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CONTENTS

NEW SCIENTIFIC SYNERGIES TO MANAGE PATIENTS WITH SEVERE RHINITIS: ALLERGY DIAGNOSIS AND TREATMENT FOR ENT SPECIALISTS

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Introduction

Allergic rhinitis (AR) is a global health problem because of its steadily increasing incidence and prevalence that currently concerns about 30% of the world's population. Although AR is not a disease that reduces the life expectancy, it is a disorder with a major impact on the quality of life of patients, resulting from an impaired social life, school performance and work productivity. Furthermore, AR produces significant costs for its treatment.

AR is a pathology of the nasal mucosa induced by IgE-mediated inflammation that follow the exposure to the culprit allergen and is clinically characterized by rhinorrhoea, sneezing, nasal itching and obstruction, which are reversible spontaneously or after treatment. The environmental allergens, after binding to specific IgE receptors on the surface of mast cells and basophils, trigger the degranulation of these cells in sensitized individuals, followed by a cascade of immunologic events resulting in the release of pre-formed mediators, with a major role for histamine, and the synthesis of further mediators that maintain the inflammatory response.

The document Allergic Rhinitis and its Impact on Asthma (ARIA), that was endorsed by WHO, has definitively certified the importance of AR in the context of respiratory disease. Recently, the international scientific community has identified a serious form of rhinitis associated with upper airways involvement: the SCUAD (severe chronic upper airways disease), a complex clinical condition that is generally poorly responsive to usual drug treatment and thus requires highly specialized management. Recent scientific evidence also confirms that patients suffering from the most severe forms of AR are turning to highly qualified specialists. The ENT specialist experienced in allergy is the physician that, more than others, is able to diagnose and consequently to cure these severe AR types. Actually, the nasal district is not an isolated compartment, but is connected with other structures that are concerned by any variations occurring in the nasal mucosa by proximity such as bronchi, lungs, paranasal sinuses and ears. It is therefore necessary to have a broader vision of the pathology and cooperation among specialists in order to manage the pathology. These were the premises leading us to organize a Master of Allergology for ENT specialists, with the aim to give a theoretical and practical background, useful in linking their expertise to allergy and immunology, enabling them to deal with clinical cases requiring both capacities. In particular, from the allergist's point of view, an appropriate use of innovative diagnostic and therapeutic tools such as molecular diagnosis, nasal cytology and their management, is more feasible; this influences when and how to use allergen specific immunotherapy to treat patients with severe rhinitis unresponsive to the usual drugs, keeping in mind that such treatment is the only one able to alter the natural history of allergy.

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HUMAN NASAL IMMUNE SYSTEM: A SPECIAL SITE FOR IMMUNE RESPONSE ESTABLISHMENT

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The mucosal immune system located in correspondence to the olfactory organs in adult humans is not well identifiable but has proven important in establishing an effective immune response against inhaled antigens, including the generation of Helper 1 (TH1)- and TH2-cells, cytotoxic T lymphocytes (CTLs), plasma cells (PCs) and memory B cells. It is constituted by a diffused network of cells of epithelial and immune origin, as well as organized lymphoid tissue, where each component has a role in the initiation and maintenance of a long-lasting immune response, which is evoked not only in the oral and nasal cavities but also in the respiratory, intestinal and genito-urinary tracts. These peculiarities, in association to the easy anatomical accessibility of such immunological site, render the nasal mucosa a good candidate for the development of vaccine, even if a better understanding of the mechanism of the immune response induction as well as finding a safe adjuvant are necessary.

NALT as a MALT subtype

The mucosal immune system represents the first line of defence against pathogens and other exogenous antigens and is constituted of a combination of innate and acquired immunity components which cooperate to ensure immunological homeostasis from the oral and nasal cavities to the respiratory, intestinal and genito-urinary tracts.

The Mucosa-Associated Lymphoid Tissues (MALT) represent the sites in which the immunological response occurs and include the Gut-Associated Lymphoid Tissues (GALT), such as Peyer's patches and isolated lymphoid follicles, the Nasal-Associated Lymphoid Tissues (NALT), and the Bronchus-Associated Lymphoid Tissues (BALT). While the NALT has been well described in rodents, where it consists of paired lymphoid structures situated above the soft palate at the entrance of the bifurcated pharyngeal duct, it is not well identifiable anatomically in the human nasal mucosa. However, many authors talk about a human nasopharynx-associated lymphoid tissue (or NALT) responsible for the mucosal immunity triggered by antigen inhalation in the upper respiratory tract. Human NALT is a disseminated lymphoid tissue constituted by lymphocyte-containing follicles in contact with overlying follicle-associated epithelium (FAE) and high endothelial venules (HEVs); it is located in the entire nasal mucosa and in the pharyngeal tonsils, called "adenoids". These last represent well-defined lymphoid structures that, together with palatine and lingual tonsils, constitute the human Waldeyer's ring (1, 2). Overall, all these diffused and/or organized nasal lymphoid tissues are responsible for the human nasopharyngeal immune response and represent a barrier for potentially

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harmful materials and microorganisms as well as an induction site for protective immune reactions, in which Immunoglobulin (Ig) A play a central role (2). The Common Mucosal Immune System (CMIS) connects NALT inductive sites with effector sites of the respiratory tract, for the generation of antigen-specific T Helper 1 (TH1) and TH2-cell, cytotoxic T lymphocyte (CTL) and B lymphocyte-dependent immune responses. In MALT-induced immunity, antigen uptake goes through the epithelium so that anatomically there is no need for afferent lymphatic but only for efferent lymphatic vessels to drain, in the case of NALT, into the cervical lymph nodes (1). Interestingly, studies on mice revealed that environmental stimulation by inhaled antigens is essential for NALT organogenesis since this tissue is not present during embryogenesis or in newborn mice (3).

NALT immunological features. Epithelial cells as immunological physical barrier and the specialized role of intraepithelial M cells

The pseudo-stratified columnar epithelium of NALT plays an important role in the initiation, maintenance and regulation of both innate and adaptive immune responses in the airways (4). Tight junctions represent the most apical of the intercellular junctions through which epithelial cells are attached to each other at their lateral membranes, ensuring the maintenance of cell polarity and are formed by a number of proteins such as claudins, occludins, and JAMs, in addition to many cytosolic scaffold proteins, such as zonula occludent (ZO) proteins, ZO-1, ZO-2, and ZO-3, multi-PDZ domain protein-1 (MUPP1) and membrane-associated guanylate kinases with inverted orientation-1 (MAGI)-1, MAGI-2, MAGI-3 (5, 6).

Epithelial cells not only provide a physical barrier for the clearance of antigens but are directly able to neutralize microorganisms through the production of enzymes (lysozyme, peroxidases, phospholipases), complement components, protease inhibitors [secretory leukocyte proteinase inhibitor (SLPI) and elafin], permeabilizing peptides [β-defensins, cathelicidins, bacterial permeability increasing protein (BPI) and palate, lung, and nasal epithelium clone (PLUNC)], collectins (such as SP-A, SP-D and MBL), binding/neutralizing proteins [mucins, serum amyloid A (SAA) and lactoferrin], pentraxins (PTX-3 and CRP), small oxygen-reactive molecules (ROS and nitric oxide) (4), whose production is regulated by the engagement of pathogen-recognition receptors (PRRs), such as Toll-like receptors (TLRs), and NOD-like family receptors [NLR]. In addition to their direct antimicrobial activity, epithelial-derived β-defensins and collectins (such as SP-A, SP-D) can also function as 'damage-associated molecular patterns' (DAMPs). In particular, β-defensin 2 activates dendritic cells (DCs) upon binding to TLR-4 (7), and β-defensin 3 to TLR-1 and TLR-2 (8), while SP-A and SP-D act through the modulation and/or direct binding of TLR-2 and TLR-4 (9).

In addition, an important role is played by taste receptors, such as the bitter taste receptor T2R38, expressed on ciliated cells, which detects acyl-homoserine lactones (AHLs) secreted by gram-negative bacteria (10), and the T2Rs expressed on solitary chemosensory cells (11).

Recently, the role of substance P (SP) in the nasal mucosa innate immune response to viral infection was described as an inducer of pro-inflammatory mediators, such as cytokines, chemokines, arachidonic acid metabolites and oxygen radicals, and of TLRs overexpression (12). SP production has been demonstrated to be induced not only by trigeminal gangliar neurons but also by human nasal epithelial cells (HNECs), upon TLR7 stimulation (13).

Exogenous antigens are transported by specialized intraepithelial M cells to antigen-presenting cells, such as intraepithelial ors and macrophages, which in turn process and present antigens to CD4+ T lymphocytes, which polarize in TH2 cells, producing IL-4, IL-5 and IL-6, which trigger an Ig A-committed B-cell development, associated to the formation of the intrafollicular germinal centres (GC). Human M cells in adenoidal tissues are identified by cytokeratin 20 (Ck20), and irregular microvilli as well as pocket-like structures, observed by scanning electron microscopy (SEM) (5).

Parafollicular DC priming, CD4+ TH activation and homing of naïve B and T cells

Interestingly, mRNAs isolated from mouse

NALT revealed the capability of CD4+ TH cells, located in the proximal follicular area, to be polarized towards a TH1 or TH2 phenotype, depending on the antigen nature (14). For example, bacterial cell-wall components or virus-associated antigens, intranasally somministrated with an adjuvant of cholera toxin, induce TH2 polarization (15), while antigen-expressing recombinant Mycobacterium bovis bacillus Calmette-Guérin (rBCG) triggered a TH1-cell-mediated immunity (14).

The nature of the immune response that occurs is strikingly dependent on the state of activation of DCs and the context in which they present antigens to T cells. Takano et al. describe HLA-DR- and CD11c- positive DCs in the epithelium of the nasal mucosa of patients with allergic rhinitis as cells that express claudin-1 and are able to penetrate beyond epithelial tight junction (16). DCs/CD4+ T interaction is mostly mediated by class II molecules of the major histocompatibility complex (HLA class II), such as the classical HLA-DR, -DQ and -DP molecules (17). Interestingly, five distinct subtypes of DCs could be identified, showing different levels of HLA-DR, CD13, and CD123, and endowed with functional differences which remain to be deeply investigated (18).

Several epithelial-derived factors, such as IL-33, an IL-1 family member (19) and IFN-α (20), regulate DC function, leading preferentially to TH2 and TH1 responses, respectively. It has been demonstrated that the epithelial-derived factor thymic stromal lymphopoietin (TSLP) acts on DCs priming their IL-12-independent activation, which leads to a TH2 response. TSLP production by epithelial cells occurs upon TLR3 engagement by double-stranded RNA, or stimuli, such as rhinovirus, parasites and TH2 cytokines (21).

Several evidence suggest the role of NALT as an inductive site for generating IgA-committed B cells ($IgM^-IgA^+B220^+$) from naive B cells ($IgM^+IgA^-B220^+$) through Class-Switch Recombination (CSR) (22) and for the generation of memory B cells, able to produce high-affinity IgA (23). Naive B and T cells reach mucosal lymphoid compartments through specialized post-capillary HEVs. This homing process is described in lymph node and tonsils where it is regulated by the binding of the lymphocyte CD62L (L-selectin) to the adhesion molecule addressin expressed by HEVs (24). The chemokines involved are stromal-produced secondary lymphoid tissue chemokine (CCL21) and Epstein-Barr virus (EBV)-induced molecule 1 ligand chemokine (CCL19) for T cell recruitment, while studies on mice revealed the role of DCs-produced CXCL13 (in humans also called B-cell attracting chemokine-1) and its CXCR5 receptor for naive B cells (25).

Intrafollicular cell interactions

In the proximal follicular area, B cells, which have already come into contact with CD4+ T cells, are ready to colonize the center of follicles for the formation of the GC. In this context, intrafollicular DCs (FDCs) play a role in the further stimulation and proliferation of B cells, through their DC-mediated interaction with antigens retained in the form of immune complexes. In addition to FDCs, CXCR5- and PD-1-expressing Follicular Helper T (F_{TH}) cells induce the differentiation of GC B cells into high-affinity plasma cells (PCs) or memory B cells, thus allowing the establishment of a long-lived antibody response (26). The interaction between F_{TH} and B cells is mainly related to binding of CD40 expressed by B cells to the costimulatory CD40 ligand (CD154) on F_{TH}, which triggers the activation of intracellular signalling pathways that blocks B cell apoptosis and induces the switching from IgM to serum IgG or mucosal IgA. Conversely, F_{TH} cell maturation and expansion require the expression of costimulatory B7 (CD80/CD86) molecules on B cells, which can bind to F_{TH} CD28 (27).

These entire events act on B cells leading to clonal expansion and positive selection of B cells producing high affinity antibodies, mostly IgA, associated to the joining (J) chain. This J-chain mediate IgA polymerization and allows dimeric or polymeric IgA to bind to the membrane poli-Ig receptor, through which IgA could be released in the mucosal secretion of the nasal passage, where they can neutralize microbial proteins, including toxins. These high-affinity IgA differ from the low-affinity type secreted preferentially in peritoneal and pleural cavities where they mediate the inhibition of adhesion of commensal microbiota,

thus establishing a favourable physiological microenvironment (28).

NALT-derived PCs and memory B cell extrafollicular dissemination

PCs and memory B-cell release from follicles is regulated by CXCR5 down-regulation and CCR7 up-regulation, allowing their attraction to extrafollicular chemokines (25).

Nasal immunization induces the production of PCs endowed with high levels of membrane CCR10 chemokine receptor and α4β1 integrin, allowing them to preferentially traffic to the respiratory and genito-urinary tracts, both expressing their corresponding ligands, CCL28 and VCAM1 (29, 30).

Other immune cells involved in NALT immune response. Cytotoxic T Lymphocytes

An acquired CTL response is also induced, resulting in the activation of CD8+ T lymphocytes which represent about the 20% of resident T cells in NALT, as documented in animal models for various viral infections of the nasal passage, such as influenza viruses, reovirus and Sendai virus (31).

Innate Lymphoid Cells (ILCs)

In addition to inducing acquired immune responses, epithelial-derived factors released upon antigen stimulation could activate innate immune cells including macrophages and innate lymphoid cells (ILCs), which can also directly influence TH cell polarization.

ILCs are a new class of innate effector cells whose development depends on signalling mediated by IL-2 receptor (IL-2R) common γ-chain and IL-7R-α (CD127). In contrast to T and B cells, ILCs do not express antigen-specific receptors and are substantially located at barrier surfaces (32). In nasal mucosa, in particular, ILC2s are present and produce type-2 cytokines. These cells have been identified in chronically inflamed human upper airways mucosa of patient suffering from nasal allergic diseases, namely chronic rhinosinusitis with nasal polyps (CRSwNP) (33). In addition, several reports underline the role of ILC2 as an effector cell in type 2 immune responses, not only through the production of TH2 mediators, such as IL-4 and IL-9 cytokines and molecules such as the granulocyte-macrophage colony-stimulating factor (GM-CSF) and the amphiregulin, all able to promote eosinophilia, mucus production, M2 macrophage development and tissue repair, but also by promoting DC activation (34) or directly interacting with CD4+ T cells.

Nasal vaccination against infections: an overview

Nasal vaccination can elicit both humoral and cell mediated antigen-specific immune responses upon exposure to lower doses of antigens than those required for oral vaccination, administered in association to immune-enhancing molecule (adjuvant), such as the Sendai-virus-associated fusion protein or the haemagglutinating virus of Japan (HVJ), encapsulated in liposome or microsphere structures (35). Further vaccination strategies consist in the use of safe toxin-based adjuvants, such as cholera toxin from Vibrio cholerae and Escherichia coli enterotoxin (36) and recently of nanoparticles, with preclinical studies mostly performed in rodents (37). In addition, it an M cell DNA Vaccine has been developed, whose application has demonstrated to be effective in mice for CTL immunity induction to HIV (38).

Only few human nasal vaccines have been licensed and commercialized, such as FluMist (Medimmune) and Nasovac (Serum Institute of India), quadrivalent and trivalent live attenuated influenza vaccines, both formulated in a spray solution (39). As expected, FluMist evokes a local mucosal IgA production to virus surface haemagglutinin (HA) and neuraminidase and low titers of serum IgG. Several new mucosal vaccines are in different stages of clinical testing but further validation on human of safe vaccine delivery system are needed (40).

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MECHANISMS OF ALLERGIC DISEASES IN OTORHINOLARYNGOLOGY

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Allergic rhinitis (AR) is an IgE-mediated hypersensitivity disease caused by inhalation of an allergen to which the patients is sensitized. Etiopathogenesis of AR comprises a sensitization phase, an immediate phase and a late phase. In the sensitization phase, inhaled allergens are processed in peptides and come into contact with the nasal mucosa cells. Antigen-presenting cells (APCs), especially represented by dendritic cells (DCs), capture them through the interaction with their own MHC class II complexes and migrate to lymph nodes. Then, allergenic peptides are presented to naïve CD4+ T lymphocytes and a differentiation of T cells in Th2 subset takes place. After Th2 lymphocyte induction due to allergen exposure, the most relevant cytokines that are produced are represented by IL-3, IL-4, IL-5, IL-9, IL-10, and IL-13 that are able to promote IgE synthesis and mast cell proliferation. The allergen reaction, when allergen meets its specific IgEs on mast cells surface, causes an early inflammatory reaction determined by mast cells and basophils degranulation with release of preformed mediators from the intracellular granules, resulting in symptoms such as rhinorrhea, itching and sneezing. This phase is followed by a late phase characterized by the release of newly formed mediators, like leukotrienes, chemokines and adhesion molecules, and by the recruitment of eosinophils, neutrophils, macrophages, mast cells, lymphocytes B and T in the nasal mucosa. Such mechanism is responsible for continuing inflammation sustained by chemoattractants, cytokines and adhesion receptors that induce cellular infiltration of eosinophils, basophils, Th2 lymphocytes and mast cells and is clinically mirrored by the prevalence of nasal congestion over sneezing, itching and rhinorrhea.

Allergic rhinitis (AR) is an allergic disorder affecting the nose, characterized by an inflammation of the nasal mucosa (1). Globally, it is one of the most common diseases, affecting up to a fifth of the general population (2). Epidemiology differs among self-reported and confirmed diagnosis, but the latter is still significant. At least 40% of adults and up to 25% of children stated to have AR compared to the established diagnosis in 17-28.5% of patients (3). AR implies an important economic burden, consequent to considerable loss of productivity and impaired school performance. Consequences of the altered sleep quality are impaired cognitive function, irritability and fatigue (4). In Europe, the annual cost for AR is estimated around €1089 per children/adolescent and €1543 per adults, with a prevalence of indirect costs (5).

Pathogenesis

AR is an IgE-mediated, type 1, hypersensitivity disease secondary to the inhalation of an allergen towards sensitive patients. AR depends upon genetic and environmental factors, including exposure to allergens and adjuvants, like immune-suppressors (6).

Etiopathogenesis of AR can be classified in a sensitization phase, an immediate phase and a late phase.

Key words: allergic rhinitis, sensitization, inflammation, early phase, late phase

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Allergic sensitization

The sensitization phase is the first step in the process of developing AR. Inhaled allergens are processed in peptides and through the mucus, come into contact with the nasal mucosa cells. Antigen-presenting cells (APCs), especially represented by dendritic cells (DCs), capture them through the interaction with their own MHC class II complexes and migrate to lymph nodes. Herein allergenic peptides are presented to naïve CD4+ T lymphocytes (7). Allergens interact with T cell through specific T-cell receptors (TCR) and the costimulatory ligation among CD28 in T cells and CD80 and CD86 on APCs. All these bonds are necessary to activate T cells and their differentiation, in case of AR, in Th2 subset (8). Polarization of T cells in Th2 is determined by strength and duration of intrinsic and exogenous signals. IL-4 plays a central rose for Th2 differentiation, but the primitive source of IL-4 is still uncertain (9). It is speculated that some genetic predisposition can favor early IL-4 production from naïve T cells, determining Th2 differentiation in susceptible patients (10). Certainly DCs do not produce IL-4, but have an indirect role in promoting TH2 polarization. In fact, DCs seems to produce IL-12, cytokine favoring Th2 pattern, in presence of some environmental signals, such as low-dose endotoxin, TLR2 ligands, glucocorticoids, and many G-protein coupled receptor agonists (e.g. histamine, PG E2, PGD2) (11).

After Th2 lymphocyte induction due to allergen exposure, the most relevant cytokines that are produced are represented by IL-3, IL-4, IL-5, IL-9, IL-10, and IL-13 that are able to promote IgE and mast cell proliferation (12). This latter is described below. Th2 cells act on B lymphocytes to induce IgE antibodies production through two mechanisms. The first is mediated by IL-4 and IL-13, able to induce ε-genes transcription. The second signal is triggered by the link among CD40 ligand on the Th2 surface and CD40 on B cells surface, that promote switching recombination for IgE production (13). Once produced, IgEs adhere to high affinity receptors (FcεRI) on the surface of mast cells and basophils present in the nasal mucosa. FcεRI are also displayed on DCs membrane, facilitating the uptake and presentation of allergens to naïve T cells (6).

The immediate phase

In an ongoing sensitization process, when allergen meets its specific IgEs on mast cells surface, an inflammatory allergic reaction occurs and two separate phases, an early and a late one, can be distinguished. The early phase starts a few minutes after the exposure to inhalant allergens to which the patient is sensitized. The interaction between allergen and IgE determines mast cells and basophils degranulation. Mast cells, in particular, release at first preformed mediators from the intracellular granules, such as histamine, leukotrienes, eosinophil chemiotactic factor and platelet activation factor (PAF). Each mediator act on specific targets. For example, histamine causes stimulation of sensory nerve endings, producing sneezing and pruritus through H-1 receptors and increases vascular permeability, determining rhinorrhea and nasal congestion through H-1 and H-2 receptors on blood vessels. The classical symptoms of early phase are, in fact, rhinorrhea, itching and sneezing, while nasal congestion is still moderate. They reach a peak within few minutes after the exposure to allergen, and, if it is eliminated, the inflammatory process is limited to one hour (14-16).

In the acute-critical phase, nasal mucosa appears hyperplastic, with an important inflammatory cells infiltration, especially mast cells and eosinophils. Macroscopically mucosa is edematous and pale, often covered with a serous translucent layer (17).

The late phase

This immediate phase is followed by an inflammatory process (late phase), characterized by the release of newly formed mediators, like leukotrienes, chemokines and adhesion molecules and by the recruitment of eosinophils, neutrophils, macrophages, mast cells, lymphocytes B and T in the nasal mucosa (6). In particular, after 4 to 8 h after allergen exposure, recruited leukocytes are activated, mediating the cellular-driven inflammatory process (18). Chemoattractants, such as eotaxin, IL-5 and RANTES are crucial, together with cytokines and adhesion receptors, for cellular infiltration of eosinophils, basophils, Th2 lymphocytes and mast cells in chronic AR. Leukocyte endothelial adhesion molecules, IL-4, IL-5, GM-CSF (granulocyte macrophage colony stimulating factor) and TNF-α

are increased in AR and may originate from several sources, including mast cells, eosinophils and T-lymphocytes, contributing to the underlying inflammatory process in rhinitis (12).

Eosinophils play a fundamental role in AR. After allergen exposure, the quickly recruited eosinophils in nasal mucosa begin to produce IL-5, a main chemoattractant and survival-favoring of eosinophils (19). Chemokine eotaxin is also of primary importance, but increased expression of endothelial adhesion molecules was demonstrated to be necessary for eosinophilic infiltration in AR (20). Tissue eosinophilia in AR is responsible for the maintenance of the inflammatory process through the production and release of lipid mediators like LTC4, thromboxane and PAF and substances able to destroy epithelial nasal cells, such as major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) (6). Tissue eosinophilia is also characteristic of allergic asthma. Braunstahl et al. demonstrated that eosinophilic infiltration secondary to the increased expression on endothelial adhesion molecules in nasal mucosa of patients with AR can be stimulated by a nasal provocation test (NPT). NPTs were performed with appropriate allergen concentration in non-asthmatic patients affected by AR, but out of season. These results are relevant to demonstrate at a pathomechanism level the interaction between upper and lower airways, even in absence of a clinical significant symptomatology (20).

Clinically, the late phase is characterized by the prevalence of nasal congestion over sneezing, itching and rhinorrhea. If the inflammatory process becomes chronic, it is possible in some cases to observe the development of nasal turbinate hypertrophy and polyps. Macroscopically, the mucosa appears as described for immediate phase. If AR is intermittent, no alterations are present in inter-critical periods (17).

Allergens

The most important allergen source inducing AR are from pollens, molds, house dust mites, animal epithelia and insects. Among pollens, the botanical species most frequently responsible are from grasses (*Lolium sp*p., *Phleum pratense*, *Dactylis glomerata*, *Anthoxanthum odoratum*, *Poa pratensis*), trees (birch, hazel, Hornbeam, alder, olive, cypress), *compositae* (mugwort, ragweed), and *urticaceae* (Parietaria). The clinically relevant molds are *Alternaria spp.* and *Aspergillus spp*. The animal epithelia of major importance are from dog, cat, rabbit, horse, laboratory animal while the insect frequently causing allergy are cockroaches (*Blatella spp*., *Periplaneta spp.*) (21).

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POLLENS CAUSING ALLERGY AND THEIR MONITORING BY AEROBIOLOGY AND PHENOLOGY

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Allergies caused by inhalant allergens, particularly pollens, are steadily increasing in urban centers. It is known that atmospheric pollution is strongly related to the inflammatory disease of the upper and lower airways but it is equally important in the development of sensitization towards pollens. Particulate Matter (PM), sulfur dioxide (SO2) and nitrogen dioxide (NO2) have an enhancement function on the persistence of pollens in the air, increasing the concentration and duration of pollinosis. It is therefore essential to use air quality control methods in urban centers to monitor the presence of pollen and fine dust that can drive the doctor and the patient to improve prevention, a step of primary importance in the treatment of allergies. Aerobiology and phenology are essential tools to monitor pollen production. The opportunity for the patients to use social media as information sources, including teletext, sms, mail and social networks, as well as a wide range of apps, allows to have reliable information on the air we breathe and therefore to better manage the methods of prevention at our disposal.

Seasonal allergy to pollens is a disease in significant expansion in large cities, caused mainly by the presence of inadequate ornamental plants and aggravated by the ever-increasing atmospheric pollution that accentuates the sensitizing action of pollen on the respiratory mucous membranes (1).

A relationship between pollen granules and atmospheric pollutants has been demonstrated: on the one hand the granules absorb and transport airborne pollutants to the respiratory tract, increasing their concentration, and on the other hand common pollutants, such as particulate emissions from diesel engines act as carriers, transmitting pollen allergens and promoting the production of antibodies of the IgE class (2). The assessment of air quality is one of the most important issues and is of particular concern in the urban environment, where gas and particulate matter sources are predominantly concentrated and where population density is a potential risk for human health (1).

Human influence on pollen production

While pollen is occurs naturally, pollen emissions are very much affected by human activities, particularly by air pollution resulting from human activity and by planting of trees that are unsuitable because of their sensitizing capacity (birch, cypress, European hornbeam, olive trees), in immediate proximity of people's homes. Tables I-III provide an overlook on the most common types of allergenic plants in Italy.

The production of allergenic pollen is also significantly affected by planting, mainly for ornamental aim, of highly allergenic trees and plants.

Key words: pollen allergies, air pollution, pollen monitoring, aerobiology, phenology

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Using birch tree as an example, approximately 25% people with hay fever are allergic to birch tree pollen, yet it is possible to see birch trees planted in many cities in central and northern Europe. Similar phenomena are observed for other trees such *Alnus*, *Corylus*, and *Cupressaceae*. In addition, many new or non indigenous allergenic species of plants arrived in Europe, for example Rome, where noteworthy changes in the local flora were observed (3). A well-known example is ragweed which is spreading across Europe and is highly allergenic. Also largely cultivated plants, such as spruce, olives and oilseed rape contribute to the aggravation of allergies. Finally, climate change represents a possible threat for a patient affected by allergy. Climate change also affects pollen season and its distribution is a major concern.

Pollution and pollinosis

According to recent studies, air pollution is significantly exacerbating the aggression of pollen. Because of higher concentrations of carbon dioxide (CO_2) in the air, plants grow faster, produce more fruits and consequently affect people with allergy more importantly. Moreover, plants stressed by air pollution produce more aggressive pollen grains because of the

Table I. *Tree pollens: name, allergens, habitat*

Tree pollen	Allergens	Habitat
Hazel (*Corylus avellana* L. - Betulaceae)	Cor a1	Hazel is commonly present in cities in parks and gardens
Cypress (*Cupressus sempervirens* L.; *Hesperocyparis arizonica*) (*Cryptomeria japonica* D. *Taxus baccata* L. *Juniperus communis* L. – Cupressaceae)	Cup a1 Cry j1 Cry j2 Jun a1 Jun a2 Jun a3	Cypress trees are commonly used in towns in parks garden, avenues, in cemeteries, in archaeological area and in sports facilities.
Alder (*Alnus glutinosa* L. – Betulaceae)	Aln g1	Alder lives near rivers or wet area.
Birch (*Betula verrucosa* Ehrh – Betulaceae)	Bet v1 Bet v2 Bet v4	Birch is common in northern Italy, not only in woods but also in cities, in parks and gardens
Plane (*Platanus hispanica* – Platanaceae)	Pla a1 Pla a2	Widely planted in alignment, in parks and schoolyards.
Olive (*Olea europaea* L. – Oleaceae)	Ole e1 Ole e2	In recent years olive trees are planted in town increasing pollen amount
Ash (*Fraxinus angustifolia* L. – Oleaceae)	Fra a1	Ash live in deciduous wood on temperate region, but sometime it occurs also in urban park
Oak (*Quercus* sp. – Fagaceae)	Que a1	Commonly present in cities in parks, green areas, avenues
Chestnut (*Castanea sativa* – Fagaceae)	Cas s1	Commonly present in cities in parks, green areas, avenues
Beech (*Fagus sylvatica* L. – Fagaceae)	Fag s1	It forms wide forest in Italy from 800 up 1700 a.s.l.
European hornbeam (*Ostrya carpinifolia* Scop. – Betulaceae)	Ost c1	Recently it is used in urban parks, in green areas

increase in the content of proteins causing reactions (4). The REVIHAAP report confirmed that other pollutants may worsen allergies as well (5). Co-exposure to grass pollen and particulate matter (PM) leads to stronger allergic responses and exposure to sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) can worsen pollen allergy and enhance lung inflammation.

To know which and how many pollen grains are present in urban air, at least 30/m^3 or more in some case, pollen monitoring activity is commonly carried out in numerous cities in Italy as well as in the world (6). In each of the sampling sites, a Hirst sampler runs the entire pollen season. For these reasons, pollutant pollen monitoring can be considered an integrated tool in the broader context of air quality assessment.

Monitoring of pollens: sampling techniques

Air sampling techniques are numerous, depending

Table II. *Weeds pollen: name, allergens, habitat.*

Weeds	Allergens	Habitat
Ragweed (*Ambrosia artemisiifolia* L. – Asteraceae)	Amb a1	They grow in dry and sunny meadows, along the rivers, on the edge of the roads and generally in abandoned grounds
Mugwort (*Artemisia vulgaris* L. – Asteraceae)	Art v1 Art v3	Very common synantropic plant, typical of the ruderal roots
Pellitory (*Parietaria judaica* L. – Urticaceae)	Par j2	It can grow in semi-shade (light woodland) or no shade. It prefers dry or moist soil. Pellitory colonises walls, ways, trap-doors
English plantain (*Plantago lanceolata* L. – Plantaginaceae)	Pla l1	It is present in uncultivated areas, along the streets, fields, vineyards, and ruderal environments

Table III. *Grass pollen: name, allergens, habitat.*

Grass (Poaceae)	Allergens	In Italy there are more than 450 different species. Grasses are annual, biennial, or perennial plants that are usually herbaceous but may be woody in some genera. They may be terrestrial or aquatic. Grasses live everywhere in our towns, in gardens, in flowerbeds, in meadows. So if these spaces are not well managed cutting grasses before flowering they produce a lot of pollen.
Cock's foot (*Dactylis glomerata L.*)	Dac g7	
Perennial ryegrass (*Lolium perenne* L.)	Lol p1 Lol p2A Lol p5B Lol p5A Lol p11	
Meadow fescue (*Festuca elatior* L.)	Fes e7	
Kentucky blue-grass (*Poa pratensis* L.)	Poa p1 Poa p10 Poa p12 Poa p13	
Yorkshire frog (*Holcus lanatus* L.)	Hol l1	
Oat (*Avena sativa L.*)	Ave s1 Ave s11s	
Timothy grass (*Phleum pratense* L.)	Phl p1 Phl p2 Phl p4 Phl p5b Phl p6 Phl p7 Phl p11 Phl p12	
Sweet vernal grass (*Anthoxanthum odoratum* L.)	Ant o1	
  Bermuda grass (*Cynodon dactylon* L.)	Cyn d1	

on the different purposes and for each particular biotic particle, it is possible to use a specific sampler. If a universal sampler does not exist, it is sure that for the same particles, for example pollens and spores, it is possible to count on a method and instrument commonly used in all the aerobiological network. This sampler is the Hirst spore trap (7) that was designed to record pollen grains and fungal spores and other biological particles. This instrument allows a morphological identification of different particles in function of time. Thus, it is possible to know pollen concentration day to day and in some cases, hour by hour. Air enters through a 2x14 mm intake orifice and impacts onto a collection surface that advances 2 mm per hour. It is possible to have daily monitoring by deposing particles on a slide, or weekly monitoring by deposing particles on transparent tape (melinex). Two Hirst sampler models are available, Burkard and Lanzoni; they are similar, the only difference is on the medium coated on tape, i.e. paraffin on the first sampler and silicon fluid on the second one (8).

The sampler sucks air with a flow of 10 litres per min, comparable to the mean breath rate of an adult in rest condition. Particle identification is carried out under a light microscope using different magnification. Pollen grains are normally stained with fuchsine for a better examination. Pollen counts are converted in pollen concentration and weekly reports are produced during the year.

The standardisation of pollen monitoring allows the comparison of data between sites, such as temporal and spatial variations in diurnal and daily concentrations and seasonal characteristics (e.g. timing and intensity of pollen seasons), as well as trends over time (e.g. in relation to land use and climate change). However, it is recognised that the pollen spectrum varies between sites in different biogeographical areas, which could still cause identification to be problematic (9).

In Italy in 2008, a regulation was written named Norma UNI 11108 2004 (10) and successively at the European Aerobiology Society, minimum requirements for monitoring activity were approved (11). More recently, a new European regulation "CEN/TS 16868:2015", is in the phase of approval (12).

Pollen grains only represent a small fraction of the total amount of viable biological particles present in the air, but pollens are the most important aeroallergens in the outdoor environment. Environmental applications of aerobic monitoring are many and the most known is certainly allergic-related: aerobiology is used in the field of allergology as a useful assessment tool for respiratory allergies. Exact knowledge of the true level of aerodynamic particles is of great importance from a diagnostic point of view, to correlate pollen presences with patient history and response to diagnostic tests and also as useful indication for proper pharmacological treatment.

Modern advances in molecular analysis could improve information for allergy sufferers and health care professionals (13, 14).

Importance of pollen monitoring

Patients and doctors should be helped in understanding how symptoms change during a pollen season; this may help to identify the individual co-factors facilitating symptomatic manifestations and consequently, disease self-management. To this end, many scales, indexes or scores have been created to measure the severity of allergic rhinitis and the impact of this disease on the patient's daily life. The most frequently used approaches are symptom scores (SS), medication scores (MS) and combined symptom-medication scores (SMS) (15, 16). Methods to measure and monitor the severity of allergic rhinoconjunctivitis are of increasing relevance for diagnosis and intervention trials.

Most methods are based on diaries filled out by the patient, who each day answers the same list of disease-related questions. These diaries generate SS, which are based on the values of the individual symptoms (e.g. by summation) (17). However, to date there is no universally accepted system to measure symptoms.

Information technology (IT) nowadays facilitates a more efficient and easier patient monitoring (18). Another important tool in aerobiological monitoring activity is the possibility to carry out phenological observations. Phenology studies the periodical rhythms of the biological events and their relationships with the environment, especially the climate. As the timing of phenological events and especially flowering is strongly affected by meteorological factors, it has been observed that plant phenological responses correlate

with climatic change. Moreover, the knowledge of the phenological stages of the plants, such as the flowering time, can be an important tool for agricultural economics (e.g. pesticides and fertilizer applications, choice of varieties of plants, crop yield) (19). For some of the pollen types (e.g. *Platanus*, *Quercus*, *Olea* and *Plantago*), there is an association between flowering phenology and airborne pollen records. In general, aerobiological observations about the pollen season are strongly influenced by the taxon, the location and the large-scale weather conditions (20-22).

Phenological studies have shown that airborne pollen results from both local and distant sources, although the pollen peaks usually appear when local sources are shedding the greatest amounts of pollen (23).

CONCLUSION

The great and constant work that is behind aerobic sampling has an important recognition in the disclosure and publication of the data. However, the increased availability of information on pollen concentration requires careful reckoning on the correct and useful ways of disseminating them from producers and of their responsible use.

The wide variety of communication modes, i.e. information panels, teletext, sms, mail, social networks, such as Facebook, My space or Twitter and a wide range of apps are the new tools that currently drive pollen information.

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THE SKIN PRICK TEST

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The skin prick test (SPT) is the most common test for the diagnosis of allergy. SPT is performed by pricking the skin, usually in the volar surface of the forearm, with a lancet through a drop of an allergen extract and is usually the first choice test in the diagnostic workup for allergic diseases because of its reliability, safety, convenience and low cost. SPT is minimally invasive and has the advantage of testing multiple allergens in 15 to 20 min. In children, SPT is far less disturbing than venipuncture and is used to obtain a sample of serum to measure specific IgE through *in vitro* tests. There is a good correlation (about 85-95%) between SPT and *in vitro* tests. Globally, SPT is an excellent diagnostic tool, with a positive predictive value ranging from 95-100%. SPTs can identify sensitivity to inhalants, foods, some drugs, occupational allergens, hymenoptera venom and latex. However, the relevance of such sensitivity to allergens should always be carefully interpreted in the light of the clinical history, because sensitization and clinical allergy may not coincide. In regards to safety, though the reports of systemic reactions, and particularly anaphylaxis, are very rare, *in vitro* IgE tests should be preferred if previous severe reactions emerge from the patient's clinical history.

Atopy is defined as the predisposition of certain allergy-prone individuals to produce specific immunoglobulin E (IgE) antibodies to environmental allergens. Atopy can manifest in childhood as infantile eczema (atopic dermatitis), allergic rhinitis and asthma. In common practice, it is critical to identify the offending allergen in atopic individuals. This will not only influence therapeutic interventions, but may also have a significant impact on the individual's quality of life. The most common test for diagnosis of allergy is the skin prick test (SPT), by which an allergen extract is directly introduced into the skin by pricking the skin with a lancet through a drop of the extract. SPT is usually the first choice in the diagnostic workup for allergic diseases because it is reliable, safe, convenient, inexpensive, minimally invasive and has the advantage of multiple allergen testing in 15 to 20 min (1-4). SPT is also far less traumatic, particularly to children, compared to venipuncture, used to obtain a sample of serum to measure specific IgE by *in vitro* tests. There is a good correlation (about 85-95%) between skin-prick testing and *in vitro* tests. Globally, SPT is an excellent diagnostic tool, with a positive predictive value ranging from 95-100 (5-7).

Skin tests were first introduced by Charles Blackley in 1865, who placed pollen on his forearm after having slightly cut the skin with a lancet. After a few minutes, that specific area presented with itching, whealing and erythema, followed by a delayed skin reaction. Mantoux introduced intradermal testing in 1908, which was then used

Key words: skin prick test, allergens, diagnosis, sensitivity, specificity, safety

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for the diagnosis of hypersensitivity type I allergy by Schloss. A few years later Lewis and Grant described the SPT, which was used for many decades with minimal modifications.

Recently, many new devices have been used for the standardizing of the conduction of skin tests (prick and puncture) (8, 9). SPT is the primary method used by allergists to diagnose IgE-mediated allergy.

Currently, several single-headed SPT devices are used internationally in allergy centers. Several studies have compared the variability in the measurements with different devices when used by a single operator (intra-user variability). However, to date no study has assessed the variability in performance of devices used by different operators (multi-user variability). Variability of SPT results performed by a single operator on the back compared with the forearm has previously been reported although multiuser variability of testing performed on the forearm *vs* the back has not been compared for different SPT devices (10, 11). The very good performance of SPT resulted in abandoning different skin test techniques previously used, due to their low reproducibility and painful effects, while others have proven useful and continue to be part of the practice. An SPT can detect tissue bound IgE and an atopic state in patients with a type 1 allergy. It can be used to provoke an immediate hypersensitivity response in the skin after the point of the device is used to prick/puncture the *stratum corneum*, resulting in exposure of the epidermis to an allergen extract solution. The allergen is then presented to tissue mast cells with cross-links surface-bound IgE, inducing the release of mediators that stimulate measurable wheal and flare reactions (12-13).

SPT is indicated if a type I (immediate type) allergy is suspected, based on the medical history and clinical symptoms. The SPT can identify sensitivity to inhalant, food, drug or occupational allergens, hymenoptera venom, and latex. SPTs, thus providing objective confirmation of sensitivity, whereas the relevance of such sensitivity to allergens should always be carefully interpreted in the light of the clinical history. The lack of confirmation by history defines asymptomatic sensitization, which reduces the specificity of STPs. Therefore, appropriate advice concerning avoidance measures should be given and, when necessary, the correct allergen(s) can be prescribed for specific immunotherapy (SIT). SPT results are related with those of nasal challenge, which may also be used as a surrogate to test clinically relevant sensitization and the severity of the disease) (14-15).

There commended method of prick testing includes the appropriate use of specific allergen extracts, positive and negative controls, and interpretation of the tests after 15-20 min from the application, with a positive result defined as a wheal ≥3 mm diameter. A standard prick test panel for Europe for inhalants is proposed and includes hazel (*Corylus avellana*), alder (*Alnus incana*), birch (*Betula alba*), plane (*Platanus vulgaris*), cypress (*Cupressus sempervirens*), grass mix (*Poa pratensis, Dactilis glomerata, Lolium perenne, Phleum pratense, Festuca pratensis, Helictotrichon pratense*), olive (*Olea europaea*), mugwort (*Artemisia vulgaris*), ragweed (*Ambrosia artemisiifolia*), *Alternaria alternata* or *tenuis*), *Cladosporium herbarum, Aspergillus fumigatus, Parietaria*, cat, dog, house dust mites (*Dermatophagoides pteronyssinus, Dermatophagoides farinae)*, and cockroach (*Blatella germanica*). Standardization of the skin test procedures and use of standard panels for different geographic areas are encouraged worldwide to permit better comparisons for diagnostic, clinical and research purposes (16).

The chief advantage of SPT as compared to an *in vitro* measurement of specific IgE antibodies is that the test can be interpreted within 15 to 20 min after the reagent is applied to the skin. The test gives a visual indication of the sensitivity towards the individual allergens. The SPT can also be used to test the common allergens, such as drugs causing reactions through the IgE-mediated mechanism and if the technique of prick-by-prick is used, fresh fruits and vegetables to which no specific IgE antibody measurements are available.

The *in vitro* measurement of specific IgE antibodies remains an important complementary tool to diagnose type I allergy, especially in subjects who cannot undergo SPT. For example,

SPT is not feasible in patients who have extensive eczema, dermographism, urticaria, or who are taking antihistamines or other medications that interfere with the proper interpretation of the test results (Table I) (17-20).

In vitro test methods may be less sensitive and/or less specific than SPT depending on the method employed and the allergens selected (21-23).

Preparation, precautions and contraindications

SPT is a safe procedure but even if systemic and life-threatening allergic reactions are very rare, a physician or other health care professional and emergency equipment should be immediately available when such tests are performed. This is especially true when foods or medications associated with anaphylactic reactions are tested (24). Systemic side effects are very unlikely for commonly available respiratory allergens. Current severe asthma may be a risk factor for respiratory reactions associated with testing. Reactions usually occur within 30 min from testing. *In vitro* IgE tests or titrated SPT are preferable for patients with suspected severe anaphylaxis from a specific allergen to be use used for testing, for example peanuts, tree nuts and shellfish. SPT should be performed with caution during the respective allergy season when the patient has severe pollen-related symptoms. In addition, patients taking a beta-blocker, or less often, angiotensin converting enzyme (ACE)-inhibitor may be at risk because of a reduced response to epinephrine that might be needed to treat as systemic allergic reaction. Relative contraindications for SPT include pregnancy, in view of a remote possibility of inducing a systemic allergic reaction that could results in uterine contractions or necessitate the use of epinephrine.

The degree of skin test reactivity may be decreased in subjects with chronic illnesses such as renal failure or cancer. In addition, chronic or acute UV-B radiation of the skin in the area to be tested may reduce the wheal size. It is important to check the stability and the expiration date of the allergen extracts. Test extracts should be stored at +2°C - +8°C when not used to maintain stability. Histamine dihydrochloride (10 mg/mlor 0.1%) can be used as a positive control and diluent, as used in the test extracts, as a negative control.

The location of each allergen can be marked with a pen or by using a test grid on the forearm to properly identify test results. Tests should be applied to the volar surface of the forearm, at least 2-3 cm from the wrist and the antecubital fossa. SPT may also be performed on the back, especially in small children. The skin on the back is more sensitive than the forearm and thus larger wheals and possibly a higher number of positive test results may occur. The distance between two SPT (≥2 cm) is critical to avoid false-positive reactions due to skin response to a nearby test or secondary to an axon reflex. A drop of each test solution should be placed on the skin in identical order for each subject tested and instantly pricked. A single-head metal lancet was found to have excellent reproducibility with few false-negative results and is thus the preferred testing device. The lancet is pressed through the drop of allergen extract and held against the skin for at least 1 sec, with equal pressure applied for each test. The epithelial layer of the skin should be penetrated without causing bleeding, which can give false-positive results. A new lancet should be used for each allergen, because wiping a previously used lancet could result in cross-contamination from the formerly tested allergen. Wiping lancets may also be a potential risk factor for the healthcare professional who perform the test. Excess solution from drops on the skin can be removed using a clean tissue. It is important to guarantee that there is no cross-contamination between drops of different allergen extracts. A timer, with an alarm, should be utilized so that all tests, including the histamine and negative control test results are read 15-20 min after application. This timing for test results is recommended, though the skin reaction to the histamine control can peak earlier (at approximately 8-10 min).

The SPT need to be managed by an allergist in the following circumstance: unavailability of specific tests required in relation to anydifficulty in interpreting the results; when complex or multiple allergies are suspected; when there were previous and severe side effects; in the case of severe angioedema; when drug-related allergies are suspected; when there is a need for food and or respiratory challenge testing.

SPT for respiratory IgE-mediated disease are cheap, easy to perform, reliable and sensitive and have the advantage of giving immediate results. Even though the risk (although minor) of developing anaphylactic reactions is still a possibility, the test remains safe to perform in a consultation facility or at the patient's bedside. SPT is still the test of choice worldwide, based on its convenience and cost-effectiveness (25-28).

CONCLUSION

The skin-prick test is currently the most effective diagnostic procedure in reaginic allergic disorders, easy to execute and safe for children and adults.

It is essential that high quality extracts are used and are well standardized and stored correctly, especially where the "cold chain" is concerned.

Table I. *Drugs that interfere with the SPT results.*

Antihistamines

• 1st generation H1-blocker +++ > 2 days
• Hydroxyzine
• 2nd generation H1-blocker +++ 7 days
• Cetirizine, Loratadine, etc.
• Ketotifen> 5 days

H2-blocker should not be taken the morning of the test

Glucocorticosteroids

▪ Topical (in test area) + > 1 week
▪ Nasal or inhaled: no limitations
▪ Systemic/short term (up to 10 days)
▪ Systemic/long term (more than 10days)

Other systemic drugs

▪ Omalizumab ++ > 4 weeks
▪ Leukotriene receptor antagonist: no limitations
▪ Cyclosporin A:no limitations
▪ Theophylline: no limitations
▪ Antidepressants
▪ Doxepin ++ 7 days
▪ Desipramine ++ 3 days
▪ SSRI: citalopram, fluoxetin, sertralin no limitations
▪ β-adrenergic agonists 0: no limitations
▪ Salbutamol, salmeterol, bambuterol: no limitations

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LABORATORY TESTS FOR ALLERGY DIAGNOSIS

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The introduction of highly purified natural and recombinant single allergenic molecules represented an important improvement in the diagnosis of IgE sensitization. The identification of specific IgE against cross-reacting molecules such as profilin, lipid transfer proteins, calcium binding proteins or against "genuine molecules", represents an added value and allows to distinguish between true and false polysensitization. *In vitro* tests add information to recognize patients with sensitization to genuine molecules that cause allergic diseases and to evaluate in childhood the spreading of sensitization for each molecule in order to choose the best treatment and to identify the ideal patient for allergen immunotherapy. Also, in order to detect patients with sensitization to pan-allergens it is important to manage the risk of anaphylaxis for patients allergic to latex and to identify IgE to particular molecules involved in occupational allergy. In patients with negative skin prick tests (SPT), that results in a lower sensitivity compared with *in vitro* tests, the negative test may be caused by the lack of some important allergenic molecules in the extract used for SPT.

Specific IgE antibodies can be detected either *in vivo* by skin prick test (SPT) or *in vitro* by specific IgE assays: both methods usually employ whole extracts from allergenic sources, which contain a mixture of allergenic and non allergenic proteins (1) but the IgE response is specifically directed only towards certain molecules (2).

Specific IgE (sIgE) for inhalant allergens (house dust mites, pollens, molds, latex) and for some food allergens that can induce respiratory symptoms (e.g. serum albumin, wheat flour, casein, fish parvalbumin and vegetables containing lipid transfer proteins (LTP, lysozyme, etc.) are measured to detect the trigger agents of allergic diseases such as conjunctivitis, rhinitis, asthma or occupational allergic respiratory diseases.

The international guidelines from the European Academy of Allergy and Clinical Immunology (EAACI) and the World Allergy Organization (WAO), as well as the national guidelines from the "Società Italiana di Allergologia Asma e Immunologia Clinica" (SIAAIC) considered *in vitro* tests for allergic diseases as a second level test, to be used after SPT, when a confirmation is needed or if the SPT cannot be carried out because of patient-related factors, such as concomitant use of antihistamines, atopic dermatitis in the site of testing, etc. The measurement of *in vitro* sIgE is an important tool, because it allows to identify in a quantitative way the sensitization towards a complete allergen and/or a specific allergy molecule. The possibility to perform a deeper analysis with the molecular diagnostics gives important information, more specific than the SPT; sIgE are usually measured for common allergens: dust mites, grass, trees, olive,

Key words: allergy diagnosis, skin prick test, in vitro IgE tests, genuine molecules, component resolved diagnosis, IgE molecules detection

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cypress, ragweed, Parietaria, Plantago, Alternaria, Cladosporium, dog and cat epithelia. Extractive preparations used for SPT usually contain cross-reactive components which are highly conserved across widely different allergen sources (3). This may complicate the interpretation of the diagnostic results, especially in polysensitized subjects. The introduction of highly purified natural and recombinant single allergenic molecules represented an important improvement in the diagnosis of IgE sensitizations and cross reactivities.

Component Resolved Diagnostics

The identification of specific IgE against cross-reacting molecules such as Profilin, Bet v1-PR10, LTP, calcium binding proteins or against "genuine molecules", represents an added value and allows to distinguish between true and false polysensitizations. A true polysensitization occurs when specific IgE against genuine components of different allergenic sources are present. The genuine molecules for grass sensitization are Phl p1, usually the first allergen to induce IgE and Phl p 5, Bet v 1 for birch, Par j2 for Parietaria, Pla l 1 for Plantago, Amb a 1 for ragweed, Der p 1, Der p 2, Der p 23, that start the sensitization towards Dermathophagoides, and Fel d1 for cat, a secretoglobin that is primarily produced in sebaceous, anal and salivary glands and is mainly present in the epidermis and fur. It is spread to the environment mainly through airborne dander, which can be detected both in homes with and without a cat. Pet allergy can induce severe symptoms of rhinitis and asthma that are difficult to control.

False polysensitizations are due to the presence of panallergens like profilins or calcium binding proteins causing the SPT positive results (4, 5). Epidemiological studies and clinical trials showed that the percentage of polisensitized ranges from 20 to 90% with a great variability depending on the populations. Polisensitization may also be associated with different clinical pictures with respect to monosensitized patients, especially those with a more impaired QoL and more severe symptoms. In addition, allergic children seem to display a higher frequency of sensitization than their parents, especially in families with polisensitization. In some cases they are true polisensitizations, in other cases they are sensitizations to panallergens such as profilin or calcium binding proteins. The component resolved diagnosis (CRD), with either recombinant or purified allergens for SPT or IgE in serum dosage, is a tool to improve the accurate identification of the sensitization allergens. A positive SPT could be due to a sensitization to major allergens or simply to a cross-reaction response to panallergens like profilin Bet v 2 or Phl p 12 present with small conformational change in both pollen species. CRD has an important impact on the management of the patients in term of accuracy of the diagnosis or decision on the therapy (for example specific immunotherapy prescription).

Recent studies demonstrated that the CRD use in the diagnostic work-up may result in changing the decision on treatment in more than 50% of the patients compared to the diagnosis based only on clinical history and SPT results (6). CRD allows the identification of specific allergens associated with the symptoms and the identification of cross-reactive molecules that are clinically significant. E.g. for Can f 6 or Fel d 4 the physician advices on whether or not to keep a household pet or which species could be tolerated. This may have a huge impact on a child's well-being. CRD gives information on the disease evolution over time; it allows to investigate the relationship between sensitization patterns to cat and dog allergen molecules during childhood and symptoms to these furry animals several years later (7); it highlights the pattern of sensitization to pet IgE components and its association with clinical symptoms.

Specific IgE to Can f 1 was significantly associated with persistent rhinitis, Can f 2 with asthma diagnosis, Can f 3 with moderate/severe rhinitis (M/S-R) and asthma diagnosis (AD) and Can f 5 with persistent and M/S-R. Positive IgE to Fel d 2 was strongly related to M/S-R and AD. Sensitization to ≥2 molecules or to pet albumins was associated with more severe respiratory symptoms (8).

Utility of in vitro tests

In patients with positive SPT in agreement with the clinical history, *in vitro* tests add further information (9) and allows:

1). to detect patients with sensitization to genuine molecules that cause allergic diseases; to distinguish among patients with positive SPT for more than one allergen, i.e. about 70% of allergic patients, the true poli-sensitization from the sensitization to pan-allergens (10-12);
2). to evaluate in childhood the "spreading" of sensitization towards each grass molecule and to obtain prognostic information about the evolution of the disease and the correlation between phenotypes of sensitization and illness severity (13-15);
3). to choose the best therapy; if only clinical history and SPT are used, without the support of CRD results, the choice of therapy may prove to be incorrect in more than 50% of cases, with an important cost increase (6);
4). to identify the ideal patient for allergen immunotherapy, i.e. the patient with sensitization towards genuine molecules with a high likelihood of improving symptoms and to improve the quality of life with the correct diagnosis (16, 17);
5). to detect patients with sensitization to pan-allergens such as profilin or calcium binding proteins, which could induce cross-reactivity with foods and pollens. Additionally, the symptoms are shown for a long period of time also if pollen grains are no longer detected in the air. The profilin, a molecule of grass, trees and ragweed pollen is able to induce nasal and bronchial inflammation for a long period of time (18);
6). to manage the risk of anaphylaxis for patients allergic to latex. To detect IgE in latex molecules inducing anaphylaxis, like Hev b 1, Hev b 3, Hev b 5, Hev b 6, or to detect IgE for Hev b8 that is not able to induce anaphylaxis because it is not present in surgery devices;
7). to identify sIgE towards particular molecules involved in allergic occupational reactions, such as serum albumin, LTPs or lysozime.

In patients with negative SPT and a clinical history suitable for allergy, CRD can be a useful tool. The lack of agreement between SPT results and clinical history may be due to the lower sensitivity of SPT compared with *in vitro* tests because the extract used for SPT could miss some important allergenic molecules such as Aspergillus like Asp f 4, Asp f6, Asp f 3 (19) or molecular wheat allergens. IgE mediated responses to wheat can be related to wheat ingestion (food allergy) or wheat inhalation (respiratory allergy). An inhalation associated, IgE mediated wheat allergy can cause baker's asthma or rhinitis, which are common occupational diseases in workers who have significant repetitive exposure to wheat flour, such as bakers. The most important wheat allergens are the α-amylase/ trypsin inhibitor subunits, which are present in all three protein fractions of raw and cooked wheat. Other important allergens are a 9-kDa LTP in the albumin/globulin fraction and several low and high-molecular-weight glutenin subunits in the gluten fraction and gliadin (alfa, beta, gamma, omega fractions). All these allergens showed heat resistance and lack of cross-reactivity to grass pollen allergens. Wheat LTP is a major inhalant allergen associated with baker's asthma caused by wheat flour sensitization. Tri a 14 and peach LTP, Pru p 3, showed a sequence identity of 45%, but the low cross-reactivity between both allergens detected reflected great differences in their 3-dimensional IgE-binding regions (20).

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ENT TESTS FOR ASSESSING THE ALLERGIC PATIENT

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Allergic rhinitis (AR) is a disease that afflicts a large percentage of the world population. It concerns both allergists and otolaryngologists, therefore it is important for both specialists to be aware of the characteristics of a patient who suffers from AR. Often, patients complain of nasal breathing difficulty only, initially not reporting any other symptoms typical of AR. In this brief review, the most important investigations, physical examination, nasal endoscopy, nasal peak flow and rhinomanometry, are described. All these investigations allow us not only to make the correct diagnosis, but also to monitor the course of the disease and the effects of therapy.

ENT tests for assessing the allergic patient

Allergic rhinitis (AR) is a pathology with a strong impact on life and it is estimated that up to 40% of the population is affected by it (1). For this reason a correct diagnosis is primary also in the ENT field. It is equally important to objectively evaluate the course of therapy, especially nowadays in which new therapies are being proposed as valid in the treatment of AR (2). A correct and exhaustive assessment of patients affected by AR is extremely complicated. In fact, this pathology is halfway between the otolaryngologist and the allergologist. Frequently, the first symptom reported by a patient with AR is the nasal breathing difficulty associated with sneezing and nasal secretion. Usually, patients also present itching and lacrimation; in the most serious cases, they present facial pain and mucous secretion caused by sinusitis.

In the absence of specific allergy symptoms, the patient first addresses an otolaryngologist to understand what the cause of their difficult nasal breathing is. For this purpose, patient history and the ENT physical examination are of great importance in the identification of typical characteristics of the allergic patient. Peak Nasal Flow and Rhinomanometry allow to quantify the nasal respiratory difficulty and successively, to monitor the effects of the therapy.

History and physical examination

In the presence of a patient who complains of nasal breathing difficulties, a properly performed anamnesis and physical examination provide numerous data that allow the physician to focus the patient's clinical condition and often formulate an initial diagnosis. ENT examination in a patient with nasal breathing difficulty should never be limited to the study of the nose because there may be numerous other signs that may lead the doctor to a correct diagnosis. Otoscopy is therefore important to identify the presence of effusion in the middle ear; this finding may be indicative of impaired tubal function secondary to nasal dysfunction or to rhino-pharyngeal mucosa hypertrophy. Further information is gathered by oropharingoscopy, which unveils the shape of the patient's palate, the bite closure and

Key words: allergic rhinitis, nasal endoscopy, nasal peak flow, rhinomanometry

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0393-974X (2018)

the size and mobility of the tongue, as well as the presence of secretion. Of course, nose examination is of crucial importance in the evaluation of nasal respiratory function. It provides numerous indications of the cause of nasal obstruction and mucous status, which may suggest the possible allergic nature of the symptoms. The inspection of nasal external structure highlights alterations of the nasal pyramid or the nasal valve, which can affect the proper nasal function. Indeed, previous traumas or surgical interventions may have altered nasal bones and cartilages, resulting in an increase in nasal resistance, especially during the inspiratory phase of breathing.

The nasal valve is the narrowest segment of the nasal cavity. Its influence on nasal breathing can be assessed by evaluating the improvement in nasal respiration while the valve is kept open; it is possible to lift the tip of the nose (3) or to perform the Cottle's manoeuvre (4) by stretching the cheek. Anterior rhinoscopy, performed with a Killian Nasal Speculum, allows the visualization of the first portion of the nasal septum medially, and the heads of lower and middle turbinates laterally. However, this exam provides only limited indications on the middle meatus and on the osteomeatal complex (OMC). Therefore, it is increasingly integrated with nasal endoscopy so that the physician has a thorough vision of the nose.

Nasal Endoscopy

Nasal endoscopy was first described at the beginning of the 1900's, when Hirshmann, Reichert and Valentin began to use a modified cystoscope to examine the nasal cavity and to remove nasal foreign bodies (5). Maltz continued the studies on nasal endoscopy in 1925 (6) but a real impulse was achieved in the 1960's, thanks to the improvement of technology and the studies by Messerklinger, a German surgeon who used a rigid endoscope to study the deep anatomy of the nose and the mucociliary clearance (7) and treated chronic sinusitis by surgical cleaning of the OMC (8). Finally, in the 1980's, studies by Kennedy and Stammberger developed the ideas of Messerklinger, making nasal endoscopy fundamental for diagnostics, but above all an indispensable instrument for surgery of the nose and paranasal sinuses. (9-12).

The use of nasal endoscopy as a diagnostic tool was confirmed through studies by Levine (13) and Lund (14) that emphasized how endoscopy allows a more accurate and complete evaluation of nasal cavities and how it detects nasal diseases better than anterior rhinoscopy. The use of nasal endoscopy as an instrument for the diagnosis of allergic disorders dates back to 1983 with Rohr, et al. (15) and more recently, "unified airways" (16), the in-depth study of nose and paranasal sinuses is also of great importance in the evaluation of asthmatic subjects. The use of nasal endoscopy in patients with chronic rhinosinusitis who undergo functional nasal surgery allows an optimal postoperative management that has improved the outcome (17). It is possible to distinguish endoscopes in flexible and rigid. Flexible endoscopes are generally preferred for their ease of handling in tight spaces. Yet, otolaryngologists seldom use them for nose assessment because they provide a lower image quality than rigid endoscopes; moreover, both hands are needed to use flexible endoscopes, whereas rigid ones leave the second hand free to perform diagnostic or therapeutic manoeuvres.

Endoscopes can be of different diameters and the largest ones have an operating channel for small biopsies. Paediatric endoscopes are smaller and are sometimes utilized in adult patients because they are easier to use; however, they suffer of a reduction in image resolution. As a rule, the smaller the diameter of the flexible endoscope, the worse the image quality. Rigid endoscopes are preferred by otolaryngologists for their greater image resolution and because they are handled by one hand, so that the dominant hand can perform tissue biopsies, nasal cavity washing and aspiration of secretions that can obstruct the view. Rigid endoscopes also have different diameters, usually from 2.7 mm to 4.0 mm; their size is chosen according to the dimension of the nose and to the space available within nasal cavities. Unlike flexible endoscopes, the rigid ones differ in viewing angles (usually 0°, 30°, 45° or 70°); larger angles display larger areas that cannot be explored with narrower optics. As a rule, the greater the

diameter of the endoscope, the higher the magnitude and the brightness of the visual field. Conversely, the 0 degree endoscope is the easiest and most intuitive to use because it provides a direct view of the area that corresponds to the tip of the instrument. Other gradations are more complex to master; in fact, the operator must take into account the refractive angle of the instrument while moving within the nasal cavity and may lose orientation during the rotation of the endoscope. Generally, 30 degree endoscopes allow better visualization of the medial and lateral walls of the nasal fossa, particularly of the maxillary sinus ostium and OMC, while 70 degree endoscopes facilitate the viewing of hidden areas, such as the frontal recess.

Both rigid and flexible endoscopes need a light source connected to the instrument through a fibre-optic cable. Xenon lights allow for greater brightness but have higher purchasing and maintenance costs. Halogen lights are generally less luminous, but also less expensive and bulky; they are adequate for diagnostic and non-surgical endoscopic procedures, but not for surgical ones. The use of monitors and cameras allow to visualize enlarged images and to save images or videos through a video capture system. At the beginning of the examination, the endoscope should be treated with an anti-fog solution to prevent fogging of the instrument by the hot and humid air present in the nose. Before starting the exam, local contact anesthesia is performed in the sitting patient; occasionally, a decongestant allows to evaluate the response of nasal turbinate mucosa to the drug by performing an endoscopy pre and post pharmacological decongestion. The endoscope is then introduced into the nasal vestibule. The operator examines the head of the lower turbinate and the mucosal features; at this stage, the operator also searches for the presence of septal deviations.

Successively, by sliding below the lower turbinate and entering the rhinopharynx, it is possible to observe the Eustachian tubes, the rhinopharyngeal mucosa and the presence of adenoid tissue. Finally, the endoscope is retracted slightly and glided over the lower turbinate with the aim of evaluating the side wall of the nasal cavity, in particular the OMC region and the medium turbinate. In the presence of large amounts of secretion, visibility decreases and it may be necessary to extract the instrument from the nose to clean it. In that case, rigid endoscopes are preferred as the operator is able to aspirate the secretion with the dominant free hand.

Nasal function

ENT physical examination and nasal cavity endoscopy are mainly to rule out the presence of anatomic alterations and of neoformations. Functional evaluation of the nose is the next step and is aimed to evaluate nasal breathing difficulty, which is the most frequent symptom reported by patients affected by AR. To achieve this purpose, two methods are available: the measurement of nasal peak flow and rhinomanometry.

Nasal Peak Flow

Nasal Peak Flow Measurement is a simple, fast, cheap and non-invasive method for assessing nasal congestion (18, 19). Inspiratory and expiratory peak flows (PNIF and PNEF, respectively) have been successfully used to evaluate nasal patency. The former is measured by the Youlten flow meter (Clement Clark International), the latter by the Wright peak flow meter (Clement Clark International); both measurements are well tolerated by patients (20). PNIF and PNEF values are correlated to each other as shown by Wihl's studies (21) and have demonstrated their validity for nasal flow measurement in patients with AR (22). In particular, PNIF has proven to be an excellent method for the evaluation of nasal resistance (23, 24). PNIF was measured for the first time by Youlten in 1980 (25) who utilized a modified Wright's flow meter (26). It is a mask that, leaning on the face, allows the patient to perform an inhalation through the nose while the mouth is kept hermetically sealed.

Measurements should be made while the patient is standing and after a forced exhalation (27). A series of measurements are performed so that patients improve their ability after a couple of attempts (28). Three valid measurements are obtained and the highest value is registered as the final value. Pediatric facial masks are available for children (29). Despite the ease of execution, the test is affected by a number

of variables, the most important one of which is the nasal patency. In the presence of complete nasal occlusion the test cannot be carried out; this is why previous nasal endoscopy is recommended to ensure that no neoformation or secretion can affect the examination. In addition, nasal valve collapse may occur during forced inhalation and cause the flow meter to register erroneously low values. A proper objective examination before performing the test allows to anticipate the problem and to evaluate the results obtained correctly.

A third factor that may affect PNIF measurement is the presence of lung diseases that limit patient's expiratory effort; a similar mechanism affects measurements performed in the immediate post-measurement period (30). Finally, other known factors that may interfere with nasal resistance are a cold and humid environment or the presence of smoke. Nasal patency follows a circadian rhythm, with values in the evening greater than in the morning.

PNIF presents critical issues that hinder the comparison of values obtained from different patients. By contrast, its quick and easy execution and low costs make it optimal to evaluate the course of the disease in the same patient. PNIF allows to compare various measurements carried out throughout the day in order to register variations in nasal obstruction (31) that in turn result from medical therapies (32), exposure to allergens (33, 34), or environmental stimuli (35). With a minimal cost, a properly trained patient can measure his or her PNIF at home and keep a diary in which PNIF values are associated to certain activities and to VAS (Visual Analogue Scale) symptomatic scores. In other words, the diary allows the physician to evaluate the progress of the nasal obstruction in order to personalize therapy analogously to the arterial pressure diary for antihypertensive therapy prescribed by cardiologists.

Rhinomanometry

Rhinomanometry measures nasal resistance by correlating the flow and pressure registered during inspiratory and expiratory phases of nasal breathing. It is the current gold-standard method on this purpose and its good reproducibility is utilized to assess the response of nasal mucosa exposed to pharmacological decongestants, as well as to powders containing specific allergens and even to saline, in order to evaluate a specific hyperreactivity of the mucosa. (36, 37). Passive Anterior Rhinomanometry was first described by Cottle in 1960's (38) but the method was successively developed by Miller (39), who employed a Fleish pneumotacograph. The combination of the pneumotacograph and the pressure gauge provided flow and pressure data at the nasal level, which are utilized to calculate the resistance to air passage. Modern rhinomanometry utilizes digitalization of recordings by computer technology (40). After having thoroughly blown the nose, the patient must sit and wear the mask properly without any air leakage from it. At least ten normal respiratory acts should be performed before initiating the test. Measurements are carried out in both nasal cavities one at a time. The result is a flow-pressure graph where the pressure is indicated on the abscissa axis and the flow on the axis of the ordinates. The curve represents resistance. According to the standard, units of measure are cm^3s^{-1} for flow and Pascals (Pa) for pressure; resistance values are conventionally given at a pressure of 150 Pa. The graph is divided into 4 quadrants numbered from I to IV counter-clockwise, starting from the top right. Quadrants I and IV correspond to the inspiratory phase, II and III correspond to the expiratory phase; quadrants I and III refer to the right nostril, II and IV to the left.

After performing a baseline recording, topical decongestants can be administered to evaluate their effect on nasal mucosa (41). It is possible to perform an aspecific provocation test using an isotonic saline solution or a specific provocation test using a specific allergen extract as a trigger. The value of the decongestion test is to evaluate the efficacy of the decongestant, and therefore to estimate the impact of nasal mucosa hypertrophy on nasal resistances opposed to that of the anatomical alterations observed during anterior rhinoscopy and nasal endoscopy. Hence, the provocation test helps to assess specific and aspecific mucosal response in patients with allergic or vasomotor rhinitis.

Rhinomanometry can also be used to express the results of surgical procedures aimed at increasing nasal patency by comparing pre and postoperative values of nasal resistance (42).

The limits of active anterior rhinomanometry, currently considered the gold standard for the objective evaluation of nasal resistances, are related to the standardization of the execution of the exam. As already noted for PNIF, nasal resistances can undergo significant changes, even at short intervals, due to patient conditions, posture (43), physical exercise (44), alcohol use (45) and the environment in which the examination takes place. For this reason, guidelines for the execution of the exam have been drawn up. Among the most important things, the examiner must be careful not to compress the nasal pyramid with the mask and to let the patient adapt to the environment by allowing him at least 15 min to acclimatise to the temperature and humidity of the study in which the exam is performed (46). Rhinomanometry is a great help for the evaluation of nasal resistance even if the bulk and high cost of the device implies its use mainly in the field of research.

CONCLUSION

Careful collection of medical history and good physical examination provide important elements for a rhinitis of allergic nature. Nasal obstruction is usually the main symptom. Anterior rhinoscopy and nasal endoscopy unveil anatomical causes of the obstruction, such as mucosal hypertrophy, a tight nasal valve, a deviation of the septum. Peak flow measurement and rhinomanometry provide objective measurements of the degree of the obstruction. Provocation tests investigate the variable component of obstruction by challenging nasal mucosa with allergens and irritants and by evaluating the effects of vasoconstrictors.

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NASAL CYTOLOGY

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Nasal cytology represents a useful, inexpensive and easy-to-apply diagnostic method to better detail the phenotypic characteristics of rhinitis. In fact, it allows to detect and quantify the cell population within the nasal mucosa at a given time. The technique involves sampling, processing and microscope reading. Sampling requires the collection of cells from the surface of nasal mucosa that is usually done by a sterile disposable curette. Samples should be collected from the middle portion of the inferior turbinate where the ratio ciliate/mucinous cells is expected to be well balanced. This totally painless procedure is performed under anterior rhinoscopy, with an appropriate light source. The sample staining is executed using the common May-Grünwald-Giemsa (MGG). The stained sample is read at optical microscopy with a 1000x objective and with oil detecting the presence of inflammatory elements (eosinophils, mast cells, neutrophils and lymphocytes) in nasal mucosa, as in the case of allergic and non-allergic rhinitis. Nasal cytology is easy to perform, non-invasive, inexpensive and repeatable in the same subject, also at short time intervals. For these reasons it represents an affordable diagnostic technique that can be applied in all age ranges, to better differentiate pathological conditions and also to evaluate the effects of various stimuli (allergens, infectious, irritants) or the effect of treatments.

Over the past 20 years, advances in technology and scientific research dramatically changed the clinical approach to diagnosis and treatment of rhinitis. Specifically, in the field of rhinology, numerous diagnostic procedures (rhinomanometry, acoustic rhinometry, fiberoptic endoscopy, immunohistochemistry) were introduced in clinical practice to refine the diagnostic accuracy (1). Among these procedures, nasal cytology (NC) (2, 3) was recently rediscovered as an attractive and promising additional diagnostic tool, also due to the fact that nasal mucosa is easily accessed, which can be associated to the standard diagnostic methods. NC represents a useful, cheap and easy-to-apply diagnostic method to better detail the phenotypic characteristics of rhinitis. In fact, it allows to detect and quantify the cell population within the nasal mucosa at a given time, to better differentiate pathological conditions and also evaluate the effects of various stimuli (allergens, infectious, irritants, physico-chemicals) or the effect of treatments (3, 4).

The NC approach subsequently provided relevant contributions to the knowledge of rhinitis from a pathophysiological point of view, also allowing to identify different phenotypes of non allergic rhinitis: non-allergic rhinitis with eosinophils (NARES), with mast cell predominance (NARMA), neutrophilic (NARNE) or mixed (non-allergic rhinitis with eosinophils and mast cell, NARESMA) (5-7).

Due to the ease in performing this procedure and the lack of invasivity, NC is repeatable in the same subject also at short time intervals. For these

Key words: nasal cytology, rhinitis, nasal mucosa, sample

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reasons it represents an affordable diagnostic technique that can be applied in all age ranges, also at the physician's office (8). The technique involves: sampling, processing and microscope reading.

Nasal sampling

Sampling requires the collection of cells from the surface of nasal mucosa, that is usually done by a a sterile disposable curette. Samples should be collected from the middle portion of the inferior turbinate where the rate of ciliate/mucinous cells is expected to be well balanced. The procedure is performed under anterior rhinoscopy, with an appropriate light source, and the procedure is totally painless. This can be made by a common sterile cotton tip or, better, with a sterile disposable curette (nasal scraping). Cotton tips can be used in infants when an anterior rhinoscopy may be considered more difficult to perform (9). The sample obtained is immediately smeared on a glass slide and air-dried.

Sample staining

The saple staining is performed using the common May-Grünwald-Giemsa (MGG) staining for its ability to correctly identify inflammatory nasal cells: in particular, MGG shows in blue the nuclei of white blood cells and the granules of basophils granulocytes, while red blood cells and eosinophils granules are red. The cytoplasm of white blood cells appears in light blue. The traditional MGG staining procedure requires about 30 minutes, but pre-mixed compounds (e.g. MGG QUICK STAIN, Bio-Optica, Milan, Italy) are available, and allow a satisfactory preparation in less than 1 minute.

Sample reading

The stained sample is read at optical microscopy, with a 1000x objective with oil immersion. Fifty fields are considered the minimum number to identify a sufficient number of cells. The count of each cell type can be expressed as a percentage of the total cells (including mucinous and ciliated cells), as an absolute value, or by a semi-quantitative grading (10).

Cytological aspects of nasal mucosa

The nasal cytogram is easy to read. The normal nasal mucosa is a pseudo-stratified ciliated epithelium, with mucinous cells. The ciliated cell is the most differentiated cell type in the nasal mucosa. The normal ciliated/mucinous cell ratio is around 4:1 in healthy individuals. only four cytotypes can be identified at NC: ciliated cells, mucinous cells, basal cells and striated cells, Only sparse neutrophils can be found occasionally. Thus, the detection of eosinophils, mast cells, bacteria or fungal ifae clearly identify a pathological condition.

In nasal citology bacterial infectious rhinitis is usually characterized by the presence of a large number of neutrophils, with intra and extracellular bacteria that can easily identified at optical microscopy. Ciliated cells are significantly reduced in favour of mucinous cells and both metaplastic and platicellular cells are observed. Lymphocytes and macrophage can accompany the neutrophilic infiltrate (11). NC allows also the identification of biofilms, i.e. organized community of bacteria or fungi adherent to an inert or living surface, embedded in a self-produced extracellular polymeric matrix (85% in volume) composed of a mixture of biopolymers, primarily polysaccharides, protein and nucleic acids. Organisms living in a biofilm are relatively protected against host defenses and antimicrobial agents. (12, 13) With NC, biofilms appear as cyan-stained 'infectious spots', whose polysaccharide nature can be confirmed by the periodic acid-Schiff staining.

The observation of bacteria in the nasal passages do not provide any specific diagnostic value for the causative pathogen but can help in suggesting a superimposed bacterial role in forms of allergic/cellular non allergic rhinitis. The patient who suffers from seasonal or perennial allergic rhinitis (AR), stimulated naturally or by specific nasal provocation tests, develops an immediate nasal response, the so-called early phase (primarily histamine-mediated), and a late phase due to the influx of inflammatory cells. From a microscopic point of view, the response is always characterized by a presence of inflammatory cells (eosinophils, mast cells, neutrophils and lymphocytes) in the nose that following the release of several chemical mediators, provoke the main symptoms (itching, nasal congestion, runny nose,

sneezing, watery eyes, etc.).

When the allergen exposure is of low intensity but persistent in time, as is typical of perennial rhinitis (for example induced by mites), it creates the cell condition defined as "minimal persistent Inflammation" (14, 15), characterized by a persistent infiltration of neutrophils overall and only minimally by eosinophils, even in the absence of symptoms. Mast cells and important signs of eosinophilic-mast cell degranulation are rarely found. This cellular condition is clinically translated in an absent or sub-chronic symptomatology that distinguishes patients suffering from these perennial forms, where the principal symptoms are nasal obstruction and mucosal rhinorrhea.

As far as seasonal AR is concerned, the rhinocytogram will change depending on whether the patient will be examined during or off the pollen season. In the first condition, the patient will present all the clinical signs of the disease: nasal cytology is characterized by neutrophils, lymphocytes, eosinophils and mast cells, largely degranulated; conversely, if assessed out of season, the patient will clearly present a clinical and cytological "silence", especially if more than thirty days have passed after the end of exposure. Rhinitis tipical symptoms without allergenic sensitization and infection are often due to "cellular rhinitis". With the exception of the neutrophilic forms, they are "congenital" disease, present at birth and they account for around 15% of all nasal disease. These are diseases with a "cellular" expression, so the diagnostic gold standard is clearly nasal cytology. These forms of rhinitis are caracherised by a chronic course, with intense symptoms, and can cause local (otitis, sinusitis) or respiratory (asthma, bronchial or/and rhino inflammation) symptoms, and over time they may evolve into nasal polyposis (16).

Little is known about the pathogenesis of these forms. For some years few studies have focused on the presence of local specific IgE, grouping these forms under the so-called local allergic rhinitis nosological entity (17). However, at present there are still many points to be clarified in this regard. Beyond the pathogenetic classification, nasal cytology represents a marker of cellular presence. The best known and first described non-allergic rhinitis was NARES. In this cellular form of rhinitis the presence of eosinophils is not only predominant but massively present with even higher expression than in seasonal rhinitis.

Another important inflammatory non allergic rhinitis is NARESMA in which, in addition to the important eosinophilic infiltrate, a mast cell component is detectable (18). NARMA is characterized by isolated mast-cell infiltration, while the feature of NARNE is a massive presence of neutrophils without concomitant bacterial colonization.

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ROLE OF IMAGING IN ALLERGIC RHINOLOGY

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Rhinitis is an underestimated clinical condition, which has a considerable impact on the quality of life of the affected patients. The subject of this review focuses on three fundamental aspects: the development of knowledge concerning anatomic landmarks, the development of radiological imaging technology, and developments that can make a difference in the treatment of allergic rhinitis. The anatomical study of paranasal sinuses has been conducted since the time of the ancient Egyptians. Development of radiological equipment from the early 1900s has helped to improve information on the morphology of paranasal sinuses, sufficient to be considered valuable information regarding frontal anatomy and its variability. Imaging has become increasingly important in the diagnosis and treatment of inflammatory diseases of the paranasal sinuses. In recent decades, radiology has helped to study this region as we have progressed from plain radiography to high-resolution Computed Tomography (HRCT). Subsequently, from radiologic imaging, digital volume tomography (DVT) has been developed, in high resolution and narrow section width. Currently, experience with third generation Cone-Beam Computed Tomography (CBCT) technologies provides useful information about bones, and it is now possible to highlight anatomical variants that involve bone structures. We still lack the ability to make a qualitative evaluation of soft tissues, as there are no Hounsfield levels in CBCT. However, this is a new area of research, and its application is evolving in an interesting manner, especially for soft-tissue allergic-inflammatory diseases.

Role of imaging in allergic rhinology

For years, it has been well established that diseases of the nasal cavity mucous and paranasal sinuses (PNS), with symptoms lasting more than 12 weeks, require instrumental investigations for proper diagnosis and treatment. Rhinitis is an underestimated clinical condition, and has a considerable impact on the quality of life of affected patients (1).

To perform the gold standard treatment for a resistant inflammation of the PNS, today we must use radiological and endoscopic imaging. The most relevant anatomical points that can be identified by radiologic imaging and which support diagnosis and treatment of allergic rhinitis are various, among them the osteomeatal unit (OMU), soft tissue thickening, and the bone morphology of the turbinate. However, all potential anatomical and pathophysiological conditions should be considered to determine the best approach for the treatment of allergic rhinopathy. It is known that nasal obstruction, one of the symptoms of allergic rhinitis, has a minimal response to medical treatment and a turbinectomy may be required to treat allergic rhinitis if medical treatment has failed.

Key words: CT, MSCT, CBCT, paranasal sinus anatomy, allergic rhinitis, diagnosis

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The types of rhinitis that we consider here include both allergic and inflammatory.

It is believed that the basis of the increased incidence of allergic forms of rhinitis is the lack of exposure to microbial species from childhood in developed countries. This involves shifting the T helper immune response from Th1, activated to attack microbiological agents, to Th2, which is activated against normally non-pathogenic agents, called allergens, to which Th2 responders become sensitized. We distinguish two phenotypes of allergic rhinitis: one characterized by rhinorrhoea and the other characterized by nasal obstruction. Both greatly alter the quality of life of affected subjects, with symptoms that occur primarily in the morning, even if they are otologic or sleep-related symptoms that disturb patients and worsen their quality of life. Nasal obstruction may be caused by hypertrophy of the turbinates. Turbinates (three in each nasal cavity: upper, lower, and middle) are bone structures covered by mucous membranes, which filter and humidify the air we breathe through the nose before it reaches the lungs, while the mucus produced by the caliciform cells captures pathogens. Nasal obstruction may have mucous or be of a bony nature. The mucosal inflammation-related form is the most common; patients complain of rhinorrhoea and obstruction. The bony form, often linked to a nasal septal deviation, must be suspected when there is no response to medical therapy. Sectional deviations alter filtration by the turbinates, with an airflow resistance below normal, which causes inferior turbinate hypertrophy to normalize the flow.

An imaging diagnosis is based not only on rhinoscopy, which is sufficient to evaluate the surfaces of the mucosa, but also on Computed Tomography (CT) scan, to evaluate the numerous bone variants that might not be clear with endoscopy (2). Chronic rhinosinusitis is an inflammation of the paranasal cavities. It can be caused by perennial allergic rhinitis, nasal seizures, hypertrophy, or nasal polyps. Inflammation can also be a cause. It could be caused by fungal, viral, or bacterial infections. Staphylococcus aureus, proliferating in the mucous membranes of these subjects, causes perennial mucosal inflammation resulting in respiratory distress. It has been noted that tobacco smoke (a little less when passive) causes mucous inflammation capable of giving rise to chronic rhinosinusitis. Another cause is gastroesophageal reflux; however, the reason for this is yet to be clarified, although it may be nasopharyngeal exposure to acid reflux. In patients with an allergy diagnosis, the symptoms of chronic rhinosinusitis are easily associated with it, which may be the underlying cause.

Direct physical examination of the nasal cavities is important, where nasal polyps, purulent secretions (indicative of infection), hypertrophy of the turbinates, and septal deviation can be identified. The rhinoscopy detects nasal polyps or purulent secretions, indicates appropriate medical therapy, or directs the patient to surgery, although it is unable to detect abnormalities under the soft tissue. Nasal endoscopy (flexible or rigid) permits review of the sinus drainage areas that are not accessible at physical examination or rhinoscopy, thus reducing the use of CT scan. Endoscopy also allows for the collection of samples for culture and provides an accurate result for the choice of antibiotic . CT scan is the standard method to detect the presence of a PNS disease; it is performed when the diagnosis is uncertain and a serious disorder is suspected, as it allows for the evaluation of both the bony and soft tissues. Through the CT scan, in the case of chronic rhinosinusitis, the distinctive sign of mucosa thickening can be detected. The PNS appear to be opaque, however, in subjects with severe and/or reactive disease (polyps), in unilateral opacification, and in 25% of cases, may be indicative of a tumour. For the diagnosis of chronic rhinosinusitis, it is crucial to detect the characteristic inflammation of the mucous membrane, so the CT scan is examined for evidence of thickening and opacification of the mucosa of the PNS. However, the CT scan can also be used to evaluate the presence of opacification in olfactory flexure and therefore olfactory disturbances of the patient (often due to nasal polyps). The primary limitation of the CT scan is that it correlates poorly with symptoms, and can show alterations even in completely asymptomatic patients.

Magnetic Resonance Imaging (MRI) has many limitations in the diagnosis of chronic rhinosinusitis.

In fact, it is very expensive and unsuitable for bone anatomy. Therefore, it is not a recommended analysis (unless a differential diagnosis is required in order to discriminate a tumour from obstructive sinusitis or a fungal infection, or the presence of a meningocele or encephalocele).

Nasal Cytology is carried out to evaluate the type of cells present in nasal secretions, in order to choose the most suitable antibiotic therapy in infectious and inflammatory forms of rhinitis (3).

The subject of this review focuses on three fundamental aspects: the development of knowledge of the anatomic landmarks, the development of radiological imaging technology, and recent developments that can make the difference for the treatment of allergic rhinitis.

A short history of anatomical knowledge

The paranasal sinuses are a region of special interest historically. Paranasal sinuses were first identified inside the bones of the skull by ancient Egyptians. Medical writings dating back from 3700 to 1500 BCE provide evidence that Egyptians were familiar with the structure of the maxillary bones, which suggests that they might also have been aware of the maxillary sinuses (4). Hippocrates (460-377 BCE), Celsus (14 BCE - 37 CE) and Galen (130-201 CE), recognized the paranasal sinuses as part of the structure of the skull but they did not describe them in detail in their works. In particular, Celsus, in his medical treatise 'De Medicina', provided a description of the surgical anatomy of the nose and the olfactory nerves passing through the cribriform plate of the ethmoid bone (5).

We had to wait for Leonardo da Vinci (1452-1519), to continue the advance in the study of the paranasal sinuses. He created anatomical drawings of the human body and recognized the close relationship of the maxillary sinus with the teeth of the upper jaw. This can be perfectly understood by the perfect representation of the projection of the teeth into the floor of the maxillary sinus (6). In the XVI Century, Andreas Vesalius made a drawing of the sphenoid bone in which the sphenoid sinuses are depicted separated by the sphenoid septum (7, 8).

Nathaniel Highmore (1613-1685) is particularly connected to the anatomy of the maxillary sinus, since it is believed that he was the first person to describe and draw it. In his treatise, Highmore included anatomical drawings depicting the maxillary sinus as well as the frontal sinus and the ethmoid. He described the close relationship of the maxillary sinus with the orbit and the teeth of the upper jaw, noticing that their roots tend to project into the sinus. Moreover, he discussed the density of the bone walls and observed that the maxillary sinus was mostly empty and only occasionally filled with mucus. According to Highmore, this mucus was a humour of the head, which drained into the maxillary sinus. It is remarkable that even Highmore was confused about the function of the maxillary sinus and even more so about the origin of the mucus that he had sometimes observed in it (4).

In 1660, Schneider established that mucus was not produced by the brain, but was produced by the same paranasal structures where it occurred (9). Another anatomist who contributed to the understanding of the anatomy of the paranasal sinuses was Emil Zuckerkandl from Austria, who described the nose and the paranasal sinuses in detail in 1870 (10). At the beginning of the 20th Century, Harris Peyton Mosher of Harvard University dissected many cadavers in order to study the anatomy of the paranasal sinuses. He is also well known for his accurate anatomical description of the ethmoid sinuses. He noted their close relation to the skull base and the orbit. Mosher's research enabled the development of the histology, embryology and surgery of the paranasal sinuses, thanks to an accurate knowledge of their anatomy (5).

The advent of imaging

On November 8, 1895, Wilhelm Conrad Röntgen discovered the X-ray and opened a new era: radiological imaging (11). It was transformed in the early 1930s, when an Italian radiologist, Alessandro Vallebona, created a radiographic technique called stratigraphy (12). Thus, the first stratigraphic exams of the paranasal sinuses were created that could see projections of the various paranasal sinuses. A large number of projections for the radiographic study of the paranasal sinuses led to the advent of

CT, although until the 1980s it was very uncertain whether it was preferred to perform one or the other diagnostic method. At the same time, the evolution of endoscopic imaging began; a recently published article describes how endoscopy opened the way for rhino-neurosurgery and details the progress made by rhino-endoscopy and related anatomic issues (13, 14). Only in 1973 did the crystallisation of the research project that started Computer Tomography occur, followed in 1979 when Godfrey Hounsfield and Allan Cormack were awarded the Nobel Prize for Medicine for developing CT (15).

The first studies that approach radiological anatomy were born and anatomical knowledge is defined with ever more accurate dissections. The X-ray technique did not add much to the anatomical knowledge of the sinuses (16); also, the CT scan does not add much to the assignment of anatomical names for the various structures. The first real impact of CT was its ability to deepen the anatomical variants and their correlations with pathology. Many generations of CT technology have rapidly evolved, and in fact, changes have become increasingly frequent, increasing from a layer acquired in 3-5 min to 4 layers in 0.4 - 0.5 seconds. For a long time, CT scan research was engaged in a 'detector war', as investigators moved from a single detector to a row of detectors until finally detector banks were developed to acquire larger study volumes in less time with more definition. Therefore, CT technology has occurred in three steps: the first step helped the development of imaging, the second provided in-depth analysis of the landmarks and anatomical variants, and we are now entering a new era in which we are enriching the diagnostics of etiopathogenetic and functional correlations.

In 2001, Roee Landsberg et al., in their retrospective study "A Computer-Assisted Anatomical Study of the Nasofrontal Region" showed that the frontal sinus opens into the middle meatus medial to the uncinate process in most patients, and lateral to the uncinate process in fewer patients. The frontal ostium dimensions on one side may differ considerably from the contralateral side. An agger nasi cell or a terminal recess (or both) is found in most cases (17).

In 2003 David L. Daniels et al., in their study "The Frontal Sinus Drainage Pathway and Related Structures", provided a visual guide to the frontal sinus drainage pathway (FSDP), associated anatomic structures, and normal variations in sinus anatomy (18, 19). In 2001, Friedman provided an update of the surgical anatomy of the frontal recess region, uncinate process, and ethmoidal infundibulum (17). They measured 288 frontal sinus sides. The mean anterior-posterior diameter was 7.22±2.78 mm, the transverse diameter was 8.92±2.95 mm, and the mean frontal ostium sectional area was 50.5 mm^2 (17). The frontal recess is a narrow cleft within the anterior ethmoid complex that forms an inverted funnel that widens in the inferior and posterior direction. This inferior third of the frontal sinus drainage pathway is the anatomical location responsible for most cases of frontal sinusitis (18).

Friedman described several variations of the superior uncinate process: 1) insertion to the lamina papyracea, 2) insertion to the posterior medial wall of the agger nasi cell, 3) insertion to both the lamina papyracea and the junction of the middle turbinate with the cribriform plate. 4) insertion to the junction of the middle turbinate with the cribriform plate, 5) insertion to the skull base, and 6) insertion to the middle turbinate. In 2009, Soraia Ale Souza et al. described the landmark of the anterior ethmoidal artery: the medial notch of the orbit (anterior ethmoidal foramen) and the anterior ethmoidal sulcus on the lateral wall of the olfactory fossae, which were characterized in almost 100% of exams. The course of the anterior ethmoidal artery was identified below the ethmoidal sinus roof in all exams in which supraorbital pneumatisation was present (20).

The study by Alper Yenigun et al. in 2016 aimed at classifying the infraorbital canal according to its position relative to the maxillary sinus as observed by axial CT. Morphologic variations of neighbouring structures were also noted and their correlations with specific canal types were investigated. Three types of infraorbital canal configurations were identified according to the canal's relationship with the maxillary sinus: Type 1, in which he infraorbital canal was totally protruding into the maxillary sinus (12.3 %); Type 2, in which the infraorbital canal was

located on the floor of the maxillary sinus or was partially protruding into the maxillary sinus (51.2 %); and Type 3, in which the infraorbital canal was totally embedded in the maxillary corpus or was bulging on the external face of the maxillary sinus (36.4 %). The study radiologically classified the infraorbital canal according to its position as related to the anterior wall of the maxillary sinus and found that the type where the canal was totally protruding into the maxillary sinus (Type 1) had a significant rate of 12.3%. The rate of the protruded infraorbital canal was doubled with the presence of maxillary sinus septa (25 %) (21). As sinus radiographs were inaccurate in a high percentage of patients, these are largely superseded by CT when imaging is necessary (22).

CT vs Cone-Beam CT (CBCT)

CT scanning provides the best preoperative information for endoscopic surgery, with excellent delineation of the complex ethmoidal anatomy, OMU, and anatomic variations, including the presence of sphenoethmoidal (Onodi) air cells, which increase the risk of injury to the optic nerves and carotid arteries (15,23). During the evolution of CT, the multislice Computed Tomography (MSCT) scanning technique has substantively improved the performance, increasing scan duration, available scan length, and spatial resolution.

Introduction of the isotropic volume and the sleep ring had led to renewed technical developments. Then begins an increase in the number of detectors for 4-slice scanners, followed by 8- to 16-slice scanners with the software using this information leading to improved multiplanar reformation techniques (MPR), and the dose also became very competitive with all of the conventional radiology techniques for the PNS. CT and MSCT imaging can also be imported into computer navigation systems for image-based guidance during endoscopic sinus surgery. The advantages of image-based guidance surgery include a reduction of surgical risks by providing real-time information regarding instrument location relative to critical structures.

The two major systems used for image-based guidance surgery are the electromagnetic and optical guidance systems. In both systems, a registration process that creates a one-to-one relationship between points in the operative field and the imaging data set is imperative, with accuracy within 2 mm (24). Non-contrast sinus MSCT is indicated for the evaluation of recurrent acute sinusitis before surgical intervention or objective confirmation in cases of chronic recurrent rhinosinusitis (22, 25). The documentation of sinonasal inflammatory diseases may also be accomplished with anterior rhinoscopy or nasal endoscopy (26).

Imaging has become increasingly important in the diagnosis and treatment of inflammatory diseases of the paranasal sinuses (27, 28). In recent decades, radiology has helped the study of this region and we have passed from plain radiography to high-resolution CT and three-dimensional reconstruction of the structures of the paranasal sinuses based on helical CT scans. Subsequently radiologic imaging has seen the development of digital volume tomography (DVT) in high resolution and narrow section width (29). CBCT became commercially available since 2001, initially for dentomaxillofacial imaging (30, 31) and has since expanded to in-office use for sinonasal evaluation. Current published estimates note a predicted exposure of approximately 0.1 to 0.2 mSv (32). However, accurate measurement of radiation dose exposure from CBCT is difficult to quantify because of the absence of accepted dose metrics. CBCT imaging may be utilized for the assessment of sinus anatomy and pathology in uncomplicated cases of sinusitis, although it has limitations in assessing soft-tissue structures. It may also aid in the diagnosis of odontogenic sinusitis, which can occur when periapical infections spread from the molar teeth into the floor of the maxillary sinus, which may be the aetiology of maxillary sinusitis in about 10% to 12% of patients (33). Appropriateness for patient selection may be made either clinically or by endoscopy (34).

The role of imaging in PNS diagnostic process

Nowadays imaging can also be used as an intraoperative guide (35) and CBCT allows for a 'near-real-time' imaging of the frontal recess (36, 37, 38). CBCT is the technology used more frequently today to perform an optimal study of the paranasal

sinus due to the image quality and radiant doses. This is especially relevant in children who are chronically ill, as this puts them in the position of requiring multiple diagnostic investigations, which may ultimately have an impact on life expectancy (39). ENT specialists have repeatedly asked for studies on the reliability of this method. Many studies have tried to respond to this request, but few have done so in vivo. However, considering the studies conducted to date, CBCT of the paranasal sinuses produces adequate images for screening, for the evaluation of sinus opacification, and for bone detail. Using the CBCT parameters mentioned in the study of Al Abduwani, the crystalline dose was found to be lower with the MSCT clinical protocol (40). However, soft tissue evaluation remains sub-optimal with CBCT imaging, especially when compared to MSCT, and therefore leads to diagnostic uncertainty. Currently, experience with third generation CBCT gives good information about bone, and it is possible to highlight the anatomical variants that involve bone structures (41).

We still do not have the ability to provide a qualitative evaluation of soft tissues. This is because CBCT lacks Hounsfield levels. However, this is a new topic of research and a very interesting application development is occurring, especially for soft-tissue allergic-inflammatory diseases. One might ask, what benefit is all of this radiological anatomical development to clinical purposes in allergic rhinitis, to the study of the pathologies underlying the mechanisms, and to improvements in therapy? We can simply answer the OMU, the turbines morphology, and their variants. As for new developments, the study of mucosal turbinate thickness and their surface are not evaluated adequately, at present we are still far from studies that correlate the anatomy to functional or therapeutic discourse. The first pilot study of how to carry out such measurements was recently done by El-Anwar et al (42). This study tried to determinate the thickness of both the non-bony (mucosa) and bony parts and their relation to nasal airspace and was conducted in asymptomatic adults. It used a 64-slice CT scan, performed with a detector width of 0.625 mm, a section width of 1.5 mm, and an interval reconstruction of 0.5 mm. The patients were in the supine position and axial images covering the paranasal sinuses were performed, with the beam parallel to the hard palate. A bone window setting of 3000 HU was used, centred at 300 HU. It was necessary to use an algorithm for enhancement of the fine bony details. After the acquisition was completed, multiplanar reconstructions in axial, coronal, and sagittal planes can proceed.

Measurement of the thickness of the medial mucosa, bones, and lateral mucosa of the inferior turbinate (IT), were taken separately on the anterior and posterior portions of ITs by hand perpendicular to the mucosal surface with a cursor on the screen. The measurements must be performed as follows: posteriorly, before disappearance of the bony part, and then anteriorly, at the level of the first part of the middle turbinate (anterior to the bone) and at the level of the second part of middle turbinate (for the mucosa). Other measurements were performed between the anterior end of the IT and the septum just before the appearance of the middle turbinate, then between the septum and posterior end at the choana, for the airspace (42, 43).

In the future, these developments will be useful for CBCT, which uses lower doses of radiation than does a 64-layer TC. Identification of the density of mucosal tissues and their thickness by CT is a great resource both before and after therapy. The evaluation and correlation between the length of the turbines and the degree of reaction that may be established could be another challenging frontier to understand how one can plan and drive, with imaging, an increasingly targeted approach towards solving the problems of patients with allergies.

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ALLERGIC CONJUNCTIVITIS: CURRENT CONCEPTS ON PATHOGENESIS AND MANAGEMENT

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Allergic conjunctivitis (AC) includes a wide spectrum of clinical entities characterized by different incidence, age of onset, natural course, clinical outcome and response to treatment. Taken together, they represent one of the most frequent ocular surface diseases affecting more than 30% of the young-adult population and show an increasing incidence over the years. Moreover, comorbidities with other systemic atopic conditions such as asthma, atopic dermatitis and rhinitis require a multidisciplinary approach. Recent advances in the knowledge of the pathogenic mechanism overcome the classic role of type I hyper-sensitivity and mast cells' activation, demonstrating an involvement of innate immunity and neuroinflammation in the pathogenesis of the most severe forms such as atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC). Ocular itching, swelling and tearing are the most frequent symptoms complained by patients with all forms of AC, while photophobia and pain are typical of the most severe forms, such as VKC and AKC, due to the frequent corneal involvement. Upper tarsal papillary reaction represents the main clinical sign of AC associated with conjunctival hyperemia and mucous secretion. Diagnosis is based on clinical history and eye evaluation and can be confirmed through allergological tests. Additional ocular exams include specific allergen conjunctival provocation tests and the presence of eosinophils in the conjunctival scraping. Current treatments of AC include the use of antiallergic eye drops for mild forms, while recurrences of ocular surface inflammations with corneal involvement in severe forms require the use of topical steroids to avoid visual impairment. Novel steroid sparing therapies such as Cyclosporine A eye drops or topical Tacrolimus have been proposed to improve VKC and AKC management.

Allergic conjunctivitis (AC) represents a frequent inflammatory disease of the ocular surface and the eyelid, characterized by type I hypersensitivity and T-helper type 2 (Th2) reaction (1). Currently, AC are classified in different clinical forms, including seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC) and atopic keratoconjucntivitis (AKC) (2, 3).

Allergic conjunctivitis has a high prevalence in the young and adult population and represents a relevant social burden due to decreased quality of life and social functioning of the affected patients (4-7). Specifically, epidemiological studies showed that AC affects 10-30% of all adults and up to 40% of children (8, 9). In addition, AC is reported to be associated with other allergic conditions such as asthma and eczema in more than 25% of cases (10). However, ocular allergic symptoms are often underestimated and underdiagnosed, at least in mild forms, in the management of atopic patients, and consequently undertreated (9, 11).

Currently, AC are managed with drugs that either treat the symptoms or suppress inflammation such as antiallergic eye drops, useful to control symptoms and topical steroids which are effective in controlling inflammatory flare-ups in the more severe forms such as VKC and AKC (9, 12). However, while

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topical steroids are effective in controlling severe inflammatory reaction, their chronic use is associated with a high risk of side effects such as cataract, glaucoma and increased risk of infection (12). The use of topical immunosuppressive drugs such as Cyclosporine A and Tacrolimus have been proposed in these forms to improve ocular symptoms and to spare topical steroids; however, their use has not yet been approved (13-16). Novel pathogenic mechanisms recently demonstrated to be involved in the allergic reaction may represent new therapeutic targets for AC such as cytokines, neuropeptdides and innate immunity (17-21).

Pathogenesis

The classical pathogenic mechanisms of AC is based on a lymphocytes T-helper type 2 (Th2) response that mostly involves IgE-mediated release of histamine by mast cells and other mediators including leukotrienes and prostaglandin D2, that stimulate the recruitment of other inflammatory cells such as eosinophils and basophiles (1, 2, 22). SAC and PAC are characterized by type I hypersensitivity reaction, while pathogenesis of VKC and AKC is more complex. In fact, antigen sensitization is present in only 50% of VKC patients and AKC shows a Th1 profile instead the typical Th2 (1, 23, 24).

The conjunctival type I reaction is triggered by direct exposure of ocular surface to allergens that dissolve in the tear film and bind to antigen presenting cells (APC) that stimulate IgE production by conjunctival B cells (1). As a consequence, IgE cross-link induces mast cells degranulation leading to the release of preformed and newly formed inflammatory mediators, such as histamine, serotonin, tryptase, ECF (eosinophil chemotactic factor) and NCF (neutrophil chemotactic factor) (25, 26). Histamine (H) is the main mediator of the early phase reaction, inducing itching through the H1 receptor activation, and hyperaemia, chemosis and lid swelling through H1 and H2 receptors activation (27). Newly formed inflammatory mediators, such as interferon (IFN), tumor necrosis factor (TNF)-alpha, Granulocyte-macrophage colony-stimulating factor, prostaglandins and leukotrienes are released later and induce eosinophils and basophils recruitment by stimulating conjunctival expression of adhesion molecules, such as ICAM-1 and ICAM-2 (22, 24). In the late phase allergic reaction, eosinophils are activated, releasing eosinophil major basic protein and eosinophil cationic protein (ECP) that cause corneal damage (28-31).

The mechanisms underlying the development and the clinical course of VKC and AKC are not completely understood. In fact, approximately 50% of VKC patients is negative to allergen skin prick-tests and other inflammatory mechanisms are involved in the pathogenesis of the disease (23, 32). VKC inflammatory reaction is characterized by Th2 driven inflammation as demonstrated by the presence of an increased number of CD4+ Th2 lymphocytes in the conjunctival tissue and in tears of VKC patients, associated with an increased expression of Human Leukocyte Antigen-D Related (HLA-DR) (32, 33). In addition, an increase of eosinophils infiltration has been demonstrated in the conjunctiva of VKC patients, and the release of eosinophil major basic protein, ECP, and eosinophil neurotoxin (EPX/EDN) has been shown to be related with the development of corneal damage (29). Several evidence demonstrated that ocular surface epithelia modulate the inflammatory reaction in VKC by expression adhesion molecules and cytokines receptors and by producing cytokines and chemokines (34). VKC is also characterized by tissue remodelling with deposition of extracellular matrix in the substantia propria, including laminin, tenascin and collagen types I, III, IV, V and VII (35, 36). Other pathogenic mechanisms of VKC have been proposed, such as the involvement of innate immunity through expressing of various membrane receptors, such as toll-like receptors (TLRs) and the increase of conjunctival natural killer cells (18-21, 37). Furthermore, changes of conjunctival expression of estrogen and progesterone receptors and alterations of levels of circulating sex hormones have been demonstrated in children with VKC suggesting a pathogenic role of sex hormones in the development of this condition (17, 38).

In regards to AKC pathogenesis, a combination of Th1 and Th2 response is hypothesized, with major implication of Th1 cytokine profile (39, 40). Calder

et al described increased conjunctival levels of IL-2, IL- 3, IL-4 and IL-5 mRNA expression in AKC patients compared to controls (24, 33). However, increased levels of IL5, a Th2 cytokine that attracts eosinophils but not IL4, correlates with ocular severity inflammation in AKC (41). Eosinophils infiltration is crucial in the pathogenesis of AKC and in particular activated eosinophils levels more than absolute number of these cells (28, 33, 42). The release of gelatinase B, eosinophil major basic protein and ECP from activated eosinophils is related to corneal damage (40, 42).

Experimental and preliminary clinical evidence showed changes of local neuromediators in all the allergic ocular conditions (SAC, PAC, VKC and AKC) suggesting that these disease share a common neuro-immune pathogenic mechanism (18, 19). In addition, most of the clinical manifestations of ocular allergy such as red, itchy eyes and tearing are secondary to changes in neuronal activity (43). It has been demonstrated that ocular allergic reaction may lead to production and release of neuromediators, such as substance P and Calcitonine-gene related peptide (CGRP) (44). These mediators participate to clinical manifestation by inducing vasodilation, increasing vascular permeability, and recruiting and activating mast cells, lymphocytes and eosinophils (18, 19).

Clinical presentation

Seasonal and perennial AC are the most common forms of ocular allergy. They are usually bilateral and the hallmark symptom is itching. Seasonal allergic conjunctivitis (SAC) shows acute or subacute clinical manifestations with seasonal onset of symptoms related to the allergens widespread in the environment (9, 22). Allergens usually involved in this form are grasses and other pollen producing plants present during spring-summer season (3, 45). Perennial allergic conjunctivitis (PAC) is characterized by persistent ocular symptoms during the year, milder than those seen in SAC, with seasonal exacerbation in 80% of cases in autumn (45). Dust mite or animal dander are the allergens usually involved in PAC. The most common symptoms of SAC and PAC are itching, tearing, and burning. The ocular signs are conjunctival hyperemia, chemosis, tarsal papillary reaction and mucous discharge without cornea involvement (3, 45-47). Diagnosis is usually based on clinical examination of the eye and clinical history (3). Skin prick tests, serum-specific IgE and conjunctival provocation test (CPT) evaluation could help to confirm the diagnosis and to identify the allergen involved (48).

VKC is a rare and severe ocular disease, with childhood onset and a duration approximatively of 10 years (23, 32, 49). This condition spontaneously resolves after puberty, whereas in rare cases it persists during adulthood. Males are mainly affected (sex ratio males:females=3:1) and it is more frequent in areas with hot weather, such as the Mediterranean, South America, India, Central Africa (23, 32, 49). Children with VKC worsen in spring/summer and mainly complain of severe symptoms of photophobia, pain, itching, redness, secretion and tearing. Allergen exposure, but also nonspecific stimuli, like sun, wind and dust, induce exacerbation of symptoms (50). VKC is divided in three forms, limbar, tarsal or mixed, based on the localization of giant papillae on limbus and/or tarsal conjunctiva (23, 32, 50). A cobblestone appearance of the upper tarsal conjunctiva is the consequence of papillar hypertrophy in the tarsal form of VKC.51 Horner-Trantas dots, multiple inflammatory nodules on the limbar conjunctiva consisting of eosinophils and epithelial cells debris, are characteristic of the limbal form (32, 49). Corneal involvement is frequent, ranging from superficial punctate keratitis to shield ulcer, called Togby's ulcer, typically located in the upper portion of the cornea (23, 52).

Atopic keratoconjunctivitis (AKC) is a bilateral chronic inflammatory disease, with onset before 5 or between 30 and 50 years of age (40, 53). Approximately 90% of patients with AKC shows the presence of associated atopic dermatitis, but conversely, only 20-40% of cases of atopic dermatitis have AKC and the ocular involvement is not related to the severity of the dermatitis (42, 53). Symptoms, usually perennial with seasonal exacerbations, are characterized by intense itching, involving the periorbital skin and eyelids, tearing and burning. The eyelids are eczematous with skin maceration

and consequent risk of bacterial superinfection (40, 53). Continued rubbing may cause thinning or loss of the outer third of the eyebrow (De Hertoege sign) (42). Conjunctival scars, sub-epithelial fibrosis until the formation of symblepharon may also occur in AKC patients. Meibomian gland dysfunction and conjunctival chronic inflammation induced tear film impairment with decrease of break up time (BUT), conjunctival squamous metaplasia and loss of goblet cells (54).

Ocular complication of AKC could lead to blindness. Cornea involvement is frequently observed, ranging from superficial punctate keratopathy to corneal ulceration, scarring and, in severe cases, neovascularization (40, 55). A long-term follow up study showed that severe corneal involvement is present in 70% of AKC patients, with corneal neovascularization in 60% of cases. 56 Others ocular associated diseases in AKC are cataract, mainly due to steroid treatments, and keratoconus (40).

Diagnosis

Differential diagnosis between the different forms of AC is based on clinical history and the evaluation of signs and symptoms (Table I). The familiar history of atopy and the association with other allergic diseases should be investigated. Patients with AC complain bilateral symptoms of itching, tearing, photophobia and mucous discharge. Symptoms may occur during season or may display a perennial course during the year. Generally, all forms of AC show the presence of conjunctival hyperaemia, chemosis and mucous secretion, while the presence of corneal damage and photophobia should address toward a diagnosis of VKC or AKC (9, 57). The age of onset may help in the differential diagnosis, in fact, VKC frequently involves children during the first decade, while SAC and PAC show a peak during the second decade and AKC after the third decade (1).

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Specific allergy tests are useful to identify the sensitization to specific allergens (57). The skin prick test (SPT) or radioallergosorbent test (RAST) are the most used exams to identify the sensitization to allergens (57). Conjunctival allergen challenge may confirm the conjunctiva sensitization to the specific allergen (conjunctival provocation test - CPT) (57, 58). CPT consists in the instillation of a specific allergen eye drop, selected on the basis of the result of SPT, at increasing concentrations to induce conjunctival itching and hyperemia (threshold dose) (48).

In the absence of positive allergological exams, the identification of eosinophils in the conjunctival cytology may help the diagnosis of allergic conjunctivitis (57, 59). Measurement of IgE and inflammatory mediators in the tears have been proposed in experimental studies but are not routinely used for clinical diagnosis (25, 57).

Treatment

Current treatments for ocular allergies aim at controlling ocular symptoms and preventing complications. Antiallergic drugs for AC include antihistamines, mast cell stabilizers and, in more severe forms, corticosteroids (60-62). Treatment of AC should be based on severity of signs and symptoms, as shown in Table II. Specifically, all patients with AC may benefit from the use of artificial tears and cold compresses. In fact, the use of ocular surface lubricants, such as preservative-free artificial tears, may provide a temporary relief of symptoms and remove allergens from the ocular surface. These agents may be used in all forms of AC associated or not with antiallergic or anti-inflammatory drugs (12, 61).

Topical antiallergic drugs are used in the presence of allergic symptoms in all patients with SAC and PAC and in mild forms of VKC and AKC. Among them, topical antihistamines are effective in inducing transient improvement of AC symptoms such as itching and tearing (12). In fact, antihistamines act by blocking histamine receptors and consequently the effects of the histamine released in the ocular surface. The first generation of H1 antagonists (pheniramine and antazoline) induces quickly response with a rapid disappearance of effects. The second generation of antihistamines such as levocabastine, azelastine and emedastine showed a better ocular tolerability and a longer effect (12).

The use of systemic antihistamines should be considered only in patients with other associated

allergic conditions such as rhinitis, while in the presence of isolated AC, topical antihistamines should be preferred (12). In fact, a randomized clinical trial showed that emedastine 0.05% ophthalmic solution was more effective than oral loratadine in improving itching and hyperemia after conjunctival allergen challenge in patients with AC (63).

Topical mast cell stabilisers act by inhibiting mast cells degranulation and addressing both early and late phases of the allergic reaction (12). These drugs, such as sodium cromoglycate, nedocromil sodium, pemirolast, and lodoxamide eye drops showed to be safe and effective in treatment of AC in order to prevent seasonal recurrences and alleviate allergic signs and symptoms.64 Since they require a preloading period, their use is mainly preventive (12, 22).

More recently, novel molecules with both antihistamine and mast cell stabilizing effects, such as olopatadine, ketotifen, epinastine, azelastine, and alcaftadine, have been introduced for AC treatment (12). These dual-action topical agents act during both ocular early and late-phase reactions providing rapid and sustained relief of ocular signs and symptoms in patients with AC (12). In addition, alcaftadine and

Table I. *Clinical characteristics of allergic conjunctivitis.*

	SAC	**PAC**	**VKC**	**AKC**
Mean age of presentation	20-30 years	20-30 years	5-15 years	<4 years 30-50 years
Seasonal/perennial presentation	Seasonal (spring-summer)	Perennial	Seasonal or perennial with seasonal exacerbations (spring/summer)	Perennial
Signs				
Conjunctival hyperemia	++	+	+++	++
Conjunctival chemosis	++	+	±	±
Mucous discharge	Clear, ±	Clear, ±	Stringy, ++	Stringy, +
Upper tarsal papillary reaction	± (micropapillae)	± (micropapillae)	++ (giant papillae)	+
Limbal papillary reaction	-	-	+	±
Trantas dot	-	-	++	±
Subconjunctival fibrosis	-	-	+	+
Lid eczema	-	-	-	++
Corneal signs	-	-	++ (superficial punctate keratitis, ulcer, plaque)	+++ (superficial punctate keratitis, ulcer)
Symptoms				
Itching	+	+	++	+
Tearing	+	±	++	+
Redness	+	±	++	+
Pain	-	-	+	+
Photophobia	-	-	++	+
Visual impairment	-	-	±	±

Table II. *Severity based therapeutic approach in allergic conjunctivitis.*

	Allergen avoidance, ocular lubricants, cold compresses	Antihistamine/ mast cells stabilizers	Short course (1-2 weeks) corticosteroid	Immunomodulators (Cycosporine A., Tacrolimus)
Mild occasional symptoms	+	±	-	-
Presence of moderate to severe symptoms	+	+	-	-
Presence of corneal involvement	+	±	+	±
Frequent flare-ups of severe ocular surface inflammation	+	-	±	+

All patients with AC may benefit from the use of artificial tears and cold compresses. In the presence of mild, occasional symptoms, topical antihistamines may be used when needed. Patients with mild to moderate symptoms should be treated daily with topical dual action agents. Topical corticosteroids should be used as short –term treatment in patients with corneal involvement, while immunomodulators can be used as steroid sparing agents in severe non-responder forms.

olopatadine eye drops are approved for once daily administration, which may improve AC patients' compliance. Recently, the effects of treatment of SAC and PAC with topical antiallergic drugs was assessed in a systematic review and meta-analysis (65). The Authors reported that topical antihistamines and mast cell stabilisers were safe and well tolerated for treatment of AC, and resulted effective when compared to placebo. However they observed a large variability in clinical outcomes and they reported that there were limited data to perform comparisons between different types of drugs (65). Only two studies resulted suitable for a meta-analysis showing that treatment with olopatadine was more effective in improving itching when compared with ketotifen, although there was high statistical heterogeneity (65).

Topical corticosteroids showed to be very effective in controlling signs and symptoms of ocular allergies, however their use is limited to patients with refractive forms of AC (VKC and AKC) associated with corneal damage and they should be used in short term course (12, 62). In fact, the chronic use of topical steroids leads to development of ocular complications, such as cataract, glaucoma and increased risk of infection (12, 23). Topical immunomodulators, such as cyclosporine A, can be used as steroid sparing agents in severe non-responder forms.

A recent survey highlighted that treatment of AC is often inappropriate, in fact, the Authors reported that most of patients with AC used decongestant/ antihistamines (43%) and corticosteroids (41%)

followed by topical (29%) or systemic antihistamines (27%), mast cell stabilizers (15%) and antibiotics in 6% (66). The use of topical vasoconstrictors for the treatment of AC should be discouraged due to the rebound hyperemia and the availability of antiallergic eye drops that resulted more safe and effective (12). Similarly, the use of topical non-steroid anti-inflammatory is limited by stinging and burning sensation upon instillation and epithelial toxicity (12).

Allergen-specific immunotherapy (SIT) showed to be effective in improving ocular symptoms in patients with AC. However, it is expensive and has potential side effects; therefore, its use should be proposed only in the presence of other associated allergic conditions (67).

Emerging therapies

Current treatments for AC include topical antihistamines and mast cell stabilizers that are able to control allergic symptoms and topical corticosteroid, which inhibit inflammation, but the long-term use is associated with development of serious side effects. Therefore, novel treatments for AC able to reduce inflammation with lower side effects are highly desired.

Among the emerging treatments for AC, the most studied is topical Cyclosporine A (CsA) that has largely demonstrated to be safe and effective in severe refractory cases of allergic conjunctivitis (VKC and AKC) (40, 68). CsA is an immunomodulatory agent that inhibits T-cell activation through calcineurin inhibition and reduces inflammatory cytokines production (69). Several studies showed that topical treatment with CsA was effective in inhibiting ocular surface inflammation without the steroids' associated side effects (68).

Topical CsA has been administered in different formulations (dissolved in olive oil, castor oil or artificial tears), concentrations (from 0.05% to 2%) and dosages in the different studies showing to be safe and effective in improving signs and symptoms of patients with severe VKC and AKC (15, 70-75). Several clinical trials also demonstrated that topical CsA was effective as steroid sparing treatment in patients with severe, refractory AKC and VKC (15, 70, 73, 76).

A systematic review and meta-analysis showed that topical treatment with CsA improved signs and symptoms of severe allergic conjunctivitis regardless of the dosage and that there was a significant reduction in the use of steroid eye drops in patients with steroid-dependent AC (68).

Actually, CsA is commercially available as a 0.05% ophthalmic emulsion in US but it is not approved for the use in allergic conjunctivitis (75). Recently, a preservative-free cyclosporine 0.01% in cationic nanoemulsion has been approved by European Medicine Agency for treatment of severe keratitis in adult patients with dry eye, which has not improved despite treatment with tear substitutes. (EMA/CHMP/473489/2014 Committee for Medicinal Products for Human Use - CHMP). Effectiveness and safety of this formulation was evaluated in a large population of 594 patients with severe VKC and AKC. This study demonstrated that topical cyclosporine 0.1% is effective for the treatment of severe VKC and AKC and can be used safely. This study reported no significant ocular and systemic adverse events related to CsA, eye burning was the most common adverse event (4.4%) and all infections (n = 10) observed developed in patients undergoing concomitant steroid (77).

Another topical immunosuppressor agent is Tacrolimus (FK506), a competitive calcineurin inhibitor drug, which decreases IL- 2, IL-3, IL-5 and TNF alpha T lymphocytes production and blocks mast cell degranulation and activation (78). Topical tacrolimus, administered either as 0.03% ointment or 0.1% ophthalmic suspension, also appears effective and safe to treat chronic inflammatory ocular disease, including ocular allergies (79, 80). The result of double masked RCT performed to evaluate the efficacy of Tacrolimus vs placebo in 56 patients with severe AC confirmed that Tacrolimus ophthalmic suspension 0.1% significantly improved signs and symptoms of ocular inflammation without severe side effects compared to placebo (81). In addition Tacrolimus 0.1% ophthalmic suspension may be more effective than cyclosporine A in resolving giant papillae in AKC and VKC patients and similar in clinical improvement of ocular symptoms (13,

70). Several studies reported that side effects of tacrolimus ointments are foreign body sensation and burning, which decrease in 2-4 weeks after drug discontinuation. Finally, Hazarika remarks that 0.03% tacrolimus eye ointment is safe ,very effective and potentially able to reduce the incidence of recurrences of ocular inflammation in patients with refractory allergic conjunctivitis (82).

In addition to the above immunosuppressive drugs, novel therapeutic agents targeting different pathogenic mechanisms are currently under investigation to identify the next class of anti-allergic therapy for patients with AC not responder to standard therapy (83).

A novel therapeutic target is interleukine-1 (IL-1) a late-phase cytokine. Specifically, experimental studies in animal models of AC showed that IL-1 induces activation and recruitment of APC, stimulates T cells and eosinophils proliferation and that administration of IL-1 receptor antagonist (Anakinra) decreased eosinophils recruitments (84). A novel IL-1 receptor antagonist, named EBI 005 (Isunakinra), was obtained by chimerism of two ligands of IL-1receptor: IL-1β and Anakinra (85). The preclinical studies in animal models of AC showed that EBI 005 is safe and has an excellent bioavailability in target tissues (85). The results of a phase II clinical trial, showed that treatment with topical EBI 005 was more effective than placebo in improving ocular symptoms (itching and tearing) in moderate-to-severe patients with AC after CPT and environmental exposure chamber (86). A Phase III multi-center, double-masked, vehicle-controlled, randomized clinical trial has been recently completed in 250 allergic subjects, but the results of this study have not yet been published. (NCT02492321)

Other studies are focusing on development of spleen tyrosine kinase (Syk) inhibitors. It has been demonstrated that SyK regulates the phosphorylation of enzymes (phopholipase-C, phosphotidylinositol-3kinase) and protein kinases which control histamine release. (87) Other therapeutic strategies targeting conjunctival mast cells in AC are currently under investigation such as the use of humanized anti-eotaxin 1 receptor (83).

Histamine H4 receptor was another target involved in T-cell mediated response and the development of new generation of H4 inhibitors could represent an advancement in the treatment of AC (83).

CONCLUSION

Allergic conjunctivitis represent a frequent ocular condition characterized by ocular surface inflammatory reaction. Mild forms of AC, SAC and PAC, are characterized by seasonal or perennial symptoms of itching, tearing and swelling associated with conjunctival hyperemia and chemosis. More severe forms, such as AKC and VKC, are characterized by intense ocular symptoms associated with frequent corneal involvement potentially leading to visual impairment. All forms of AC are frequently associated with other allergic conditions and require multidisciplinary management. Diagnosis of AC is based on clinical history and eye evaluation and may be confirmed by allergological tests. Currently, mild forms of AC may be treated with topical antihistamines, mast cell inhibitors and/or dual-action agents that are effective in provide relief of ocular symptoms. More severe forms, with corneal involvement, require more aggressive approach with short course of topical steroids. In fact, long term use of topical steroids is associated with development of ocular complications such as glaucoma and cataract and should be avoided. In patients with refractory AKC and VKC, the use of topical CsA showed to be effective in improving signs and symptoms and in decreasing steroid use. However, CsA is not yet approved for the use in pediatric population and in allergic conjunctivitis. Novel molecules, targeting different pathogenic mechanisms of AC are under investigation in order to develop novel, safe and effective treatments for AC.

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ALLERGIC RHINITIS

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Allergic rhinitis (AR) was long considered a quite trivial disease, but the advance in epidemiological and clinical knowledge, with a major role for Allergic Rhinitis and its Impact on Asthma (ARIA) initiative, substantially changed the scene. Now we know that AR has significant effects on patients' quality of life and also has a relevant economic burden. The ARIA phenotypes related to the duration of symptoms and to the severity of AR are very useful in establishing the optimal strategy in each patient with AR, also according to the kind of allergens that cause rhinitis. When traditional allergy testing, including skin prick tests and *in vitro* of specific IgE antibodies are not sufficient for the diagnosis, modern techniques such as molecular diagnostics may be used. Also the management of AR may be tailored to single patients according to the clinical expression of AR, that may vary from mild to moderate-severe stage, with the aim of achieving the best possible control of the disease.

Epidemiology of allergic rhinitis

Allergic rhinitis (AR) is a major health issue, as defined by a continuously increasing prevalence worldwide. In the 2000's, a population-based survey in Western Europe (including Belgium, France, Germany, Italy, Spain, and the UK) found a prevalence ranging from 17% in Italy to 29% in Belgium, with an overall value of 23% (1). Concerning children, the International Study of Asthma and Allergy in children (ISAAC), which acquired data from 37 countries worldwide, reported a prevalence of 10 to 40% (2). In recent years, further studies confirmed the trend of increasing prevalence. In a cross-sectional, nationwide survey in Portugal on subjects aged 65-years or more, the data obtained from 3678 responders defined a prevalence of current AR of 29.8% (3). In a Japanese study including 1472 residents aged from 20- to 81-years-old, AR impacted 23.2% of males and 25.4% of females in 2011, compared to 17.6% and 23.0%, respectively, in 2006 (4). A more recent survey was conducted in Greece, where 675 school-aged children were enrolled. Of them, 231 (34.2%) had AR (5). In the same year, Maio et al. published the data from 3 cross-sectional surveys performed in Central Italy in 1985-88 (3865 subjects), 1991-93 (2841 subjects), and 2009-11 (1620 subjects). An increase in prevalence of all respiratory symptoms/diseases was observed and AR corresponded to a raise from 16.2% to 37.4% (6). In some studies the prevalence was also assessed according to the classification of the Allergic Rhinitis and its Impact on Asthma (ARIA) document (7). In the Portuguese study cited above, 49.1% of patients had mild-intermittent, 7% mild-persistent, 27.5% moderate-severe-intermittent, and 16.4% moderate-severe-persistent rhinitis (3). Data are available from two surveys performed in China: in a group of 1,067 pre-school children in Bejing, 67.1% had

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intermittent and 32.9% had persistent AR, with moderate to severe symptoms in 41.5% (8), while in a group of 5,236 adult patients in Ningxia Area, persistent AR concerned 27.6% and intermittent AR 72.37% of the population (9). All these data make it clearly apparent that the worldwide prevalence of AR is steadily and globally increasing.

Factors associated with the increased prevalence of allergy

The reasons underlying such an increase are not yet defined, but some hypotheses based on genetic and environmental factors, are currently considered. That genetic predisposition is related to the development of allergy is understandable if one considers the risk of becoming allergic associated to familiar atopy. It is possible to state, according to the available data, that a systemic condition driven by an imbalance between T-helper cell types 1 and 2 lymphocyte populations that results in the preferential production of IgE antibodies is under genetic control (10). Also, gene defects were identified that favor the development of allergy including AR. This is true for filaggrin, a protein which maintains an effective skin barrier against the environment, blocking transcutaneous entering of allergens (11). However, the level of understanding of the precise genetic mechanisms underlying allergy is far from comprehensive.

As far as environmental factors are concerned, pollution is long suggested as an important cause of the increasing prevalence of allergy. Some data seem quite convincing. As reviewed by Brandt et al. in 2015 "there is considerable evidence that exposure to traffic-related air pollutants (TRAP) is associated with childhood asthma symptoms and exacerbations" (12). However, based on the presence of additional factors related to host, that include genetic, obesity, nutrition and co-morbidities or to environment, the authors acknowledge that "additional studies are needed to fill the remaining gaps including identification of the key host factors associated with enhanced susceptibility". For example, a study comparing West German and East German cities before the unification of Germany had the opportunity to evaluate two genetically homogeneous populations exposed to different kind of pollution. In fact, in West Germany pollution was mainly associated to high car density and NO2 exposure, while heavy industrialization and private coal burning for house heating purposes were dominant in East German cities. However, no significant difference in the prevalence of asthma between the two parts of the country was detected when atopy was taken into account (13). A pivotal source of pollution is tobacco smoking. In a systematic review and meta-analysis including 97 studies, AR was not associated with active smoking in adults, while a modest increased risk was detected in children and adolescents for both active and passive smoking. According to authors, "to further explore the role of smoking in allergic diseases additional studies with detailed measurement of exposure and better case definition are needed" (14).

The so-called "hygiene hypothesis" was proposed by Strachan in 1989, based on the possible capacity to prevent allergy exerted by infections in early childhood and exposure to endotoxins (such as bacterial lipopolysaccharide) that commonly occur in farm dust. The striking decrease of these aspects by higher standards of personal cleanliness, declining family size, and modern households could result in the rise of atopic diseases (15). In the face of a number of publications in favor of the hygiene hypothesis and others denying it, a revisited hypothesis was proposed in 2005, more centered on the innate immune response and immunoregulatory mechanisms (16). Recently, Schuijs et al. argued that epidemiological evidence of the hygiene hypothesis is mainly based on cross-sectional studies that cannot demonstrate a cause-effect relationship. Indeed, the repeated stimulation of the immune system by pathogens is crucial in the development of a balanced response and should remain the core issue of the hypothesis (17). According to Bloomfield et al., though interaction with microbes occurring in the natural environment and human microbiome play an essential role in immune regulation and changes in lifestyle and environmental exposure have certain effects on the human microbiome, the term hygiene hypothesis is misleading. They propose that promoting a risk assessment approach (targeted hygiene) provides a framework for

warranting "protection against pathogen exposure while allowing spread of essential microbes between family members" restoring public perception of hygiene as a means to prevent infections (18). Also, to join the concept of the protective role of the farming-rural environment to genetic factors has produced the recent experimental observation that chronic exposure to low-dose endotoxin or farm dust protects from developing asthma caused by house dust mites by reducing the epithelial cell cytokines that activate dendritic cells and in turn suppressing type 2 immunity to mites. Based on the discovery that the ubiquitin-modifying enzyme A20 in lung epithelium abolished the protective effect, children living on farms were studied as it was possible to observe that a single-nucleotide polymorphism in the gene encoding A20 was associated with allergy and asthma risk in these children (19). Still, the new European study on Mechanisms of the Development of ALLergy (MeDALL), which included a very large number of children prospectively followed after puberty, provided a larger approach. In fact, along with a new standardized MeDALL Core Questionnaire, estimates of air pollution exposure were available for 10,000 children. A microarray for allergen molecules with increased IgE sensitivity for 3,292 children, omics data for 23,000 children and DNA methylation for 2,173 children. Using classical epidemiology and machine-learning methods in 16,147 children aged 4 years and 11,080 children aged 8 years, MeDALL assessed the multi-morbidity of eczema, rhinitis, and asthma and found that only 38% of multi-morbidity was associated to IgE sensitization. Monosensitization and polysensitization were recognized as two distinct phenotypes and the allergic phenotype characterized by polysensitization and multi-morbidity was related with the frequency, persistence and severity of allergic symptoms (20).

The economic burden of allergic rhinitis

Tough AR is often regarded by the lay (and also by many physicians) as a trivial disease, the healthcare costs produced by its management are very high. In a study performed in U.S., patients with a diagnosis of AR were extracted from the 2007 Medical Expenditure Panel Survey medical conditions file and compared to non-AR patients calculating the differences in healthcare utilization and healthcare expenditures based on demographically and comorbidity adjusted multivariate models. AR concerned 17.8±0.72 million adult patient (7.9±0.3% of the U.S. population). The additional incremental healthcare utilizations associated with AR compared with non-AR patients resulted in additional healthcare expenses of $1.492±346, $461±122, $876±126, and $168±25 for total healthcare expenses, office-based visit expenditures, prescription expenditures, and self-expenditures, respectively. The authors concluded that AR is a commonly prevalent and costly disease that would be a prime target for guideline development and standardization of care (21). A study performed in Italy estimated the average annual cost incurred by the National Health Service (NHS), as well as society, due to respiratory allergies and their main co-morbidities in Italy; direct costs were estimated multiplying the hospitalization, drugs and management costs derived by the literature with the Italian epidemiological data. Indirect costs were calculated based on lost productivity according to the human capital approach. Overall, the total economic burden associated with respiratory allergies and their main co-morbidities was €7.33 billion, a percentage of 27.5% being associated with indirect costs (€2.02 billion) and 72.5% with direct costs (€5.32 billion). In AR, the model estimated an average annual economic burden of €1.72 billion. According to authors, these data should be considered an efficient tool for public decision-makers to correctly understand the economic aspects involved by the management and treatment of respiratory allergies-induced diseases in Italy (22). A recent study from France evaluated the costs of perennial allergic rhinitis (PAR) with or without asthma by medical resource utilization (MRU); Electronic Health Records (EHRs), allowed to identify in 2010 two cohorts of PAR patients. For each patient, the EHRs were connected to corresponding claims data with MRU and costs during 2011 to 2013. Subgroup analyses were performed according to severity of PAR and level of asthma control. The results of the analysis showed that an average annual cost reimbursed by

social security system for a patient with PAR and no asthma was €159 in 2013, with a range from €111 to €188, depending on PAR severity. A clear rise in costs with severity of PAR and control of asthma was found (23).

Impact of allergic rhinitis on quality of life

Patients with AR often report that disturbed sleep, fatigue and irritability, that are associated with an impairment in quality of life (QoL), may vary according to the severity of AR. QoL can be measured by specific questionnaires assessing the effects of AR on various domains of daily life, including practical functions, social interactions, emotional well-being, and economic activity (25). In a survey on 3635 patients from 6 countries in Europe and U.S. it was observed that they were affected for a considerable part of each day, with the most severe symptoms occurring in the morning. The data from the survey showed the range of allergic symptoms that negatively impact the lifestyle of subjects with AR and their will to recover the capacity to cope with activities of daily living (26).

In a recent study the association between nasal and extranasal symptoms of AR with general health-related QOL was prospectively investigated in 150 adults with persistent AR. QOL was measured by the five-dimension EuroQol QOL survey and the severity of nasal and extranasal symptoms was measured by the 22-item Sino-Nasal Outcome Test (SNOT-22). In addition, each subject completed a Rhinitis Control Assessment Test (RCAT). The results showed that SNOT-22 score was significantly correlated with RCAT ($p<0.001$) and EuroQol ($p<0.001$). Sleep-related symptoms and otologic symptoms were associated with the maximum reduction in health-related QOL. This suggested that sleep and otologic symptoms should be routinely assessed in their clinical evaluation (27).

Clinical characteristics of allergic rhinitis

AR may present various clinical phenotypes that indicate the diversity of its nature, depending on genetic and environmental factors. The distinction of a phenotype characterized by prevalence of rhinorrea and sneezing (runners) or nasal obstruction (blockers) was the first to be proposed (28). The ARIA document cited above introduced phenotypes related to duration of symptoms and to the severity of AR. In regards to duration, intermittent AR is defined by symptoms less than 4 days a week or for less than 4 weeks, while persistent AR is defined by symptoms more than 4 days a week or for more than 4 weeks. Concerning severity, mild AR is defined by the absence of sleep disturbance, impairment of daily activities, school or work activities, and troublesome symptoms, while moderate-to-severe AR by the occurrence of one or more of these items (7). Studies on the prevalence of the different clinical presentations of AR are available. In a survey including 2623 children from 35 centers in Italy, 2319 had AR, while 304 had other kinds of rhinitis. In patients with AR, 597 (25.7%) had mild intermittent, 701 (30.2%) mild persistent, 174 (7.5%) moderate-severe intermittent, and 773 (33.3%) moderate-severe persistent AR. The allergens most frequently responsible were grass pollen and dust mites (29). A similar survey from the same centers included 3383 adults patients with rhinitis, 2788 of whom (82.4%) had AR, 311 (11.5%) had a mild intermittent, 229 (8.8%) a mild persistent, 636 (23.5%) a moderate-severe intermittent, and 1518 (56.1%) a moderate-severe persistent AR (30).

CONCLUSIONS

The epidemiological and clinical observations from studies performed in the 2000's with a major role for the ARIA initiative, define AR as a pathology of general interest (31) that warrants adequate consideration, according to its impact on patients' quality of life and its economic burden, in order to perform the most adequate diagnostic and therapeutic procedures.

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ASTHMA ASSOCIATED TO RHINITIS

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A large amount of data show that AR and asthma are associated both epidemiologically and clinically, introducing the definition of "united airway disease". The mechanisms underlying such association were initially suggested to start from the nose, including the loss of the protective and homeostatic effects of nasal function, the activation of a naso-bronchial reflex and the spread of allergic inflammation from the nose to the lower airways. Later, other factors such as microbial stimuli and systemic inflammatory mechanisms, involving bloodstream and bone marrow, were advocated. The advance in knowledge made it clear that the link between asthma and AR is multifactorial, with particular importance for inflammatory cells and especially eosinophils. By the model of nasal challenge, important immunological responses were revealed, with particular importance for the increased expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin) and of cytokines such as interleukin (IL)-13, that was accompanied by a rise of eosinophils in blood and developement of bronchial hyper-responsiveness. The occurrence in AR of a concomitant sinusitis is frequently associated with worse asthma outcomes, as assessed by a lower pulmonary function, increased asthma symptoms and poorer quality-of-life compared to patients with asthma alone.

It is known that allergic rhinitis (AR) is associated with several multi-morbid disorders that are based on a common allergic origin. Such disorders may be anatomically related to the nose, as occurs for conjunctivitis, sinusitis, turbinate hypertrophy, middle ear and laryngeal dysfunctions, but may also have no such a relationship (1). This is true under the anatomical point of view for atopic dermatitis or anaphylaxis, while solid functional bases support the association between AR and asthma. The epidemiologic and clinical evidence of this association suggested the definition of "United airway disease" (2) or "United allergic airway" (3). Moreover, the increasing understanding of the mechanisms which underline the clinical expression of the two diseases confer further strength to this concept. Since 2001 (4), the influence of AR on asthma is the object of the initiative "Allergic Rhinitis and its Impact on Asthma" (ARIA), which is regularly updated, the latest revision being published on 2017 (5), and represents a firm reference for the issue.

Epidemiological data on the association between AR and asthma.

A study estimated that more than 75% of patients with asthma have AR, while up to 40% of patients with AR are affected by asthma. The authors discovered that, following adjusting for age, gender, atopy, duration in years of follow-up, and smoking status, AR still stands as an independent risk factor for asthma (6). In a cohort of 11.540 Finnish adults

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the prevalence and incidence of asthma and AR was studied during a 15-year period. The prevalence of asthma increased during the observation period from 2% in men and 2.2% in women to 2.9% in men and 3.1% in women. The increase of prevalence of AR was larger, with values from 6.8% to 11.8% in men and from 9.8% to 15.3% in women. Concerning incidence, no significant rise was observed for asthma, while the incidence of AR was lower during the latter period in men. The increase in prevalence of asthma and AR over time was comparable, and AR was found to be a strong predictor of asthma (7). In the majority of cases, AR precedes asthma, as shown by a 10-year long study in children with AR, asthma was developed later in 19% of cases (3). In a 23-year follow-up study of around 2000 college students, subjects with AR had a likelihood approximately three times higher to develop asthma than control subjects without AR (8).

The pathophysiological link of the association of AR with asthma

The effect of nasal inflammation on bronchial asthma were noted in the 1980's, when bronchial hyperresponsiveness (BHR) to methacholine in patients with AR was observed, especially during the pollen season (9). In the 1990s, based on the introduction of the concept of united airways, Mygind et al. hypothesized that antigen presentation in the nose could induce cell recruitment and activation not only in the nasal mucosa but also in the lower airways (10).Analyzing the available data, Togias proposed possible mechanisms to explain the relationship between AR asthma. Such mechanisms were the loss of the protective and homeostatic effects of nasal function, the activation of naso-bronchial neural interactions (induced by histamine and bradykinin) stimulating the afferent nasal sensory nerves that in turn activates the vagus nerve, finally resulting in bronchial smooth muscle hyperreactivity and the spread of allergic inflammation from the nose, either directly or through the systemic circulation, to the lower airways (11).

By observing new studies in 2006, Braunsthal and Hellings expanded the triggers to local tissue factors, such as microbial stimuli and systemic inflammatory mechanisms involving bloodstream and bone marrow as well (12). The development of knowledge made clearer that the link between asthma and AR is probably multifactorial, but that local factors such as naso-bronchial reflex and dripping of nasal contents into lower airways are less convincing compared with systemic response (3). The effects on inflammatory cells are particularly important, as indicated by studies on eosinophils. Actually, a nasal challenge with the specific allergen in non-asthmatic patients with pollen-induced AR, along with the occurrence of BHR, was related to an increase of eosinophil counts in the sputum (13). Moreover, nasal challenge in AR patients induced not only an influx of eosinophils on both nasal and bronchial epithelium but also the increased expression of adhesion molecules, including ICAM-1, VCAM-1 and E-selectin in nasal and bronchial tissues. Interestingly, the number of mucosal eosinophils was correlated with the local expression of ICAM-1, E-selectin and VCAM-1, suggesting that contact with the specific allergen by nasal challenge in patients with AR, results in generalized airway inflammation through up-regulation of adhesion molecules (14). A reversed model also confirmed that inflammation in any of the two parts of airways (upper or lower) induces influx of eosinophils in the other. In fact, in asthmatic patients without AR, biopsies from nasal mucosa revealed high eosinophil counts, that were significantly correlated with cell counts in the airways (15).

The occurrence of the same inflammatory mechanisms in nose and bronchi was further confirmed by a study on 66 patients, investigating the effects of natural pollen exposure on inflammatory cytokines. Pollen-allergic patients showed a significantly higher increased in levels of interleukin (IL)-13 compared to a group of patients with nonallergic rhinitis. This was accompanied by an increase of eosinophil numbers in the blood in patients who developed BHR during pollen season. By contrast, the level of IL-10, which is typically produced by tolerogenic Treg lymphocytes, was higher in patients without BHR than those with BHR (16).

Another factor supporting the association of AR

and asthma is the outcome of medical treatment of AR on asthma. The first observations on the improvement of seasonal asthma in patients were casual, as reported by Welsh et al, who noted that in patients with ragweed pollen-induced AR treated with topical beclomethasone or flunisolide nasal solution, the symptoms of seasonal asthma considerably reduced (17). This observation was confirmed by the results from another study (18) although in this larger study on 262 patients randomized to receive intranasal fluticasone, inhaled fluticasone or placebo for 6 weeks, only inhaled fluticasone was able to reduce bronchial reactivity (19). In 2003, a Cochrane systematic review analyzed the issue, including 14 trials involving 477 patients.

Meta-analysis for asthma outcomes was unsuccessful in finding a statistically significant benefit of intranasal corticosteroids on asthma. Nevertheless, a trend favoring a beneficial effect on symptom scores and pulmonary function as assessed by the improvement in forced expiratory volume in one second, was found (20). Therefore, the issue remains controversial, as it does for antihistamines. In fact, the theoretical basis of their systemic effects that follow oral administration targeting the histamine receptors on mast cells and T cells and then reducing inflammation not limited to the nose was confirmed by studies showing a positive effect of antihistamines on asthma (21, 22). To date, there is no indication from any research to treat asthma with antihistamines.

When nasal disorders worsen asthma: the role of sinusitis

Based on the strict anatomical connection, sinusitis in quite common in patients with AR (23). Sinusitis may be also induced in patients with AR through a nasal challenge with the causative allergen, as demonstrated by Baroody et al, who observed a significant increase in allergic patients compared with controls, in maxillary sinus eosinophils and levels of eosinophil cationic protein and histamine during the challenge (23). A multifactorial etiology for sinusitis is also suggested, including the edema of nasal mucosa, the damaged nasal cilia and the mucus excess resulting in the blockage of ostial drainage from the sinuses along with a spreading inflammation triggered by recruitment of eosinophils (3).

Importantly, when tissue cytokine expression and inflammatory cells infiltrate occurring in chronic hyperplastic sinusitis were investigated in patients with allergy *vs* patients without allergy, distinct mechanisms were detected. Actually, the allergic mechanism was associated to the production of TH2-type cytokines, including GM-CSF, IL-3, IL-4, and IL-5, whereas the non-allergic involved production of GM-CSF, IL-3, IFN-gamma and non-allergic eosinophilia was independent compared to allergic eosinophilia, of IL-4 and IL-5 (24). Clinically, it is known that in children undergoing functional endoscopic sinus surgery, the recovery time is significantly longer if they suffer from of AR (25). As far as imaging stage is concerned, in severe sinusitis the extent of the disease as assessed by computed tomography (CT) was significantly correlated with peripheral eosinophilia and the presence of atopy (26).

A strong association between asthma and chronic sinusitis was reported, with the highest level in patients suffering from both sinusitis and AR (27), as well as a high prevalence of chronic sinusitis was found in patients with asthma. In particular, sinusitis, as evaluated by clinical symptoms and CT, was significantly more common in patients with severe steroid-dependent asthma than in those with mild-to-moderate asthma (28). Several studies reported that chronic sinusitis is associated with worse asthma outcomes, as indicated by a lower pulmonary function, increased asthma symptoms and worse quality-of-life scores compared to patients with asthma alone (29-32). As for AR and asthma, a research line investigated whether the successful treatment of sinusitis could be beneficial also for asthma but, similarly to AR, no definite evidence is available. The possible outcomes of the biologic treatment approach are currently under investigation, particularly concerning the use of anti–IL-5 targeted therapies (including mepolizumab and reslizumab) in patients with severe asthma and chronic sinusitis (33).

CONCLUSIONS

A bulk of data supports the epidemiological and clinical association between AR and asthma. Still, the pathophysiological mechanisms underlying this association are not definitely elucidated. Papadopoulou et al. argued that there is no clear demonstration that this association is sustained by common mechanisms or is a comorbidity or even an epiphenomenon. In particular, they mentioned that in preschool children the two diseases seem to coexist, while in older children they are apparently the manifestation of one united syndrome (34). On the other hand, a different visual was provided by an European prospective cohort study assessing in 16147 children aged 4 years and 11080 aged 8 years, current eczema, rhinitis, and asthma from questionnaires and serum-specific IgE to six allergens, with comorbidity defined as coexistence of two or three diseases in the same child. Relative and absolute excess comorbidity was calculated by comparing the observed and expected occurrence of diseases at 4 years and 8 years.

The data obtained showed that the absolute excess of any comorbidity was 6% for children aged 4 years and 2% for children aged 8 years and that 44% of the observed comorbidity at age 4 years and 50% at age 8 years was not a result of chance. Concerning IgE, though sensitization was independently associated with excess comorbidity of eczema, rhinitis and asthma, its presence occurred only in 38% of comorbidity. According to authors, IgE sensitization should no longer be considered the dominant causal mechanism of comorbidity for these diseases (35). This suggests to further expand the spectrum of research on the issue.

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