medications existing on the market, integrated into the AIMED model; ii) a rapid healing, iii) a prompt pain relief, iv) reduced complications, v) reduced healthcare expenditure, vi) a better adherence and patient's and family's satisfaction, and vii) a reduced rate of recurrence. With the aid of the AIMED protocol, this study has tried to harmonize the clinical approach to the patient with skin lesions by putting him/her at the care core. However, difficulties may be found both in the hospital, where there is organizational excellence (at I, II, and III levels), and territory, where the patient often does not find adequate answers. In addition, during the Repair 1.0 and 2.0 years, the healthcare expense reached excessive levels regarding materials, aids, and devices to treat the wounds. However, there was no real action on the systemic and local processes that determined the onset of the lesion. The costs for the human resources also increased as the organization was dispersive. As a result, the only outcome was limited to maintaining a sterile dressing process but not decisive.

With the Regeneration 3.0 phase, a lot has changed regarding the healing rate. For example, the introduction of negative pressure wound therapy (NPWT) has radically accelerated the healing of complex lesions. However, the organizational difficulties persisted, mainly concerning territorial medicine. So that the NPWT may increase the costs if managed at the primary care level (for example dressing should be changed up to 4 days, often impossible in the doctor's office). For example, a lesion infected by *Pseudomonas aeruginosa* or *Escherichia coli* can significantly worsen the patient's local and systemic conditions. Thus, success can be wasted as a never-ending task when the management moves from the hospital to the primary care setting. Therefore, the need arises to reconsider the care model and propose a new, such as the Wound Regeneration 4.0, in which the international guidelines must be able to combine a reflection on the strategic choices for optimizing care with two main objectives: reduction of healing times, pain and reduction of both health and social costs (in both cases with benefits for the patient and his family). In this perspective, it seems strategic to reflect on the pharmaceutical equipment for outpatient clinics dedicated to the care and treatment of lesions.

Wound Regeneration 4.0 experience

The current study had the following objectives:

disseminating, among all professionals, the knowledge of innovative methodologies and protocols shared by the hospital network and territory, together with an analysis of clinical and economic indicators;

also guarantee to patients treated at home the bio-photonic system, shortening the healing time and hospitalizations so having a cost reduction, also for the integrated house assistance (IHA);

use a shared language for a correct management of patients with complex injuries;

create an integrated pathway between all health workers and embrace a new way of thinking that we could define;

reduce the burden on hospitals by avoiding the improper use of emergency and first aid services for patients who always more often use them because they do not find adequate responses at home and territory from well-trained and qualified personnel;

spread the organizational model to facilitate an interchange network with a rapid circulation of patients inside;

reduce the global healthcare spending on difficult injuries.

In the specific pathway of the patient (treated in consultation when hospitalized, then followed as an outpatient, and then managed at home), it has been reached the objective to demonstrate the importance of strengthening the management process when in the hospital there is the involvement of a specialized team that interacts with the department staff and broadens the skills and methodology transversally towards the patient. In order to reduce the healing time and social health expenditure, the bio-photonic system was

initially used by the RMC team, which involved and trained colleagues of the ward and emergency room in the immediate use of the LumiHeal® device (4).

We are convinced that biophysical therapies are essential because these natural resources, including light at various frequencies, really help the patient at a systemic and topical level. The Multi-LED KT-L Lamp is a certified CE marked class IIa electromedical device, emitting non-coherent blue light with a single peak emission wavelength between 440-460nm. The device was delivered to the CRM free of charge (a loan for use).

The protocol used at home was the same employed for outpatients. The medical and nursing staff of the hospital and territory shared and applied the home protocol twice a week for two months. In this period, there was a careful verification of the progress carried out by the CMR team, to guarantee the bio-photonic therapy even at home. The various phases of the protocol have been previously reported in detail (4). In particular, step 3 consisted of photobiomodulation (PBM), such as biophysical therapy. Namely, PBM is an adjuvant treatment for stimulating regenerative cellular processes, reducing inflammation and pain, and biochemical modulation of molecular response. In addition, PBM is particularly indicated in patients with difficult-to-treat wounds. In this regard, personal experience used the LumiHeal® protocol.

The LumiHea® protocol involves using the LumiHeal System comprised of two components: a non-coherent multi-light-emitting diode (LED) light source (primary device); and a topical chromophore-containing photo converter gel. The LumiHeal System produces fluorescent light energy (FLE), a form of photobiomodulation (PBM), to stimulate healing (4-9). The multi-LED light source delivers non-coherent blue light with a single peak wavelength between 440 and 460 nm. The LumiHeal gel, applied to a thickness of 2 mm, contains chromophores able to absorb the blue light emitted from the multi-LED light source and, through a Stokes shift, emit FLE in the range of 450-610 nm. Different wavelengths have different skin penetration capacities and are known to modulate the healing response (4-9). Treatment consists of using the multi-LED light to illuminate the LumiHeal Photo converter gel applied on the wound for the duration of the treatment, after which the multi-LED light is turned off, and the gel removed. The LumiHeal® method allows an effective and rapid improvement of treated lesions with pain reduction of the inflammatory process combined with other treatments (10) through the modulation of the immune response and cell proliferation (5). Moreover, in the immediate clinical course, the lesion changes in appearance at the biofilm level, at the bottom level of the lesion, and at the margins.

LumiHeal® a new frontier in wound healing

Lumiheal® is a photo converter used in combination with a LED lamp (KT-L or KT-P50) to heal acute and chronic wounds. It can stimulate mitochondrial biogenesis and modulate the three healing phases (inflammation, proliferation, and remodelling).

Photobiomodulation

Visible light has been historically used to treat a vast range of skin and soft tissue disorders and wounds. The therapy with light at a low energy level is effective as it exerts a stimulating activity on biological processes, such as the PhotoBiomodulation (PBM). The PBM rationale is that the photons activate endogenous photo-acceptors that induce a cascade of molecular events (11,12). Usually, PBM techniques include low-level laser, LED, and broadband light lamps. The PBM therapy positively affects all phases of wound healing, including the growth of granulation tissue, collagen synthesis, and nitric oxide, acting as an anti-inflammatory agent and pain-reliever (13).

The biomodulation by fluorescent light energy (FLE) is a form of PBM type that can stimulate mitochondrial biogenesis (14). FB Dermatology Srl introduced the patented system, known for its emitted blue light and FLE to penetrate the skin and promote wound healing (15, 16).

A peculiarity of LumiHeal® is its capacity of acting on every phase of the healing process. LumiHeal® can have chronic wounds progress out of their stalled, chronically inflamed status and respond to treatment like acute wounds would do. Namely, the preclinical data demonstrated that PBM modulates the wound healing process. LumiHeal® gel re-activates the healing of difficult-to-treat wounds and

acute wounds, including traumatic wounds, surgical wounds, burns, sutures, and skin grafts. In particular, LumiHeal® gel induces cellular proliferation, promote the synthesis of healing proteins, increases collagen production, stimulates angiogenesis, relieves pain, and physiologically remodels the collagen.

The indications of LumiHeal® gel are chronic wounds (i.e., venous ulcer, diabetic foot, pressure ulcer, and ischemic ulcers); acute wounds (i.e., burn, post-surgical wounds, and trauma); and cosmetics (cosmesis for scars). The contraindications include photosensitivity, pregnancy, breastfeeding, and childhood). The first effects can be observed after 15 days, or in any case, a mean 50% reduction of wound area is usually observable after 4-6 weeks.

Clinical records

The last experience included 2836 patients visited in a 5-year timeframe (Fig 1-5). In particular, the national healthcare service reimbursed 2154 patients (assuring 4067 performances), whereas 682 (with 967 performances) were private patients. Vascular lesions accounted for 72% of the global sample, rheumatological lesions for 5%, and bedsores for 23%.

Pharmacoeconomic outcomes

The global cost for the supply of LumiHeal® and medical devices was 21.272 %; in the graduated scale, the income deriving from the regional reimbursement was 75.782%. Thus, the gross operating revenue was 54.509 % for the hospital organization. Moreover, the advantages for the patients, and indirectly for the society, were also more significant. The 62% of patients with non-responder wounds obtained a 50% reduction of lesions within three months. Furthermore, a complete recovery was observed in 18% of patients with non-responder wounds within three months.

The global saving is impressive if it is considered a series of direct and indirect costs. Even if a precise estimate is not possible as many variables have to be considered, the mean cost for a hospitalization day may be more than €1000. The cost of a day job is about €200. Moreover, medications, rehabilitation, transportation, and company costs may be costly, mostly in severe patients. Last but not least, the quality of life and wellbeing, also of the family, have a considerable burden. Therefore, this new approach could guarantee great savings and a significant improvement for the people.

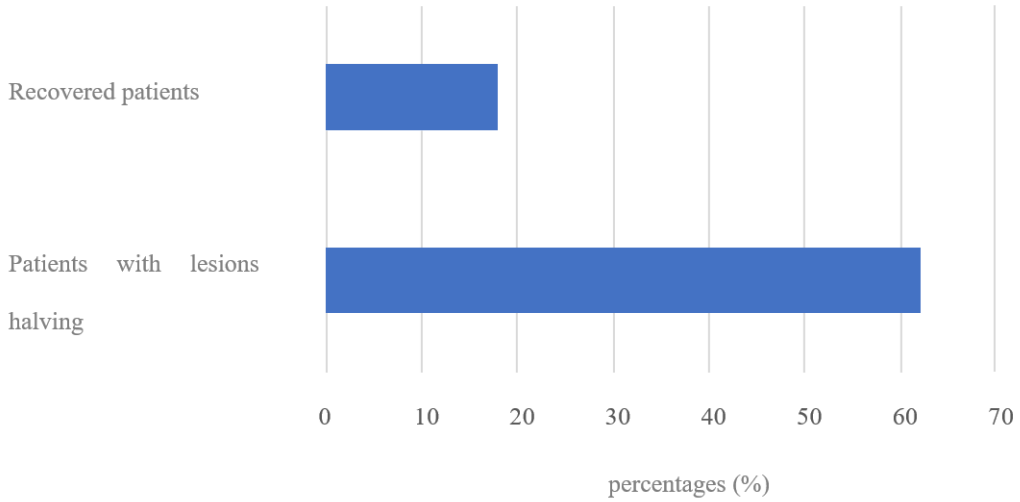

Fig. 1. *Pharmacoeconomic outcomes obtained in patients treated with Regeneration Medicine 3.0 protocol after 3 months*

Recent findings

In February 2020, shortly before the start of the COVID-19 pandemic, results of the clinical activity of the RMC were reported in a Plenary of the Hospital group ("Regenerative medicine: the patient with disabilities at the centre of care").

The analysis covered the three-year activity, detailing the number of patients enrolled, services reimbursed by the national healthcare service (NHS), insurance premium, or freelance work. In addition, the report described the cost analysis performed by the competent offices and the ongoing evaluation of economic performances. In particular, biophysical therapies, mostly Lumiheal®, were evaluated in terms of expenditure/revenues and potential savings of healing times and the use of additional materials for complete healing. The Key Performance Indicators (KPI) were:

a new consideration of RMC that went beyond the concept of the classic outpatient clinic but could share clinical experiences with the best technologies and be the reference for the patient and family;

a replicable and effective methodology;

the divulgation of methodology;

the need for clinical and economic outcomes;

an accurate data evaluation according to the RMC strategy;

an appropriate and responsible management of KPIs

a communication about KPIs and how they can help us change performance

a dialogue with the Purchasing Department for the budget definition;

the use of cutting-edge technologies and innovative therapeutic approaches to reduce healing times;

a direct relationship with GPs and IHA to rationalize home management with a reduction in healthcare expenses for the NHS.

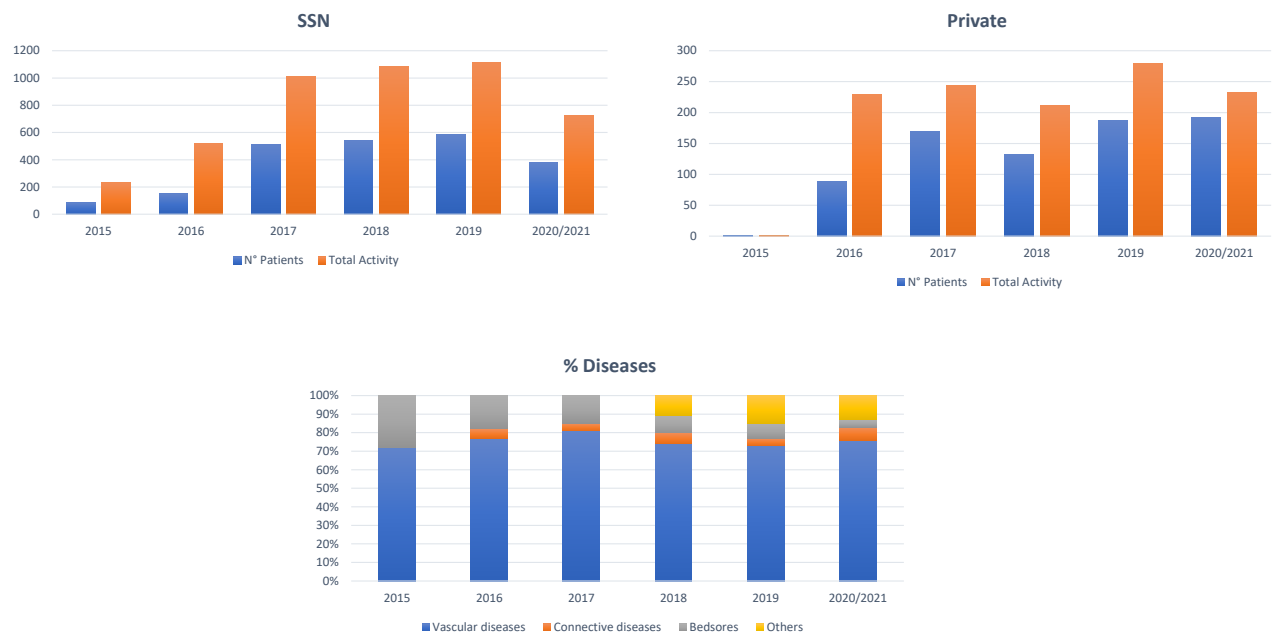

Fig 2. *Number of treated patients and performances during some years SSN and Private and types of diseases.*

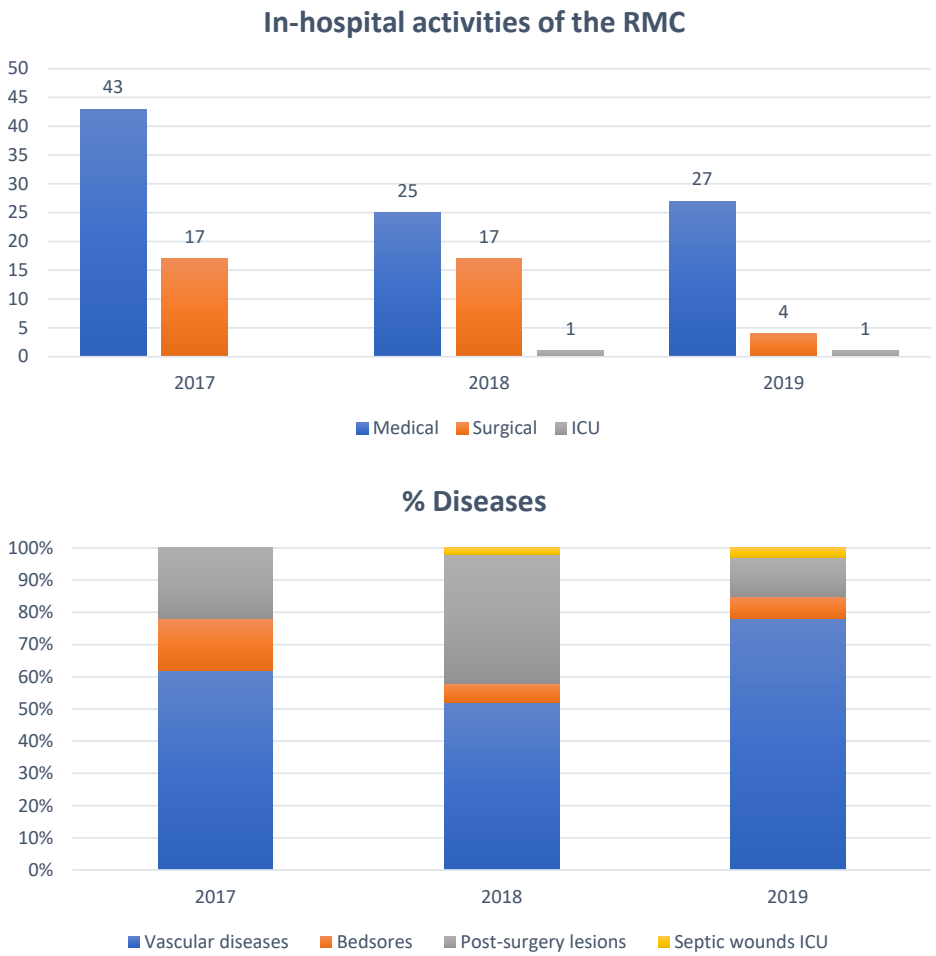

Fig. 3. *The number of patients and activities considering different conditions.*

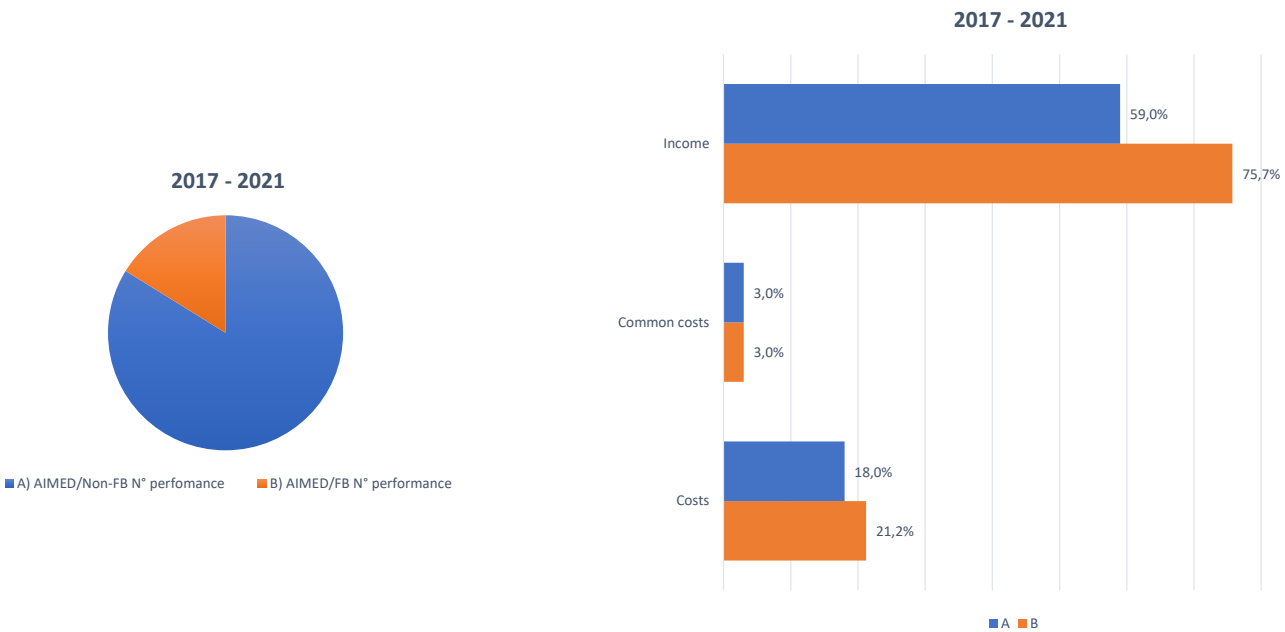

Fig 4 . *.The number of patients and activities considering different conditions AIMED /non FB VS AIMED/ FB.*

The last outcomes

The COVID-19 pandemic significantly affected the care for the other diseases and had a relevant impact on psychological issues. In this context, the present experience demonstrated that the new technology with bio-photonic therapy could also be applied at the primary care level, mainly concerning home care. Namely, the biophysical therapy had an excellent clinical performance due to the results obtained about the healing time and reduction of complications.

The cost/income analysis allowed an engaging projection on the savings for the GP concerning the reduction of the global costs for materials and better performance on the total number of hospital admissions (- 30/33%) and on working days lost (-23/32%) in working patients. In addition, the recovery time diminished and material and staff costs (Figure 1). In particular, figures 2, 3, 4 and 5 detail the pharmacoeconomic issues in patients cured with different protocols. The cost-saving, clinical improvement, and pain reduction were relevant as reported. So, this experience demonstrated that the management of chronic patients could be guaranteed along the entire pathway from hospitalization to the outpatient clinic, primary care setting, and at home. As a result, this protocol ensures personalized medicine to reduce inconvenience for the patient and the family.

CONCLUSION

The wound healing process is a complex process intertwined with the biological mechanisms causing individuals to become ill. In addition, systemic and local factors combine to cause the process to become chronic and perpetuate itself in the skin wound, an expression of it all. Regenerative Medicine provides

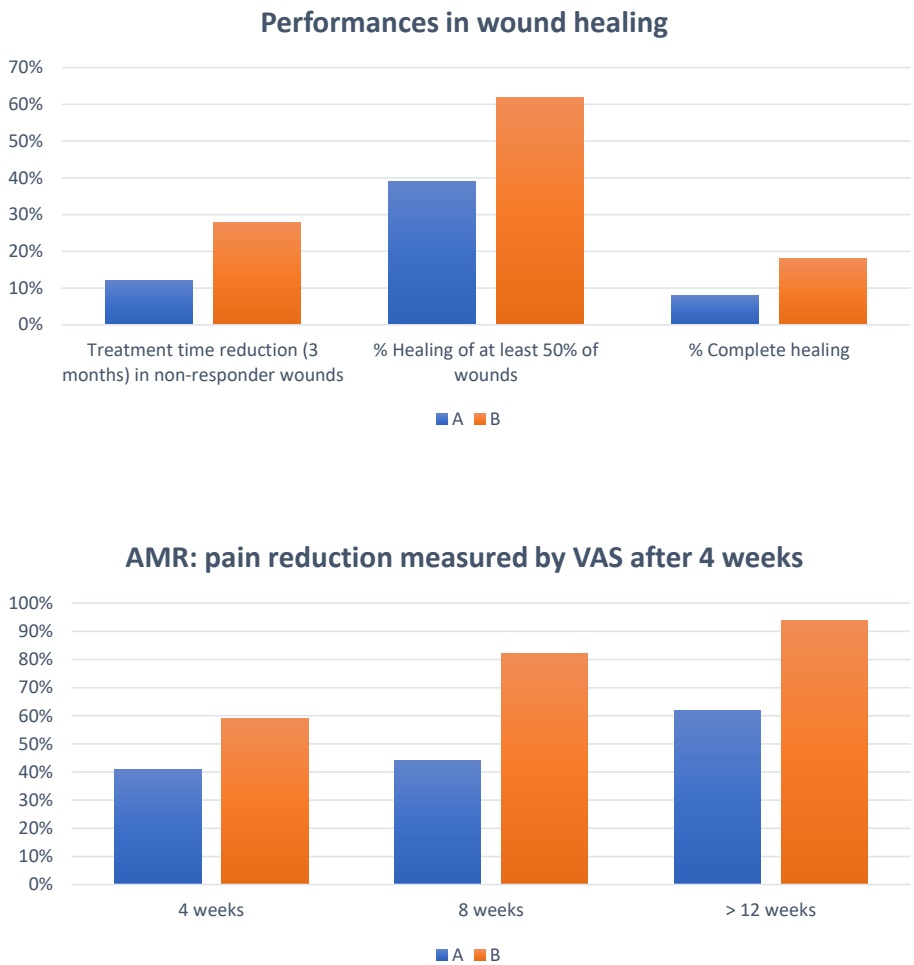

Fig. 5. *Results on wound healing rate and pain reduction*

new approaches to manage wound healing, mainly concerning difficult to treat lesions. An innovative protocol using multidisciplinary management and original medical devices, including the LumiHeal®, allows achieving relevant savings and optimal patient care. The Wound Regeneration 4.0 ensures an optimal pharmaceutical expenditure and pays attention to technological innovations supported by documentable results on clinical efficacy, healing speed, and spending review. The LumiHeal® technology and method play a major role in determining a treatment protocol for chronic non-responding skin lesions for the near future.

Conflict of interests:

All authors state that there is no conflict of interest.

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A pilot surveillance investigation on the influence of a probiotic combination on side-effects caused by common anti-cancer therapies

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***Objective*: This study aimed to investigate the possible reduction of the side effects caused by cycles of anti-cancer therapies using a probiotic combination of *Lacticaseibacillus rhamnosus* LR04 (DSM 16605), *Lactiplantibacillus pentosus* (formerly *Lactobacillus pentosus*), LPS01 (DSM 21980), *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) LP01 (LMG P-21021), *and Lactobacillus delbrueckii subsp. delbruekii* LDD01 (DMS 22106).**

***Methods*: 180 patients were enrolled and grouped into four categories according to different cancers: breast (n = 38), lung (n=22), colon (n=55) and prostate (n=65). They received the probiotic combination with the first anti-cancer cycle (T0) for 30 days (T1) and reported the symptoms (nausea, vomit, etc.) using an absent-to-severe score.**

***Results*: Patients with prostate cancer registered a marked reduction in some symptoms after receiving the probiotic combination. Intestinal-related disorders showed a statistically significant decrease during the use of the probiotic (30 days). Nausea and vomit were reduced from 32.3% to 2.1% ($p<0.0001$), intestinal swelling from 53.8% to 21.6% ($p<0.01$), mucositis from 29% to 0 ($p<0.001$), diarrhea decreases from 38.4% to 8.1% ($p<0.001$), abdominal pain was reduced from 44.6% to 10.8% ($p<0.001$) and constipation from 40% to 13.5 % ($p<0.05$). Patients with colon cancer reported a decreased intestinal swelling over time (54.4% - 38.4%, $p<0.05$). No statistical differences were found in the two last groups (breast and colon cancer) for all the symptoms after introducing the probiotic.**

***Conclusion*: With a continuous focus on safety, a specific probiotic combination in contrasting the side effects of the anti-cancer therapies for the four different cancers here analysed should be considered. This pilot investigation was expected to provide only general indications that must be further validated with numerically larger studies and under more strict parameters.**

keywords: anti-cancer therapy, probiotics

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Gastrointestinal toxicity is one of the most frequent side effects caused by anti-cancer therapies (1, 14, 21). In addition, chemo- and radiotherapy can cause severe and debilitating intestinal damages, which can clinically lead to diarrhea, mucositis, and inflammatory (or ulcerative) alteration of the gastrointestinal mucosa (17, 19).

Anti-cancer therapies are also responsible for a long series of additional side effects such as nausea and vomiting, hair loss, constipation, and cutaneous pigmentary changes (4, 12, 13). In addition, vascular, dermatologic, endocrine, immunologic, and pulmonary toxicities have emerged for targeted cancer therapy (8).

The oncological patient is, by definition, a chronic patient who must undergo both antineoplastic and supportive cycles of therapy. Therefore, the main objective of clinicians is to contrast the numerous undesirable side effects caused by anti-cancer treatments and the development of more effective, less detrimental, and patient-specific therapies (24, 25).

Recent studies investigated the possibility of targeting microbiota components to enhance anti-cancer treatment efficacy while preventing toxicity; in this panorama, probiotics represent one of the most promising and valuable intervention tools (11, 22).

Probiotics are known to modify and protect the intestinal microbiota exerting a contrasting action on pathogens adhesion, enhancing mucosal barrier function, modulating the innate and adaptive immune response, and secreting bioactive metabolites (10). They have a beneficial role in diverse severe conditions such as inflammatory bowel diseases, multiple sclerosis, and rheumatoid arthritis (3, 6, 7, 15, 26).

An ever-growing number of studies show that probiotics might be implicated in protecting and maintaining the functionality of the intestinal microbiota with a positive outcome on cancer prevention, onset, and progression (5, 9, 23, 27). In addition, regular consumption of oral probiotics has been positively linked with the clinical efficacy of anti-cancer treatments and the capacity to mitigate the adverse and even life-threatening side-effects of chemo- and radiotherapy (18).

This study aimed to measure the probiotics' capacity in limiting the toxicity and reducing the severity of some common symptoms caused by the conventional anti-cancer treatments.

MATERIALS AND METHODS

Study design

Data were collected throughout a survey. A specific questionnaire was dispensed to each patient who answered the questions in a self-reported document deposited in an online electronic repository of data managed by Medical Doctors (MDs). The Information was collected from MDs together with the anamnestic and clinical status. This study represents a data collection that can be considered a preliminary step to plan a subsequent clinical trial. This type of study does not require Ethical approval according to Italian law; however, it had been approved by the Authorship Internal Board, which included several oncologists. Informed consent has also been requested from the patients during the first visit. The biases and limitations of this type of study have been considered and broadly discussed in the text.

Questionnaire and outcome

Patients included in this study were diagnosed with different cancers and grouped according to the tumour location: breast (n =38), lung (n =22), colon (n=55), prostate (n=65). All these individuals (n = 180) were at the beginning of the anti-cancer treatment when enrolled (T0). The patients were subjected to different therapies: chemotherapy, radiotherapy, immunotherapy, hormone and target therapy.

This study aimed to evaluate whether the daily administration (as recommended by the manufacturer's instructions) of the probiotic combination "Abivisor" (AURORA Biofarma, Milan, Italy) containing *L. rhamnosus* LR04 (DSM 16605; >=10^9 Colony Forming Units/ Active Fluorescence Unit [CFU/AFU]), *L. pentosus* LPS01 (DSM 21980; >= 8×10^8 CFU/AFU), *L. plantarum* LP01 (LMG P-21021; >=3×10^9 CFU/AFU), and *L. delbrueckii* subsp. delbruekii LDD01 (DMS 22106; >= 2×10^8 CFU/AFU) with N-acetylcysteine, recommended at a >= 5×10^9 CFU/AFU per day, or in combination with standard anti-cancer therapy for 30 days could clinically improve the adverse symptoms associated with the anti-cancer treatments. Each strain used in the probiotic is patented by Probiotical SpA (Novara, Italy) and selected for gastric pH tolerance and

synergy. Each patient delivered the scheduled self-reporting sheets during the first visits (T0-time of enrolment) and one month later (T1). The probiotic was administered upon enrolment (together with anti-cancer therapy); moreover, all the subjects signed the informed consent and privacy. The participation in the survey was on a volunteer basis, and the reasons behind patient withdrawal can be diverse (e.g., improvement or worsening of their health condition; surgeries or other treatments incompatible with probiotic administration; transfer to other facilities). The drop-out was generally independent of the probiotic treatment; no opportunistic infections were registered due to the probiotic combination intake or other adverse reactions.

The primary outcome focused on evaluating the presence and severity of specific clinical symptoms at the beginning (T0) and the end of the study (T1). The symptoms considered were: 1) burning epigastric pain, 2) nausea and vomiting, 3) belching flatulence and borborygmi, 4) intestinal swelling, 5) aphthosis and halitosis, 6) mucositis of esophagus and stomach, 7) mucorrhea, 8) colitis, 9) fatigue, 10) hydroelectric alterations, 11) diarrhea, 12) recurrent abdominal pain, 13) constipation, 14) dehydration needs, 15) cutaneous dyschromia, 16) cutaneous alterations. The severity of clinical symptoms was self-reported by patients using a progressive multiple choices questionnaire with three options: mild, moderate, and severe. The symptoms were considered absent when not included in the previous categories.

Statistical analysis

Statistical analyses were performed by *Chi*-square test and Fisher's exact test comparing the severity of symptoms at T0 and T1. The comparison was made by several patients reporting specific symptoms as mild, moderate, or high. A *p-value* less than 0.05 was considered statistically significant. The statistical analysis and graphic representation were performed with GraphPad Prism version 8.01 for Windows (GraphPad Software®, San Diego, USA, www.graphpad.com).

RESULTS

Overall data

The study enrolled 180 patients divided in four different categories depending on cancer types: breast (n = 38), lung (n=22), colon (n=55), prostate (n=65). The drop-out is consistent since 86 patients (47.4%) decided not to undergo the second visit at the end of the survey (T1). Safety issues remain of key importance, considering certain cancer patients' weakened and immunocompromised health status. Negative effects due to the utilisation of the probiotic were never reported. Chemotherapy, radiotherapy, immunotherapy, hormone therapy and target therapy for each cancer type administered before and during this study are reported in Table I.

Main outcomes

In patients with prostate cancer, the overall symptoms of nausea and vomiting decreased from 30.7% at T0 to 2.7% at T1 ($p<0.001$). Eructation, flatulence and borborygmi were reduced from 36.9% to 10.8% ($p<0.01$). The intestinal swelling decreased from 61.5% to 21.6% ($p<0.001$).

The prevalence of patients reporting aphthosis and halitosis decreased from 27.6% at T0 to 2.7% at T1 ($p<0.01$). Diarrhea was reduced from 38.4% at T0 to 8.1% at T1 ($p<0.001$) and abdominal pain from 44.6% at T0 to 10.8% at T1 ($p<0.001$). The percentage of

Table I. *Anticancer treatment prescribed to each patient included in this pre-clinical study.*

Cancer location	CT	RT+CT	RT	HT	TT	IT	Others
Breast (N=38)	14 (36.9%)	1 (2.6%)	4 (10.6%)	5 (13.1%)	2 (5.3%)	1 (2.6%)	11 (28.9%)
Lungs (N=22)	10 (45.4%)	2 (9%)	0	1 (4.5%)	0	3 (13.6%)	6 (27.3%)
Colon (N=55)	32 (58.2%)	4 (7.3%)	3 (5.4%)	0	3 (5.4%)	0	13 (23.7%)
Prostate (N=65)	2 (3.1%)	0	36 (55.4%)	19 (29.2%)	3 (4.6%)	0	5 (7.7%)

*Abbreviations: **RT**= Radiotherapy; **CT**= Chemotherapy; **RT**+**CT**; **HT**=Hormone therapy; **TT**= Target therapy; **IT**= Immunotherapy*

individuals reporting constipation decreased from 40% at T0 to 13.5% at T1 ($p<0.05$). Dehydration was reduced from 24.6% at T0 to zero% at T1 ($p<0.001$).

After using the probiotic combination for 30 days (T1), the overall number of patients reporting moderate and severe symptoms decreased. Only one patient reported nausea and vomiting at T1; statistically significant differences were found at a mild level, reducing from 26.1% to 2.7% ($p<0.01$). Eructation, flatulence and borborygmi showed a statistically significant difference at a moderate level, from 50% to 0 ($p<0.01$). Intestinal swelling was reduced to zero from a rather high-moderate (42.8%) and severe (22.8%) levels ($p<0.0001$ and $p<0.05$, respectively). Patients reported mucositis only at a moderate level, reducing from 100% at T0 to zero at T1 ($p<0.001$). The only statistically significant difference was at a moderate level for colitis, with a reduction from 25% at T0 to zero at T1 ($p<0.05$). A difference in hydroelectric alteration was reported at T0, at a mild level (93.3%), which was reduced to zero at T1 ($p<0.001$). Diarrhea decreased at a moderate level from 52% at T0 to zero ($p<0.001$). Abdominal pain decreased at a moderate level from 51.7% at T0 to 50% ($p<0.05$). Dehydration was reduced at a mild level from 93.7% at T0 to zero ($p<0.05$). Alteration of skin annexe was reported at a mild level, reducing from 100% at T0 to zero at T1 ($p<0.01$). For a comprehensive overview, including non-significant results, refer to Table II.

In the colon cancer group, the symptoms of intestinal swelling decreased from 54.5% at T0 to 38.4% at T1 ($p<0.05$). After using the probiotic for 30 days, the patients reported a decreased prevalence of symptoms at moderate and severe levels, but the differences with T0 are rarely significant. On a couple of occasions, we also noticed an increase in the severity of the symptom. The prevalence of patients reporting burning epigastric pain at a severe level increased from zero to T0 to 23.2% at T1 ($p<0.05$). The prevalence of mild symptoms for fatigue increased from 20.8% at T0 to 57.2% at T1 ($p<0.05$). For a comprehensive overview, including non-significant results, please refer to Table III.

In the remaining two groups (breast cancer and lung cancer), there were no statistically significant differences for all the considered symptoms between T0 (enrolment and beginning of probiotic administration) and time T1 (end of the study).

DISCUSSION

Anti-cancer therapies cause severe side effects to patients, including gastrointestinal dysbiosis (30). However, several clinical trials have highlighted the efficacy of using probiotics for reducing general discomfort and specific side-effects in cancer patients under anti-cancer treatment, therefore, improving the general clinical conditions and health status of such patients (29).

Among the most drastic gastrointestinal side-effects, often associated with anti-cancer treatments, diarrhea and mucositis are two common symptoms that can often be alleviated using oral probiotics (2, 20, 31).

In this study, we reported positive effects of the probiotic on patients affected by two different types of cancer: prostate and colon. In addition, statistically significant results were observed between T0 (enrolment) and T1 (end of the study), with a marked amelioration of some symptoms' severity.

For patients affected by prostate cancer, there was a consistent reduction ($p< 0.001$-0.0001) of nausea and vomiting, intestinal swelling, diarrhea and abdominal pain at moderate and/or severe levels after 30 days of using the probiotic combination. The cancer patients affected by nausea and vomiting, eructation, flatulence and borborygmi, constipation, colitis, and fatigue seem to receive some benefits, but the difference was less significant ($p< 0.05$-0.01). The remaining four symptoms (burning epigastric pain, mucorrhea, fatigue and dyschromia) seem not to receive benefits after using probiotics.

For patients diagnosed with colon cancer, intestinal swelling was the only symptom that showed a consistent reduction from 54.5% at T0 to 38.4% ($p< 0.05$). Remarkably, there are two cases in which the specific symptoms worsened after using the probiotic for 30 days. A statistically significant increase was registered for burning epigastric pain at severe level (3 patients 23.2% at T1 compared to zero at T0) and fatigue at mild level (from 20.8% at T0 to 57.2% at T1, $p<0.05$). The remaining symptoms (epigastric burning pain, belching, flatulence and borborygmi, aphthosis and

Table II. *The number, prevalence, and severity of specific symptoms for patients with different prostate* cancers.

Symptoms	Prevalence T0 (%)	Prevalence T1 (%)	*p*-value
PROSTATE CANCER	N = 65	N = 37	
Burning epigastric pain	29 (44.6%)	11 (29.7%)	Ns
Mild	15 (51.7%)	8 (72.2%)	Ns
Moderate	11 (38%)	3 (27.8%)	Ns
Severe	3 (10.3%)	0	Ns
Nausea and vomiting	20 (30.7%)	1 (2.7%)	P<0.001
Mild	17 (85%)	1 (100%)	P<0.01
Moderate	3 (15%)	0	Ns
Severe	0	0	Ns
Eructation, flatulence and borborygmi	24 (36.9%)	4 (10.8%)	P<0.01
Mild	9 (37.5%)	4 (100%)	Ns
Moderate	12 (50%)	0	P<0.01
Severe	3 (12.5%)	0	Ns
Intestinal swelling	40 (61.5%)	8 (21.6%)	P<0.001
Mild	17 (48.6%)	8 (100%)	Ns
Moderate	15 (42.8%)	0	P<0.0001
Severe	8 (22.8%)	0	P<0.05
Aphthosis and halithosis	18 (27.6%)	1 (2.7%)	P<0.01
Mild	11 (61.1%)	1 (100%)	Ns
Moderate	6 (33.3%)	0	Ns
Severe	1 (5.6%)	0	Ns
Mucositis	16 (24.6%)	0	P<0.001
Mild	16 (100%)	0	P<0.001
Moderate	0	0	Ns
Severe	0	0	Ns
Mucorrhea	5 (7.6%)	1 (2.7%)	Ns
Mild	0	0	Ns
Moderate	2 (40%)	1 (100%)	Ns
Severe	3 (60%)	0	Ns
Colitis	28 (43%)	11 (29.7%)	Ns
Mild	19 (67.8%)	9 (81.8%)	Ns
Moderate	7 (25%)	0	P<0.05
Severe	2 (7.2%)	2 (18.2%)	Ns
Fatigue	30 (46.1%)	19 (51.3%)	Ns
Mild	13 (43.3%)	13 (68.4%)	Ns
Moderate	13 (43.3%)	2 (10.5%)	Ns
Severe	4 (13.4%)	4 (21.1%)	Ns
Hydroelectric alterations	15 (23%)	0	P<0.001
Mild	14 (93.3%)	0	P<0.001
Moderate	1 (6.7%)	0	Ns
Severe	0	0	Ns
Diarrhea	25 (38.4%)	3 (8.1%)	P<0.001
Mild	11 (44%)	3 (100%)	Ns
Moderate	13 (52%)	0	P<0.001
Severe	1 (4%)	0	Ns
Abdominal pain	29 (44.6%)	4 (10.8%)	P<0.001
Mild	15 (51.7%)	2 (50%)	P<0.05
Moderate	13 (44.8%)	2 (50%)	Ns
Severe	1 (3.5%)	0	Ns
Constipation	26 (40%)	5 (13.5%)	P<0.05
Mild	14 (53.8%)	3 (60%)	Ns
Moderate	10 (38.4%)	2 (40%)	Ns
Severe	2 (7.8%)	0	Ns
Dehydration	16 (24.6%)	0	P<0.001
Mild	15 (93.7%)	0	P<0.001
Moderate	1 (6.3%)	0	Ns
Severe	0	0	Ns
Dyschromia	15 (23%)	4 (10.8%)	Ns
Mild	14 (93.3%)	4 (100%)	Ns
Moderate	1 (6.7%)	0	Ns
Severe	0	0	Ns
Alteration skin annex	14 (21.5%)	0	P<0.01
Mild	14 (100%)	0	P<0.01
Moderate	0	0	Ns
Severe	0	0	Ns

Table III. *The number, prevalence, and severity of specific symptoms for patients with different colon cancers.*

Symptoms	Prevalence T0 (%)	Prevalence T1 (%)	*p*-value
COLON CANCER	N = 55	N = 26	
Burning epigastric pain	16 (29.1%)	13 (50%)	Ns
Mild	8 (50%)	8 (61.5%)	Ns
Moderate	8 (50%)	2 (15.3%)	Ns
Severe	0	3 (23.2%)	P<0.05
Nausea and vomiting	22 (40%)	12 (46.1%)	Ns
Mild	11 (50%)	10 (83.3%)	Ns
Moderate	10 (45.5%)	2 (16.7%)	Ns
Severe	1 (4.5%)	0	Ns
Eructation, flatulence and borborygmi	17 (30.9%)	9 (34.6%)	Ns
Mild	7 (41.2%)	4 (44.4%)	Ns
Moderate	10 (58.8%)	3 (33.3%)	Ns
Severe	0	2 (22.3%)	Ns
Intestinal swelling	30 (54.5%)	10 (38.4%)	P<0.05
Mild	12 (40%)	3 (30%)	Ns
Moderate	15 (50%)	6 (60%)	Ns
Severe	3 (10%)	1 (10%)	Ns
Aphthosis and halithosis	13 (23.6%)	4 (15.3%)	Ns
Mild	7 (53.8%)	4 (100%)	Ns
Moderate	4 (30.8%)	0	Ns
Severe	2 (15.4%)	0	Ns
Mucositis	11 (20%)	2 (7.6%)	Ns
Mild	8 (72.7%)	2 (100%)	Ns
Moderate	2 (18.2%)	0	Ns
Severe	1 (9.1%)	0	Ns
Mucorrhea	9 (16.3%)	8 (30.7%)	Ns
Mild	0	0	Ns
Moderate	6 (66.6%)	5 (62.5%)	Ns
Severe	3 (33.4%)	3 (37.5%)	Ns
Colitis	17 (30.9%)	8 (30.7%)	Ns
Mild	6 (35.3%)	5 (62.5%)	Ns
Moderate	8 (47%)	1 (12.5%)	Ns
Severe	3 (17.7%)	2 (25%)	Ns
Fatigue	24 (43.6%)	14 (53.8%)	Ns
Mild	5 (20.8%)	8 (57.2%)	P<0.05
Moderate	12 (50%)	3 (21.4%)	Ns
Severe	7 (29.2%)	3 (21.4%)	Ns
Hydroelectric alterations	8 (14.5%)	4 (15.3%)	Ns
Mild	6 (75%)	3 (75%)	Ns
Moderate	2 (25%)	1 (25%)	Ns
Severe	0	0	Ns
Diarrhea	25 (45.4%)	9 (34.6%)	Ns
Mild	9 (36%)	6 (66.7%)	Ns
Moderate	13 (52%)	3 (33.3%)	Ns
Severe	3 (12%)	0	Ns
Abdominal pain	20 (36.3%)	9 (34.6%)	Ns
Mild	8 (40%)	5 (55.5%)	Ns
Moderate	9 (45%)	1 (11.2%)	Ns
Severe	3 (15%)	3 (33.3%)	Ns
Constipation	12 (21.8%)	7 (26.9%)	Ns
Mild	5 (41.7%)	6 (85.7%)	Ns
Moderate	7 (58.3%)	1 (14.3%)	Ns
Severe	0	0	Ns
Moderate	0	0	Ns
Severe	1 (10%)	0	Ns
Dyschromia	12 (21.8%)	2 (7.6%)	Ns
Mild	7 (58.3%)	2 (100%)	Ns
Moderate	3 (25%)	0	Ns
Severe	2 (16.7%)	0	Ns
Alteration skin annex	6 (10.9%)	3 (11.5%)	Ns
Mild	5 (83.4%)	3 (100%)	Ns
Moderate	1 (16.6%)	0	Ns
Severe	0	0	Ns

halitosis, fatigue, hydroelectric alterations, dehydration, cutaneous dyschromia and cutaneous alterations) do not show any statistically significant difference after the 30 days of probiotic administration. Two groups of patients in two other cancer groups (breast and colon cancer) did not show any statistically significant results. However, most lung cancer symptoms (except for burning epigastric pain and mucositis) registered a reduction from T0 to T1. In the breast cancer group, in addition to the two previously mentioned symptoms, the overall occurrence of mucorrhea, fatigue, constipation, dyscromia and alteration of skin annexe did not improve after assuming the probiotic combination for 30 days.

From a clinical perspective, the absence of statistically significant differences must be interpreted with caution. The non significant outcome might be easily connected with the small sample, representing a clear limitation of this study and is expected to influence statistical results.

We can presume that the patients' health might not be extremely compromised by the anti-cancer therapy, since they are in an early stage of the treatment; this aspect is important as late administration of the probiotic might not be effective in a compromised health status (affected either by the progression of cancer and/or several cycles of anti-cancer therapies).

Our study's higher number of individuals diagnosed with prostate cancer might not be the only factor influencing statistically significant differences after using the probiotic combination. Prostate cancer was often treated with radiotherapy (55.4%) compared to the other types of cancer (breast: 10.6% and colon 5.4%) considered in this study. Despite the continuous effort to reduce or limit radiotherapy treatment side effects for cancer patients, heavy gastrointestinal disorders such as nausea and vomiting, flatulence, abdominal discomfort, and diarrhea are frequent (16,28).

The high number of patients who decided to withdraw their study participation might have negatively affected our results. The constant drop-out over time (from T0 to T1) could have implications for the statistical significance of the differences, especially in numerically smaller groups (breast and lung cancer). From the initial 38 individuals (T0) in the breast cancer group, only 16 were still engaged after the study (T1). In the lung cancer group, the patients at the beginning of the study were 22 (T0), but only 7 reached the end of the study (T1). Therefore, non-significant differences might have been caused by a low number of patients included in this study.

Despite the encouraging clinical results confirming the role of probiotics in contrasting diverse side-effects of anti-cancer therapies will require further studies with a higher number of patients and the application of more strict and well-defined parameters.

In conclusion, the current study laid out the premise for considering probiotics as add-on therapies in Oncology to improve the overall condition of patients.

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Bifidobacterium animalis subsp. *lactis* BB-12 and cow milk-related symptoms in infants: a real-life experience

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In infancy, cow's milk allergy (CMA) is a common medical problem. However, there is no simple diagnostic tool, so a symptom-based score has been developed in clinical practice and parents. The Cow's Milk-related Symptom Score (CoMiSS) includes the assessment of symptoms associated with intestinal, cutaneous, and respiratory complaints. The probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) is the world's most documented probiotic *Bifidobacterium.* The present study aims to evaluate the effects exerted by a two-month BB-12 course on CoMiSS scores in infants with artificial feeding (partial or absolute). Infants were supplemented with six drops ($1x10^9$ CFU; ABINAT12®) daily (Group A; 499 subjects) or without any probiotic (Group B; 461 subjects) for two months. Paediatricians performed a baseline visit (T0) and after 30 (T1) and 60 (T2) days. In addition, the CoMiSS questionnaire was administered to the parents. BB-12 significantly improved near all items of CoMiSS both at T1 and T2. In conclusion, this experience demonstrated that symptoms potentially related to CMA represent a relevant medical problem in infants. In addition, a two-month BB-12 supplementation (ABINAT12®) significantly reduced the symptom severity of infants.

Formula-fed infants present more frequently with gastrointestinal (GI) discomfort symptoms than breastfed infants. For example, rates have been reported to be as high as 67% for regurgitation at four months of age (1), 56% for flatulence at two months (2), 17% for constipation in infants younger than six months (3), and 10–40% for colic and/or heavy crying in infants younger than six months (4).

These functional digestive symptoms may or may not be attributed to the ingestion of cow's milk. Consequently, long-term dietary measures are prescribed in more than 70% of patients. However, a positive cow's milk allergy (CMA)

Keywords: cow's milk allergy; infants; paediatricians; Bifidobacterium animalis subsp. lactis BB-12; CoMiSS questionnaire

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diagnosis has rarely been established after adequate implementation and reporting of diagnostics (5).

In addition, there is no simple and readily available test for CMA. However, a symptom-based score to quantify the number and severity of suspected CMA-related symptoms has been validated (6). Therefore, the Cow's Milk-related Symptom Score (CoMiSS), which considers general manifestations, dermatological, gastrointestinal, and respiratory symptoms, was developed as an awareness tool for cow's milk-related symptoms (7). CoMiSS could be fruitfully used by healthcare providers and parents of children with suspected CMA.

It was also reported that infants having a low CoMiSS (median, 5) after one-month dietary treatment free from intact CM protein had a significant risk of having a positive challenge test, such as an odds ratio of 0.83 (8).

Further, it has been indicated that CoMiSS could also be useful in non-exclusively breastfed infants (9). Another study demonstrated that the median CoMiSS score was 3.0 in healthy infants ≤ six months (10). The convenience and simplicity for parents were also tested, and the study concluded that parents could reliably score CoMiSS without additional training (11). Finally, a real-life study used CoMiSS as a parameter to investigate a formula in infants with functional gastrointestinal disorders (12).

The probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) is the world's most documented probiotic *Bifidobacterium* (13). In addition, there is a relevant body of evidence that supports its ancillary use in different pediatric conditions, including infant colic, functional digestive disorders, and infections (14).

Therefore, the current experience aimed to investigate the potential effect of a two-month supplementation with BB-12 in infants with a partial or absolute formula diet.

MATERIALS AND METHODS

The study was a randomized, controlled study involving 17 Italian paediatricians distributed across the country, assuring comprehensive and complete national coverage. Accordingly, the Ethical Committee approved it of the "Ospedale Policlinico" (Bari), where the coordinating centre was allocated.

The inclusion criteria were to be a healthy infant (at term-born or late preterm), with age at enrollment ≤ 4 weeks, and to be fed with formula (exclusively or predominantly). Exclusion criteria were acute gastrointestinal diseases, including infections.

The experience consisted of three visits: at baseline (T0) and after 30 (T1) and 60 (T2) days. Infants were randomly (ratio 1:1) subdivided into two groups: Group A was supplemented with a probiotic (BB-12), administered once daily (six drops), and Group B did not receive any supplementation and was considered the control group. The probiotic ABINAT12® contained $1x10^9$ CFU per six drops.

The first visit included a detailed history and demographic and clinical data collection. At the end of this visit, paediatricians instructed the parents to use the CoMiSS questionnaire to assess symptoms potentially related to cow's milk problems; this is based on scoring daily duration of crying, number and volume of regurgitation episodes, stool pattern, presence and severity of cutaneous and respiratory manifestations unrelated to infections. The score ranges from 0 to 33 points (Vandenplas 2015).

The CoMiSS awareness tool is composed of different items from different categories. In this instance, the crying duration was used as a parameter, the longer the crying, the higher the score. Colic was defined as more than 3 h of crying, for at least 3 days a week and at least one week. Up to 3 h of crying per day accounted for a score of three, which is only half of the maximum score for crying.

The scoring atopic dermatitis (SCORAD) score is the best and most widely validated method to describe atopic dermatitis. More recently, the patient-oriented SCORAD score (PO-SCORAD) has been developed. However, a good correlation between both scores could only be obtained if the patients received an illustrated tutorial from the investigator and the PO-SCORAD questionnaire to guide them in grading atopic dermatitis severity. However, primary healthcare providers have limited time, and the CoMiSS score is easy to apply and based on an estimation of the surface covered by dermatitis. The presence of urticaria was also considered. Furthermore, the CoMiSS considers the respiratory symptoms. Each item is weighted according to its severity.

The Wilcoxon and Mann U Whitney tests were used.

Analyses were performed using GraphPad Prism software, GraphPad Software Inc, CA, USA.

RESULTS

The study included 960 infants who completed the treatment period: 499 (52%) in Group A and 461 (48%) in Group B. The mean gestational age was 38.2 weeks in Group A and 38.1 in Group B. The mean birth weight was 3.2 Kg in both Group A and Group B. Table I shows the comparison between groups at T1. Group 1 experienced less severe symptoms than control subjects for all items of the CoMiSS questionnaire. Table II shows the comparison between groups at T2. Infants of the Group 1 experienced less severe symptoms than control subjects for all items of the CoMiSS questionnaire but the questions 5. Fig. 1 shows the changes over time of the single questions of the CoMiSS questionnaire. The probiotic ABINAT was safe and well-tolerated.

DISCUSSION

The current study investigated the use of a questionnaire adapted for CMA in infants fed with formula and supplemented with BB-12 for 2 months. The use of a well-known probiotic, BB-12, was associated with significant improvement of symptoms related to digestive, cutaneous, and respiratory problems. These findings underscore the multifaceted activities exerted by BB-12. Furthermore, a relevant

Table I. *Items reported in CoMiSS questionnaire in Group A and Group B at T1*

	Group A	**Group B**	**p-value**
	N=499	N=461	
I-CoMISS score			
1. How much he/she cries a day (0-6), mean (SD)	0.77 (0.85)	1.05 (1.02)	<0.001
2. Atopic dermatitis on the face / neck / trunk (0-3), mean (SD)	0.23 (0.47)	0.42 (0.62)	<0.001
3. Atopic dermatitis on arms / hands / legs / feet (0-3), mean (SD)	0.20 (0.45)	0.35 (0.61)	<0.001
4. Urticaria, n(%)	4 (0.8%)	13 (2.8%)	0.018
5. Respiratory symptoms (0-3), mean (SD)	0.12 (0.35)	0.22 (0.52)	0.009

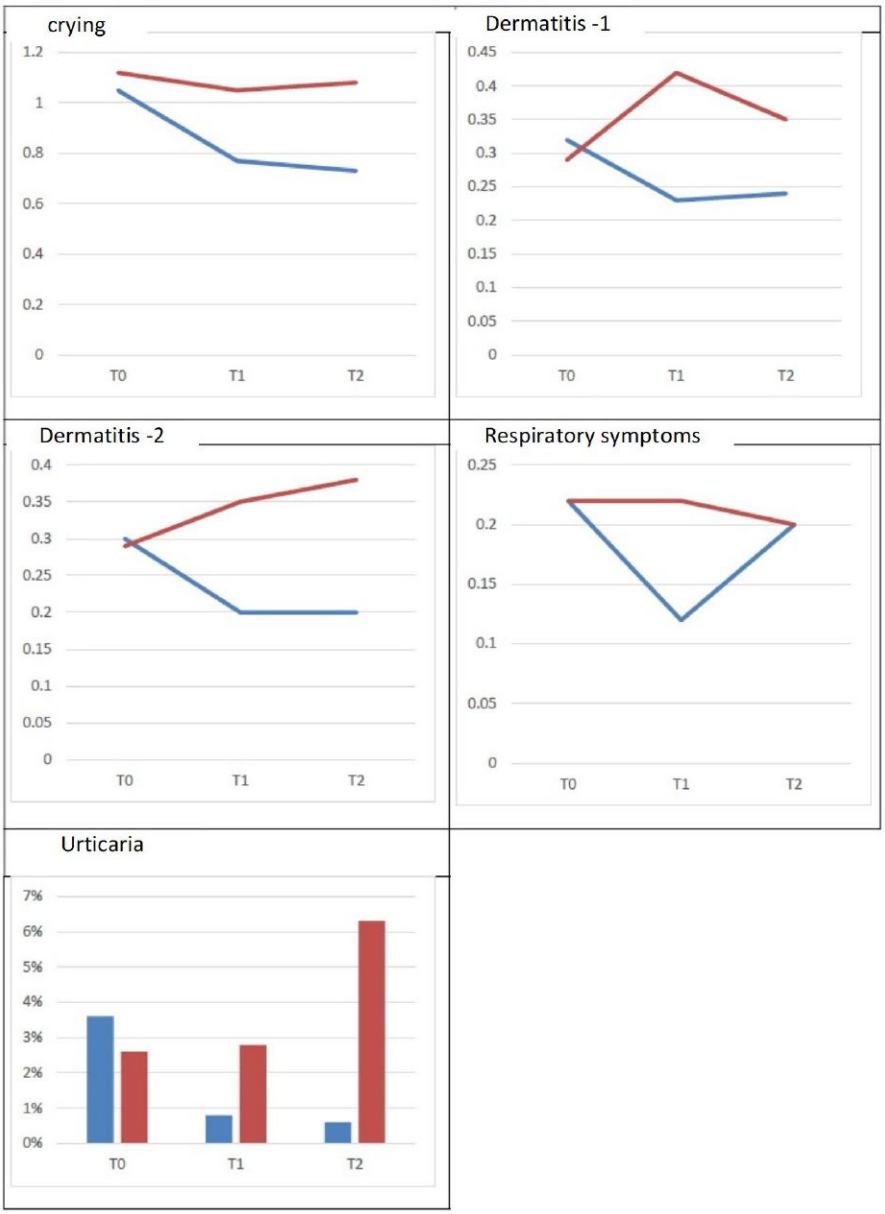
Table II *Items reported in CoMiSS questionnaire in Group A and Group B at T2*

	Group A	**Group B**	**P-value**
I-CoMISS score			
1. How much he/she cries a day (0-6), mean (SD)	0.73 (0.86)	1.08 (1.11)	0.006
2. Atopic dermatitis on the face / neck / trunk (0-3), mean (SD)	0.24 (0.50)	0.35 (0.52)	0.026
3. Atopic dermatitis on arms / hands / legs / feet (0-3), mean (SD)	0.20 (0.48)	0.38 (0.65)	0.014
4. Urticaria, n(%)	1 (0.6%)	9 (6.3%)	0.006
5. Respiratory symptoms (0-3), mean (SD)	0.20 (0.50)	0.20 (0.42)	0.555

body of evidence demonstrated its beneficial effects on the immune system, digestive tract, microbiota, and respiratory airways (13, 14). Namely, the reported mechanisms of action allow to BB-12 of exerting multi-organ efficacy.

The present experience confirmed these aspects as consistent with previous studies on different disease models, including infant colic, functional digestive disease, and respiratory and intestinal infections (15-20). However, some limitations should be noted, including the open design, the lack of microbiota assessment, and biomarkers measurement. The strength of this experience was the relevant number of infants who participated and the clinical setting, such as neonatal clinics. Thus, the findings could faithfully reflect what occurs in daily practice.

Fig. 1. *The trend of changes over time for different items of the CoMiSS questionnaire in Group A (blue) and Group B (red). The CoMiSS items: crying, dermatitis (1-2), respiratory symptoms, and urticaria.*



In conclusion, this experience demonstrated that symptoms potentially related to CMA represent a relevant medical problem in infants. In addition, a two-month BB-12 supplementation (ABINAT12®) significantly reduced the symptom severity of infants.

Conflict of interest:

All authors state that there is no conflict of interest.

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Bifidobacterium animalis subsp. *lactis* BB-12 and infant regurgitation: a real-life experience

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Infant regurgitation is the most common functional gastrointestinal disorder (FGID) worldwide and causes parental concern with relevant direct and indirect costs for families and the healthcare system. *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) is a well-known studied probiotic with evidence in managing another FGID, such as infantile colic. This real-life study evaluated the efficacy of BB-12 in decreasing functional regurgitation symptoms when supplemented in formula-fed infants (partial or absolute). In 17 outpatient services of the Italian National Pediatric Health Care System, formula-fed infants with persisting regurgitation symptoms were randomly (1:1) allocated to receive six drops ($1x10^9$ CFU of BB 12; ABINAT12®) daily (Group A) or any probiotic (Group B) for two months. Regurgitation symptoms were evaluated through the Infant Gastroesophageal Reflux Questionnaire-Revised (I-GERQ-R), performed at the baseline visit (T0) and after 30 (T1) and 60 (T2) days. A positive response was defined as a total score ≥ 16. Nine hundred and sixty infants were randomly allocated to receive BB12 (Group A; 499 subjects) or any probiotic (Group B; 461 subjects). At baseline, 25.8% in Group A and 31.7% in Group B responded positively to the I-GERQ-R questionnaire. At T1, 16% in Group A and 45.8% in Group B ($p<0.001$) had a positive I-GERQ-R. At T2, 14.7% in Group A and 50.7% in Group B ($p<0.001$) had a positive I-GEQ-R. Consistently, the total scores significantly decreased in Group A. In conclusion, this real-life study demonstrated that a two-month BB-12 supplementation (ABINAT12®) significantly reduced regurgitation prevalence and severity in formula-fed infants.

Infant regurgitation is the most common of the functional gastrointestinal disorders (FGIDs) and one of the leading causes of parental concern and anxiety in the first months of life, with relevant direct and indirect costs for families and the healthcare system (1-3). Diagnostic criteria for infant regurgitation include at

Keywords: infant regurgitation; formula-fed infants; Bifidobacterium animalis subsp. lactis BB-12; I-GERQ-R questionnaire

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least due episodes of regurgitation per day for at least three weeks in an otherwise healthy infant 3 weeks to 12 months of age without retching, hematemesis, aspiration, apnea, failure to thrive, feeding or swallowing difficulties, or abnormal posturing (4).

Although infant regurgitation must be considered a self-limited manifestation that spontaneously resolves during the first year of life, a low-grade mucosal inflammation, leading to dysfunctional gut motor activities, have been described in infants with persistent regurgitation (5).

Recent evidence underlines the pivotal role of intestinal microbiota in the modulation of intestinal inflammation, and many researchers have hypothesised how using some probiotic strains in infancy could be beneficial in managing functional gastrointestinal disorders (6, 7).

The probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) is the world's most documented probiotic *Bifidobacterium* (8). Indeed, more than 300 scientific publications concerned this probiotic, and more than 130 publications regarded human clinical studies. In addition, the complete genome sequence of BB-12 has been determined and published (9).

Strain characteristics and mechanisms of BB-12 have been established through extensive *in vitro* testing. BB-12 exhibits excellent gastric acid and bile tolerance; it contains bile salt hydrolase and has strong mucus adherence properties, all valuable probiotic characteristics (8). Pathogen inhibition, barrier function enhancement, and immune interactions are mechanisms that all have been demonstrated for BB-12. BB-12 has proven its beneficial health effect in numerous clinical studies within gastrointestinal health and immune function. Clinical studies have demonstrated the survival of BB-12 through the gastrointestinal tract, and BB-12 has been shown to support a healthy gastrointestinal microbiota (8).

Furthermore, BB-12 has been shown to improve bowel function, protect against diarrhoea, and reduce side effects of antibiotic treatment, such as antibiotic-associated diarrhoea. In terms of immune function, clinical studies have shown that BB-12 increases the body's resistance to common respiratory infections and reduces the incidence of acute respiratory tract infections (8). Finally, BB-12 safety has been widely reported (10).

However, it is essential to note that the beneficial effects of a given probiotic are specific to that strain and cannot be regarded as general for other strains of the same species, or other species, of bacteria or yeast.

The current study aimed to evaluate the efficacy of BB-12 supplementation in decreasing regurgitation symptoms in formula-fed infants.

MATERIALS AND METHODS

This was a multicenter, perspective, randomised, and controlled study performed in 17 Italian pediatric outpatient services distributed across the country and assuring comprehensive and complete national coverage. The study was approved by the Ethical Committee of the "Ospedale Policlinico" of Bari, which was allocated to the coordinating centre.

Inclusion criteria were: 1) at least two episodes of regurgitation per day for 3 or more weeks, according to Rome IV criteria (4); 2) gestational age > 34 weeks; 3) age at enrollment ≤ 4 weeks; and 4) fed with exclusively or predominantly not-thickened formula. Exclusion criteria were: 1) acute gastrointestinal diseases; 2) current infections; 3) chronic diseases, and 4) malformations.

All parents of eligible infants answered the Infant Gastroesophageal Reflux Questionnaire-Revised (I-GERQ-R), a validated questionnaire to evaluate GER and GERD-related symptoms in infants (11,12). We considered persistent regurgitation when I-GERQ-R total score was above the cut-off limit, such as ≥16, as previously reported (13). Infants with I-GERQ-R ≥16 were enrolled and randomly allocated (ratio 1:1) into two groups: Group A was supplemented with a probiotic (BB-12), Group B did not receive any supplementation and was considered the control group. The probiotic (ABINAT12®) was administered once daily (six drops = $1x10^9$ CFU). I-GERQ-R has been repeated at follow-up visits: after 30 (T1) and 60 (T2) days during probiotic supplementation.

The Wilcoxon and Mann U Whitney tests were used. Analyses were performed using GraphPad Prism software, GraphPad Software Inc, CA, USA.

RESULTS

The present study included 960 infants who completed the treatment period: 499 (52%) in Group

A and 461 (48%) in Group B. The mean gestational age in Group A was 38.2 weeks and 38.1 weeks in Group B. The mean birth weight was 3.2 Kg in both Group A and Group B. The two groups were well matched at baseline.

Table I reports the clinical data concerning the anthropometric measures and breastfeeding details. At baseline, 129 (25.8%) infants of Group A and 146 (31.7%) of Group B had an I-GERQ-R total score ≥ 16 (Table II). The mean total score was 22.3 (SD 5.3) in Group A and 23 (SD 5.3) in Group B (Table III). At T1, 16% of infants of Group A and 45.8% of Group B had an I-GERQ-R total score ≥ 16 ($p<0.001$). The mean total score was consistently 14.6 (SD 5.2) in Group A and 22.3 (SD 7.8) in Group B. At T2, 14.7% of infants of Group A and 50.7% of Group B had an I-GERQ-R total score ≥ 16 ($p<0.001$). The mean total score was consistently 11 (SD 4.5) in Group A and 21 (SD 7.6) in Group B.

Figures 1 and 2 show the over-time changes of infants with a positive response to I-GERQ-R and severity of regurgitation, such as the total score. The oral probiotic was safe and well-tolerated.

DISCUSSION

Regurgitation is a common medical problem in infants with well-defined and standardised diagnostic criteria (14-15). In the present study, I-GERQ-R was used to consider the relevance of regurgitation symptoms when the total score was equal to or more than 16 (16).

Table I. *Clinical data in the groups of infants*

	Group A	**Group B**	**p-value**
	N=499	N=461	
Weight, kg, mean (SD)	3.1 (0.5)	3.1 (0.5)	0.675
Height, cm, mean (SD)	49.7 (3.7)	49.5 (3.4)	0.127
Cranial circumference, cm, mean (SD)	34.3 (1.8)	34.2 (1.8)	0.238
Type of breastfeeding - formula alone - mixed	 134 (26.9%) 365 (73.1%)	 112 (24.3%) 349 (75.7%)	 0.364
Number of daily suckings, mean (SD)	6.4 (2.9)	6.6 (3.3)	0.642
Quantity of milk for each sucking, mL, mean (SD)	41.4 (26.8)	42.4 (27.0)	0.584

Table II. *Percentages of infants with positive I-GERQ-R in both groups at different visits*

Visits	**Group A**	**Group B**	**p-value**
T0	25.8%	31.7%	0.046
T1	16%	45.8%	<0.001
T2	14.7%	50.7%	<0.001

Table III. *Mean (+ Standard Deviation) total scores of I-GERQ-R in both groups at different visits*

Visits	**Group A**	**Group B**	**p-value**
T0	22.3 (5.3)	23 (5.3)	0.204
T1	14.6 (5.2)	22.3 (7.8)	<0.001
T2	11 (4.5)	21 (7.6)	<0.001

According to our results, the prevalence of relevant regurgitation symptoms at baseline was about 25-30% in an unselected population of formula-fed infants. This data was consistent with others, although the prevalence of regurgitation has a wide range in literature, according to the different populations studied (1, 17, 18).

The current guideline does not recommend pharmacological treatment but only reassurance and behavioral approach as a first steps strategy (19). However, we speculated that nutritional supplementation with probiotics could improve microbiota colonisation and represent a strategy to reduce the prevalence of relevant regurgitation symptoms in formula-fed infants.

According to the current data, the percentage of relevant regurgitation symptoms significantly decreased over the time when a probiotic supplementation with BB-12 was scheduled.

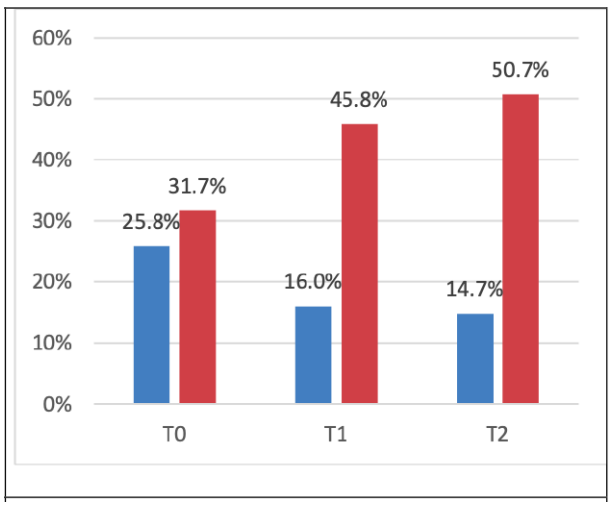

Fig. 1. *Changes over time for prevalence of persistent regurgitation (total score > 16 for I-GERQ-R) in Group A (blue) and Group B (red).*

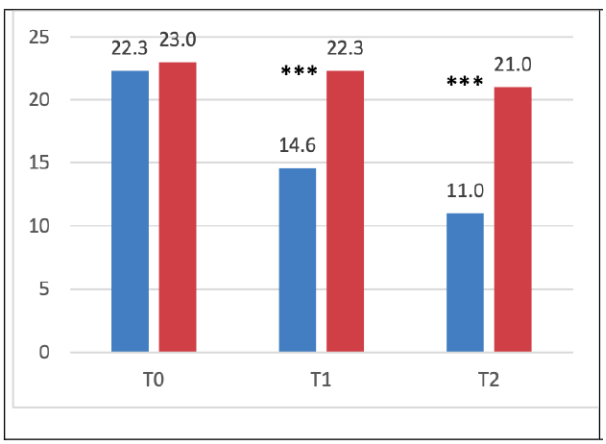

Fig. 2. *Changes over time for the total score of I-GERQ-R questionnaire in Group A (blue) and Group B (red).*

These results were consistent with some previous studies investigating the role of probiotics in managing infants with regurgitation. The first placebo-controlled study explored the effects of *Lactobacillus reuteri* DSM 17938 on gastric emptying in infants with regurgitation (20). Thirty-day probiotic supplementation with *L reuteri* significantly reduced the fasting antral area, increased the Δ gastric emptying rate, and, consequently, reduced the regurgitation episodes. A further randomised study, conducted by the same researchers, investigated the prophylactic use of *Lactobacillus reuteri* DSM 17938 during the first 3 months of life for reducing the onset of colic, gastroesophageal reflux, and constipation in term newborns (21). The probiotic supplementation significantly reduced the duration of crying time, regurgitation episodes, and evacuation number. Another study demonstrated that the early administration of *Lactobacillus reuteri* DSM 17938 for 4 weeks in full-term breastfed infants significantly reduced daily regurgitation (22).

The previous study investigated the effect of a partially hydrolysed whey infant formula supplemented with starch (as prebiotic) and the probiotic *Lactobacillus reuteri* DSM 17938 on regurgitation and gastric motility in infants with functional regurgitation (23). The formula containing the probiotic significantly decreased the regurgitation frequency and improved the gastric emptying rate. Therefore, this body of evidence underscores the potential benefit of probiotics in managing infants with functional digestive disorders. Namely, the present study confirmed previous positive outcomes in infants supplemented with oral probiotics.

In addition, BB-12 supplementation exerted positive effects in colic infants (24,25), increased the production of intestinal short-chain fatty acids in infants (26), improved the microbiota composition in preterm infants (27), and reduced the gastrointestinal infections in early childhood (28). Consequently, the outcomes obtained in the current study conducted in infants with regurgitation confirmed the multifaceted efficacy of BB-12 in pediatric disorders.

However, the present study had limitations, including the open design, the lack of microbiota assessment, and biomarkers measurement. Nevertheless, the strength of this experience was the relevant number of infants who participated and the clinical setting, such as neonatal clinics. Thus, the findings could faithfully reflect what occurs in daily practice.

In conclusion, this study demonstrated that persistent regurgitation is a relevant medical problem in infants. In addition, a two-month BB-12 supplementation (ABINAT12®) reduced the prevalence and severity of persistent regurgitation in formula-fed infants significantly.

Conflict of interest:

All authors state that there is no conflict of interest.

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