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CONTENTS

The management of upper airway diseases: an ongoing challenge for the clinician

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Upper airway diseases are widespread in clinical practice. However, some aspects are still debated. The current supplement presents and discusses the most common disorders encountered in daily medical activity. The COVID-19 dramatic pandemic requires an urgent solution. Promising non-pharmacological agents are discussed. Chronic diseases are frequent in childhood, so to know risk factors is useful in their management. Allergic rhinitis and chronic rhinosinusitis should be treated with anti-inflammatory drugs, but complementary compounds should be alternated to preserve health. Empty nose syndrome is a frequent complication of nasal surgery and requires adequate staging and hydrating procedure. Lastly, laryngopharyngeal reflux is an intriguing challenge for the clinician. Alginates represent a safe and effective way to relieve LPR symptoms.

Upper respiratory diseases (URD) include many disorders caused by many potential causes (1). URD may be classified into two main groups in clinical practice, such as acute and chronic diseases. Infectious and immune-mediated pathways are the most common pathogenic mechanisms involved in these diseases; however, mechanical and congenital factors also contribute to their development. The current Supplement is aimed at presenting and discussing some updated topics in this field.

Firstly, COVID-19 has dramatically interfered with the daily life of all of us (2). COVID-19 is still an unknown disease as the exact pathogenesis and clinical presentation is still obscure. Likewise, prevention and treatment still require a precise definition. In this regard, there are initial suggestions that non-pharmacological remedies could be useful in reinforcing the immune system. Lactoferrin, glycyrrhizin, zinc, vitamin D, and local bacteriotherapy could be promising candidates in tackling COVID-19. In this regard, some multi-component dietary supplements and medical devices are subject to intense attention and investigation, as reported in some current articles.

Upper airway diseases are widespread in childhood, mainly concerning infectious diseases and chronic disorders (3). In this Supplement, two primary topics are discussed: sleep-disordered breathing and recurrent acute otitis media. Two clinical studies, conducted on a vast population, investigated the potential factors associated with these problems. The clinical relevance of these two studies relied on the study design, such as the real-life setting. Real-life studies mirror what occurs in clinical practice; consequently, outcomes are applied to the daily medical activity. The knowledge of pathogenic mechanisms associated with these disorders is useful as treatment could be addressed to resolve the implicated factors. In this context, the treatment of allergic rhinitis could be convenient

Keywords: upper airways diseases, COVID-19, allergic rhinitis, chronic rhinosinusitis, empty nose syndrome, laryngopharyngeal reflux

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in children with this comorbidity. Intranasal corticosteroids alternating with glycyrrhizin could be a reasonable therapeutic strategy finalized to resolve type 2 inflammation and at the same time to prevent side effects.

On the other hand, allergic rhinitis also affects adulthood (4). An International survey involved a group of expert otorhinolaryngologists. A questionary included specific queries concerning the management of allergic rhinitis. Impressive outcomes highlighted the relevance of adequately perform the work-up and the treatment using appropriate medications and allergen immunotherapy. Chronic rhinosinusitis is another common disease and requires adequate attention (5).

For this reason, a survey was conducted on an international panel of experts. There is a need to correctly performing diagnostic procedures and adopting a consolidated therapeutic strategy. Anti-inflammatory drugs should include corticosteroids and inhibitors of an alarmin, HMGB-1. Namely, HGMB1 exerts pro-inflammatory signals that promote, amplify, and maintain the cascade of pathogenic events involved in chronic rhinosinusitis. In this regard, glycyrrhizin binds HMGB1 blocking negative signals.

A common consequence of allergic rhinitis and chronic rhinosinusitis is the hypertrophy of turbinates. Treatment of hypertrophic turbinates requires medical and surgical strategies. However, surgery may induce a problematic condition, such as empty nose syndrome. Empty nose syndrome deserves careful attention in clinical practice (6). Staging the grade of empty nose syndrome is useful to define the correct therapeutic approach. In this regard, hyaluronic acid and vitamins could improve nasal mucosa's hydration and relieve annoying symptoms.

Lastly, laryngopharyngeal reflux (LPR) is a frequent disease consequent to the leaking beck of gastric material out of the esophagus (7). LPR diagnosis primarily consists of a thorough history and laryngoscopy. However, management is still debated. Consequently, a national survey explored the most common controversies in this topic. Participants answered a series of practical queries concerning the work-up and the treatment, mainly regarding the use of proton pump inhibitors (PPI) and alginates. PPIs are often overused and sometimes are ineffective but are burdened with adverse events. Alginates are effective and safe (8). A study analyzed a medical device containing magnesium alginate and simethicone in patients with LPR. The medical device was significantly effective in relieving dysphonia, dysphagia, and cough and was safe.

In conclusion, upper airway diseases are a multifaceted challenge for the clinician in daily practice. However, appropriate and safe remedies are available to treat these disorders.

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Prevention and treatment of upper respiratory diseases in the pandemic COVID-19 era

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In the pandemic coronavirus disease 2019 (COVID-19) era, the need to use preventive-curative treatments is compelling. A series of non-pharmacological compounds, including oligo-elements, vitamins, nutraceuticals, and bacteriotherapy, might affect the risk of COVID-19, both reinforcing the immune system and improving the inflammation resolution during respiratory infections. Non-pharmacological remedies are very popular and usually have no relevant side effects. Bacterial and natural products may potentiate the immune system against respiratory viruses. Moreover, these compounds also exert anti-inflammatory and antioxidant activity. Consequently, these non-chemical remedies could be prescribed to build up the immune defence and adequately treat the upper respiratory infection. In this way, natural compounds could be used to manage people in the pandemic COVID-19 era.

Background

Coronavirus disease 2019 (COVID-19) is a new emerging health problem with dramatic consequences. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first cases occurred in Wuhan (China) at the end of 2019. Then, the plague spread worldwide, and the World Health Organization (WHO) declared the pandemic on March 11, 2020 (1). This dramatic pandemic is continuously in progress, and there is a severe concern for a second wave. In this scenario, there is the awareness that no specific treatment nor a vaccine is currently available.

COVID-19 presents a broad clinical spectrum that ranges from asymptomatic disease to acute respiratory distress syndrome and multiorgan failure (2). COVID-19 is, therefore, a multifaceted, multiorgan, multi-system disease and affects every age.

From a pathophysiological perspective, the pathogenesis of severe COVID-19 entails both a hyper-inflammatory response and hyperstimulation of the immune system (cytokine storm), as widely pointed out (3). Several pharmacological treatments have been used, including antiviral drugs, anti-inflammatory drugs (corticosteroids and heparin), anti-cytokine biologics, and hyperimmune plasma. Numerous experimental trials have been performed or are ongoing, but definitive evidence still lacks the gold standard therapy.

On the other hand, preventive hygienic precautions, such as social distancing, facial mask, and handwashing, are undoubtedly essential for mitigating the infection's dissemination, but cannot be considered sufficient. In this regard, natural substances, such as non-chemical compounds, could be a reasonable way to prevent COVID-19, modulating the immune system, and resolving the inflammation associated with upper respiratory infections. The outcome could be to increase the defenses against infections. Moreover, non-pharmacological remedies have a consolidated

Keywords: COVID-19, upper respiratory infections, microbiota, bacteriotherapy, oligo-elements, nutraceuticals

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efficacy combined with optimal tolerability and safety. These remedies include bacteriotherapy, nutraceuticals, and food supplements.

Bacteriotherapy

Recently, increasing attention has been paid to the concept of microbiota (4, 5). The concept that an infection changes the healthy composition of microbiota has long been well known (6, 7). Consistently, the healthy composition of bacteria in the upper airways could inhibit pathogens' growth (8). As a proof of concept, the recolonization with "interfering" bacteria could restore the physiological microbioma and prevent RRI (9). A possible mechanism is the capacity of some strains of α Streptococci of producing bacteriocin-like inhibitory substances (BLIS) able to contrast pathogens (10). Therefore, modulation of upper airways microbioma could represent an intriguing option (11). The term "bacteriotherapy" has been coined over 70 years ago (12). The first experiences were collected in the early '50s (13-15). Bacteriotherapy has been re-evaluated later using bacteria as probiotics throughout the maintenance or restoration of a physiological microbiome. The mechanism involves the interference and/or inhibition of pathogens from the production of antimicrobial proteins and immunomodulating mediators (12). In this regard, one strain, *Streptococcus salivarius* 24SMB, has been evaluated. *S salivarius* is a non-pathogenic species that colonize the oral cavity and is a primary BLIS (16). A study demonstrated that this strain had potent activity against *S pneumoniae*, was harmless to other *S salivarius* species, was non-pathogenic, and adhered to human larynx cells (17). A further study provided evidence that the topical administration of *S salivarius* by nasal spray colonized the nasopharynx (18). Therefore, these preliminary studies paved the way for a new approach based on the local administration of "friend" bacteria. In particular, the local bacteriotherapy approach re-colonize upper airways with healthy microbes and displace pathogens by bacterial interference (10).

Nutraceuticals

In COVID-19 patients, exaggerate inflammatory response, impaired innate and acquired immune activity, and oxidative stress are frequently detected. Oxidative stress and inflammation are closely associated as well as coagulative disorders are frequently observed in severe COVID-19. There are medical devices and food supplements claiming anti-inflammatory, antioxidant, and immunomodulatory activities. In this regard, we focus the attention on some substances, including lactoferrin, β-glucan, glycyrrhetic acid, vitamin C and D, and D-panthenol. All these natural components could exert synergistic effects providing a good effect in preventing respiratory infections and potentially for COVID-19.

Lactoferrin

Lactoferrin is a glycoprotein of human secretions that is part of the non-specific defensive system, such as the innate immunity (19). Lactoferrin exerts a relevant activity against microbial and viral infections and provides anti-inflammatory effects (20,21). Its anti-inflammatory activity depends on its ability to enter, through receptor-mediated endocytosis, inside host cells, and to translocate into the cell nucleus (22). Lactoferrin regulates, in fact, the pro-inflammatory gene expression at this level (23). Consequently, lactoferrin down-regulates the production of pro-inflammatory cytokines, reinforcing the acquired immune response as demonstrated in *in vitro* and *in vivo* studies and clinical trials (24-27). There is convincing evidence that lactoferrin exerts important antiviral activity against many viral families, including *Retroviridae*, *Papillomaviridae*, *Herpersviridae Adenoviridae*, *Pneumoviridae*, *Orthomixoviridae*, *Hepadnaviridae*, *Picornaviridae* (28). Lactoferrin antagonizes the viral entry into host cells through its competitive binding to the cell surface receptors (29). Moreover, lactoferrin prevents viral infections activating dendritic cells (30). Therefore, lactoferrin acts in the early phase of viral infections.

B-glucans

B-glucans are natural molecules that have highly conserved structures, which act as PAMPs (31). Glucans are polysaccharides exerting immunomodulatory activity, mainly concerning

cellular immunity. Macrophages are the principal target of glucans and monocytes, dendritic cells, and NK have receptors for them. Glucans modulate transcription factors and dampen the release of pro-inflammatory cytokines, mostly IL-6, IL-8, and TNF-α (32). Moreover, glucans promote a type 1 immune response increasing interferon production (33). Therefore, β-glucan plays a crucial role in enhancing the immune response against infections.

Glycyrrhetic acid

Glycyrrhetic acid (GA) is the most active glycyrrhizin component, a glycoside alkaloid present in *Glycyrrhiza glabra* roots (34). GA inhibits the HMGB1 chemotactic and mitogenic functions, without impeding DNA binding, which exerts important anti-inflammatory activity. GA is well tolerated, even at high concentrations (35).

Vitamin C

Vitamin C is an antioxidant; consequently, it is most evident under conditions characterized by elevated oxidative stress. A paradigmatic example is provided by the infections in which activated phagocytes release an abundant quantity of oxidizing substances, such as reactive oxygen species (36). Vitamin C is an efficient water-soluble antioxidant and may protect host cells against these agents' actions released by phagocytes. Moreover, vitamin C promotes interferon production (37). Therefore, vitamin C plays a relevant adjuvant activity during infections.

Vitamin D

Vitamin D (VD) is an essential hormone for humans as exerts pleiotropic effects, including anti-inflammatory activity (38). Throughout the body, many cells express the VD receptor (VDR) and the enzyme 1α-hydroxylase (39). 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 (40). Low VD status (< 50 nmol/L) is an independent risk factor for treatment failure and delayed recovery from severe lower RI in children (41). 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 (42).

Panthenol

Panthenol (also called pantothenol) is the alcohol analog 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 (43). It improves hydration, reduces inflammation, and accelerates mucosal wounds' rate of healing (44). 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.

CONCLUSIONS

The pandemic COVID-19 era taught us that there is a compelling need to identify a potential preventive strategy to avoid infection and, if infected, minimize inflammatory consequence. In this regard, the use of non-chemical remedies should be welcome. There are several products, but the choice should be oriented toward compounds with adequate evidence. Lactoferrin is a natural component, so it is safe and well-tolerated at any age, mainly in children. In particular, lactoferrin is an essential physiologic immunomodulant in early life able to act on different targets, including the immune system, cellular replication, virus, bacteria, parasites, and fungi (Fig. 1). A new multi-component medical device contains other natural biological agents, including β-glucan, glycyrrhetic acid, vitamin C and D, and D-panthenol. All these substances have synergistic activity in preventing and fighting a respiratory infection (Fig. 2). In the absence of specific vaccines and medications, this new therapeutic strategy could

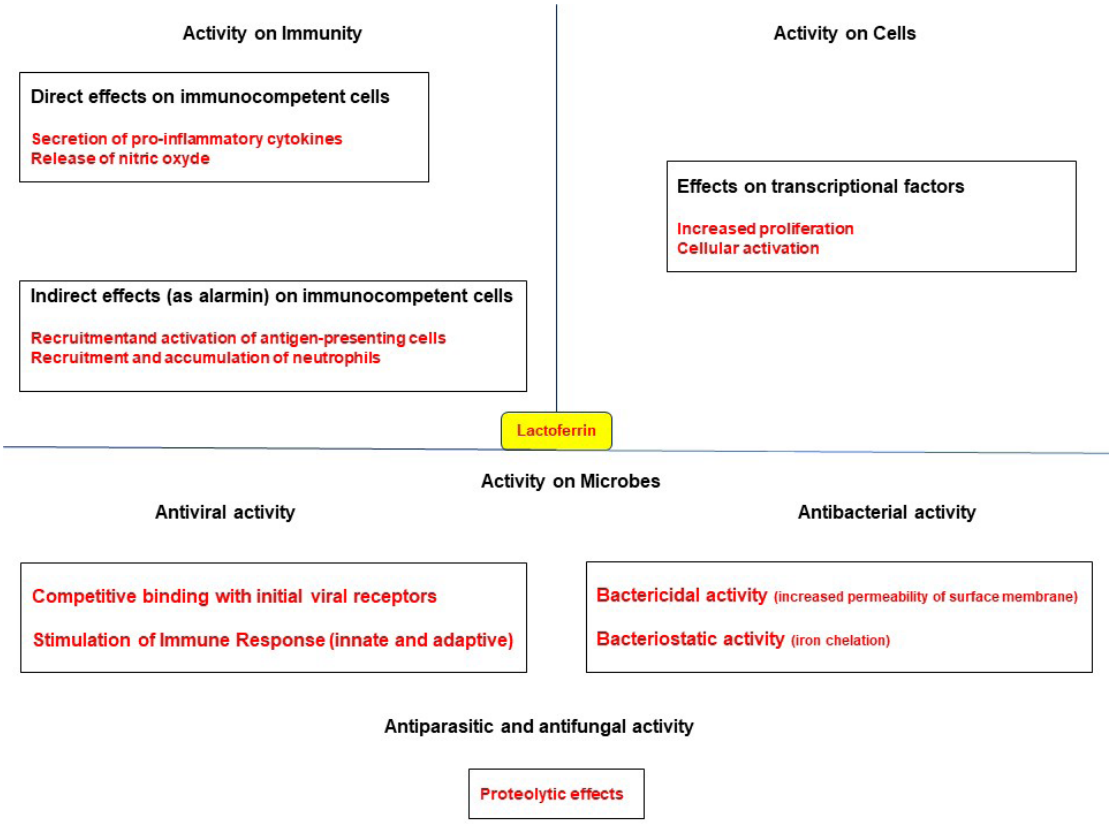

Fig. 1. *Mechanisms of action of Lactoferrin on immunity, cells, viruses, bacteria, and fungi*

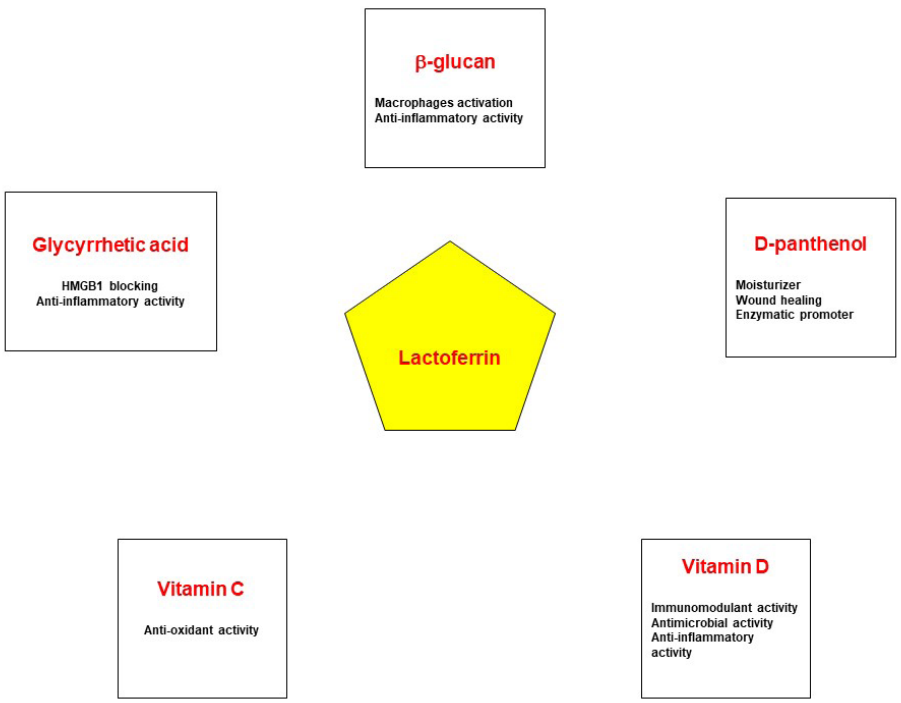

Fig. 2. *Synergic effects exerted by the components contained in a new medical device*

also be useful from an emotional point of view, as people are looking for valid preventive options. Of course, there is a need to provide adequate evidence to support this opportunity.

In conclusion, our opinion is that preventive bacteriotherapy and other natural substances, as well as early treatment of upper airway infection with anti-inflammatory and antioxidant compounds, could represent an appropriate strategy to potentially prevent COVID-19 in the general population and overall in at-risk subjects, mainly concerning children, elderly subjects, and people with fragility.

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Lactoferrin: a potential candidate to fight respiratory infections in the pandemic COVID-19 era

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Respiratory infections are a significant burden at any age, but especially in childhood and aging. The COVID-19 pandemic has worsened the issue since there is no specific treatment and vaccine is not available. Moreover, respiratory symptoms cause social stigma in subjects suffering from an infection of any kind. As new drugs require a very long time to be marketed, a natural compound's interest is growing. In this regard, lactoferrin is a multifunctional protein present in secretions, mainly in breast milk. Lactoferrin has marked antimicrobial activity, including antibacterial, antiviral, antiparasitic, and antifungal. Moreover, lactoferrin strongly affects immune response and cellular control activity. Therefore, this natural component could provide a promising effect in preventing respiratory infections and potentially also for COVID-19.

Background

A new coronavirus (SARS-CoV-2) caused the coronavirus disease, which emerged in late 2019 (COVID-19). COVID-19 started in China and rapidly spread worldwide, so becoming a pandemic. Being a virus new to humankind, everyone is potentially susceptible to this infection. Consequently, the situation has evolved so rapidly that over 10 million people have been infected worldwide, and hundreds of thousands have died until now. Moreover, there is no specific treatment for COVID-19 at present, and a traditional drug requires 10-15 years to be available for clinical use. Active immunization depends on a safe and effective vaccination, but the SARS-CoV-2 vaccine is still under investigation. Therefore, there is a need to identify a potential candidate among the various therapeutic options available to date (1). In this regard, it is, above all, fundamental to know in detail the immunological response to SARS-CoV-2. This virus belongs to the *Coronaviridae* family (2). It has been reported that the genome of SARS-CoV-2 corresponds to 80% of SARS-CoV-1 (3), the etiologic agent for the severe acute respiratory syndrome (SARS). Therefore, the available information for the pathogenesis SARS-CoV-1 infection could help define potential treatments for COVID-19.

The SARS emerged in 2003 (4), and the genome sequence has been described in detail (5). Reghunathan and colleagues investigated the expression profile of immune response genes in patients suffering from SARS (6). Surprisingly, there was no expression of genes coding for cytokines nor a specific adaptive immune response against CoV, but several genes, involved in innate immunity, were overexpressed, including the genes coding for lactoferrin (6). Lactoferrin (LF) expression was elevated by approximately 150 fold in SARS patients compared with healthy controls. That study also demonstrated that LF enhanced NK cell activity and stimulated neutrophil

Keywords: respiratory infections, COVID-19, lactoferrin, food supplement, prevention

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aggregation and adhesion. Therefore, it has been speculated that Lactoferrin (LF) could be a candidate in the fight against COVID-19 (7, 8).

On the other hand, there is growing concern about the possibility of getting other respiratory infections. Furthermore, another source of concern is the stigma of those who complain of respiratory symptoms such as sneezing, coughing, and fever potentially attributable to COVID-19. Also, contracting a respiratory infection during this period can severely affect the work and school presence. Fever (>37.5°) and respiratory symptoms are factors that preclude access to public places pending diagnostic tests. As a result, never as now is the need to be can prevent respiratory infections. So even in this case, the use of substances that modulate the immune response appears attractive.

Based on this background, lactoferrin could be a potential biological agent able to modify the immune response.

Lactoferrin

LF is a multifunctional protein present in external secretions, including saliva, tears, milk, nasal, and bronchial secretions, gastrointestinal fluids, and urine mucosal secretions, and is an essential constituent of the neutrophilic granules of leukocytes (9). In particular, it has been believed that LF is the most polyvalent protein in vertebrates (10). LF was initially identified in 1939 as a "red protein" in the whey (11). The most abundant source of LF is human and bovine milk (12). The concentration varies with the lactation stage as colostrum contains up to 8 mg/mL, whereas mature breast milk about 2-3 mg/mL.

LF is a glycosylated globular protein and binds iron due to its sequestration of Fe^{2+} and Fe^{3+} free ions; therefore, it is included in the metalloproteins family (13). LF is produced by different cell populations, including glandular epithelial cells, neutrophils, lymphocytes, and macrophages (14). LF is rapidly and abundantly secreted during an inflammatory response (15).

LF has an extraordinary multitasking ability, such as has metabolic activity, modulates innate and adaptive immunity, has antimicrobial activity against bacteria, viruses, parasites, and fungi, exerts antioxidant and anti-inflammatory effects, and repairs damaged tissues (16).

LF and immune response

LF is a relevant modifier of innate and adaptive immunity and significantly affects the immune system's maturation during the first stages of life (13). Consistently, the breast milk and mostly colostrum are plenty of LF. Adequate maternal LF intake guarantees the immune response's physiological plasticity and defends from infections (9). LF supplementation provided beneficial results (17), but conflicting outcomes were reported, probably influenced by methodological bias, including timing and patients' selection (18).

LF activates antigen-presenting cells (APC), namely dendritic cells, macrophages, and B cells, so increasing their phagocytosis and release of interleukin(IL)-12 that amplifies APC activity (19). LF stimulates dendritic cells to release IL-8, but reduces IL-6 and IL-10, modulating the immune response (20). LF promotes B lymphocyte differentiation and maturation, antigen presentation to T cells, and IgG and IgA (19). Moreover, LF advances Type 1 response and dampen type 2 inflammation, typical of allergic disorders, balancing a physiologic immune response (21).

LF and inflammatory response

LF down-regulates pro-inflammatory production, dampening acute inflammation and facilitates inflammation resolution (22). LF blocks the detrimental persistence of inflammation leading to chronic inflammation. LF exerts a pivotal anti-inflammatory activity in several aseptic inflammation diseases, including iron-deficient chronic anemia, type diabetes mellitus, Alzheimer's disease, atherosclerosis, and septic inflammation which experience patients with cystic fibrosis, inflammatory bowel disease, and bacterial infections (22). LF supplementation provided beneficial anti-inflammatory effects, as recently reviewed (15).

LF and infections

LF displays antimicrobial activity against bacteria, viruses, fungi, and parasites. It possesses a dual antibacterial activity, such as bacteriostatic, chelating Fe^{3+}, limiting bacterial growth, and bactericidal, disrupting bacterial cell wall and increasing membrane permeability, and so causing bacterial death (10). Moreover, LF interferes with bacterial adhesion to mucosal tissues; consequently, LF reduces virulence (23). LF, interacting with fractions of microbial origin, such as pathogen-associated molecular patterns (PAMPS), promotes the release of pro-inflammatory cytokines (IL-1, IL-6, IL-

8, IL-12, TNF-α), lipid-derived mediators, and reactive oxygen molecules, that antagonize bacteria. Finally, LF interacts with toll-like receptors amplifying the immune response against microbes.

LF plays different antiviral activity, mainly inhibiting the viral binding to host cells, hindering the intracellular replication, and enhancing immune response. In particular, LF blocks the glycosaminoglycans, mainly heparan sulfate, which are initial viral receptors. LF also stimulates NK activity and type 1 cytokines, that fight virus infection.

LF has a wide-spectrum activity against fungi and antiparasitic activity, modulating the immune response and increasing T $CD4^+$ cell effects.

LF and COVID-19

LF, as mentioned above, interacts with the virus in the early stages of exposure. LF interferes with the first anchoring of CoV acting on heparin sulfate glycosaminoglycan (HSPG) cell receptor. HSPG molecules provide the preliminary docking sites on the cellular surface. In other words, HSPG functions as a storage site for CoV, mediating an "in trans" infection and presenting it to the target cells. CoV, after initial anchoring to HPSG, is accumulating on the cell surface where can recognizes specific receptors, namely angiotensin-converting enzyme 2 (ACE2): a metallopeptidase hooking the virus to spike proteins and so allowing virus penetration and internalization into host cells, the so-called "viral surfing" (24). In this regard, Lang performed an elegant study that provided evidence concerning the LF ability to inhibit SARS-CoV binding to HSPG (25). LF protects against coronavirus, indirectly enhancing NK activity and neutrophil aggregation, and directly blocking intracellular entry.

In this way, LF may protect the host against coronavirus invasion. Therefore, based on this evidence, LF could be an intriguing candidate to fight respiratory infections in the COVID-19 era. A proof of concept has been recently provided to support this hypothesis by a preliminary study conducted in Spain (26). Serrano and colleagues enrolled 75 patients with typical COVID-19 symptoms. Patients were treated with a liposomal bovine lactoferrin nutritional syrup food supplement, containing 32 mg of LF/10 mL and 12 mg of vitamin C, 4-6 times/day per 10 days. In some patients, an additional zinc solution was administered. Family members, cohabitant with patients, were also treated with a half dose. This treatment ultimately resolved the COVID-19 in all patients within the first 4-5 days. Equally, preventive treatment in family members was effective. The authors proposed different mechanisms of actions, including LF anti-inflammatory effects by balancing digestive microbiota, increasing "good" cytokines (IL-4, IL-10), reducing pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), and downregulating transcription factors (NFkB). Of course, the open design and the lack of robust methodology require further rigorous studies to confirm these exciting outcomes.

LF future applications

As discussed, LF could represent a promising biological agent potentially able to prevent and cure a respiratory infection, mainly of viral origin. LF properties ensure a multifunctional activity carrying out the immune response, inflammatory reaction, and microbial infection.

Conclusions

Lactoferrin could be a promising candidate to prevent and cure respiratory infections, mainly in the pandemic COVID-19 era. Lactoferrin is a multifunctional agent, providing anti-inflammatory, antimicrobial, and immunomodulatory effects. Moreover, block virus docking and enhances immune response. In particular, severe COVID-19 is characterized by hyper-inflammation and high virulence (27). In this regard, lactoferrin effectively counteracts both inflammation and infection (28).

On the other hand, lactoferrin is a natural component, so it is usually safe and well-tolerated at any age, mainly in children. In particular, it seems to be one of the essential physiologic immunomodulant in early life. Lactoferrin could be, therefore, able to prevent and fight a respiratory infection. In the absence of specific vaccines and medications, this new therapeutic strategy could also be useful from an emotional point of view, as people are looking for valid preventive options. Of course, there is a need to provide adequate evidence to support this opportunity.

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Glycyrrhizin for topical use and prophylaxis of COVID-19: an interesting pharmacological perspective

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COVID-19, the disease caused by the SARS - CoV - 2 pathogen, is currently a pandemic. At the moment there is not an available vaccine, so, scientific community is looking for strategies and drugs to implement prevention and prophylaxis. Several compounds are examined for this purpose. Glycyrrhizin, an alkaloid extracted from licorice plant (glycyrriza glabra), is one of the most studied molecules, both for its peculiar biological functions and for its pharmacological effects. This brief review aims to highlight the characteristics of glycyrrhizin for topical use on the nasal and ocular surfaces. The anti-inflammatory activity, the ability to inhibit the accumulation of ROS, the antiviral property, but, above all, the ability to bind the ACE receptor and the SARS - CoV-2 protein S in the extracellular environment make Glycyrrhizzin for topical use a compound with a high prophylactic potential for SARS - CoV - 2 infection, also due to its low cost and the absence of significant side effects.

Background

In the last quarter of 2019 in Wuhan city, Hubei Province of China, a syndrome of unknown etiology, characterized by fever associated with pneumonia, spread with impressive speed, so much so that in December the Chinese health authorities made known to the world the presence of an epidemic outbreak. On January 7, 2020, Chinese researchers isolated the pathogen: an unknown new beta coronavirus. WHO named it SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) (1). Although the infection was initially confined to China, it has spread rapidly worldwide since February 2020. On 11 March 2020, WHO declared a pandemic.

At the moment there isn't any vaccine against SARS - CoV - 2 syndrome (named by WHO COVID- 19): the management of the pandemic is therefore founded on the prevention of infection, on the use of antiviral drugs blocking replication of the virus, and on the use of drugs attempting to mitigate the inflammatory response and its effects on the host organism in which the virus has replicated and which leads to the most serious and fatal complications in 6.7% of people affected, especially the older ones and those with significant comorbidities (2). Every therapeutic strategy is therefore at this moment urgent and important.

SARS - CoV - 2

The pathogen virus responsible of COVID - 19 was named SARS - CoV - 2 by the WHO as it is extremely similar to the pathogen responsible for the 2003 Chinese SARS epidemic, SARS - CoV - 1. The two pathogens share 79.5% of the genetic sequences. Like the other CoVs, SARS - CoV - 2 own as genome a single strand of RNA and four structural proteins: protein S (spike); protein E (envelope), protein M (membrane) and protein N (nucleocapsid).

Keywords: COVID-19, SARS CoV-2, Glycyrrhizin, Prophylaxis

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At the same way of SARS - CoV -1, SARS - CoV-2 uses the ACE2 receptor (angiotensin - conversion - enzyme 2) as a gateway for to enter in the human cell, as its protein S has the ability to bind this cellular receptor. ACE2 receptor is a cell membrane protein with the active site domain exposed on the extracellular surface of the cell membrane (1).

Micheal Letko et al., also demonstrated that SARS - CoV-2 has the ability to enter and replicate only and exclusively in human cells expressing the ACE2 receptor, and not into the others, pointing out the important role of the ACE2 receptor in the therapeutic management of COVID 19 (3). Compared to SARS - CoV- 1, SARS - CoV - 2 has a binding affinity to the ACE2 receptor 10 - 20 times higher, and it is due to structural differences in the protein S of the two viruses (4).

Once inside the cell, SARS - CoV-2 begins the replication phase, usually in the respiratory system, inducing the host's immune response. Fever, cough and pneumonia are the most common clinical result of the virus replications. In some subjects the immune response caused by virus is abnormal and violent. In this situation patients develop a cytokine storm syndrome (5).

Cytokine storm has been observed in patients with severe SARS - CoV-2 disease and it is characterized by an overproduction of proinflammatory cytokines such as interferons (IFNs), tumor necrosis factors (TNFs), interleukines (ILs) and chemokines. The overproduction of these factors leads to serious tissues damage: the subjects affected by the cytokine storm progress rapidly towards acute respiratory distress (ARDS) and towards shock up to multi-organ failure, because of disseminated intravascular coagulation (DIC). Often all that leads to the *exitus* (6, 7). Therefore, recognizing of the reasons of cytokine storm and, above all, protecting people who may develop cytokine storm early is the crucial challenge.

At the moment the evidence indicates that the elderly subjects, the subjects with other important comorbidities and the subjects exposed to high viral load are the most interested by the phenomenon, however this aspect needs further studies to be completely understood (6, 7).

Glycyrrhizin for topical use

Glycyrrhizin is an alkaloid extracted from the licorice plant (Glycyrriza glabra) made up of glycyrrhetic acid linked to two carbohydrate residues, the glucuronic acid and the glycyrrhetinic acid. For systemic use, glycyrrhizin has been widely demonstrated to have several pharmacological relevant properties, and this places it among the leading molecules in both traditional Chinese and Ayurvedic medicine (8-9).

Several scientific works demonstrate that Glycyrrhizin is endowed with anti-inflammatory activity, with ability to modulate the production of inflammatory cytokines (10), with ability of inhibit ROS accumulation (11), with ability of inhibit thrombin activity (12), with ability of reduce inflammatory exudates and with ability of induce production of endogenous interferon (13). However, the focus of this work is pointing out the attention on the glycyrrhizin for topical use, in particular on its action on the nasal mucosa and ocular surface (15, 16).

Topical glycyrrhizin has anti-inflammatory properties, as it is able to bind to a pocket of HMGB1 protein, preventing the binding with its receptor. HMGB1 protein is a nuclear protein belonging to the group of DAMP (damage associated molecular patterns). These proteins are released into extracellular liquid after a cell damage and they amplify the message of damage occurred, inducing the activation of the immune cells, in particular the macrophages and the monocytes, because of the binding to their surface's receptors RAGE, TLR - 2 and TLR -4 (14). Thus activated, macrophages and monocytes release cytokines and chemokines, creating an inflammatory process. In addition, having the immune activated cells the property of releasing HMGB1 in the extracellular liquid, inflammation tends to be fueled due to the chemotaxis induced by HMGB1.

In this context, the topical use of glycyrrhizin on the nasal mucosa and on the ocular surface, can significantly reduce the amount of two important pro-inflammatory cytokines, TNF-alpha and IL-beta, confirming the fact that the bond Glycyrrhizin - HMGB1 effectively reduces the inflammatory infiltrate and counteracts its chronicization (17).

Recent studies have also highlighted that

Glycyrrhizin could inhibit the production of free radicals by neutrophilic granulocytes, although it does not involve an inhibiting action on chemotaxis and although Glycyrrhizin is not a ROS scavenger substance. The decrease in ROS amplifies the anti-inflammatory activity of glycyrrhizin for topical use (18).

Antiviral activity of Glycyrrhizin has been known for years (19). However, it is worth noting that in vitro studies for to observe the effect of Glycyrrhizin on replication of SARS - associated coronavirus have shown that this activity occurs even before the adsorption of virus into the cell where the replication cycle begins, and, that, this activity, is actually more effective than the activity of viral anti-replication inside the cell that the molecule is in any case able to exercise (20). Therefore, based on these observations, we can see that topical glycyrrhizin could play an important role as antiviral substance.

Glycyrrhizin and SARS-COV-2

Glycyrrhizin is one of the molecules that the scientific community is studying to find out an effective molecule for the prophylaxis of COVID-19. There are many studies that highlight how Glycyrrhizine for systemic use can represent an interesting molecule and for its potential effectiveness in counteracting SARS-CoV-2 that for the low amount of side effects (21, 22). However, this work aims to highlight how the topical use of glycyrrhizine also has the potential to represent an important weapon against SARS-CoV-2 infection.

It has now been observed and widely demonstrated that the SARS-CoV-2 virus uses the upper respiratory tract and the ocular surface to enter into the human body and that it uses the ACE 2 cellular receptors to enter into the cell and begin the replication phase.

The important work of Ziegler et al., an international collaboration between some of the major research centers in the world, highlighted how, in addition to being present in lung and intestine tissue, ACE 2 receptors are expressed by secretory globets cells of the nasal mucosa, and that this expression is amplified by the contact with some types of virus (23).

Similarly, a recent work of Lingli Zhou et al. at the John Hopkins University of Baltimora, analyzed the ocular surface conducting both in vivo and on cadaver studies, and it showed that ACE2 receptors are also expressed at the conjunctiva, limbus and cornea level, with particular incidence on the conjunctival and corneal epithelial surface (24).

We know that topical Glycyrrhizin used both on the nasal mucosa and on the ocular surface has anti-inflammatory, antiviral and anti-ROS action because of the reasons discussed above in this paper, but recent studies highlight two very interesting aspects.

In silico docking and drug likeness studies conducted on different molecules to predict their capacity for binding ACE2, which may prevent the SARS-CoV-2 infection, have clearly highlighted the ability of Glycyrrhizin to bind to sites ARG-559, GLN-388, ARG393 and ASP-30 of ACE2 receptors present at the external surface of cellular membrane with a selectivity index greater than 65 and with an estimated binding force of -9 Kcal / mol (25).

Moreover, in silico docking and drug likeness studies conducted on different molecules to predict their capacity for binding SPIKE protein of SARS-CoV-2 showed that Glycyrrhizin and Glycyrrethic Acid have significant binding affinity with various SARS-CoV-2 proteins, but especially with S protein, the one who interacts with the host ACE2 receptor. Their predicted binding energies are around - 8 kcal /mol (26).

Another very interesting evidence is due to a recent Italian study conduct in silico and in vitro. An important finding of this study has been that several steroidal molecules were effective inhibitors of the binding of the ACE2 receptor in silico and *in vitro, but* in particular, the glycyrrhetinic and the oleanolic acid showed good agreements in terms of docking AD score and in their ability to inhibit the spike/ ACE2 interaction *in vitro* (27).

This observation indicates that Glycyrrhizzin for topical use can interfere with the interaction of the virus with the host cells and can slow down the viral entry. Moreover, binding of Glycyrrhizin with the viral S protein may also minimize the immune response mediated by it.

CONCLUSION

The urgency of drug development for treating

COVID - 19 means that the attention of science has turned to all the molecules that have a pharmacological potential against this type of virus.

Glycyrrhizin is clearly considered a molecule with a great potential for its peculiar characteristics. However, little attention has been given to topical Glycyrrhizin so far. Quite the opposite, we observed that, Glycyrrhizin for topical use can be used both on the nasal surface and on the ocular surface with no side effects and it has antiviral and anti-inflammatory activity. ACE2 receptors are present on the nasal and ocular surface and the fact that Glycyrrhizin has the ability to bind the receptor ACE2 and the Spike protein of the virus and the fact that it is a low-cost and low-risk molecule make it a real interesting option as agent for blocking the virus infection. Other studies will have to be conducted, but we believe that topical Glycyrrhizzin could play a major role in SARS - CoV- 2 infection prophylaxis.

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Can *Pseudomonas aeruginosa* growth be modulated by natural compounds?

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***Pseudomonas aeruginosa* is an opportunistic human pathogen that frequently induces antibiotic resistance, as it mainly tends to form biofilms. Iron chelation may be an intriguing strategy to contrast bacterial growth. Lactoferrin is a natural compound able to chelate iron. A new multi-component medical device also contains lactoferrin. This study analyzed this compound investigating the *in vitro* capacity to inhibit *Pseudomonas aeruginosa* growth. In conclusion, this study demonstrated that a multi-component medical device (Saflovir), also containing lactoferrin, could inhibit the *in vitro* growth of *P. aeruginosa.* This activity could be positively used in the prevention of respiratory nasal infections.**

Pseudomonas aeruginosa is a common opportunistic Gram-negative pathogen causing infections in a wide range of tissue (especially in immunocompromised hosts). However, it exhibits a particular predilection for soft tissues, where the resulting infections can become either acute or chronic (1). It can be isolated from plants, fruits, soil, and water environments, such as rivers, lakes, and swimming pools. *Pseudomonas aeruginosa* typically infects airways and urinary tracts, causes blood infections, and is the most common cause of burn injury infections, hot-tub dermatitis, and outer ear infections, so-called swimmer's ear (2). *P. aeruginosa* is also the most common colonizer of medical devices (catheters, nebulizers, humidifiers). Moreover, *P. aeruginosa* commonly causes nosocomial infections, including ventilator-associated pneumonia, meningoencephalitis, and sepsis (3).

P. aeruginosa is an opportunistic pathogen; it rarely causes disease in healthy persons but can growth easily in immunocompromised patients (4). Patients with burn wounds, AIDS, and cystic fibrosis (CF) are at high risk of developing severe Pseudomonas infection, which accounts for a high death rate in this population (5). Pseudomonas can become resistant to antibiotics, which further complicates the treatment of its infections. This resistance arises from its ability to form a biofilm, a bacterial community embedded in an exopolysaccharide matrix (6). This microoganism can colonize many natural and artificial surfaces such as the mucus plugs of the CF lung, catheters, and contact lenses. Exopolysaccharide matrix is made of polysaccharides, proteins, and extracellular DNA, promoting the formation of three-dimensional structures that give the bacteria increased access to nutrients and advantages of multicellular living.

Quorum sensing (QS) plays an essential role in biofilm formation. It is an intercellular signaling system in which bacteria communicate and regulate

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gene expression by releasing small compounds called autoinducers in the environment (7). The process of *P. aeruginosa* biofilm development involves the attachment of planktonic cells to a solid surface which form microcolonies (8). QS regulated rhamnolipid production aids in microcolony formation. Cells migrate and spread over the substratum, resulting in a flat, uniform mat; the microcolonies later grow, forming stalk and mushroom-like structures. Rhamnolipids are responsible for maintaining open channels and mushroom cap formation. EPS matrix is produced aided by eDNA release and Pel polysaccharide production, which are under QS control. Cells disperse from biofilm with the help of rhamnolipid during various biofilm maturation stages and can resume the planktonic mode of growth (9).

The characteristic feature of *P. aeruginosa* biofilms is their ability to develop resistance against antibiotics, including aminoglycosides, quinolones, and β-lactams (10). Generally, the primary mechanisms of *P. aeruginosa* used to counter antibiotic attacks can be classified into intrinsic, acquired, and adaptive resistance. The intrinsic resistance of *P. aeruginosa* includes low outer membrane permeability, expression of efflux pumps that expel antibiotics, and the production of antibiotic- inactivating enzymes. The acquired resistance of *P. aeruginosa* can be achieved by either horizontal transfer of resistance genes or mutational changes (11). The adaptive resistance of *P. aeruginosa* involves the formation of biofilm in the lungs of infected patients, where the biofilm act as a diffusion barrier to limit antibiotic access to the bacterial cells (12).

Based on this background, many attempts have been made to contrast *P. aeruginosa* biofilm formation. In this regard, attention has been given to the iron chelation. Namely, iron is essential for bacterial growth. It is involved in various cellular processes, such as energy production, DNA replication, and electron transport (13). Thus, limiting the concentration of extracellular iron or disrupting iron uptake is a strategy to counter *P. aeruginosa* infections. For these reasons, lactoferrin could be an intriguing candidate as this secretory protein is characterized by iron chelating capacity (14).

A new multi-component medical device containing lactoferrin, β-glucan, glycyrrhetic acid, vitamin C and D, and D-panthenol (Saflovir, DMG Italy), could be useful to prevent infections. Therefore, we aimed to investigate whether this compound can *in vitro* contrast *P. aeruginosa* growth.

MATERIALS AND METHODS

Microbiological procedures

Culture media and reagents were purchased from Becton-Dickinson (BD Diagnostics-Difco, Franklin Lakes, NJ, USA). Tissue-culture treated 96-well polystyrene plates (Nunc) were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). *Pseudomonas aeruginosa* ATCC 27853 was grown on Columbia Agar supplemented with 5% sheep blood. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. A suspension of each microorganism was obtained in Brain Heart Infusion (BHI) after overnight incubation at 37 °C in a 5% CO_2 atmosphere. The bacterial cells were collected by centrifugation (2200 rpm, 19 °C, 5 min), washed twice in phosphate-buffered saline (PBS), and resuspended in the same buffer. Each suspension was sonicated to disperse the bacterial aggregates (Sonifier model B-15, Branson, Danbury, CT, USA, 7W for 30 s), and adjusted to 0.3 optical density units (OD) using a spectrophotometer at 550 nm (Genesys 10-S, Thermo Spectronic, Rochester, NY, USA). This value corresponds to an approximate microbial concentration of 6.00×10^8 cells / mL.

Evaluation of MCMD *effect on P. aeruginosa biofilms*

Three independent experimental runs were performed in three different weeks in order to exclude day-to-day variability, and data from the three runs were averaged. A total of 180 µl of BHI and 20 µl of the bacterial suspension were inoculated into each well of 96-well plates. The plates were incubated at 37 °C and 5% CO_2 - supplemented atmosphere for 24 h to allow for biofilm development. After that, the surnatant broth was gently removed from the wells ans 12 replicate wells were inoculated with a total of 50 µl of either the Saflovir (test), sterile PBS (negative control), or 1 wt% freshly prepared chlorhexidine solution (positive control). After 5 min, the solutions were removed, the wells gently rinsed three

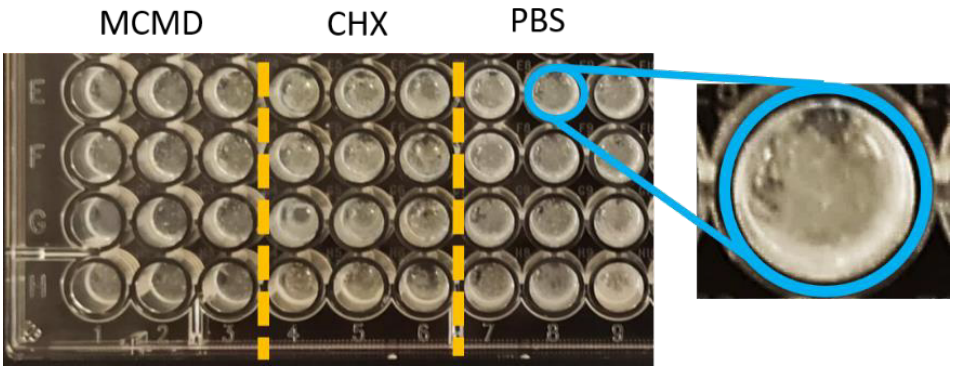

Fig. 1. *View of the 96-well plate showing the high biofilm formation expressed by P. aeruginosa after 24 h of incubation.*

times with sterile PBS to remove non-adherent cells and dilute remnants of the tested solutions, then the viable biomass adherent to the substrate was assessed using a colorimetric assay based on the reduction of a tetrazolium salt, as follows (Fig. 1).

Viable Biomass Assessment

Viable and metabolically active biomass adherent to the specimen surface was assessed using a tetrazolium-based assay as described previously (15). In brief, a tetrazolium salt stock solution was prepared by dissolving 5 mg/mL 3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) in sterile PBS; a phenazinium salt stock solution was prepared by dissolving 0.3 mg/mL of N-methylphenazinium methyl sulphate (PMS) in sterile PBS. The solutions were stored at 2 °C in light-proof vials until the day of the experiment when a fresh measurement solution (FMS) was prepared by diluting 1:10 v/v of MTT stock solution and 1:10 v/v of PMS stock solution in sterile PBS. A lysing solution (LS) was prepared by dissolving 10% v/v of sodium dodecyl sulphate (SDS) and 50% v/v dimethylformamide in distilled water and stored at 2 °C until the day of the experiment when it was warmed at 37 °C for 2 h before use. The plates were inoculated with a total of 100 µL of FMS, then immediately incubated at 37 °C under light-proof conditions for 3 h. During incubation, electron transport across the microbial plasma membrane and, to a lesser extent, microbial redox systems, converted the yellow salt to insoluble purple formazan crystals. The conversion at the cell membrane level was facilitated by the intermediate electron acceptor (PMS). The unreacted FMS was gently removed by aspiration, and the formazan crystals were dissolved by adding 100 µL of LS to each well. The plates were stored for 1 h under light-proof conditions at room temperature; 80 µL of the solution was then transferred into new 96-well plates. The absorbance of the solution was measured using a spectrophotometer (Genesys 10-S, Thermo Spectronic, Rochester, NY, USA) at a wavelength of 550 nm; results were expressed as relative absorbance in optical density (OD) units corresponding to the amount of adherent, viable, and metabolically active biomass.

Statistical analysis

All statistical analyses were performed using statistical software (JMP 12.0, SAS Institute, Inc., Cary, NC, USA). The OD data were reported as means and standard errors calculated from the natural values. The normality of distributions was preliminarily checked using Shapiro-Wilk test, and homogeneity of variances was preliminarily checked using Bartlett's test. Oneway ANOVA was used on the dataset, and Student-Newman-Keuls post-hoc test was used to highlight significant differences ($p < 0.05$).

RESULTS

Saflovir significantly ($p<0.05$ in comparison with negative control) reduced the viability of a *Pseudomonas aeruginosa* biofilm, to the same extent as the positive control, as reported in Fig.2.

DISCUSSION

P. aeruginosa is an opportunistic human pathogen associated with an ever-widening array of life-threatening acute and chronic infections, including cystic fibrosis, ventilator-associated pneumonia, urinary tract infections, otitis externa,

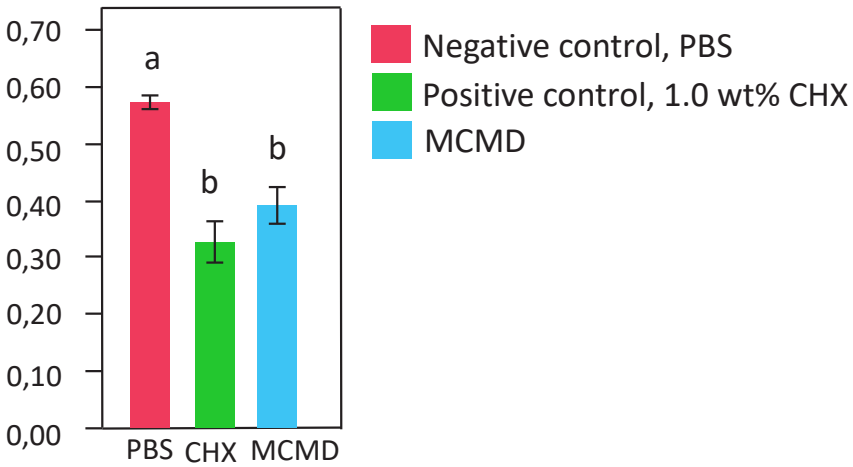

Fig. 2. Amount of adherent, viable biomass after treatment with the tested solutions. Different superscript letters indicate significant differences between groups (Student-Neuman-Keuls, $p < 0.05$).

burn and wound injuries, bone and joint infections, and systemic infections (16). *P. aeruginosa* is also one of the "ESKAPE" pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa,* and *Enterobacter* species, involved in nosocomial infections, which can "escape" the activity of antibacterial drugs (17).

On the other hand, *P. aeruginosa* has the absolute requirement for iron for infection success. By influencing cell-cell communication (by QS) and virulence factor expression, iron is a potent regulator of *P. aeruginosa* behavior (18). The management of iron metabolism is a critical factor in the interplay between host tissues and bacterial pathogens. Consequently, iron acquisition systems imposed perturbation has been proposed as a novel therapeutic approach to contrast *P. aeruginosa* biofilm infection. Moreover, consistent evidence has been provided by demonstrating that cigarette smoking produces factors that increase bacterial growth and biofilm formation in the lung by disrupting the iron-to-lactoferrin in the airways (19).

Therefore, the current study demonstrated that a new multi-component medical device could inhibit the *in vitro* growth of *P. aeruginosa*. Notably, this inhibitory activity was not shown by other phytotherapeutic agents. Probably, the reported inhibitory activity could depend on lactoferrin contained in the medical device. In this regard, there is evidence that increased iron concentration in the airways correlates with the severity of lung disease in cystic fibrosis and chronic bronchitis (20). Consequently, it has been proposed that changes in iron homeostasis can affect the susceptibility of the airway to develop infections (21).

This study has been conducted *in vitro*, so further studies should be performed clinically to confirm these preliminary outcomes.

In conclusion, this study demonstrated that a multi-component medical device, also containing lactoferrin, could inhibit the *in vitro* growth of *P. aeruginosa.* This activity could be positively used in the prevention of respiratory infections.

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Sleep-disordered breathing in the otorhinolaryngological practice

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Sleep-disordered breathing (SDB) is a common disorder in childhood. Snoring and obstructive sleep apnea represents a demanding challenge for both paediatricians and otolaryngologists. This real-life study investigated the association of demographic and clinical factors on snoring and sleep apnea in children consecutively visited. In this study, 1,002 children (550 males, mean age 5.77 ± 1.84 years), complaining upper airway symptoms, were prospectively enrolled during 2015-2017. Medical history, clinical examination, and fiberoptic nasopharyngoscopy were performed in all children. Tonsil hypertrophy significantly predicted sleep apnea (OR 95.08) and snoring (OR 5.44). Asthma comorbidity significantly predicted snoring (OR 2.26). Breastfeeding could be a protective factor for sleep apnea (OR =0.37). SDB is a frequent disorder observable in otorhinolaryngological practice. Tonsil hypertrophy and asthma could be considered predicting factors for both snoring and sleep apnea, whereas breastfeeding was a protective factor for SDB.

Sleep-disordered breathing (SDB) has been described as prolonged partial upper airway obstruction and/or intermittent complete pharyngeal obstruction that disrupts the normal ventilation during sleep (1-4). SDB ranges in severity from simple snoring to severe illness, such as obstructive sleep apnea syndrome (OSAS) (5). The European guidelines on obstructive SDB define a spectrum of clinical entities, including i) primary snoring, ii) upper airway resistance syndrome, iii) obstructive hypoventilation, and iv) OSAS (6).

Snoring is defined when there is habitual snoring (>3 nights per week) without apneas, hypopneas, frequent arousals from sleep, or gas exchange abnormalities (6). OSAS is defined as recurrent events of partial or complete upper airway obstruction (hypopneas, obstructive or mixed apneas) with disruption of normal oxygenation, ventilation, and sleep pattern (6).

The prevalence of habitual snoring is about 7.5%, and OSAS ranges between 1 and 4% of the general pediatric population (6,7). In clinical practice, snoring and OSAS are indeed commonly observed. Therefore, SDB represents a demanding challenge for both the paediatrician and the otorhinolaryngologist.

SDB management is based on a " step by step" diagnostic and therapeutic approach (8). The first step should recognize the most critical risk factors for SDB occurrence. In this regard, the work-up of SDB includes fiberoptic nasopharyngoscopy as a pivotal investigation. Therefore, the current study aimed to identify the effect of a series of demographic and

Keywords: sleep-disordered-breathing, children, sleep apnea, snoring, tonsil, asthma, breastfeeding.

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clinical factors on snoring or sleep apnea in children visited in an otorhinolaryngological clinic.

MATERIALS AND METHODS

In this study, 1,002 children (550 males, mean age 5.77 ± 1.84 years) complaining upper airway symptoms were consecutively assessed during 2015-2017. Inclusion criteria were: i) age between 3 and 10 years; ii) to have upper airways (i.e., nasal obstruction, rhinorrhea, otalgia, sore throat, cough, snoring). Exclusion criteria were: i) a craniofacial anomaly, micro/macrognatism, neuromuscular disorders, and other congenital malformation syndromes, ii) recent facial trauma, and iii) any treatment that may have influenced the findings of the otolaryngological exam. The study procedure, consisting of thorough medical history, clinical examination, and nasal endoscopy (routinely performed in all subjects during a visit), was approved by the Internal Review Board, and the parents gave informed written consent.

The clinical visit included medical history, mainly concerning prematurity, feeding (type and duration), passive smoking, family history of atopic disease, and documented diagnosis of asthma and allergic rhinitis previously performed by paediatricians.

Complaining of upper airway symptoms, i.e., snoring and sleep-associated apnea, to whose parents had concerns about upper airway related symptoms were reported by parents who, in our experience, are usually reliable in this regard. In particular, snoring was considered if present >4 nights a week, and sleep apnea when parents observed respiratory break lasting >3 seconds for more than three days a week.

Endoscopy was performed with a pediatric rigid endoscope diameter 2.7 mm with a 30° angle of vision (Karl Storz cod 7207 ba) with a 300-W cold light source (Storz Xenon Nova, cod. 20134001), and a light cable of 1.8 mm length. The complete description of the procedure was previously described in detail (9,10).

Tonsils size was assessed according to validated criteria (11), as follows: grade 1: tonsils in the tonsillar fossa barely seen behind the anterior pillar; grade 2: tonsils visible behind the anterior pillar; grade 3: tonsils extended three-quarters of the way to med-line; grade 4: tonsils completely obstructing the airway (also known as kissing tonsils).

According to Parikh's classification, the adenoid size was graded based on the anatomical relationships between the adenoid tissue and the following structures: vomer, soft palate, and torus tubarius (12). Turbinate hypertrophy was defined by the contact of inferior and/or middle turbinate with the adjacent structures, as previously described (10). Continuous variables are given as means with standard deviations and categorical variables as the number of subjects and percentage values. The SDB was considered as primary outcome measurement. The univariate Multinomial Logistic regression models were performed to screen the effect of the clinical and demographic variables on the SDB. The Likelihood Ratio test was used as a statistical significance test, and the estimated p-values were adjusted for multiple comparisons by the Bonferroni correction method. The covariates with a p-value <0.05 were then selected for the multivariate analysis, where SDB was the dependent variable. Multivariate analysis was performed using the Multinomial Logistic regression again, and the Akaike and Information Criterion made the model selection. The odds ratios associated with SDB were calculated with their 95% confidence interval. With a p-value <0.05, differences were selected as significant, and data were acquired and analyzed in the R v3.5.2 software environment (13).

RESULTS

A total of 1,002 (450 females, 552 males, mean age 5.77 ± 1.84 years) children were assessed, and the data were analyzed. SDB was observed in about 75% of subjects: 55% with snoring and 18% with sleep apnea.

Descriptive statistics of demographic and clinical factors in the levels of SDB are reported in Table I. Patients were stratified in 3 groups: without SDB, with snoring, and with sleep apnea. The univariate logistic regression analysis (Table I) demonstrated that there were significant differences among groups about the age ($p<0.0001$), the feeding ($p<0.0001$), passive smoking ($p=0.0001$), asthma comorbidity ($p=0.0002$), allergic rhinitis comorbidity ($p<0.0001$), tonsil and adenoid size ($p<0.0001$ for both), and middle turbinate hypertrophy ($p=0.0001$).

The multivariate analysis (Table II) confirmed a statistically significant effect of feeding, asthma, and

tonsillar hypertrophy on SDB (p-values: <0.0001, 0.0046, and <0.0001, respectively). In particular, breastfeeding significantly reduced the chance of having sleep apnea (OR=0.37; 95% CI 0.21 : 0.64). On the contrary, asthma comorbidity significantly increased the chance of having to snore (OR 2.26; 95% CI 1.35 : 3.8). Equally, tonsil hypertrophy increased the chance of having both snoring (OR

Table I. *Contingency tables and Output of the univariate analysis. Characteristic: variable taken into account; p-value: Likelihood Ratio p-value adjusted using the Bonferroni method. *Variables entering the multivariate analysis (see the text for abbreviations and further details).*

Characteristic	***Sleep-disordered breathing (SDB)***			**p-value**
	No SDB	*Snoring*	*Sleep apnea*	
Age *	6.12 (2.22)	5.87 (1.7)	4.99 (1.39)	<0.0001
Gender				0.9999
Female	129 (28.73%)	229 (51%)	91 (20.27%)	
Male	133 (24.18%)	322 (58.55%)	95 (17.27%)	
Prematurity				0.9999
No	237 (25.68%)	515 (55.8%)	171 (18.53%)	
Yes	25 (32.47%)	37 (48.05%)	15 (19.48%)	
Feeding *				<0.0001
Artificial	57 (24.26%)	113 (48.09%)	65 (27.66%)	
Breastfeeding 0 to 6 months	188 (29.47%)	357 (55.96%)	93 (14.58%)	
Breastfeeding 6 to 12 months	17 (13.39%)	82 (64.57%)	28 (22.05%)	
Passive Smoking *				0.0001
No	243 (26.19%)	529 (57%)	156 (16.81%)	
Yes	19 (26.03%)	24 (32.88%)	30 (41.1%)	
Family Atopy				0.2989
No	55 (20.22%)	157 (57.72%)	60 (22.06%)	
Yes	207 (28.51%)	393 (54.13%)	126 (17.36%)	
Asthma *				0.0002
No	238 (27.29%)	458 (52.52%)	176 (20.18%)	
Yes	24 (18.75%)	94 (73.44%)	10 (7.81%)	
Allergic rhinitis *				<0.0001
No	99 (21.9%)	226 (50%)	127 (28.1%)	
Yes	158 (29.04%)	327 (60.11%)	59 (10.85%)	
Tonsils size *				<0.0001
1	90 (38.63%)	138 (59.23%)	5 (2.15%)	
2	118 (38.19%)	158 (51.13%)	33 (10.68%)	
3	46 (15.65%)	202 (68.71%)	46 (15.65%)	
4	6 (3.68%)	55 (33.74%)	102 (62.58%)	
Adenoid size *				<0.0001
1	140 (37.84%)	216 (58.38%)	14 (3.78%)	
2	76 (35.02%)	112 (51.61%)	29 (13.36%)	
3	35 (16.28%)	140 (65.12%)	40 (18.6%)	
4	11 (5.56%)	85 (42.93%)	102 (51.52%)	
Inferior Turbinate Hypertrophy				0.3022
No	94 (28.06%)	166 (49.55%)	75 (22.39%)	
Yes	165 (24.96%)	387 (58.55%)	109 (16.49%)	
Middle Turbinate Hypertrophy *				0.0001
No	116 (24.58%)	238 (50.42%)	118 (25%)	
Yes	146 (27.7%)	314 (59.58%)	67 (12.71%)	

Table II. *Multivariate analysis, the predictor effects on the SDB. Results are expressed as odds ratio (OR) with 95% confidence interval (95%CI); p-value: Likelihood Ratio p-value.*

Characteristic	*Sleep-disordered breathing (SDB)*		p-value
	Snoring** versus **No SDB	***Sleep apnea** versus **No SDB***	
(*Intercept*)	3.74 (2.41 : 5.79)	1.22 (0.67 : 2.20)	<0.0001
Feeding			<0.0001
Artificial	1	1	
Breastfeeding	0.78 (0.52 : 1.16)	**0.37 (0.21 : 0.64)**	
Asthma			0.0046
No	1	1	
Yes	**2.26 (1.35 : 3.8)**	1.51 (0.59 : 3.83)	
Tonsil Hypertrophy			<0.0001
No	1	1	
Yes	**5.44 (2.9 : 10.19)**	**95.08 (39.34 : 229.8)**	

5.44; 95% CI 2.9 : 10.19) and sleep apnea (OR 95.08; 95% CI 39.34 . 229.8).

DISCUSSION

SDB is a common problem in childhood and represents a frequent reason for a medical visit. Both paediatricians and otorhinolaryngologists should manage children with SDB daily. The work-up of SDB is a step by step pathway; in this context, medical history, clinical examination, and fiberoptic nasopharyngoscopy are first-line investigations.

The current study aimed to evaluate potential risk factors associated with SDB, namely snoring and sleep apnea. The setting was a private otorhinolaryngological Unit in an Italian metropolis. This point is crucial as it means that the present investigation has been conducted in a real-life model. Also, the work-up was based on history, clinical examination, and fiberoptic nasopharyngoscopy, such as a fundamental approach. If this may constitute a study limitation, conversely, this pragmatic pathway may mirror the common daily practice occurring in primary care. From this point of view, this study shows an impressive outcome, such as about ¾ of the cohort has SDB, mostly snoring (55%) and sleep apnea (18%). Of course, this study evaluated a selected cohort of children visited by an otorhinolaryngologist because of complaining of upper respiratory symptoms. However, it underlines the high prevalence of this disorder in otorhinolaryngological practice.

The multivariate analysis provided information about predictor effects on SDB in our sample. Although many factors were associated with SDB at the univariate analysis, only three variables could significantly predict SDB outcome at the multivariate analysis. The most relevant factor was the tonsil hypertrophy, concerning both snoring and mostly sleep apnea. If tonsil hypertrophy could be involved in snoring has a conceivable explanation, finding the strong association between tonsil hypertrophy and sleep apnea is intriguing. This result is conflicting with the recent literature. As stated in the European guidelines (6), the association between tonsillar size and OSAS severity is weak at best, as reported by a meta-analysis and a randomized study (14,15). OSAS severity was determined, however, by polysomnography. Thus, the current findings could be mitigated after an in-depth investigation. Asthma comorbidity was significantly associated with snoring. This outcome is consistent with the literature that recognizes a frequent SDB coexistence with asthma, probably due to common pathogenic mechanisms (16,17).

On the other hand, breastfeeding proved to a protective factor for SDB. This finding is also consistent with a recent study demonstrating that

breastfeeding for longer than one month decreased the risk of habitual snoring and witnessed apneas (18). Those authors concluded that the underlying mechanism remained unclear, but the finding would be consistent with a beneficial effect of the breast in the mouth on oropharyngeal development with consequent protection against upper airway dysfunction, causing sleep-disordered breathing.

The current study has some limitations, including the lack of information about obesity, the selected population, the cross-sectional study design, and the lack of an appropriate and complete SDB work-up, mainly concerning polysomnography current setting was a screening visit. However, it has been reported that the history and the clinical examination with fiberoptic nasopharyngoscopy are sensitive tools, even though relatively non-specific, to screen children with SDB using a decision-making algorithm for suspected apnea that reserved polysomnography to selected cases (19). Moreover, the study did not use validated questionnaires because they are not available in Italian, and the aim was to evaluate children following a real-life approach employed in daily practice. The SDB classification was arbitrary but based on a previous study that proposed a practical approach in the primary care setting.

On the other hand, this study was conducted in a large sample and a real-life setting. Further studies should therefore be performed to confirm these results.

The current study substantially confirmed the literature evidence, but its strength, based on an impressive number of patients, suggested that it is possible to define some predicting factors associated with SDB during a simple routine ENT visit. In other words, a simple ENT visit, performed in a primary care setting, could be able to predict clinical risk factors associated with SDB.

CONCLUSION

SDB is a frequent disorder observable in otorhinolaryngological practice. Tonsil hypertrophy and snoring could be considered predictive factors for sleep apnea, and asthma was a confounder, whereas breastfeeding was found to be protective.

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Risk factors for recurrent acute otitis media: a real-life clinical experience

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Acute otitis media (AOM) is the most common bacterial infection in children. Some children with AOM tend to be otitis-prone, such as frequent recurrence of AOM (RAOM). Possible RAOM risk factors are widely debated. The current study was performed in a real-life setting, such as an otorhinolaryngologic (ORL) clinic, to identify predictive factors, including clinical data and endoscopic findings, for RAOM in children. In this study, 1,002 children (550 males, 452 females, mean age 5.77 ± 1.84 years) complaining of upper airway symptoms were consecutively visited. Detailed clinical history and nasal endoscopy were performed. Throughout the ORL visit, it was possible to define some factors involved in the recurrence of AOM, including female gender, artificial feeding, tonsillar and adenoid hypertrophy. Adenoid and tonsillar hypertrophy, female gender, and artificial are factors significantly associated with RAOM. Therefore, reducing adenoid and tonsil size, also using topical corticosteroids or glycyrrhizin, could be a reasonable strategy to potentially reduce adenoid and tonsil size. The current study suggests that also in a primary care setting, it is possible to achieve meaningful information that is relevant in clinical practice.

Acute otitis media (AOM) is an ear disease defined by acute infection signs or symptoms [1]. AOM is the most common bacterial infection in children [2-6]. Consequently, AOM is the most common reason for antibiotic prescription in the pediatric age [7,8]. Almost all children experience at least one episode of AOM during childhood. Therefore, the burden of AOM is relevant concerning the direct (healthcare expense) and indirect cost (loss of school and workdays) and the impact on the quality of life of children and their parents. Moreover, antibiotic overuse is the leading cause of the increase of multidrug-resistant microbes and the occurrence of adverse reactions [9,10]. For these reasons, several guidelines on AOM management were performed to optimize therapy [2-4].

Notably, some children with AOM tend to be otitis-prone, such as frequent recurrence of AOM (RAOM). International guidelines on AOM management define RAOM as when at least three episodes occur in the preceding six months or at least four episodes in the preceding year [3-6]. So, the identification of factors involved in the recurrence may have a beneficial interest. In particular, allergy is still a controversial and debated risk factor for RAOM. Therefore, the current study was performed in a real-life setting, such as an otorhinolaryngologic (ORL) clinic, to identify predictive factors, including clinical data, allergy, and endoscopic findings, for RAOM in children.

Keywords: recurrent acute otitis media; tonsils, real-life, predictive factors, children

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MATERIALS AND METHODS

Patients

1,002 children (550 males, 452 females, mean age 5.77 ± 1.84 years), complaining of upper airway symptoms, were consecutively referring to an ORL clinic during 2015-2018. They were consecutively enrolled in the study. Inclusion criteria were: age between 3 and 10 years and complaints of upper airways. Exclusion criteria were: current disorder(s) and treatment(s) able to interfere with the findings. The study was approved by the local Review Board and informed the parents obtained written consent.

All children were evaluated by detailed medical history (concerning RAOM, premature birth, feeding type (breastfeeding or artificial), familiar atopy, passive smoking, wheezing, recurrent respiratory infections); clinical visit; nasal endoscopy (assessing turbinate, tonsillar, and adenoid hypertrophy); and skin prick test.

Tonsil hypertrophy was defined according to Friedman's classification [11]. Adenoid hypertrophy was defined according to Parikh's classification [12]. Turbinate Hypertrophy was considered as previously described and validated [13].

Skin Prick Test was performed, as stated by the European Academy of Allergy and Clinical Immunology [14].

Statistical analysis

Continuous variables were expressed as means with standard deviations (S.D.) and categorical variables as the number of subjects and percentage values. The univariate Logistic Regression models were performed to screen the effect of clinical and demographic variables on the RAOM. The odds ratios associated with RAOM were calculated with their 95% confidence interval for each factor from the Logistic model. The Likelihood Ratio (L.R.) test was used as a statistical significance test, and the estimated p-values were adjusted for multiple comparisons by the Bonferroni correction method. The covariates with a p-value <0.05 were then selected for the multivariate analysis, where the RAOM was the dependent variable. Possible multicollinearity was assayed using Intraclass Correlation Coefficient (ICC), and the variables with an ICC >0.5 were considered associated. Multivariate analysis was performed using the Logistic Regression model again, and the model selection was made by the Akaike an Information Criterion. Moreover, multiplicative interaction terms were used to test whether the feeding type was different according to the risk factors.

The multivariate model performance was assayed using K-fold cross-validation. In particular, the dataset was split into a training set (95% of the data) and a test set (5% of the data) randomly for k different times and then the percentage of total items classified correctly, false positive and false negative rate were estimated using a confusion matrix.

Stratified analysis was then performed based on that variable using the Penalised Logistic Model for the results suggestive of an interaction with the feeding type factor (p-value <0.05).

Differences with a p-value less than 0.05 were selected as significant, and data were acquired and analyzed in the R v3.5.3 software environment [15].

RESULTS

A total of 1002 (550 males) children were consecutively visited and included in this study. The demographic and clinical characteristics of the study participants are summarised in Table I. Briefly, the mean age was 5.77 years (SD=1.84). The majority of children (N=765) received breastfeeding, while 236 received artificial feeding time. About the primary outcome, 210 (20.96%) children had RAOM, while 792 (79.04%) had no RAOM, so children were subdivided into two groups: with and without RAOM.

Descriptive statistics of demographic and clinical factors in comparison to RAOM are reported in Table II. The mean age of children in the two groups was quite similar (5.84 and 5.52 years, respectively). In patients without RAOM, artificial feeding time was received by 169 (71.61%) children, while 623 (81.44%) children received breastfeeding. Instead, in children with RAOM, 67 (28.39%) and 142 (18.56%) had artificial feeding and breastfeeding, respectively.

The univariate logistic regression analysis (Table II), using the complete set of data, demonstrated a significant association among gender, feeding type, wheezing, recurrent respiratory infections, turbinate hypertrophy, tonsillar hypertrophy, adenoid hypertrophy, and RAOM (p<0.05).

The multivariate analysis (Table III) confirmed

Table I. *Demographic and clinical characteristics of study participants (n= 1002). The results are expressed as mean with standard deviation or number of subjects with the percentage*

Characteristic	Overall
Recurrent Acute Otitis Media	
Absence	792 (79.04%)
Presence	210 (20.96%)
Age (years)	5.77 (1.84)
Gender	
Female	450 (45%)
Male	550 (55%)
Prematurity	
No	924 (92.31%)
Yes	77 (7.69%)
Feeding type	
Artificial	236 (23.58%)
Breastfeeding	765 (76.42%)
Passive Smoking	
No	929 (92.71%)
Yes	73 (7.29%)
Family Atopy	
No	273 (27.33%)
Yes	726 (72.67%)
Allergic rhinitis	
No	453 (45.44%)
Yes	544 (54.56%)
Wheezing	
No	872 (87.11%)
Yes	129 (12.89%)
Recurrent respiratory infections	
No	364 (36.51%)
Yes	633 (63.49%)
Turbinate Hypertrophy	
No	288 (28.74%)
Yes	714 (71.26%)
Tonsillar Hypertrophy	
No	233 (23.3%)
Yes	767 (76.7%)
Adenoid Hypertrophy	
No	370 (36.96%)
Yes	631 (63.04%)

a statistically significant effect of gender, feeding type, recurrent respiratory infections, tonsillar hypertrophy, and adenoid hypertrophy on RAOM (p: 0.0004, 0.0117, <0.0001<0.0001, and 0.0313, respectively).

Children with tonsillar hypertrophy had a chance three times more likely to have RAOM than children without tonsillar hypertrophy, maintaining constant the other covariates (OR = 2.97). Consistently, children with adenoid hypertrophy had a chance of having MAOR about 1.4 times more likely than children without adenoid hypertrophy, maintaining constant the other covariates (OR = 1.36).

Finally, the multivariate model performance showed an excellent model average accuracy (accuracy = 0.81). All the accuracy scores are greater than 0.66, and they ranged from 0.66 to 0.96. Moreover, low false positive and negative rates were 0.01 and 0.18, respectively.

DISCUSSION

RAOM represents an intriguing challenge in the clinical practice for both the pediatrician and the ORL specialist. The AOM diagnosis requires adequate procedure and precise differential diagnosis, mainly concerning OME. There is current debate concerning the identification of risk factors associated with RAOM. Allergy is a controversial candidate. Moreover, AOM therapy is controversial as many guidelines suggest watchful waiting for mild-moderate episodes in children > 2 years. The prevention of RAOM is overwhelmingly desirable, even though it is debated. At present, there is no convincing evidence of preventing RAOM by the proposed treatments, both conventional and not (2-6). Therefore, as there is no effective preventing and effective preventive treatment for RAOM, knowing predictive factors for RAOM could be fruitful from a pragmatic point of view.

Therefore, this real-life study aimed to evaluate whether some clinical data and/or endoscopic findings may be predictive markers of Recurrent Acute Otitis Media (RAOM) in children during an ORL visit. In other words, the current study would identify easy and straightforward factors that could be achieved during an ORL consultation.

The data analysis allowed us to define some variables to predict RAOM in children with upper respiratory complaints. In particular, adenoid and tonsillar hypertrophy were relevant risk factors for RAOM. Consistently female gender and artificial feeding were associated with RAOM. On the other hand, male gender, breastfeeding, and recurrent respiratory infections were protective factors for RAOM.

These outcomes confirm partially known mechanisms involved in RAOM, even though they reinforce the value of a thorough ORL visit, including endoscopy. In particular, anatomic and mechanic features play a relevant pathogenic role in favoring the recurrence of AOM. Tuba compression/obstruction is a crucial factor in promoting infections in the middle ear. Also, adenoids and tonsils are a reservoir for pathogens; enlargement increases the odds of re-infection (16).

Table II. *Contingency tables and summary output of the univariate analysis. Characteristic: variable taken into account; OR (95% CI): Odd Ratios with 95% Confidence Interval; p-value: Likelihood Ratio p-value. *Variables entering in the multivariate analysis (see the text for abbreviations and further details).*

Characteristic	Descriptive statistic		Univariate analysis	
	Recurrent Acute Otitis Media			
	Absence *792 (79.04%)*	Presence *210 (20.96%)*	OR (95% C.I.)	p-value
Age	5.84 (1.87)	5.52 (1.69)	0.93 (0.85 : 1.01)	0.9407
Gender *				0.0037
Female	333 (74%)	117 (26%)	1	
Male	457 (83.09%)	93 (16.91%)	0.56 (0.41 : 0.77)	
Prematurity				0.8649
No	736 (79.65%)	188 (20.35%)	1	
Yes	56 (72.73%)	21 (27.27%)	1.66 (0.95 : 2.78)	
Feeding type *				0.0070
Artificial	169 (71.61%)	67 (28.39%)	1	
Breastfeeding	623 (81.44%)	142 (18.56%)	0.54 (0.39 : 0.77)	
Passive Smoking				0.9999
No	739 (79.55%)	190 (20.45%)	1	
Yes	53 (72.6%)	20 (27.4%)	1.51 (0.85 : 2.58)	
Family Atopy				0.8737
No	207 (75.82%)	66 (24.18%)	1	
Yes	583 (80.3%)	143 (19.7%)	0.73 (0.53 : 1.03)	
Wheezing *				0.0492
No	676 (77.52%)	196 (22.48%)	1	
Yes	115 (89.15%)	14 (10.85%)	0.46 (0.25 : 0.79)	
Allergic rhinitis				0.1039
No	341 (75.28%)	112 (24.72%)	1	
Yes	451 (82.9%)	93 (17.1%)	0.66 (0.48 : 1.01)	
Recurrent respiratory infections *				<0.0001
No	254 (69.78%)	110 (30.22%)		
Yes	538 (84.99%)	95 (15.01%)	0.42 (0.3 : 0.57)	
Turbinate Hypertrophy *				0.0058
No	207 (71.88%)	81 (28.12%)	1	
Yes	585 (81.93%)	129 (18.07%)	0.56 (0.4 : 0.77)	
Tonsillar Hypertrophy *				<0.0001
No	213 (91.42%)	20 (8.58%)	1	
Yes	579 (75.49%)	188 (24.51%)	2.32 (1.67 : 3.34)	
Adenoid Hypertrophy *				<0.0001
No	325 (87.84%)	45 (12.16%)	1	
Yes	466 (73.85%)	165 (26.15%)	1.84 (1.44 : 2.4)	

Interestingly, recurrent respiratory infections seem to be negatively associated with RAOM. This finding confirms the dichotomy between respiratory and ear infective recurrence as it is a common experience to observe children with RAOM without recurrent respiratory infections, such as RAOM is a separate issue. Also, recurrent respiratory infections often are treated, if not overtreated, with antibiotics and immunomodulators so that RAOM could be diminished (17).

Breastfeeding is an essential protective measure to promote the global well-being of the child and immune system maturation (18). Male gender may be another protective factor, probably for hormonal influence on the immune response (19). Finally, an allergy could represent a protective factor for adenoidal hypertrophy; consequently, it might represent a factor not associated with RAOM (20).

On the other hand, the current study has some limitations, including the cross-sectional design,

Table III. *Multivariate analysis, the predictor effects on the Recurrent Acute Otitis Media (N=986). Results are expressed as odds ratio (OR) with a 95% confidence interval (95%CI); p-value: Likelihood Ratio p-value.*

Characteristic	Multivariate analysis	
	OR (95%C.I.)	p-value
(Intercept)	0.52 (0.35 - 0.77)	0.0013
Gender		0.0004
Female	1	
Male	0.55 (0.39 - 0.77)	
Feeding type		0.0117
Artificial	1	
Breastfeeding	0.62 (0.43 - 0.9)	
Recurrent respiratory infections		<0.0001
No	1	
Yes	0.33 (0.24 - 0.47)	
Tonsillar Hypertrophy		<0.0001
No	1	
Yes	2.97 (2.05 - 4.45)	
Adenoid Hypertrophy		0.0313
No	1	
Yes	1.36 (1.03 - 1.81)	

the lack of biomarkers measurement able to identify specific pathogenic mechanisms. However, the study's strength is the high number of enrolled children and the real-life setting, so the outcomes may mirror what happens in daily clinical practice.

CONCLUSIONS

The current study showed that adenoid and tonsillar hypertrophies were a significant risk factor for RAOM and female gender and artificial feeding. Therefore, reducing adenoid and tonsil size, also using topical corticosteroids or glycyrrhizin, could be a reasonable strategy to potentially reduce adenoid and tonsil size. This study also demonstrated that, during an ORL visit, it was possible to define some factors involved in the recurrence of AOM.

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The impact of Allergic Rhinitis in clinical practice: An International Survey

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Abstract Allergic rhinitis (AR) is a frequent disease caused by an IgE-mediated inflammation of the nose and characterized by typical symptoms. Diagnostic workup is directed to document the production of specific IgE (sensitization). Clinical management aims to relieve symptoms, resolve allergic inflammation, use medications, and potentially induce allergen tolerance, using allergen immunotherapy (AIT). The current survey was conducted in 17 International ear nose throat experts using a questionnaire with 20 questions concerning the practical management of AR patients. It was administered in the 2020 summer. The large majority (94%) of participants use the ARIA classification in clinical practice. On average, subjects with suspected AR represent half of the patients who turn to the ENT experts; 80% have the confirmed diagnosis. Most of the experts use both cutaneous and serum assay to document IgE production. Antihistamines are prescribed in 59% of AR patients, intranasal corticosteroids in 69%, non-adrenergic decongestants in 88%, nasal lavage in 88%, and AIT in 22%. About 68% of AR patients had turbinate hypertrophy, which requires surgery in 62% (mostly surgical decongestion). In conclusion, the current International Survey demonstrated that AR is a common disorder worldwide, the diagnostic workup is mainly based on IgE assessment, and the therapeutic approach is also based on non-pharmacological remedies.

Allergic rhinitis (AR) is a type 2 inflammation of the nasal membrane (1). AR is characterized by symptoms, including nasal itching, sneezing, watery rhinorrhea, and nasal obstruction (2). Nasal obstruction depends on allergic inflammation, whereas itching, sneezing, and runny nose ("irritative

Keywords: Allergic rhinitis, Italy, Survey, general population, questionnaire, on the road

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symptoms) are histamine-associated symptoms (3). AR may be classified considering the duration of symptoms (seasonal and perennial AR) or their persistency and severity (intermittent and persistent mild or moderate-severe AR) according to the ARIA (allergic rhinitis and its impact on asthma) guidelines, mainly concerning the rhinitis control (4).

The diagnosis of AR is based on the demonstration of the production of allergen-specific IgE and the concordance between allergy testing and history, such as the symptom that occurs after the inhalation of the sensitizing allergen (5). Allergen-specific IgE can be measured by cutaneous and/or serologic tests (6).

The International guidelines proposed pharmacological treatments, mainly concerning antihistamines, intranasal corticosteroids, and allergen-specific immunotherapy (AIT), the only disease-modifier treatment still now (5-7). In particular, antihistamines are most effective on "irritative symptoms" (8), whereas intranasal corticosteroids preferably relieve nasal obstruction (9). AIT aims to restore immunologic tolerance toward the causal allergen (10). However, the practical management of AR patients could vary between countries. Therefore, a Survey evaluated the behavior of a group of International ear nose throat (ENT) experts managing AR patients in clinical practice.

MATERIALS AND METHODS

The current survey was performed using a questionnaire administered and completed in 17 Countries, including Albania, Azerbaijan, Belarus, Croatia, Germany, India, Iran, Iraq, Italy, Japan, Kazakhstan, Macedonia, Malaysia, Mexico, Moldova, Philippines, Romania, and South Chorea. The International Survey was performed, using a questionnaire, in August 2020. The questionnaire included 20 queries, reported in detail in Table I. The analysis of the data was descriptive. Data were expressed as absolute numbers or frequency.

RESULTS

Globally, 17 ENT international experts participated in the survey, equally distributed along with the world. The results are reported in detail in Table I. The large majority (94%) of participants use the ARIA classification in clinical practice. All participants believe that the AR prevalence was increasing in the last year. About 50% of patients referring to ENT clinics have the suspect of AR; in 80.5% of them, the AR diagnosis is confirmed. Skin prick tests and serum IgE assay are performed in most patients (71%). Quality of life is measured in about 80% of ENT clinics. However, only one expert considers nasal cytology in the AR workup. The most prevalent symptoms in AR patients are those "histamine-dependent" (82%), whereas inflammation-dependent complaints affect 71%.

Antihistamines are prescribed in 59% of AR patients, intranasal corticosteroids in 69%, non-adrenergic decongestants in 88%, nasal lavage in 88% (70.5% using hypertonic saline solution and 23.5% isotonic one), and AIT in 22%. The 82% of ENT experts consult an allergist in selected cases, whereas 47% consult a pulmonologist in selected cases. About 68% of AR patients had turbinate hypertrophy, which requires surgery in 62% (mostly surgical decongestion 60% or coblation 40%).

DISCUSSION

Allergic rhinitis is a disease affecting many people. AR is usually classified using the ARIA criteria, such as considering the symptoms' duration and severity. Moreover, its prevalence is unanimously considered increasing. Consistently, the patients with suspected AR represent about half of the people referring to ENT clinics. This outcome is relevant and underlines the social importance of AR in the healthcare scenario. ENT experts are successful in confirming AR diagnosis in most patients. This outcome could depend on professional expertise and proper workup. Namely, 71% of participants consider both cutaneous and serologic assessment of specific IgE. This way allows us to obtain optimal diagnostic performance. Quality of life is a particular aspect that deserves adequate attention in managing AR patients (11). Accordingly, three-quarters of ENT experts consider QoL in AR patients. Instead, nasal cytology is very rarely used in clinical practice.

From a pathophysiological point of view, AR

symptoms may depend on two main pathogenic mechanisms, such as involving mediators, essentially histamine, or cellular pathways of allergic inflammation (12,13). The expert panel believes that histamine-dependent symptoms are preponderant in AR patients, even though inflammation-associated complaints assume an essential remark. Consequently, antihistamines and intranasal corticosteroids are the most commonly prescribed medications as they target AR's pathophysiological events.

Table I. *Questionnaire with answers*

QUESTION	ANSWER
Do you prefer to classify Allergic Rhinitis using the classical definition (Seasonal and Perennial) or the ARIA one (Intermittent and Persistent)?	SAR-PAR 6% ARIA 94%
Do you believe that the prevalence of AR is increasing in the last years?	Yes 100%
Which is the percentage of patients visited in your clinic with suspected AR?	48.5%
Which is the percentage of patients with suspected AR who have the diagnostic confirm of AR?	80.5%
Which method do you prescribe to assess allergen-specific IgE	Skin prick test 29% Both cutaneous and serologic assay 71%
Do you consider nasal cytology in the AR workup?	No 94%
Which are the most relevant symptoms in your patients?	Histamine-dependent (Itching, Sneezing, Watery rhinorrhea) 82% Inflammation-dependent (Nasal Obstruction) 71%
Which is the percentage of your patients treated with oral antihistamines?	59%
Which is the percentage of your patients treated with intranasal corticosteroids?	69%
Which is the percentage of your patients treated with the combination of oral antihistamines plus intranasal corticosteroids?	30%
Which is the percentage of your patients treated with allergen-specific immunotherapy?	22%
Which is the percentage of your patients treated with decongestants?	24%
Do you prescribe non-adrenergic decongestants (e.g., natural compounds, osmotic agents)?	88%
Do you prescribe nasal lavage with a saline solution?	Yes with isotonic saline 23.5% Yes, with hypertonic saline. 70.5% No 12%
Do you consider the assessment of Quality of Life in AR patients?	Yes 76.5%
Do you consult an Allergist?	Never 18% In selected cases, 82%
Do you consult a Pulmonologist?	Never 53% In selected cases, 47%
Which is the percentage of patients with turbinate hypertrophy?	68%
Which is the percentage of patients who need surgery for turbinate hypertrophy?	62%
Which surgical technique do you prefer to treat turbinate hypertrophy?	Surgical decongestion 60% Coblation 40%

Unfortunately, AIT is scarcely used in AR. This fair use could depend on the long duration and costs. Notably, adrenergic decongestants are barely prescribed. The relief provided by adrenergic agents has a short duration, but overall, these agents are also burdened with severe side effects and can easily induce abuse and addiction. For these reasons, non-adrenergic compounds are prevalent in ENT clinics. Many products are available, including a medical device containing glycyrrhetic acid and mannitol. This product exerts both anti-inflammatory effects and osmotic activity and, in randomized clinical trials on adult and paediatric patients with allergic rhinitis, resulted equivalent to mometasone furoate in symptoms relief, with no reported adverse effects (14,15). Also, nasal lavage is a frequent remedy for AR patients. Hypertonic saline solution is preferred to isotonic, probably because the first may also exert decongestant and anti-inflammatory effects (16). Saline irrigation could provide a cheap, safe, and acceptable alternative to intranasal steroids and antihistamines (17). Concerning the consultation with other specialists, allergists are consulted more frequently than pulmonologists. It depends on the allergic nature of the disease.

Turbinate hypertrophy (TH) is very common in AR patients, affecting 68% of patients. TH reflects the intense inflammatory reaction and may be associated with inadequate response to decongestants (18). Namely, it has been proposed that patient non-responder to decongestants should be considered a candidate for surgery, whereas patients responder to decongestants could be initiated for medical therapy (19). Moreover, different surgical techniques are available in clinical practice (20). Interestingly, surgery could be considered the fourth way to treat AR besides prevention, medications, and AIT (21). Surgical decongestion and coblation, such as high frequencies, are the most used (22). In this regard, the panel of experts believes that surgical decongestion is more common than coblation.

The outcomes of the current International survey are consistent with another recent survey (23). In particular, the previous survey provided evidence that the prevalence of AR was between 15%–25%, asthma, sinusitis, conjunctivitis, and nasal polyposis were frequent comorbidities, AIT was prescribed for both perennial and seasonal allergens (32.69%) via sublingual swallow (46.15%) and subcutaneous (32.69%) routes. The most prescribed drugs were intranasal corticosteroids (86.54%) and oral H1-antihistamines (82.69%).

The current survey has some limitations, including the cross-sectional design, the lack of a methodologically correct definition of the questions, and the answers based only on experts' opinions. On the other hand, the strength of this study is based on the worldwide origin of participants. Further issues to be addressed could be the role of biologics in patients with AR and other allergic comorbidities as recently advanced (24). In conclusion, the current International Survey demonstrated that AR is a common disorder worldwide, the diagnostic workup is mainly based on IgE assessment, and the therapeutic approach is also based on non-pharmacological remedies.

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The management of chronic rhinosinusitis in clinical practice: An International Survey

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Chronic rhinosinusitis (CRS) is a common disease and is currently classified in two main phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). A panel of international experts conducted the present survey. A questionnaire, containing 25 questions, was completed by each member of the panel. About half of patients with suspected CRS had confirmed diagnosis. CRSwNP affected 31% of CRS patients. Endoscopy and CT were ever performed. Rhinitis and asthma were frequent comorbidities. Intranasal corticosteroids were prescribed on average in 86% of patients. Non-adrenergic compounds were prescribed by 71% of experts. Surgery for CRSwNP was performed in about half of patients; repeated intervention occurred in about one/third. In conclusion, the current survey demonstrated that CRS requires thorough diagnostic work-up, and the most common therapeutic approach is mainly based on intranasal corticosteroids, non-adrenergic decongestants, and surgery.

The term chronic rhinosinusitis (CRS) defines an inflammatory disease affecting the nose and paranasal sinus [1]. It has to be noted that CRS may concern any age. For a definition, CRS lasts more than 12 weeks [1,2]. Rhinorrhea, nasal congestion, facial pain, and olfaction impairment are the most common symptoms [3–5]. The diagnosis of CRS initially relies on the clinical ground. However, there is evidence that fiber-optic endoscopy and computerized tomography (CT) must confirm the

Keywords: Survey, chronic rhinosinusitis, nasal polyps, intranasal corticosteroids, non-adrenergic decongestants, surgery

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diagnosis [6–9]. According to the endoscopic and/or radiological findings, there are two main phenotypes: CRS with nasal polyposis (CRSwNP) and CRS without nasal polyposis (CRSsNP).

CRS is frequently associated with rhinitis and asthma; CRS may also be frequent comorbidity in patients with immunodeficiency, cystic fibrosis, and aspirin intolerance [9–11]. CRSwNP is a common trigger and/or worsening factor in patients with asthma [12,13]. Consistently, it has been documented that 36.7% of asthmatic patients had CRSwNP. Notably, a significant association between CRSwNP and asthma severity has been reported [14–18]. CRS is also frequent in patients with poorly controlled asthma [19]. CRS may cause hospital admission for asthma exacerbation [20]. Further, about 50% of children with persistent asthma presented concomitant CRS [21]. Therefore, CRS should be ever suspected in patients with rhinitis and asthma [22].

Recently, an Italian Survey has been conducted in patients with rhinosinusitis, recruited on the road; the study showed impressive outcomes useful in clinical practices [23]. Therefore, a panel of international experts of the ear nose throat (ENT) specialization participated in a survey by completing a questionnaire devoted to known the pragmatic approach to patients with CRS.

MATERIALS AND METHODS

The current survey was performed using a questionnaire administered and completed in 24 Countries, including Albania, Azerbaijan, Belarus, Congo, Croatia, El Salvador, Germany, India, Iran, Iraq, Italy, Japan, Kazakhstan, Macedonia, Malaysia, Mexico, Moldova, Philippines, Romania, Slovenia, South Chorea, Sweden, Turkey, and Vietnam. The International Survey was performed using a questionnaire in August 2020. The questionnaire included 25 queries, reported in detail in Table I. The analysis of the data was descriptive. Data were expressed as absolute numbers or frequency.

RESULTS

Globally, 25 International experts participated in the survey, equally distributed along with the world. The results are reported in Table I. The large majority (87.5%) of participants retain that CRS's prevalence increases in the last years. The patients with CRS make up about 30% (range 1-80%) of the cases relating to their clinical structure. On average, half of the patients with suspected CRS have diagnostic confirmation. The patients with CRSwNP are 31.2% of all patients with CRS.

All experts use both nasal fiber-optic and CT in the work-up of patients with suspected nasal polyps. Ancillary examinations, including nasal cytology and olfaction assessment, are less usually used (29% and 54.2% respectively); instead, quality of life evaluation is commonly performed (83.3%). On the other hand, olfaction impairment may affect about half of patients with CRSwNP, Asthma, and rhinitis comorbidity are relatively common: 20.6% and 41%, respectively.

Concerning the treatment, one third (range 1-100%) of CRSwNP patients are treated with oral corticosteroids, whereas intranasal corticosteroids are used in most patients (86.2%). Combined corticosteroids (oral and intranasal) are used in 30% of patients with nasal polyps. Nasal decongestants, such as α-adrenergic molecules, were used in 20.6% of patients. However, experts prescribe non-adrenergic decongestants, such as natural products and osmotic agents, in 71% of CRS patients. Also, nasal lavage is very common in clinical practice: 79% of patients use isotonic saline solution and 41.7% hypertonic saline solution.

An allergist is ever consulted by 16.6% of experts, in selected cases by 83.4%. A pulmonologist is ever consulted by 8.3% of the participants, in selected cases by 91.7%.

Surgery for nasal polyps is a therapeutic strategy for 55.2% of patients. About one-third of patients should repeat the nasal operation.

DISCUSSION

CRS is a chronic inflammation of both the nose and the sinus. From an epidemiological perspective, it is estimated that CRS affects 5%-12% of the general population worldwide [24-26]. The European Position Paper on Rhinosinusitis and

Table I. Questionnaire

Question	Answer
Do you believe that the prevalence of CRS is increasing in the last years?	Yes 87.5%
Which is the percentage of patients visited in your clinic with suspected CRS?	28.9% (range 1-80%)
Which is the percentage of patients with suspected CRS who have the diagnostic confirm of CRS?	53.3% (range 8-100%)
Which the percentage of CRS patients with CRSwNP?	31.2% (range 3-70%)
Do you consider nasal fiber-endoscopy in the CRSwNP work-up?	Yes for 100%
Do you consider CT in the CRSwNP work-up?	Yes for 100%
Do you consider nasal cytology in the CRSwNP work-up?	Yes for 29%
Do you consider olfaction assessment in the CRSwNP work-up?	Yes for 54.2%
Do you consider Quality of Life assessment in the CRSwNP work-up?	Yes for 83.3%
Which is the percentage of your CRSwNP patients with olfaction impairment?	48.1% (range 10-100%)
Which is the percentage of your CRSwNP patients with comorbid asthma?	20.6% (range 3-60)
Which is the percentage of your CRSwNP patients with comorbid allergic rhinitis?	41% (range 10-90%)
Which is the percentage of your CRSwNP patients treated with oral corticosteroids?	36.2% (range 1-100%)
Which is the percentage of your patients treated with intranasal corticosteroids?	86.2% (range 35-100%)
Which is the percentage of your patients treated with the combination of oral corticosteroids plus intranasal corticosteroids?	29.8% (range 1-100%)
Which is the percentage of your patients treated with decongestants?	20.6% (range 0-60%)
Do you prescribe non-adrenergic decongestants (e.g., natural compounds, osmotic agents)?	Yes for 71%
Do you prescribe nasal lavage with an isotonic saline solution?	Yes for 79%
Do you prescribe nasal lavage with a hypertonic saline solution?	Yes for 41.7%
Do you ever consult an Allergist?	Yes for 16.6%
Do you consult an Allergist in selected cases?	Yes for 83.4%
Do you ever consult a Pulmonologist?	Yes for 8.3%
Do you ever consult a Pulmonologist in selected cases?	Yes 91.7%
Which is the percentage of patients treated with surgery?	55.2% (range 30-90%)
Which is the percentage of patients who need repeated surgery?	31.9% (range 8-80%)

Nasal Polyps (EPOS) proposed a statement about CRS diagnosis that is clinically based on symptoms supported by signs of mucosal inflammation found on imaging or with nasal endoscopy [27]. The prevalence of clinically-based CRS diagnosis usually ranged between 3% and 6.4% [28,29]. Using patient questionnaires, the prevalence of CRSwNP was 2.1% (France) to 4.3% (Finland) in Europe and 1.1% in China [30]. Based on this background, the current survey was conducted involving 25 International experts and a specific questionnaire.

The outcomes were impressive, as reflected in the standard practice in the management of CRS patients worldwide. There was a shared conviction that the prevalence of CRS is increasing worldwide. Consistently, CRS is a common disorder, representing about 30% of the admissions to ENT clinics, even with a vast range (1-80%). However, the suspected CRS diagnosis was confirmed in about half of the patients.

There was complete certainty that nasal fiber-endoscopy and CT are gold standard diagnostic tools in the CRS work-up. The quality-of-life assessment is also considered a relevant aspect that deserves adequate attention; more than 80% of participants measured it. As olfaction impairment is another relevant symptom affecting about half of CRS patients, its evaluation is performed by the 54%

of ENT experts. On the contrary, nasal cytology is rarely investigated (<30%).

The current survey reported that rhinitis and asthma are a common comorbidity in CRS patients as their prevalence is about 40 and 20%, respectively. Consistently the consultation of an allergist or a pulmonologist is common.

Regarding CRSwNP management, oral corticosteroids are prescribed in about one-third of patients, but the variability is vast (1-100%). Instead, intranasal corticosteroids are more frequently prescribed as the mean was 86.2% of CRSwNP patients. The combination of oral and intranasal corticosteroids is used in 30% of CRSwNP patients, but also, in this case, the range is vast, ranging from 1 to 100%. These crucial differences may depend on local aspects, including socio-economic issues, doctors' and patients' beliefs, mainly concerning the concept of corticosteroid phobia [31-33]. Consistently, using a-adrenergic decongestants is relatively scarce as prescribed in 20% of CRS patients, even though with wide variability (0-60%).

On the other hand, non-adrenergic decongestants are very popular, as 71% of ENT experts prescribed products containing natural compounds and/or osmotic agents. In this regard, a medical device is frequently used as exerts both anti-inflammatory and decongestant activities, as it contains glycyrrhetic acid and mannitol [34]. Also, nasal lavage with isotonic or hypertonic saline solution is frequently prescribed in CRS patients.

Surgery is a therapeutic option in about half of CRSwNP patients, but in one-third of cases has to be repeated over time.

Globally, the scenario that appears from this survey represents quite faithfully reflects the ENT specialist's behavior in the world. Namely, CRS is a common disease that frequently is managed by the ENT specialist. Its work-up requires adequate diagnostic procedures and thorough evaluation of particular aspects, including quality of life and olfaction. The therapeutic approach is mainly based on intranasal corticosteroids, non-adrenergic decongestants, and nasal lavage with saline solutions, as recently pointed out [35]. Surgery option is chosen in about half of CRSwNP patients, but post-surgery clinical relapses are uncommon.

The current survey has some limitations, including the cross-sectional design, the lack of a methodologically correct definition of the questions, and the answers based only on experts' opinions. On the other hand, the strength of this study is based on the worldwide provenience of participants.

In conclusion, the current survey demonstrated that CRS is a common disorder worldwide, the diagnostic work-up deserves the correct approach, and the therapeutic options are usually consistent with International guidelines.

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A practical classification of the Empty Nose Syndrome

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The empty nose syndrome (ENS) entails different clinical conditions usually caused by nasal surgery. Many pathogenic factors contribute to the disease progression. Symptoms may be very bothersome and significantly affect the quality of the life. Many therapeutic strategies have been proposed. In this regard, a new multicomponent medical device, containing hyaluronic acid, D-panthenol, vitamin A and E, and biotin, seems to provide promising results.

The term primary atrophic rhinitis defines an umbrella condition including different nasal disorders with unknown origin. On the other hand, secondary atrophic rhinitis is caused by either surgical or non-surgical nasal trauma or may follow a nasal manifestation of a specific systemic disease.

The patient with secondary atrophic rhinitis patients usually presents a number of predisposing factors involved in the symptom complex of atrophic rhinitis. Firstly, surgery for nasal turbinates, mainly concerning the inferior turbinate, represents a leading factor for atrophic rhinitis. In fact, any type of surgical procedure for reducing the turbinate volume, to alleviate nasal breathing obstruction, may induce the "empty nose" syndrome (ENS). ENS produces a secondary atrophic rhinitis which may not occur instantaneously but only materialize years following the initial surgical trauma. Moreover, ENS encompasses multiple pathological forms that are characterized by different anatomy and different clinical pictures. Eugene Kern and Monika Stenkvist initially coined the term ENS in 1994 as a rare but debilitating clinical syndrome that typically occurred after surgical therapy (1). The main symptom is a paradoxical perception of nasal obstruction, despite normal nasal patency (2). Other troublesome complaints include nasal crusting, dryness, nasal discharge, and facial pain (3). Patients with severe ENS may also experience bothered sleep, impaired mental concentration, and choking (4). Psychiatric symptoms may also occur until a suicide attempt (4) as well as neurological dysfunction (5).

From a pathophysiological point of view, ENS follows on from functional interventions on the nasal cavities sometimes performed with too much guilty aggression. Moreover, ENS could also be included in the umbrella definition of secondary atrophic rhinitis. Anyone of the procedures for reducing the turbinate volume, to alleviate nasal breathing obstruction, may induce the ENS, yielding and producing secondary atrophic rhinitis, which may not occur instantaneously

Keywords: empty nose syndrome, turbinates hypertrophy, surgery, classification, phenotyping, medical device

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but only materialize years following the initial surgical trauma.

The correct nasal physiology, mainly concerning the function of heating and moistening the inspired air, resides in the lower, middle, and upper turbinates fundamental elements whose conservation must always be considered indispensable. When the nasal physiology, especially in the ventilatory function, is upset by irreversible hypertrophy of the turbinates, it is necessary to proceed with the intervention that must prefer, among the dozens of methods proposed in the literature, that or those useful for unclogging but the most conservative as possible at least respecting the mucosa.

Since anatomical and clinical pictures can be very different, there is the need to define a shared and scientifically based common language for performing comparisons and statistics. Therefore, it seems to be appropriate to propose a classification of ENS that can, at least in general, differentiates post-surgical situations due to the different clinical pictures.

Our proposal derives from hundreds of interventions performed by us in a suitable, safe, and secure environment. This classification is based on endoscopic objectivity and considers five grades (with sub-grades) according to macroscopic features.

Table I shows different characteristics. Figure 1 describes the different grades according to the type of turbinate surgery. Each picture can correspond to a symptom severity ranging from the annoying comes to be unbearable that can even indicate extreme solutions.

The diversity of symptom characteristics requires differentiation of the term Empty Nose Syndrome so far unable to fully define how important it is to know nor to define the border with the most common Atrophic Rhinitis of different origins. Every grade corresponds to different symptoms. In this regard, a study is ongoing to define the clinical features characterizing the different ENS grades.

Form a clinical point of view; it is clinically relevant to phenotyping patients with ENS to personalize the more appropriate treatment. Lubricants, moisturizing, cytoprotective agents could restore the perception of physiological breathing. In this regard, a new multi-component medical device seems to be promising, as it contains D-panthenol, hyaluronic acid (HA), vitamin E, vitamin A, and biotin (Rinocross, DMG, Italy).

D-panthenol is the alcohol analog 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. D-panthenol is used as a moisturizer to improve wound healing in pharmaceutical and cosmetic products (6). It improves hydration, reduces inflammation, and accelerates mucosal wounds' rate of healing (7). D-panthenol readily penetrates the mucous membranes (including the intestinal mucosa), quickly oxidized to pantothenic acid. It is also used in

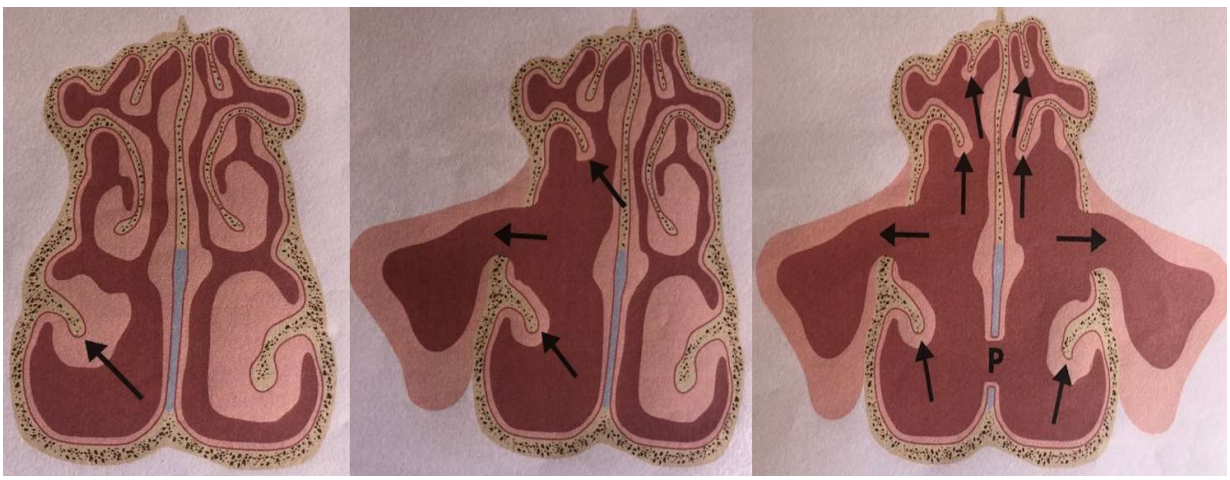

Fig. 1. *Different grades according to the type of surgical turbinate resections. Left panel (grade I); middle panel (grade II); right panel (grades III-V) with arrows indicating the sites of surgical turbinate resection.*

Table I. *Classification of Empty Nose Syndrome based on grades.*

Grade	Characteristics
I A	Single-sided resection of the inferior turbinate
I B	Bilateral resection of the inferior turbinates
II A	Unilateral resection of the inferior and middle turbinates
II B	Bilateral resection of the inferior and middle turbinates
II C	Unilateral resection of the inferior and middle turbinates with ipsilateral meatotomy
II D	Bilateral resection of the inferior and middle turbinates with bilateral meatotomy
III A	Ipsilateral resection of all turbinates
III B	Bilateral resection of all turbinates
III C	Unilateral resection of all turbinates with ipsilateral meatotomy
III D	Bilateral resection of all turbinates with bilateral meatotomy
IV	Resection of all turbinates with the removal of mucosa
V	Resection of all turbinates with septum perforation

the biosynthesis of coenzyme A, which controls a wide range of enzymatic reactions. HA is a fundamental component of the connective tissue. HA can modulate the inflammatory response, cellular proliferation, and remodeling of the extracellular matrix (8).

Moreover, HA has important lubricant and moisturizing properties. Vitamin E, such as tocopherol, is a fat-soluble antioxidant that can protect the polyunsaturated fatty acids in the membrane from oxidation, regulate the production of reactive oxygen species, and reactive nitrogen species, and modulate signal transduction (9). Moreover, vitamin E has eutrophic property and immune stimulation (10). Vitamin A is a retinoid and is an essential micronutrient for the body and is associated with the proper functioning of the visual system, maintenance of epithelial integrity, red cell production, growth and development, immune and reproductive function (11). In particular, topical application stimulates epithelial growth, fibroblasts, granulation tissue, angiogenesis, collagen synthesis, epithelialization, and fibroplasia (12). These activities concur to repair the mucosal wound. Biotin, such as vitamin H, is a water-soluble B-complex vitamin and is well-known as a co-factor for five essential carboxylases (13). In particular, biotin exerts anti-inflammatory and immune- modulatory activities, relevant in repairing mechanisms of mucosal damage (14). At present, two studies investigated the role of this medical device in patients with functional impairment after nasal surgery or patients with dystrophic rhinitis (15,16). The outcomes are promising, as this medical device was effective and safe in the large majority of treated patients.

In conclusion, ENS is a complex disorder that challenges the rhinologist. Phenotyping patients could be a reasonable strategy in the workup and management of ENS. Topical medical device with lubricant and hydrating activity could be useful to treat ENS.

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Laryngopharyngeal reflux management in clinical practice

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Laryngopharyngeal reflux (LPR) is an inflammatory reaction of the mucosa of the pharynx, larynx, and other associated upper respiratory organs, caused by a reflux of stomach contents outside the esophagus. LPR is considered a relatively new clinical entity with a vast number of clinical manifestations that are sometimes treated empirically and without a correct diagnosis. Alginate is a reasonable therapeutic option as a first-line or add-on option. A survey included 35 Italian otorhinolaryngologists. The survey considered ten practical queries. LPR is a common disease in clinical practice. History and fiber-optic endoscopy constitute the main diagnostic tools. Alginates represent a frequent medication to treat LPR both as first-line and add-on. The mean effectiveness rate is 44% for first-line choice and 76% for the add-on. In conclusion, the current survey provided exciting information about the management of LPR in clinical practice.

Laryngopharyngeal reflux (LPR) is considered an extraesophageal manifestation of gastroesophageal reflux disease (GERD). Both GERD and its extraesophageal manifestation are very common in clinical practice. Both disorders have a relevant burden on society.

LPR is most commonly manifested as laryngeal symptoms such as coughing, hoarseness, dysphagia, globus, and sore throat, but there can be signs of the nose, sinus, ear, and eye involvement (1). Epidemiological studies have shown that the prevalence of this LPR may be too 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, pediatricians, pulmonologists, allergists, and psychiatrists (2-5). LPR is a multifactorial syndrome with a vast clinical representation during the disease and complications, requiring a multidisciplinary approach. From a pathophysiological point of view, the oesophageal mucosa has protective mechanisms against aggressive factors of the stomach content (mucosal barrier), and it remains intact when physiological reflux occurs, which usually happens at night.

On the contrary, laryngeal and pharyngeal mucosa do not possess the oesophageal protective mechanisms, so acid and peptic activity of the stomach content quickly leads to mucosal lesions. Notably, laryngopharyngeal reflux occurs most

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commonly during the day due to the upper oesophageal sphincter dysfunction. This aspect is intriguing as typical GERD symptoms usually occur in a supine position and overnight. However, the acidity of the stomach content is not the only cause of LPR. Pepsin, with its proteolytic effects, can be the determining factor. Other possible etiological factors are pancreatic proteolytic enzymes, bile salts, and bacteria (5,6). In clinical practice, LPR is mostly not recognized because it may be a "silent reflux," and diagnostic and therapeutic protocols are still inadequate, so proper treatment is usually delayed. Laryngeal symptoms are the most common, so otolaryngologists manage patients.

Indeed, otolaryngologists have developed the diagnostic Reflux Symptom Index (RSI) questionnaire based on the importance of specific disease symptoms and the Reflux Finding Score (RFS) based on the frequency of pathological changes determined by laryngoscopy (7). Knowledge of pathogenic mechanisms and clinical manifestations helps physicians create an adequate program for the prevention, early diagnosis, and adequate therapy for LPR. In particular, it has to be considered that untreated LPR can be one of the etiological causes of laryngeal cancer. Laryngeal pathological changes could be discovered with fiberoptic endoscopy. These changes may include edema, hyperemia, or erythema of the vocal cords and laryngeal edges, ventricular obliteration, granulation, presence of dense endo-laryngeal secretion, and hypertrophy of the posterior commissure (2,7).

As a consequence, an appropriate diagnosis of LPR represents a challenge for the general practitioner and specialists. Many clinical studies confirmed low specificity and sensitivity of diagnostic tests such as laryngoscopy, esophagogastroscopy, proximal pH monitoring (hypopharyngeal and oropharyngeal). Evaluation of symptoms using the Reflux Symptom Index is considered to be the necessary diagnostic procedure.

From a diagnostic point of view, LPR diagnosis may be performed clinically as there is no gold-standard diagnostic tool. In this regard, some questionnaires may very fruitful in clinical practice: Reflux Finding Score (RFS) based on signs viewed by laryngoscopy and Reflux Score Index (RSI) based on reflux symptoms.

LPR therapy may be complex and also requires modification of the patient's lifestyle and habits. Bodyweight reduction and physical activity, quitting cigarettes, and alcohol use are the first steps in lowering the intensity of symptoms in patients (8). Nutritional interventions with correct food choices and bowel movement regulation lead to lowering dyspeptic complaints and lower the number of reflux episodes. High BMI is an independent factor in stomach reflux because of its specific effect mechanism on the gastroesophageal juncture (8). LPR treatment and management is supposed to reduce the acidity or stomach contents and neutralize acid-peptic activity in the larynx, pharynx, and esophagus. Acid suppression is relevant to reduce gastric reflux, and proton pump inhibitors (PPIs) are the most potent drug in this field (9,10). However, some patients are refractory to PPI, and PPI may cause relevant adverse effects over time (11). Therefore, 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 so-called "acid pocket" pathogenic role. The acid pocket is a short zone of unbuffered highly acidic gastric juice that accumulates in the proximal stomach after meals. Serving as the acid reflux source, the acid pocket increases the propensity for acid reflux (12,13). Alginate is an anionic polysaccharide occurring naturally in brown algae and has a unique property in gastric reflux treatment by eliminating the acid pocket.

The alginate-antacid formulation can reduce postprandial symptoms by neutralizing the acidity of gastric contents. In addition to neutralizing gastric acidity, alginate and bicarbonate, usually contained in an alginate-based formulation, form a foamy gel, like a raft floating on the surface of gastric contents after interacting with gastric acid. 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 (14-17). Like an antacid, an alginate-based formulation

demonstrates an immediate onset of effect within one h of administration, faster than a PPI and H2RA (18). Compared with antacids, an alginate-based formulation is more effective than an antacid in controlling postprandial oesophageal acid exposure and quickly relieving reflux symptoms, including heartburn, regurgitation, vomiting, and belching, with longer duration (19-21). Alginate-based formulations are also non-inferior to omeprazole in achieving a heartburn-free period in moderate episodic heartburn (22). Therefore, alginate has the unique properties of protecting the oesophageal and upper respiratory mucosa from acid and non-acid reflux and displacement of the acid pocket away from the esophagus. Furthermore, adding alginate to a PPI can significantly relieve heartburn compared to using a PPI alone in patients poorly sensitive to PPI, suggesting an additional benefit of alginate as add-on therapy in the management of refractory symptoms (24).

Considering this background, a survey was conducted on 35 Italian otorhinolaryngologists using a simple questionary, including ten queries reported in Table I.

Suspected LPR was a common reason for accessing an otorhinolaryngologist, representing 40% of the global visits. An LPR diagnosis was confirmed in about 60% of cases, even though with a wide range (10-100%), probably depending on the single doctor's characteristics.

History and fiberoptic endoscopy were the most common diagnostic tools for the diagnosis and for assessing the effectiveness of LPR therapy. Almost all the participants prescribed alginate to treat LPR, mostly as a first-line choice. The follow-up usually lasted three months, but one-third of doctors evaluated patients after two months. The specialists believed that alginate monotherapy's effectiveness rate was 44%, but it increased up to 76% if administered as an add-on.

The current survey highlighted the relevance of LPR in clinical practice. History and fiberoptic endoscopy are the diagnostic cornerstones. Moreover, both are outstanding in the follow-up to measure the effectiveness of the treatment. Alginate is a common medication in the LPR treatment, mainly as a first-line option. Close follow-up represented a common practice as all visits were planned by three months.

Table I. Questions and answers concerning the survey conducted on a group of Italian otorhinolaryngologists

QUESTION	ANSWER
What is the percentage of patients whom you see with the suspect of Laryngo-Pharyngeal Reflux (LPR)?	41% (range 10-75%)
Considering these patients, how many patients have a confirmed diagnosis of LPR?	62% (range10-100%)
To diagnose LPR, which test do you consider?	
a) History	100%
b) Fiberoptic endoscopy	100%
c) Reflux finding score	29%
d) Reflux Score Index	43%
What is the gold standard to evaluate the effectiveness of LPR therapy?	History 40% Fiberoptic endoscopy 60% pH-metry 17% RFS/RSI 9%
Treating patients with LPR, do you use alginate?	91%
Do you use alginate as the first-line choice?	57%
Do you use alginate as an add-on?	34%
After how long do you re-evaluate the patient?	One month 23% Two months 34% Three months 43%
What is the percentage of effectiveness of alginate monotherapy?	44% (range 10-90%)
What is the percentage of effectiveness of add-on alginate?	76% (range20-90%)

The effectiveness of alginate monotherapy was good as 44%. This quote increased up to 76% when prescribed as an add-on.

These outcomes were consistent with a recent study which analysed magnesium alginate plus simethicone, zinc hydroxide, and sodium bicarbonate (Gastrotuss®) in patients with LPR. The results showed that the alginate compound significantly reduced the perception of dysphagia, dysphonia, and cough (in press).

In conclusion, the current survey provided exciting information about the management of LPR in clinical practice.

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Magnesium alginate in patients with laryngopharyngeal reflux

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Laryngopharyngeal reflux (LPR) is a common disease caused by the leaking beck of gastric material out of the esophagus. The main symptoms are dysphonia, dysphagia, and cough. There is an established use of proton pump inhibitors (PPI) in patients with suspected LPR in common practice. This habit is translated by the standard strategy to use PPI in treating patients with gastroesophageal reflux. However, PPI can not wholly inhibit all types of reflux and are burden by adverse effects. Alginate, a derivative from algae, is devoid of side effects and effectively counteracts gastric material reflux forming a foaming gel in the stomach. The current study enrolled 100 outpatients with LPR. Alginate treatment was administered for two months. Patients underwent four visits (at baseline and 15, 30, and 60 days after treatment). A visual analog scale assessed the perception of dysphonia, dysphagia, and cough. Alginate significantly ($p<0.0001$) reduced all parameters. Therefore, the current study demonstrated that magnesium alginate was effective and safe in LPR treatment.

Laryngopharyngeal reflux (LPR) is an extra-esophageal manifestation of gastroesophageal reflux (1). The gastroesophageal reflux frequently becomes a disease (GERD) (2,3). Many patients experience unusual sensations in the laryngopharynx (4,5). Consequently, LPR has a relevant impact on otolaryngologist practice, namely, up to 50 % of patients referring to dysphonia have LPR (6). Moreover, LPR is associated with different diseases, including reflux laryngitis and reflux cough. LPR's main symptoms are hoarseness, throat clearing, choking sensation, dysphagia, dysphonia, laryngeal globus, sore throat, and laryngospasm (7).

The LPR diagnostic work-up is pragmatically based on history, clinical examination, and laryngoscopy. Moreover, a protonic pump inhibitor (PPI) test, such as an empiric course of this medication, is very popular in the clinic setting (8). Altman suggested that empirical PPI therapy for 1–2 months is a reasonable initial approach in patients with LPR symptoms (9). Therefore, LPR diagnosis usually results from history, fiberoptic endoscopic outcomes, and empiric trial (10). So, patient-reported outcome measures are currently a primary method of diagnosing LPR and monitoring prescribed treatments' effectiveness. In this regard, the symptom perception measurement fruitfully relies on the visual analog scale (VAS). VAS may reasonably reflect the symptom severity and is a reproducible measure over time.

Keywords: laryngopharyngeal reflux, magnesium alginate, dysphagia, dysphonia, cough

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From a therapeutic perspective, PPIs are overprescribed, expensive, and there are some safety concerns. On the contrary, alginate is an anionic polysaccharide occurring naturally in brown algae and has a unique property in gastric reflux treatment. The alginate-antacid formulation can reduce postprandial symptoms by neutralizing the acidity of gastric contents. In addition to neutralizing gastric acidity, alginate and bicarbonate, usually contained in an alginate-based formulation, form a foamy gel-like raft floating on gastric contents' surface interacting with gastric acid. 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 (11-14). Like an antacid, an alginate-based formulation demonstrates an immediate onset of effect within one h of administration, which is faster than a PPI and antagonist of the histamine-2 receptor (15). Compared with antacids, an alginate-based formulation is more effective than an antacid in controlling postprandial oesophageal acid exposure and quickly relieving reflux symptoms, including heartburn, regurgitation, vomiting, and belching, with longer duration (16-18). Alginate-based formulations are also non-inferior to omeprazole in achieving a heartburn-free period in moderate episodic heartburn (19). Therefore, alginate has the unique properties of protection of the oesophageal and upper respiratory mucosa from acid and non-acid reflux and displacement of the acid pocket away from the esophagus (20).

Based on this background, the current study aimed at investigating the efficacy and safety of a medical device containing magnesium alginate, simethicone, Dex-panthenol, zinc hydroxide, and sodium bicarbonate (Gastrotuss®) in patients with LPR.

MATERIALS AND METHODS

This study included 100 outpatients (52 males, mean age 54.4 ± 12.5 years) with LPR. Inclusion criteria were adulthood, both sexes, and documented LPR diagnosis. Exclusion criteria were comorbidities and concomitant treatments able to interfere with the interpretation of the results. All patients signed informed consent. The local Review Board approved the procedure. The perception of dysphagia, dysphonia, and cough severity was measured by VAS, where 0 was no symptom, ten was very bothersome symptoms. Each outpatient took magnesium alginate-simethicone (20 mL/three times a day) for two months. Patients underwent a medical examination at baseline and after 15, 30, and 60 days after treatment. Safety was also considered by reported side effects. The statistical analysis was performed using the Wilcoxon test.

RESULTS

All patients completed the study. The active treatment was tolerated, and no clinically relevant adverse event was reported. Table I shows the mean VAS scores for dysphonia, dysphagia and cough in patients with LPR treated with alginate-simethicone at baseline and after 15, 30 and 60 days after treatment. Active treatment significantly ($p<0.0001$) diminished the VAS values over time.

Table I. Mean VAS scores for dysphonia, dysphagia and cough in patients with LPR treated with alginate-simethicone at baseline and after 15, 30 and 60 days after treatment.

MEAN VAS SCORES					
	Baseline	Day 15	Day 30	Day 60	Willcoxon
dysphonia	6.2	4.65	3.79	3.23	P<0.0001
dysphagia	6.35	4.52	3.64	3.22	P<0.0001
cough	6.71	3.82	2.85	2.24	P<0.0001

DISCUSSION

The current study demonstrated that a 2-month magnesium alginate-simethicone course significantly reduced the perception of dysphonia, dysphagia, and cough in patients with LPR. The treatment was also safe and well-tolerated. Interestingly, this study is the first report concerning the efficacy and safety of this medical device in treating patients with LPR.

The obtained outcomes depend on the mechanisms of action of the various components of the medical device. Magnesium alginate is well-known alginate that is effective in counteracting gastric reflux. Simethicone is an inert silicone type substance and reduces the effects of excessive gas in the digestive tract. The Dex-panthenol and zinc hydroxide repair mucous wounds due to aggressive refluxate. Sodium bicarbonate is a buffer system for acid material.

The present study has some limitations, including the open design, the lack of objective assessment, and sample size calculation. However, the study was conducted in a real-world setting, such as an outpatient clinic. Thus, the outcomes can mirror what occurs in clinical practice. Moreover, the halving of symptom severity is an optimal outcome for a medical device, such as a product that does not require a medical prescription, so it is easily available.

In conclusion, the current study demonstrated that magnesium alginate was effective and safe in LPR treatment.

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