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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Surfing the third wave

Surfando a terceira onda

Pedro Giavina-Bianchi[1]

The COVID-19 pandemic reaches two years in March with the staggering figures, officially reported, of more than 400 million infections and about 6 million lives lost in all parts of the world.[1] In Brazil, 28 million infections and 650 thousand deaths have been confirmed so far, in addition to possible underreporting.[1] It will take a few decades to have a more realistic perception of the impact of the pandemic on public health, the economy and society, as well as the changes triggered by COVID-19 in our lives.

Health professionals and scientists have never studied, learned and worked so hard before. In PubMed, the term "COVID-19" was identified in 230 thousand publications, corresponding to more than 300 manuscripts per day. Many questions were answered and much knowledge gained. The well-deserved recognition also came, including the appreciation of Clinical Immunology and Allergy.

However, facing the pandemic also has its obscure pages written by: politicians who are not up to the challenges and demands required by the pandemic; opportunistic and irresponsible people; crooks who invent and spread false information; criminals who appropriate funds earmarked for health; among other evil forces. It is very sad and exhausting the energy wasted on unfruitful discussions, but they are necessary to better guide the population about the fact that COVID-19 is not only a "little flu", that many of the medicines used in the acute phase of the disease do not have scientific evidence, and that vaccines are safe and effective.

The influence of socioeconomic inequality and the heterogeneity of health systems was also evident on the outcomes of the pandemic. We analyzed the presence of antibodies against SARS-CoV-2 in healthcare workers with no previous history of infection and who were providing care to patients with COVID-19 during the first wave of the pandemic. We observed a seroprevalence of 14%. Factors statistically associated with higher seropositivity were: lower schooling (aOR of 1.93), use of public transport to travel between home and work (aOR of 1.65) and cleaner professionals or hospital security (aOR of 10.1).[2] Another study showed that

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mortality from COVID-19 in Manaus in people over 70 years old was double and triple compared to those observed in Rio de Janeiro and São Paulo, respectively.[3]

In the beginning, hygiene and social distancing measures were the interventions that contributed to the control of the pandemic. Subsequently, the anti-SARS-CoV-2 vaccination became the turning point in the fight against COVID-19. About 10 billion doses of vaccines have been administered worldwide, although heterogeneously distributed among different countries, with the lowest vaccination rates observed in Africa. In Brazil, approximately 75% of the population is fully vaccinated and 10% others received at least the first dose.[4]

As the months passed, but before the pandemic completed a year, we found that we would have more than one wave of coronavirus infections. At least three factors contribute to the resurgence of COVID-19 and with the increase in cases: decrease in hygienic habits and social distance; decreased immune response over time after natural SARS-CoV-2 infection or vaccination; and, mainly, the emergence of variants of the virus. While the vast majority of countries faced four distinct waves, in Brazil we are experiencing our third wave as a result of infections with the Omicron variant (Figure 1).[1] Here there was a combination of infections caused by more than one variant, in addition to observing greater circulation and impact of the Gamma variant (P1) in relation to Delta, during our second wave.

As with other viruses, SARS-CoV-2 undergoes random mutations of its genome and continually adapts to changes in the environment through the process of natural selection. Most mutations are neutral or harmful to the virus; however, few mutations can provide advantages, such as increased transmissibility, escape from the immune system of a host previously activated by vaccination or previous infections, resistance to antivirals, escape from diagnostic detection, among others. The World Health Organization (WHO) calls these SARS-CoV-2 variants with such characteristics as variants of concern (VOCs). Variants that have specific genetic mutations that can translate into adaptive advantages to the virus need to be monitored and are called variants of interest (VOIs). The WHO system decided to name variants after letters of the Greek alphabet in order

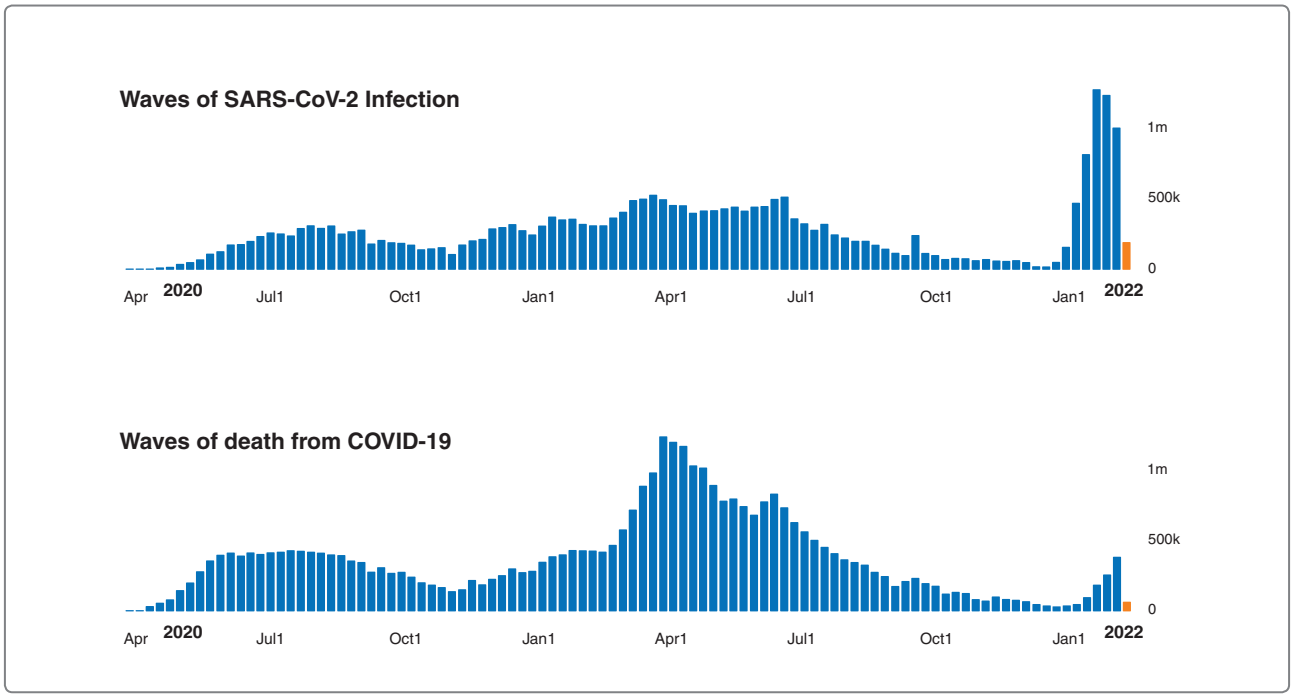

Figure 1
SARS-CoV-2 infections and COVID-19 deaths per week from 2020 to 2022
m = million, k = thousand.
Source: WHO (https://covid19.who.int/)

to facilitate the sharing of research knowledge with a wider audience, but also provide a platform to enable uniform global discourse around VOIs and VOCs, and avoid stigmatization of places where variants were discovered.

The Omicron variant of SARS-CoV-2 (B.1.1.529) was identified in Africa in late 2021. Compared to the other four previously identified VOCs (alpha, beta, gamma and delta), the Omicron variant is the strain with the highest number of mutations, with 97 mutations accumulated throughout the genome, including at least 33 mutations in the spike protein. Studies have shown that the different mutations of this variant confer increased infectivity with greater affinity of the virus for the ACE2 receptor and immune escape compared to the wild-type initial strain and the other VOCs.[5] Changes in viral antigenicity causes significant coronavirus evasion to the therapeutic monoclonal and polyclonal neutralizing antibodies induced by the original two-dose vaccine schedule. A third dose with mRNA vaccine rescues and extends neutralization.[6] The Omicron variant has become the dominant strain, accounting for record new infections per day worldwide, and brings additional challenges to the prevention and control of COVID-19.

Fortunately, we observed that, despite the rapid increase in new cases of SARS-CoV-2 infection with the circulation and dominance of the Omicron variant, there is not a proportional increase in the number of deaths. Scientific evidence in experimental animal models suggests that this variant has some intrinsic characteristics that make it less virulent, pathogenic and lethal. The Omicron variant has a lower capacity to cleave the spike protein and to bind to the human TMPRSS2 receptor, two properties that help in viral invasion, in addition to a lower activity of inducing multinucleated syncytia in host cells.[5,6] Consequently, there is less tissue damage compared to other variants, especially in human cells that express the TMPRSS2 receptor, such as pneumocytes.[2]

Which factor differentiates the third wave of COVID-19 from others? This time the virus is facing a population with some degree of immunity triggered by vaccination and/or previous infections by other variants. A study by the VISION network showed that the efficiency of mRNA vaccines in preventing infections by the Omicron variant that lead to hospitalization reaches 91% during the first two month of the third vaccine doses, and 78% after four months.[7] According to CDC data, at the end of 2021, the risk of individuals aged 18 years and over without vaccination of having a fatal infection with SARS-CoV-2 was 68 times greater than in those vaccinated with three doses.[8] Therefore, what is the main message to be highlighted and disseminated? Keep the vaccination for COVID-19 up to date to minimize the risks of contracting the disease and its serious evolutions.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Special Article

Latin American Guideline on the Diagnosis and Treatment of Ocular Allergy – On behalf of the Latin American Society of Allergy, Asthma and Immunology (SLAAI)

Diretriz Latino-americana sobre o Diagnóstico e Tratamento da Alergia Ocular – Em nome da Sociedade Latino-americana de Alergia, Asma e Imunologia (SLAAI)

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ABSTRACT

Ocular allergy, also known as allergic conjunctivitis, is an immunoglobulin E-mediated hypersensitivity reaction of the eye triggered by airborne allergens, primarily house dust mites and grass pollen. Symptoms usually consist of ocular or periocular itching, watery eyes, and red eyes that may be present year-round or seasonally. Ocular allergy has a high frequency, is underdiagnosed, and can be debilitating for the patient. It is potentially harmful to vision in cases of severe corneal scarring, and in most patients, it is associated with other allergic conditions, especially rhinitis, asthma, and atopic dermatitis. It is classified as perennial allergic conjunctivitis, seasonal allergic conjunctivitis, atopic keratoconjunctivitis, and vernal keratoconjunctivitis. Diagnosis seeks to identify the etiologic agent, and confirmation is given by conjunctival provocation testing. Treatment is based on avoiding contact with triggers, lubrication, topical antihistamines, mast cell stabilizers, immunosuppressants, and specific immunotherapy with the aim of achieving control and preventing disease complications.

Keywords: Ocular allergy, allergic conjunctivitis, rhinoconjunctivitis.

RESUMO

A alergia ocular, também conhecida como conjuntivite alérgica (CA), é uma reação de hipersensibilidade mediada por imunoglobulina E (IgE) do olho desencadeada por aeroalérgenos, principalmente ácaros da poeira doméstica e pólen de gramíneas. Os sintomas geralmente consistem em prurido ocular ou periocular, lacrimejamento e olhos vermelhos que podem estar presentes durante todo o ano ou sazonalmente. A alergia ocular tem frequência elevada, é subdiagnosticada e pode ser debilitante para o paciente. É potencialmente danosa para a visão, nos casos em que ocasiona cicatrização corneana grave, e na maioria dos pacientes associa-se a outros quadros alérgicos, principalmente rinite, asma e dermatite atópica. É classificada em conjuntivite alérgica perene, conjuntivite alérgica sazonal, ceratoconjuntivite atópica e ceratoconjuntivite vernal. O diagnóstico procura evidenciar o agente etiológico e a confirmação se dá pela realização do teste de provocação conjuntival. O tratamento baseia-se em evitar o contato com os desencadeantes, lubrificação, anti-histamínicos tópicos, estabilizadores de mastócitos, imunossupressores e imunoterapia específica com o objetivo de obter o controle e prevenir as complicações da doença.

Descritores: Alergia ocular, conjuntivite alérgica, rinoconjuntivite.

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Introduction

Ocular allergy, also known as allergic conjunctivitis (AC) is an immunoglobulin E (IgE)-mediated hypersensitivity reaction of the eye triggered by airborne allergens, primarily house dust mites and grass pollen. Symptoms usually consist of ocular or periocular itching, tearing, and red eyes, which may be present year-round or seasonally.[1]

Ocular allergy is highly frequent, underdiagnosed, and can be debilitating for the patient, and often challenging. It is potentially harmful to vision in cases where it causes severe corneal scarring, and in most patients it is associated with other allergic conditions, especially rhinitis, asthma and atopic dermatitis[1].

Eye anatomy

The eye in general can be divided into three parts: the outer layer (composed of the cornea and sclera), the middle layer (uveal-iris tract, ciliary body and choroid), and the inner layer, composed of the retina.

Conjunctiva

The conjunctiva is a thin, transparent mucous membrane that covers the sclera and also the eyelids, internally (a region known as the cul-de-sac).

It has rich vascularization, composed of connective and lymphoid tissue. Between the episclera and the conjunctiva there is Tenon's capsule, a thick collagenous membrane that surrounds the eye from the optic nerve to the limbus (Figure 1).

The first layer is the epithelial. Composed of goblet cells, single-celled glands that secrete mucin.

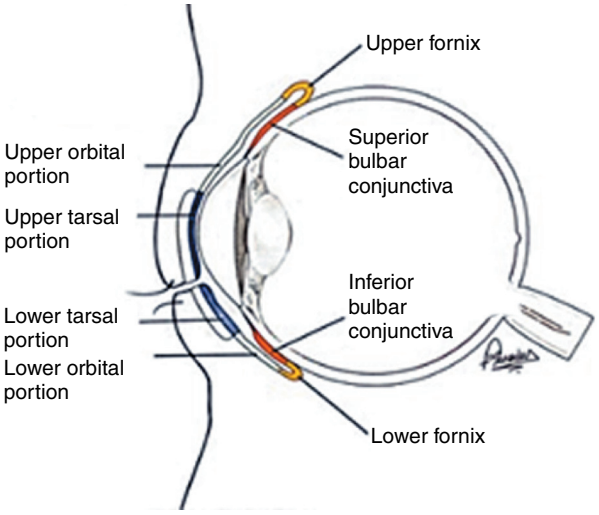


Figure 1
Anatomical aspect of the conjunctiva

Goblet cells can produce up to 2.2 mL of mucus per day. Mucus is essential for the integrity of the ocular surface as it lubricates and protects the epithelial cells. Mucin reduces the surface tension of the tear film to ensure its stability. Melanocytes: Melanocytes are mainly seen in the limbus, fornix, plica semilunaris, caruncle, and at the perforation sites of the anterior ciliary vessels. Sometimes they give the conjunctiva a brownish tinge. Langerhans cells: Langerhans cells are actually dendritic cells. Its main function is to process the antigen material and present it on the surface to other cells of the immune system. So, they function as antigen-presenting cells. The highest density of Langerhans cells was found in the tarsal conjunctiva, followed by the fornix and bulbar conjunctiva. The number of cells decreases with age.

The second layer is the substance itself. It consists of a superficial lymphoid layer and a deeper fibrous layer. It is rich in mast cells, lymphocytes, plasma cells and neutrophils.

Lymphocytes, mainly T lymphocytes, are found in abundance in the conjunctiva. They are present in the substantia propria and in the epithelium in a ratio of 2:3. Lymphoid aggregations similar to the mucosa-associated lymphoid tissue (MALT) found in the intestine and bronchi are also seen in the conjunctiva. These lymphoid aggregations consisting of T and B lymphocytes are known as conjunctival-associated lymphoid tissue (CALT).

Mast cells are basophils similar to granulocytic cells. The conjunctiva contains a large number of mast cells in the substantia propria. The total number of mast cells in the conjunctiva and adnexal tissue is approximately 50 million. Allergic conjunctivitis is a typical mast cell-mediated hypersensitivity reaction. In patients with allergic conjunctivitis, mast cells were also found in the conjunctival epithelium.

The third layer is fibrous, contains vessels, nerves and Krause's glands.

The function of the conjunctiva is to protect the ocular surface from external agents and maintain ocular lubrication, as the mucus produced by the conjunctiva helps to prevent eye dryness. It is divided into palpebral, bulbar and fornitial conjunctiva.

The palpebral conjunctiva is further subdivided into marginal conjunctiva, tarsal and orbital conjunctiva. The marginal conjunctiva is a transition zone between the eyelid skin and the conjunctiva proper. It begins in the intermarginal bands of the eyelid as a continuation of the skin. It consists of stratified epithelium. The marginal conjunctiva continues on the posterior surface of the eyelid for a short distance of 2 mm, to a shallow crease or crease, where it fuses with the conjunctiva proper. This sulcus is called the subtarsal sulcus.

The fornicial part is a fold that lines the cul-de-sac formed by the conjunctiva that covers the posterior surface of the eyelids to the conjunctiva that covers the anterior surface of the globe, this portion is thicker and weakly fixed to allow movement of the globe. It is divided into four regions, as described below.

The superior fornix lies between the upper eyelid and the globe. It extends 8 to 10 mm from the upper edge of the limbus. The inferior fornix lies between the lower eyelid and the globe. It extends to a distance of 8 mm below the bottom of the limbus. The lateral fornix is situated between the lateral corner of the eye and the globe. It extends a distance of 15 mm from the side of the limbus. The medial fornix is the shallowest and contains the caruncle and the plica semilunaris.

The bulbar conjunctiva is the thinnest of all parts of the conjunctiva, and so transparent that the sclera and underlying white vessels are clearly seen. It is loosely attached, except for a 3 mm zone near the limbus and near the insertions of the rectus muscles. The limbic conjunctiva is the part of the bulbar conjunctiva that covers the limbic region and fuses with the corneal epithelium.

Semiology of allergic conjunctivitis

Faced with a patient with suspected allergic conjunctivitis, we must establish the etiological origin of their condition and establish whether their symptoms are due to a primary condition or are secondary to other diseases, such as dry eye or blepharitis, which are frequent causes of conjunctival inflammation and whose treatment should focus on treating the underlying causes.[2]

For this, it is important to value the tools of the medical clinic, which include a good anamnesis, clinical exploration and the performance of complementary exams that allow us to establish an accurate diagnosis, and thus treat the patient correctly. The initial evaluation should include relevant aspects of the general eye examination and then focus on the specific examination of the allergic condition.

Anamnesis

You need to ask about the topics listed below.

- Duration of symptoms, their evolution and recurrence.
- Factors that exacerbate them.
- Single or two-sided presentation.
- Type of secretion: serous, mucous, purulent, filamentous, etc.
- Exposure to different environments and/or substances.
- Rubs ("friction") the eyes.
- Contact lenses: regimen use, lens type, hygiene, cleaning fluids, etc.
- History of allergies, asthma, eczema, atopy, etc.
- Topical and/or systemic drugs in use.
- Immune compromise: immunosuppression, chemotherapy, transplants, etc.
- Systemic diseases: atopy, Stevens Johnson syndrome, cancer, etc.
- Exposure to tobacco smoke: active or passive smoker, use of illegal drugs.
- Occupations and hobbies, exposure to air pollutants, travel, exercise habits, diet, etc.

Physical exam

The initial physical examination of the patient with allergic conjunctivitis includes assessment of visual acuity, external examination, and examination with a slit lamp.

It should include a detailed exploration of:

- regional lymphadenopathy: especially pre-auricular, in order to rule out infectious disease;
- skin: looking for signs of rosacea, eczema, seborrhea, etc.;
- abnormalities of the eyelids and appendages: inflammation, discoloration, ulcerations, nodules, loss of eyelashes, etc.;
- symptoms and signs: hyperemia, presence of follicles and papillae, adherence of eyelids, itching, irritation, pain, photophobia, blurred vision, epiphora, etc.

In addition, through the use of the slit lamp (biomicroscope), attention should be paid to:

- eyelid edges: inflammation, hyperedema or hypopigmentation, changes in the meibomian glands, keratinization, etc.;
- conjunctiva: laterality of symptoms, type of conjunctival reaction (follicular, papillary), distribution (diffuse, local), etc.;
- eyelashes: loss, trichiasis, secretions, parasites (demodex, lice);
- lacrimal system: puncture and channels;
- tarsal conjunctiva and fornix: follicles, scars, foreign bodies, etc.;
- bulbar conjunctiva: follicles, edema, nodules, chemos, laxity, papillae, ulcers, foreign bodies, keratinization, etc.

Complementary diagnostic tests[3]

In vivo diagnostic stains of the ocular surface

There are different substances with staining properties that can be used to study the ocular surface and that are very useful for the diagnosis and monitoring of patients with allergic conjunctivitis. These are fluorescein, rose bengal and lysamine green.

Target crops

Biopsy/brushing/shaved conjunctival

Blood/tear tests

Conjunctival provocation

Other diagnostic methods

Pathophysiology of allergic conjunctivitis

Allergic eye diseases are caused by direct exposure of the ocular mucosa to environmental allergens, which are dissolved in the tear and penetrate the conjunctiva to attach to IgE antibodies bound to the surface of mast cells.

Since the conjunctiva is an area of mucosa similar to the nasal mucosa, the same allergens that trigger allergic rhinitis may be involved in the pathogenesis of allergic conjunctivitis. Common airborne antigens, such as dust, fungi, pollen, and grasses, can cause the symptoms of acute allergic conjunctivitis, such as itching, redness, burning, and tearing.

In sensitized individuals, Th2 cells release proinflammatory cytokines (IL-4, IL-5, IL-13) that stimulate IgE production by B cells.[4]

The reactions produced can be divided into an initial stage lasting 20 to 30 minutes, which is related to the specific activation of conjunctival mast cells that causes their degranulation, releasing histamine, proteoglycans, proteases (tryptase, chymases), as well as the formation of mediators. lipids (prostaglandins and leukotrienes), interleukin (IL4, IL5, IL6, IL8, IL13) and tumor necrosis factor alpha (TNF-α). This reaction follows a late phase originated by the stimulation of epithelial cells and fibroblasts with the release of proinflammatory cytokines and chemokines and characterized by infiltration of inflammatory cells (neutrophils, eosinophils, lymphocytes and macrophages), with consequent persistent conjunctival inflammation. Unlike other allergic diseases, there is little eosinophilic infiltration in acute forms, which increase as the pathology becomes chronic.

Since the discovery of two functionally different subpopulations of CD4+ T cells (TH1 and TH2) some 30 years ago, it has quickly become evident that TH2 cells play a crucial role in the development of allergic respiratory tract inflammation. It has commonly been assumed that a th2 immune response and type I hypersensitivity form the basis of allergic conjunctivitis. The main factors contributing to the severity of AC are believed to be the allergen load on the ocular surface and locally produced specific IgE. Furthermore, there is a very significant correlation between the presence of allergen-specific IgE in tears and ocular allergy symptoms. This continuous release of histamine, together with the increased allergic load, leads to an expanding population of mast cells residing in the conjunctival tissue, thus perpetuating the allergic response. There is a general correlation between the degree of cellular infiltration and the severity of the disease. In addition, cell infiltration products are known to promote conjunctival irritation. In addition, connective epithelial cells and fibroblasts generate the allergic response, producing cytokines and other factors that maintain local inflammation and lead to tissue remodeling.[5]

It was found that new subpopulations of helper T cells–Th17 cells that produce interleukin-17 (IL-17) – play an important role in the pathogenesis of Th2-mediated conjunctivitis. Studies have shown that Th17 cells are involved in a variety of immune inflammation, including psoriasis, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, and asthma. However, the role of Th17 and IL-17 in allergic conjunctivitis is still unclear[6].

Epidemiology

There is a lack of international data on the prevalence of ocular allergy. In the United States, ocular allergy (OA) is estimated to affect 15-20% of the general population.[7] Ocular symptoms are believed to occur in 30-70% of patients with allergic rhinitis (AR),[8] and are more commonly triggered by intra-household than extra-domiciliary allergens.[9]

In Sweden, the estimated prevalence of allergic conjunctivitis was 19%, in Turkey, the prevalence of allergic conjunctivitis (AC) in children aged between 6 and 14 years was 7%. In Pakistan, the prevalence of conjunctivitis in patients aged 5 to 19 years was 19%.[10]

The prevalence of ocular allergy symptoms in Brazilian adolescents in 3,120 schoolchildren was 15.5%, considering the criterion of more than three episodes of ocular itching in the last 12 months. In this study, the prevalence of AC was higher in females, with 17.4% compared to males, with 13.3%. Genetic, hormonal factors and use of cosmetics are being investigated as possible causes.[11] In other studies, all eye allergy symptoms surveyed were significantly more prevalent in female adolescents, including eye itching, tearing, sensitivity to light, and feeling of grit in the eyes. Girls had more eye symptoms but lower sensitization rates than boys.[1,8]

In most studies, the most frequently related symptoms are: tearing (74%), followed by photophobia (50%) and foreign body sensation (37%). In these, the prevalence of allergic conjunctivitis was 20%, affecting more females than males (56% versus 46%; $p = 0.01$).[10] More recent studies show a trend towards a change in the prevalence of AC symptoms in relation to sex, being more frequent in childhood in boys and, after puberty, in girls.[8,9]

Allergic conjunctivitis (AC) is often underdiagnosed in patients with rhinitis and asthma. The diagnosis of conjunctivitis was recorded by the attending physician in 16% of 1549 asthmatics (mean age 4.3 years), however, 618 (44%) had at least one eye symptom that suggested ocular allergy (OA).[12]

AC alone has been estimated to be 6-30% of the general population. Seasonal allergic conjunctivitis is the most frequent form; however, studies from tertiary referral centers for ophthalmology report that the chronic forms, such as vernal and atopic keratoconjunctivitis, are the most frequently seen by ophthalmologists. A survey of 304 ophthalmologists showed that most patients with allergic conjunctivitis suffer from a few

episodes of mild, intermittent conjunctivitis annually. However, 30% of patients experience frequent episodes with intense and persistent symptoms. Treatment is often inappropriate.[9]

A study carried out in a Brazilian ophthalmology center evaluated 207 patients, of which 38% were diagnosed with spring keratoconjunctivitis; 39% as atopic keratoconjunctivitis; 13% as perennial allergic conjunctivitis and in 10% of patients there was no definite diagnosis. The presence of extraocular allergy was higher in patients with atopic conjunctivitis (91%), and lower in patients with spring (32%). The most intense symptoms were itching and tearing in patients with keratoconjunctivitis and there was a positive correlation between the intensity of symptoms and clinical signs.[13]

Triggers of conjunctivitis

The most common forms of allergic conjunctivitis (AC) are related to the type of allergen to which the patient was exposed and previously sensitized, and include perennial (PAC) or seasonal (SAC) allergic conjunctivitis. The difference between the two is given by the periodicity or persistence of symptoms. PAC is usually caused by indoor allergens such as house dust mites, pet dander, cockroaches, mold spores, or others. CAE is usually caused by pollen and is described more frequently in temperate climates, which are characterized by having four well-differentiated seasons during the year and presenting rains in the winter months, a climate present in Anglo-Saxon America, where a higher prevalence of SAC is reported.

The presence of pollens and other seasonal allergens such as extra-domestic fungi (eg *Cladosporium*, *Aspergillus*) depends on geographic and meteorological factors; mainly on the temperature and relative humidity of the environment. Minor household allergens, such as those from cats, dogs, and rodents, tend to become volatile and remain airborne longer, giving them the ability to produce severe or bothersome symptoms.[4] In contrast, house dust mites or cockroach allergens, due to their larger size, can only remain suspended for a few minutes in the air. Many patients are sensitized to more than one allergen, that is, they are polysensitized and may experience permanent symptoms with seasonal exacerbations. Being polysensitized seems to be associated with the persistence and severity of allergic diseases.[14]

The role of IgE-mediated allergy was clearly demonstrated in SAC and PAC, and two phases of inflammation were characterized. The initial phase, mediated by IgE, begins a few seconds or minutes after exposure to the allergen and lasts for 20 to 30 minutes. The late phase, which begins a few hours later, is responsible for ongoing inflammation, with persistent symptoms and risk of damage. Conjunctival allergen provocation tests are reproducible and used to confirm the diagnosis of AC by seasonal and perennial allergens, both in research studies and in clinical practice.

Ocular allergy to pollen in Latin America

Ocular symptoms (OS) associated with SAC occur when pollen dissolves in the tear film and passes through the conjunctiva. Twenty percent of individuals with pollinosis submitted to nasal pollen challenge present OS, suggesting that the same can occur without the direct contact of the allergen in the conjunctiva.[15]

Pollinosis is common in regions where there is a harsh winter, with low temperatures, followed by an exuberant spring (temperate or sub-tropical climate). Thus, in Latin America, it is prevalent in countries such as Argentina, Chile, Uruguay and southern Brazil (PR, SC, RS). However, it can be considered, eventually, also in tropical regions where there are high altitudes compensating for low latitudes.

Most studies focus on rhinitis, and few have examined ocular symptoms as an independent entity. OS is often described as nasal allergy symptoms, using the expression “allergic rhinoconjunctivitis” or simply “allergic rhinitis” or they combine all eye symptoms as OS.[16] This naturally complicates studies on the epidemiology of SAC, not only in Latin America, but also in other parts of the world.[17,18]

In Brazil, grass pollination is found basically in the southern states (PR, SC, RS), where the subtropical climate predominates, with well-defined seasons, unlike the rest of the country, with a tropical climate. Grasses are the main agent associated with changes in the external environment in recent decades, due to the increase in population and natural vegetation being replaced by agricultural and pastoral activities. Here, *Lolium multiflorum* (ryegrass) with intense allergenic activity can be included, in addition to *Cynodon dactylon* and *Paspalum notatum*.

Retrospective study on SAC, involving 876 patients with pollinosis in the area of Caxias do Sul, RS, Brazil, where all were sensitized to mixed grass pollen antigens, SAC occurred in 86.2% of cases, being classified as severe in 24.8%, with "symptoms difficult to tolerate interfering with daily activities and sleep".[19]

In the city of Bahía Blanca (AR), air pollen began to be recorded in 1995. Over the following years, intermittent and continuous aerobiological studies were carried out in the cities of Buenos Aires, Bariloche, Córdoba, Neuquén (Valle del Rio Negro), Mar del Plata, Paraná, Santa Fe, Santa Rosa, Mendoza and, recently, in the city of Trelew. In the city of Bahía Blanca, as in Buenos Aires, the skin sensitivity through allergen tests corresponded to the aerobiological counts and the geographic-regional representation of the studied species. Currently, data collection from some cities follows, and new challenges and questions are opening to be revealed in future research.

Many of the studies carried out so far can be found summarized in the Alergopalinological Atlas of the Argentine Republic, published in 2019 by the Argentine Association of Allergy and Clinical Immunology.

The prevalence of different plant species is mentioned according to aerobiological studies. Allergic skin sensitivity to pollen, or serological sensitivity, is associated with allergic rhinoconjunctivitis and/or asthma.

The subdivision of *Gymnospermae* in terms of aerial pollen is represented by the order *Pinales*, which comprises the *Cupressaceae*, which are a family of conifers of the order *Cupressales* of great economic and landscape importance worldwide. These species are present in almost all cities in Argentina and stand out mainly in the Patagonian mountain range, being one of the most abundant pollen species in the city of Bariloche. *Nothofagus*, also known as "southern beeches", is a genus of several species of trees and shrubs native to the southern hemisphere of America and Australasia.

In general, *Cupressaceae* pollen sensitivity represents low sensitivity to skin tests; however, they become symptomatically important, as they are the first pollens to appear in abundance. The incipient appearance of these pollens causes patients to experience the first symptoms of the pollen season, which sometimes cause the priming effect, which is increased sensitivity to other species that flower later. In addition, in the *Cupressaceae* family, there are many species that maintain a pollination of several weeks. In the study, there is allergic sensitivity to *Nothofagus* proteins, which still needs to be determined.[20-23]

The order *Lamiales,* which includes several subfamilies, is represented by the *Oleaceae*, consisting of varieties of *Fraxinus spp.* (ash), with high air pollination, but low skin sensitivity. Here, *Olea europae* (olive tree) and several species of *Ligustrum* (privet) were found, which represent a large number of pollen grains and accompany the flowering of grasses such as that early in the beginning of summer. Privet and olive showed significant sensitization in allergic patients both regionally and globally. The olive tree is important in the Bahía Blanca region for its use at olive oil production and in urban afforestation. The same occurs in the city of Mendoza, predominantly for commercial purposes. High sensitization to olive tree pollen was demonstrated in the city of Bahía Blanca. In other cities, such as Rosario and Buenos Aires, privet represents a higher prevalence in skin sensitivity and air pollen.[24-27]

The order of *Proteales*, represented by *Platanus spp.*, is an abundant species in the cities of Buenos Aires and La Plata, mainly in urban linear trees. Despite having a moderate amount of grains in the air, they showed that pollen causes greater sensitivity in patients with allergic rhinoconjunctivitis in CABA and, in the city of London, is responsible for moderate to severe allergic symptoms. Its pollination period (on the order of two weeks) may be a factor that aggravates or induces a greater sensitization to other pollens, as it seems to give continuity to the flowering of ash and cypress trees.[28,29]

The order *Fagales*, within the family *Betulaceae*, representatives such as Castanea native (chestnut, *Quercus spp.* - oak) and *Alnus spp.* (alder), have pollination periods of less than one month, and in some cases, only two weeks. Therefore, none of them represent a persistent causative agent of rhinoconjunctivitis, however in polysensitized patients, due to cross-reactions, they may contribute to the exacerbation of allergic symptoms.[30]

Poaceae, or ragmines, are the most representative pollens in aerobiological studies in almost all cities in Argentina, as well as in the rest of the world, where they are shown to be the most common cause of seasonal allergies.[31]

It is considered to be the most common cause of rhinoconjunctivitis symptoms, especially in early

summer, caused by the *Pooideae* subfamily and then, in late summer, by the flowering of the *Chloridoideae* and *Panicoideae* subfamilies. The *Chloridoideae* subfamily is well represented by *Cynodon dactylon*, and also by *Distichils spicata* in the city of Bahía Blanca, which demonstrated cutaneous sensitivity in patients with allergic rhinoconjunctivitis. This last herb is a perennial herbaceous species, native to America, Canada, Chile and Argentina. The second family, *Panicoideae*, is represented by *Paspalum notatum* and *Sorghum halepense*, called "elatior" in modern taxonomic classification. The species of the last two subfamilies are called "subtropical", have different photosynthesis and phenological behavior, with flowering later than that of the *Pooideae*, which are called "temperate". Subtropical grasses have a significantly similar grade to temperate grasses. Recent studies of these subtropical species describe their own allergens that they do not share with the *Pooideae*.[32,33]

There is a wide variety of species that do not belong to trees and grasses, which is often called a "weed group", or weeds, in English. This is a group that encompasses a large number of genera, making it difficult to use a single taxonomic name. In this group are the *Asteraceae*, the Angiosperm family with the greatest richness and biological diversity. In the Republic of Argentina, are prevalent, especially in regions pampeana and patagonia, on the east coast of the continent: *Salsola kali*, *Kochia scoparia*, *Chenopodium spp.*, and others stand out among them.

In other Latin American countries, several researchers can be listed who show results in aerobiological counts and in studies of skin sensitization to pollens. In Chile, Pedro Mardones demonstrated, in several studies, the prevalence of trees and grasses. In Uruguay, there are several multidisciplinary works on allergenic flora and air pollen in the cities of Montevideo and Concepción del Uruguay.[34] In Mexico, there is the Center for Aerobiological Studies that has several centers where they monitor the pollen in the air of different species. It should be mentioned that this Latin American country is located in the northern hemisphere.

Countries like Colombia, along with Venezuela and Ecuador, are located in Ecuador proper, and have a great plant diversity as a heritage, which is under study.

In Paraguay, for three years, in the city of Asunción, a group of allergists formed by Perla Alcaráz, Cinthia Perez, Rosmary Stanley and Pedro Piraino, has studied air pollen and presented important data such as the moderate presence of pollen grains along the year. The pollen of the species *Cecropia adenopus* stands out especially, which was previously classified as a tree of the *Moraceae*, but is currently in the family *Urticaceae*. Also called ambay, it is a tree belonging to the botanical family of *Cecropiaceae*. It grows in the marginal forests of the rivers of Brazil, in the Amazon region of Bolivia and Paraguay and in the northeast of Argentina. It can measure up to 15 meters in height, and its trunk has a diameter between 20 and 30 centimeters. The pollen of this species is the most prevalent in quantity and frequency, with an almost annual persistence. To date, there are no serological or skin sensitivity studies.

In Peru, Oscar Calderón has found pollen in several cities for over a year, the results of which will be published soon. Similarly, in Bolivia, Fabiola Ramallo recently started to monitor pollens from Santa Cruz de la Sierra. In a short time, these publications should be complemented with studies of allergic sensitivity to the pollens found.

Climate change, pollution and conjunctivitis

The ocular conjunctivae are richly vascularized and are constantly exposed to external factors. They are vulnerable to the adverse influence of bacteria, viruses, allergens, chemicals, and air pollutants, which can cause their inflammation.[35] Air pollution is one of the most important risk factors affecting people around the world,[36] especially the most vulnerable: children and the elderly.[37]

The eyes are vulnerable to air pollution, either by acute, short-term, or chronic exposure.[38] individuals who live in areas with a high level of pollution experience symptoms of conjunctivitis more often. Human eyes are only protected by a thin layer of tear film, and innervations present on the ocular surface are very sensitive to environmental chemicals.[39,40]

Air pollutants include: particulate matter (PM10, PM2.5), diesel exhaust particles (DEPs), gases (NOx, SO_2, O_3, CO and oxidants) in addition to organic compounds and metallic particles.[8] In large cities, the main source of pollutants is related to motor vehicle traffic, and the resulting pollutants (TRAPs) are a mixture of combustion-derived PM, DEPs and gaseous emissions, NOx, CO, oxidants and organic aerosols.[41]

The increase in exposure to high concentrations (> 10 μg/m^3) of CO, NO_2, SO_2, O_3 or PM, whether in the days before or on the day of emergency room care, has been accompanied by a significant increase in visits for conjunctivitis, as well as symptoms.[42-47] In addition, hot weather and strong winds, combined with the spread of pollen allergens, can potentiate these effects leading to ocular surface instability.[48]

Generally, the most severe forms of conjunctivitis are associated with greater damage related to environmental pollution.[41] Recent meta-analysis confirms air pollution as an important factorrisk for conjunctivitis. NO_2 followed by O_3 were related to the greatest impact, especially among women under 18 years old.[49]

Weather factors (humidity, temperature) can also affect eye health. Exposure to a controlled environment with low humidity has documented adverse effects on the evaporation rate, lipid layer thickness, stability and tear production on the tear film, generating significant ocular discomfort.[50] On the other hand, increased temperature may not directly induce eye discomfort like low humidity, but may lead to exacerbation of allergic conjunctivitis by raising pollen levels.[51]

Relationship between conjunctivitis and pollution: mechanisms

To date, the underlying pathophysiological mechanisms of conjunctivitis caused by air pollutants are still unclear. As human eyes are directly exposed to air pollution, some studies speculate that PM2.5[46,52,53] and PM10[54] particles could easily cause intraocular epidermal cells to become unadaptable, leading to cell death and inflammation tissue.[49]

Second, NO_2 and O_3 have strong oxidative stress effects, which can stimulate conjunctival cell inflammation[49]. NO_2 is an acidic gas, which upon entering the eye, easily changes the environment of the inner cells of the ocular epidermis, disrupts the function of eye cells, and causes inflammation.[55,56] It is plausible that the association between air pollution and the risk of conjunctivitis events is due to these potential mechanisms.[49]

Chronic exposure to air pollutants favors cellular damage, such as hyperplasia of the goblet cells of the epithelium on the ocular surface.[57] Exposure to DEPs increases the expression of cytokines, chemokines and growth factors in conjunctival epithelial cells, which means activation of conjunctival inflammation.[39]

All of these pollutants can directly damage the ocular surface by reducing the pH of the tear fluid or by oxidizing it. In addition, PM10 can cause dysregulated T cell responses and inflammation,[41] and is significantly associated with reduced Treg cell counts, as demonstrated in a birth cohort stud.[58]

Diseases associated with air pollution

Conditions associated with air pollution are mainly: eye irritation, discomfort, conjunctivitis, red eye syndrome and meibomian gland dysfunction.

Dry eye syndrome

Dry eye syndrome (SOS) is the most common eye condition[59] and has a frequency of 11% to 58%. Many factors can influence the occurrence of SOS symptoms: smoking, alcohol consumption, low humidity, air pollution, exposure to sunlight, sociodemographic factors, past eye surgery anduse of contact lenses (in this group the influence of air pollution is more noticeable).[60]

Among individuals living in large cities, tear film disorders occur more frequently.[61] A study carried out in Brazil showed an association between exposure to high concentration of NO_2 andtear film disorders, in addition to a sensation of ocular discomfort.[40]

Meibomian gland dysfunction

Meibomian glands are sebaceous glands, whose excretion is the outermost component of the tear film and prevents tears from evaporating from the surface of the eye. The symptoms reported by patients with Meibomian Gland Dysfunction (MGD) are: eye itching, feeling of discomfort, feeling of dry eyes and redness of the eyes. GMD is one of the most common causes of SOS.

Many factors influence gland function disorders, among others: chronic blepharitis, contact lens wear, Sjögren's syndrome, acne rosacea, humidity and air quality,[57] particularly high concentrations of NO_2.[40]

Blepharitis

Blepharitis is an inflammatory condition that covers the edges and skin of the eyelids, tarsal glands, and hair follicles eyelashes. The cause of blepharitis is mechanical (eg by dust, smoke), and also by bacterial infection. The connection between blepharitis and air

pollution is not well examined. A study carried out in Brazil showed a significant correlation between exposure to high concentrations of PM10 and CO in the air and an increased incidence of blepharitis on the day of exposure.[62]

Influence on the cornea

The cornea is constantly exposed to external factors such as atmospheric pollutants, ultraviolet radiation and cigarette smoke. Oxidative stress, which is an effect of these factors, favors corneal damage and visual impairment. PM2.5 concentrations between 20 µg/mL and 200 µg/mL are genotoxic, stimulate DNA damage and decrease the efficiency of corneal epithelial cells.[52]

Cataract

The frequency of cataracts is higher in developing countries, and the factors that help their formation are: age, sex, active smoking, exposure to ultraviolet radiation and diabetes. Individuals exposed to domestic burning of biomass (coal, wood, animal feces), especially for cooking, had a higher frequency of cataracts, especially among women[63]. In the case of using stoves powered by liquefied petroleum gas or biogas, the risk of cataracts was much lower. Lack of ventilation in the kitchen was an independent risk factor for cataract.[64]

Influence on retinal microcirculation

Air pollution has been identified as an independent risk factor for the development and progression of cardiovascular disease, however its influence on microcirculation is still poorly studied. Compared to changes in coronary macro and microcirculation, pathological lesions in the retinal vessels are justified, indicative of atherosclerosis.

A Brazilian study evaluated the association between air pollution and retinal vessel narrowing and demonstrated a narrowing of the central retinal arteriolar equivalent (CRAE) of 0.8 µm in response to chronic exposure (2 years) to PM2.5 (3 µg/ m^3) in more than 4,500 individuals.[65] At acute exposure, on the day before the evaluation, the highest concentration of PM2.5 (7 µg/m^3) determined a narrowing of the CRAE by 0.4 µm. Louwies et al.[66] measured retinal vessel diameter in 85 healthy subjects and assessed how short-term exposure to PM10 and carbon dust affects them. CRAE narrowing by 0.93 µm and central retinal venular equivalent (CRVE) narrowing by 0.86 µm was observed with every 10 µg/m^3 increase in PM10 concentration.

These findings draw attention to the fact that not only chronic but also short-term exposure to air pollutants causes abnormalities in the retinal microcirculation, which can cause disturbances in retinal nutrition and oxygenation, which can lead to visual impairment. Retinal microcirculation disorders may also impact cardiovascular incidents in the future.[38]

Comorbidities

AC can have a significant impact on quality of life, affecting sleep and causing emotional problems and impairment of activities of daily or social living, such as productivity at work or school performance. AC occurs concurrently with allergic rhinitis (AR) and other allergic diseases in most patients, at least 60% of patients with allergic conjunctivitis may have rhinitis.[67] Ocular symptoms may also be present without nasal involvement in 2 to 7% of patients with AC.[68,69] Asthma and atopic dermatitis are other common comorbidities. In adolescents, most epidemiological data, including data from different phases of the International Study of Asthma and Allergies in Childhood (ISAAC), associate ocular and nasal symptoms, making it difficult to separate the prevalence of AC from allergic rhinitis.

A study carried out in Shanghai analyzed the prevalence of symptoms of ocular allergy, allergic rhinitis, asthma, atopic dermatitis and sensitization to dust mites, pollen and food in a population of children and adolescents. The overall prevalence of symptoms of AR, diagnosed asthma, and diagnosed atopic dermatitis was 40.4%, 11.6%, and 16.7%, respectively. Young children had a higher prevalence of being diagnosed with allergic rhinitis and atopic dermatitis than adolescents. There were gender-associated differences in the prevalence of allergic rhinitis and asthma among young children, but not among adolescents. Sensitization to mites, food, and pollen was associated with a higher prevalence of allergic diseases.[70]

A survey conducted by the American College of Allergy, Asthma and Immunology found that 35% of families surveyed had allergies, of which more than 50% reported associated eye symptoms.[71] The importance of AC is mainly due to its frequency, which varies from 5% to 22% of the population.[72]

A study carried out by Geraldini M. et al., which included 3,120 patients between 12 and 18 years of age, found a prevalence of symptoms of allergic conjunctivitis of 20.7%. At least one comorbidity (asthma, rhinitis, or atopic eczema) was reported by 75.3% of children with AC. Rhinitis was the most frequent comorbidity (64.6%). Asthma appeared in 31.4%, and atopic eczema in 13.1%. The percentage of children with none, one, two or three comorbidities related to allergic conjunctivitis was 24.7%, 44.7%, 27% and 3.6%, respectively. Those patients with perennial symptoms were more frequently associated with the three comorbidities compared with those with seasonal symptoms (66.7% versus 56.9%; p = 0, 034), while asthma and topical eczema did not differ between the two groups (33.1% versus 24.8%; p = 0.062) and (12.3% versus 16%; p = 0.25), respectively. The probability of an adolescent with AC having asthma, rhinitis and atopic eczema was (OR = 5.7; 95% CI: 4.5 to 7.1); (OR = 3.6, 95% CI 3.0 to 4.3) and (OR = 2.6, 95% CI 2.0 to 3.5), respectively. The association between asthma and AC was greater among those with AC and rhinitis than among those with AC alone (36.8% versus 20.5%; p < 0.01).[1]

Classification of allergic conjunctivitis

To date, several methods and nomenclatures have been proposed to classify the different forms of ocular hypersensitivity, the most accepted and used classification being adopted by the European Academy of Allergy and Clinical Immunology (EAACI),[73] which encompasses two groups: hypersensitivity non-allergic ocular and ocular allergy, which in turn is subdivided into IgE-mediated forms – such as seasonal (SAC) and perennial (PAC) allergic conjunctivitis and more than half of the cases of vernal (VKC) and atopic (AKC) keratoconjunctivitis) – and non-IgE-mediated forms, which include some cases of the latter two forms, as well as contact blepharoconjunctivitis (CBC) (Figure 2).

The different ocular hypersensitivity disorders range from mild situations, usually intermittent and with acute symptoms (SAC, PAC and CBC) to moderate to severe forms, usually chronic and that can affect vision (giganto-papillary conjunctivitis, VKC and AKC).

The main differentiating characteristics of the different ocular hypersensitivity disorders are summarized in Table 1.

Allergic conjunctivitis

Allergic conjunctivitis is the most frequent form of ocular allergy and involves IgE-mediated mechanisms, causing bilateral ocular symptoms that are usually associated with the presence of rhinitis. The most characteristic symptom is ocular pruritus, and tearing, conjunctival hyperemia, eyelid edema and mild papillary hypertrophy of the tarsal conjunctiva may also occur.[73] Corneal involvement is rare, but blurred vision may occur.[8]

Depending on the frequency of symptoms, it is subdivided into seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC).

In SAC, symptoms are intermittent and more prevalent in spring and autumn, when pollen levels are highest. In PAC, symptoms are persistent and arise related to exposure and sensitization to perannual allergens, such as dust mites, dust mites and fungi, or due to the presence of multiple sensitizations.

Vernal keratoconjunctivitis

Vernal keratoconjunctivitis (VKC) is a rare, persistent and severe form of ocular allergy, which occurs mainly in areas with a warm climate, such as the Mediterranean basin, North Africa and the Middle East. It is typically seasonal (spring to late summer) and most commonly affects male children (male/female sex ratio 3/1), aged 4-12 years, disappearing after puberty. Approximately 50% of patients have no history of atopic disease or allergic sensitization, which suggests that VKC is not a fully IgE-mediated pathology.[8]

The first symptom to appear is intense bilateral eye itching, usually triggered by non-specific stimuli such as wind, dust, strong light or physical exertion, followed by extreme photophobia, burning and foreign body sensation and, often, blurred vision. Conjunctival injection, ptosis, mucous and creamy secretion and blepharospasm are observed.

There are three major forms of the disease: tarsal, limbic and mixed. In western countries, the tarsal form is the most frequent, while in subtropical countries the limbic form, also known as tropical endemic limboconjunctivitis, predominates. The tarsal form is characterized by the presence of giant papillae (between 7 and 8 mm in diameter) in the upper tarsal conjunctiva that resemble cobblestones, infiltrated by fibrin and mucus (pseudomembrane). In the limbic form, there are limbic papillae with white outgrowths

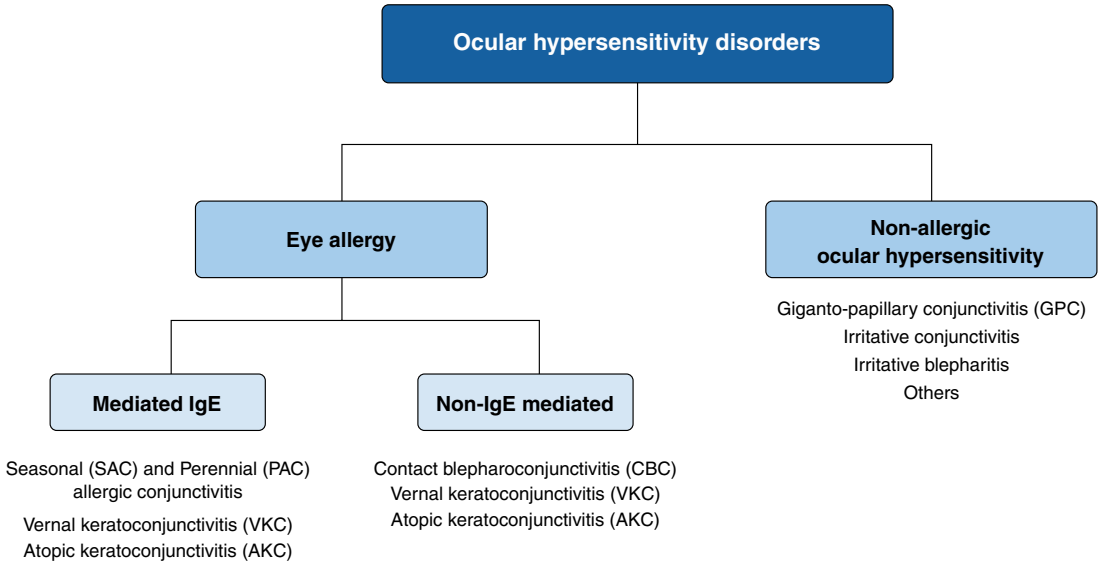


Figure 2
Classification of ocular hypersensitivity disorders
Adapted from Leonardi A et al.[73]

at the apexes, with a gelatinous appearance – Horner-Trantas nodules. Corneal involvement arises: punctiform keratopathy or round "escutcheon" ulcer, and there is a high incidence of keratoconus in these patients, and the most severe cases can lead to blindness.

Atopic keratoconjunctivitis

Atopic keratoconjunctivitis (AKC) is a chronic inflammatory ocular pathology, with bilateral involvement in the eyelids, conjunctiva and possibly the cornea. It corresponds to the entity with the highest risk of blindness and occurs in adults (18-50 years) who have systemic manifestations of atopy, namely atopic dermatitis. Its prevalence in patients with atopic dermatitis varies between 20% and 77%, and corresponds to one of the most serious ophthalmological complications of atopic dermatitis. There is usually a family history of other atopic diseases with elevated serum IgE levels. In its pathogenesis, IgE, Th2 and Th1 mediated mechanisms are involved. Unlike VKC, which rarely goes beyond 5-10 years of evolution, AKC can last for decades.

Clinically, it is similar to vernal conjunctivitis, with formation of (minor) papillae on the upper tarsus. A characteristic sign is eyelid eczema which tends to harden and crack, the eyelids are often inflamed, macerated and crusted – chronic blepharitis. It may be associated with eyelid colonization by *Staphylococcus aureus* and meibomian gland dysfunction. The development of keratopathy with neovascularization is particularly severe and is relatively frequently complicated by cataracts, herpes simplex, keratoconus, chronic blepharitis, conjunctival fibrosis, and retinal detachment, with sustained deterioration of vision.

Contact blepharoconjunctivitis

Contact blepharoconjunctivitis (CBC) is a form of non-IgE-mediated ocular allergy in which contact dermatitis occurs on the eyelids, with or without extraocular dermatitis and with possible involvement of the conjunctiva. It often affects middle-aged or older women.[9]

It arises associated with the repeated use of eye medication "drug conjunctivitis", due to toxicity or contact sensitization to the constituents of the drugs, usually preservatives. In addition to the constituents and preservatives of eye topicals, also various cosmetic products and metals may be involved as causal agents. Cosmetics applied to the hair, face or nails can be inadvertently transferred to the eyes, causing sensitization, sometimes without any symptoms at the point of origin, where the epidermis is thickest.

The most frequent symptoms are itching and burning sensation in the eyelids, with detection on physical examination of edema, erythema, eczema or lichenification of the eyelid skin, conjunctival hyperemia and papillae.

Table 1
Characteristics of main ocular hypersensitivity disorders

	SAC	PAC	VKC	AKC	CBC	GPC
Incidence	++++	++	+	+/–	–	–
Presentation	Intermittent	Persistent	Persistent with exacerbations flashing	Chronic	Chronic with exacerbations flashing	Persistent
Mechanism immunological	IgE	IgE	IgE/Non-IgE	IgE/Non-IgE	No IgE	Not allergic
Clinical context	Rhinitis allergic	Rhinitis allergic	Atopy	Dermatitis atopic	+- dermatitis contact at other places	No atopy
Age/Sex	Start childhood/ young adult	Start childhood/ young adult	++ Children sex M (M/F = 3/1); resolution > 20	++ Adults (18-50 years old)	++ Women middle-aged or older (F/M = 2/1)	–
Eyelids	Edema	Edema	Edema, ptosis	Eczema, blepharitis	Eczema	–
Conjunctivitis	Papillae	Papillae	Papillae giants	Papillae giants	Hyperemia	Papillae giants
Cornea	–	–	Nodules of Horner-Trantas, ulcers, CPS	Nodules of Horner-Trantas, ulcers, CPS	–	Rare

SAC = seasonal allergic conjunctivitis, PAC = perennial allergic conjunctivitis, VKC = vernal keratoconjunctivitis, AKC = atopic keratoconjunctivitis, CBC = contact blepharoconjunctivitis, GPC = giganto-papillary conjunctivitis, CPS = superficial punctate keratitis.

Non-allergic ocular hypersensitivity

Non-allergic ocular hypersensitivity includes several entities, namely giganto-papillary conjunctivitis (GPC), irritative conjunctivitis, irritative blepharitis, among others.

Giant papillary conjunctivitis

Giant papillary conjunctivitis (GPC) arises in the context of non-allergic hypersensitivity to products that chronically contact the ocular surface, most often contact lenses and their cleaning products and preservatives, ocular prostheses or postoperative sutures. The mechanism involved is related to chronic mechanical trauma.

Clinically, it resembles other forms of ocular hypersensitivity, and may cause symptoms of eye itching, foreign body sensation, blurred vision and production of mucous secretions. Sometimes there is a worsening of symptoms in the spring season. The patient develops a papillary reaction in the upper eyelid (with or without keratopathy), which is more common with soft contact lens wear (5-10%) compared with hard contact lenses (4%).

Usually, GPC resolves when contact lens wear is discontinued or when the foreign body that is in contact with the ocular surface is removed.

Diagnosis of allergic conjunctivitis

Serological and skin tests in the assessment of ocular allergy

The diagnosis of different conjunctivitis is clinical. The etiology is determined by screening for aeroallergen-specific IgE. In vivo test, allergic skin prick test, must be performed by a trained professional, being easy to perform, low cost, high sensitivity and specificity. Specific IgE is identified for mites, pollens, animal epithelia, cockroaches and fungi. In vitro serum specific IgE testing can also be used to identify allergy to the same aeroallergens as the skin test. More recently, single or multiple IgE detection platforms have been developed for specific proteins from allergen sources, called components, and this has brought us closer to Precision Medicine. There is a need for a specialized laboratory, higher cost, but also with good sensitivity and specificit.[74]

Skin prick tests are still the main tool for diagnosing the allergic phenotype of airway disease in the allergist's daily practice. Total serum IgE, in turn, seems to be correlated with the complexity of the IgE repertoire,[75] and specific serum IgE is very useful in the pediatric population and as an adjunct or replacement to skin tests.[76]

However, the vast majority of commercially available diagnostic tests and vaccine extracts use solid phase allergens obtained from the original source through protein extraction and purification. The availability of a broad panel of recombinant or highly purified allergenic molecules for in vitro diagnosis has profoundly changed the basic knowledge in the area, but also the conduct in clinical practice.[77] Furthermore, it is now possible to define the patient's IgE repertoire using these species-specific and cross-reactive allergenic molecules.[78] This has become a brilliant solution, particularly in so-called “polysensitized” patients, and not only in food allergy or hymenoptera, but also in respiratory allergy.[76] Until the present moment, There are few data in the literature regarding the use of component resolved diagnosis (CRD), particularly with the multiplex technology, in allergic diseases of the upper airways (AR and allergic conjunctivitis), especially with regard to the so-called “perennial allergens”, such as mites, fungi and animal epithelium. In this section, we will review the available publications on CRD in allergic rhinoconjunctivitis (RCA), particularly studies on the sensitization profile in these patients, what there is data divided by allergen sources, and some news on methods that can help in the management of more severe conjunctivitis. by detecting specific IgE in the tear. Especially with regard to so-called “perennial allergens”, such as mites, fungi and animal epithelia. In this section, we will review the available publications on CRD in allergic rhinoconjunctivitis (RCA), particularly studies on the sensitization profile in these patients, what there is data divided by allergen sources, and some news on methods that can help in the management of more severe conjunctivitis. by detecting specific IgE in the tear. Especially with regard to so-called “perennial allergens”, such as mites, fungi and animal epithelia. In this section, we will review the available publications on CRD in allergic rhinoconjunctivitis (RCA), particularly studies on the sensitization profile in these patients, what there is data divided by allergen sources, and some news on methods that can help in the management of more severe conjunctivitis. by detecting specific IgE in the tear.

Sensitization profile in patients with rhinoconjunctivitis

As we have already highlighted, the literature is scarce in relation to RCA, in particular with regard

to the sensitization profile of this population using this method. Two recently published series in Asian populations evaluated the multiplex component IgE detection technique in atopic patients and compared it with traditional skin tests or the FEIA method.[79,80]

In the Singapore study, atopics with RA, asthma and AD were evaluated and it was found that sensitization to mites was associated with the RA phenotype, while asthma and AD did not have a predominance of a single Ag. Furthermore, the ISAC did not prove to be useful as a screening tool if the major suspicion (clinical or epidemiological) was monosensitization.[80] In the survey carried out in Korea, it was shown that the agreement between ISAC and FEIA is quite high for *Dermatophagoides* mites, but the same was not true for birch pollens and the fungus *Alternaria alternata*, for which the sensitivity of FEIA was higher to ISAC.[79]

In a British cohort of live births, reassessed at the age of 11 years, the multiplex technique was used to assess patterns of sensitization and its relationship with atopic diseases presented by children. It was found that children with rhinoconjunctivitis were more sensitized to mites and pollens, while asthmatics were sensitized not only to these two sources of Ag, but also to animal epithelia. Finally, those with eczema were more sensitized to eggs and bovine Ag.[81]

A study in elite athletes participating in the Beijing Olympics evaluated 72 polysensitized individuals (defined based on skin test results) using the ImmunoCAP-ISAC method. The athletes were classified into four groups according to the clinical picture presented: allergic rhinoconjunctivitis, asthma, food allergy and asymptomatic. In addition to being useful in differentiating true polysensitized patients from those with positive cross-reactivity Ag tests, the method confirmed that the rhinoconjunctivitis phenotype was more associated with monosensitization, whereas asthmatics were more frequently polysensitized.[82]

In a retrospective study carried out in the western part of the Czech Republic, using the ImmunoCap-ISAC® multiplex platform for the detection of specific IgE, the results of 1,331 patients treated between December 2011 and June 2013 were analyzed. Results show that in 826 patients with a median age of 32.6 years old, the samples tested positive for at least one pollen-derived component, 62% of them were diagnosed with rhinitis. The highest level of sensitization was to grass components (81%), with Phl p 1 (69.6%) being the most frequent, followed by components derived from *Betulaceae* (54.8%), where Bet v 1 (54 .2%) was the most prevalent. Sensitization to components derived from *Cupressaceae* (14.1%), *Oleaceae* (10.8%) and Pla a 2 trees (15.5%), were less prevalent.[83]

In a study with 120 atopic patients with rhinoconjunctivitis and/or asthma, the microarray technique (ISAC CRD 103) was compared with FEIA (ImmunoCap) for the diagnosis of allergy to grass and cypress pollens. Both microarray and Cap showed high sensitivity (S) and specificity (E) in detecting grass allergy (ISAC: S = 97.7% and E = 92.3%; Cap: S = 95.3% and E = 96.1%) showing agreement between the two techniques. In the detection of allergy to cypress, ISAC showed similar sensitivity to Cap, but higher specificity (ISAC: S = 91.7% and E = 91.3%; Cap: S = 91.7% and E = 80.4%, p = 0.034).[84]

In South Korea, 168 adult patients with allergic rhinitis were evaluated and the detection of specific IgE by ImmunoCap and ImmunoCap-ISAC® for birch and mugwort pollens, among other aeroallergens, was compared. ImmunoCap sensitivity for birch was 86.9% versus 43.6% for ImmunoCap-ISAC®,κ = 0.511. In the case of mugwort, the sensitivity by ImmunoCap was 92.3% against 69.2% by ImmunoCap-ISAC®, κ = 0.670.[79]

In a study of 101 adults with rhinoconjunctivitis in Germany, the detection of specific IgE for 8 grass components (rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5b, rPhl p 5b, rPhl p 6, rPhl p 7, rPhl p 11 and nPhl p 4) single and multiplex platform, ImmunoCap and ImmunoCap-ISAC®. The correlation coefficient was significant in seven components [0.88 (rPhl p 1), 0.96 (rPhl p 2), 0.70 (nPhl p 4), 0.94 (rPhl p 5b), 0.92 (rPhl p 6), 0.85 (rPhl p 11), and 0.78 (rPhl p 12)], the exception being rPhl p 7.[85]

In Curitiba, 101 children with allergic rhinitis underwent skin test for *Lolium multiflorum* pollen, among other aeroallergens, and specific IgE measurement by multiplex platform ImmunoCap ISAC version 103. Allergic sensitization to *Lolium multiflorum* pollen determined by allergic skin test was 14.9%, whereas the most frequent sensitization to grass components was Cyn d 1 in 16.8%, Phl p 1 and Phl p 4 in 14.8% and 12.9%, respectively.[86]

Clinical application of the components in conjunctivitis

As already mentioned, the use of the multiplex chip in the RCA in relation to sensitization to dust mites,

both *Dermatophagoides* and *Blomia tropicalis*, showed accuracy comparable to the FEIA and prick tests. In addition to these data, Der p 1 and Der p 2 components have been shown to correlate with atopy and anti-Der p IgE serum levels, unlike Der p 10 and MUXF3 (bromelain) components.[87,88] Another application of ImmunoCAP-ISAC in relation to mite sensitization was demonstrated in a French study published in 2012, in which it was shown that sublingual immunotherapy with extract of *Dermatophagoides pteronyssinus* + *farinae* for one year did not induce new sensitization to the three Der p allergens (1, 2 and 10).

Regarding sensitization to fungi, there are no studies particularly carried out in RCA using the multiplex microarray technique. A retrospective study in children with ACR or asthma compared the Alt a 1 component measured by the FEIA method and the *Alternaria* allergen source, and showed that the accuracy of both is quite comparable, allowing either to be used to diagnose this sensitization.[89]

As much as allergens from so-called "furry" animals are more associated with asthma, there are some published data regarding RCA and the use of the multiplex technique. The main fact to be known by the attending physician to the patient exposed or sensitized to domestic animals, particularly dogs and cats, is that albumins (examples Fel d 2, Can f 3) are proteins that do not cause symptoms and have cross-reactivity between si, whereas lipocalins (Can f 1 and Can f 2) and secretoglobins (Fel d 1) are associated with clinical reactivity and do not have molecular mimicry. In this context, it has been suggested that the ImmunoCAP-ISAC could be indicated with a view to differentiating sensitization from allergy, or even to assess individuals at greater risk for future reactivity according to the degree of exposure.[90] A recently published Swedish cohort study showed that the Fel d 1 and Can f 1 components documented by the multiplex chip were better predictors of allergy evolution over time than cat and dog extracts.[91]

The multiplex platform has been used to detect early sensitization to pollen components and subsequent development of allergic rhinitis. In a German birth cohort, followed up to 13 years of age, a questionnaire was applied and blood samples were collected from 820 children aged 1, 2, 3, 5, 6, 7, 10 and 13 years. Diagnosis of pollen-related seasonal allergic rhinitis was performed according to nasal symptoms. Specific IgE antibodies to *Phleum pratenses* and 8 components were performed by FEIA and multiplex platform. One hundred and seventy-seven developed seasonal allergic rhinitis. Sensitization to Phl p 1 was early and the most common (78%)[92] in children with allergic rhinitis. Sensitization at 3 years predicts allergic rhinitis at 12 years, PPV = 68% and PPV = 84%.[93] In another study in children that used a multiplex platform for the detection of specific IgE, the serum of 764 individuals was evaluated to investigate the pathogenesis of IgE positive for proteins of the PR-10 family and birch allergic rhinitis at 16 years of age. Questionnaire and serum were collected at 4, 8 and 16 years of age from children in Stockholm, and performed ImmunoCap-ISAC. The risk of persistent allergic rhinitis to Bet v 1 at age 16 years was eight times greater (OR = 8.2) when the child had PR-10 sensitization at age 4 years.

Detection of specific IgE in the tear

Two recent studies used the multiplex microarray technique to detect allergenic components in tears in patients with ocular allergy, one being a case report[94] and the other a series of 10 patients with vernal keratoconjunctivitis.[95] Both showed that component-specific IgE is quantifiable by the ISAC method in the tear and may differ from the serum-specific IgE results. These data suggest that, in the near future, CRDs in secretions may be incorporated and collaborate in the etiological diagnosis of allergic diseases, particularly those that are difficult to define, such as vernal keratoconjunctivitis, for example.

Conjunctival allergen provocation test

The conjunctival allergen provocation test (CAPT) is a diagnostic tool to investigate IgE-mediated external ocular surface hypersensitivity diseases by evaluating the inflammatory effects caused by the conjunctival application of the allergen in a previously sensitized individual.[96] The purpose of the test is to accurately reproduce the signs and symptoms (eye itching, conjunctival redness, tearing, chemosis, and conjunctival/eyelid swelling) of allergic conjunctivitis (AC). Allergen-specific conjunctival hyperreactivity (eHRC) triggered by CAPT is a cascade of inflammatory events typical of an IgE-mediated hypersensitivity, which affects the conjunctival mucosa of individuals previously sensitized to the tested allergen and genetically predisposed. Positive CAPT causes the same signs and symptoms of AC in the tested eye that occur during natural exposure to the allergen. By triggering HRCe, CAPT confirms the allergen tested as the etiologic factor of ocular allergy.[97]

Conjunctival provocations with pollen grains to diagnose pollinosis (hay fever) and measure patient tolerance during desensitization experiments with pollen extracts were performed by Blackey in 1873. Until the 1970s, CAPT was used mainly for diagnostic purposes, but currently it has also been used to study the pathophysiology of AC, to evaluate the efficacy of topical ocular medications and of allergen-specific immunotherapy.[98] Another indication of CAPT is as an alternative test to assess mucosal reactivity in other IgE-mediated allergic diseases such as rhinitis, asthma, food allergy, and latex allergy.[99]

Recently, a publication by the EAACI Ocular Allergy Interest Group gathered recommendations for the clinical practice of CAPT.[99]

When to perform

Most cases of ocular allergy are benign, IgE-mediated conjunctivitis, of intermittent (seasonal allergic conjunctivitis) or persistent (perennial allergic conjunctivitis) evolution, for which an allergic cause must always be sought. Identification of aeroallergens is generally performed by skin prick tests (TCA) or by serum IgE-specific to the total allergen (S-IgE) or its molecular components.[100]

TCA and serum allergen IgE levels show good correlation with CAPT. Abelson et al.[97] observed 84% of positive CAPT in 396 individuals with positive TCA and suggestive history of AC, suggesting the routine use of the allergic skin test as a diagnostic tool for ocular allergy. Reactivity to the same allergen that presented positive TCA was detected by CAPT in 94% of subjects with eye complaints, which demonstrates a high positive predictive value of TCA in predicting a positive conjunctival challenge to the allergen, when there is a suggestive clinical history.[101]

However, positive TCA and elevated S-IgE only indicate sensitization to a specific allergen. Leonardi et al.[102] found an agreement of 81% of positive results of TCA and S-IgE to the same allergen with positive CAPT. In cases where there was no agreement, there was greater positivity of skin tests and/or serological tests (23%) in relation to ocular provocations (6%). Thus, the use of skin and serological tests, without proof by ocular provocation, could lead to an increase in the number of false positive cases of AC. Importantly, systemic sensitization can occur without clinical allergy, and local symptoms can occur without evidence of systemic sensitization. In another study, a significant correlation of elevated tear levels of allergen-specific IgE occurred only with positive conjunctival provocation, and not with TCA and S-IgE. These findings suggest the possibility that the conjunctiva is the only sensitized target organ in allergic individuals.[103]

For diagnostic purposes, CAPT is the only method that confirms a specific conjunctival response to a suspected allergen based on the clinical history in cases of SAC and especially PAC.

CAPT is particularly indicated when sensitization is inconsistent with clinical history, when a patient is polysensitized, or when skin or serological tests are negative or contradictory despite a medical history strongly suggestive that a specific allergen is involved in the ocular pathology. For SAC and PAC, detection of the most relevant allergen is critical before initiating allergen-specific immunotherapy.[99]

In vernal keratoconjunctivitis (CCV), SCPT is not routine practice and is used to identify allergens for immunotherapy in drug-resistant cases.[104]

CAPT is also widely used to study the pathophysiology of different forms of ocular allergy, for cell collection, measurement of chemical mediators, cytokines and other inflammatory biomarkers.[105]

Another indication of CAPT is for the follow-up of allergen-specific immunotherapy and for the evaluation of the antiallergic effect of topical drugs for the treatment of AC, being a method recognized by the Food and Drug Administration (FDA) for these purposes.[106]

There are some studies of the use of CAPT to confirm some cases of occupational latex allergy and food allergy. In a population of 174 children with suspected food allergy to cow's milk, egg, peanuts, and fish, negative CAPT excluded clinical food allergy, regardless of the serum IgE value to the allergen. Likewise, positive CAPT confirmed IgE-mediated food allergy.[107]

Practical recommendations for carrying out the CAPT

Prior to conjunctival provocation, patients must be asymptomatic, without active conjunctivitis or rhinitis, and unstable asthma. CAPT should be avoided in individuals with a history of eye surgery and recent infectious and inflammatory ocular pathologies (< 6 months), pregnant and lactating women, immunodeficiencies, neoplasms, liver or kidney failure, autoimmune diseases, cardiovascular diseases

using beta-blockers or who may decompensate if adrenaline is needed to treat a possible anaphylactic reaction.[100]

A complete eye examination must be performed before the challenge to rule out any conjunctival inflammation, being essential in cases of chronic conjunctivitis, when the presence of an ophthalmologist during the ocular provocation, in addition to the allergist, is necessary. Examination of the conjunctiva for mild chemosis visible only to the slit lamp can prevent high doses of allergens from being used during challenge, and thus prevent the onset of a late-phase reaction that could cause severe exacerbation of chronic conjunctivitis.[99]

There is no recommendation for performing CAPT in ocular surface pathologies that are not caused by IgE-mediated hypersensitivity, such as keratoconjunctivitis sicca, blepharitis and blepharoconjunctivitis, giant papillary conjunctivitis due to intolerance to contact lenses or foreign body, contact blepharoconjunctivitis with suspected allergy to eye drops, medications and cosmetics preservatives.[96]

CAPT cannot be performed during the period of natural exposure to the allergen, such as during pollinosis.[97]

Oral and topical topical medications that may interfere with the outcome of the conjunctival reaction should be discontinued prior to CAPT. H1 antihistamines, mast cell stabilizers, and topical ocular corticosteroids should be discontinued for at least two days prior to challenge. For cyclosporine and topical nonsteroidal anti-inflammatory drugs, the withdrawal period is for at least one week. Oral antihistamines should be discontinued one week prior to CAPT (three weeks for ketotifen), oral corticosteroids for two weeks, and antileukotrienes for three weeks.

As with other types of in vivo provocation, the consent form informing the risks and benefits of CAPT must be provided, discussed and signed by patients or legal guardians prior to conjunctival provocation. CAPT can be performed on children and adults, but requires cooperation and understanding of the procedure for a reliable result.[96,99]

The place for performing the CAPT (offices and hospitals) must have a structure for the treatment of possible serious adverse effects, such as asthma exacerbation, acute urticaria and anaphylaxis. Rescue medication, such as H1 antihistamine and topical and systemic corticosteroids, short-acting bronchodilators, and adrenaline, should always be available. Any positive CAPT should be treated with a topical ocular H1 antihistamine, and the subject should remain at the test site for 2 hours or until symptoms have completely disappeared. Ocular corticosteroids and oral H1 antihistamine should be considered in severe reactions that may progress to a late-phase reaction. These individuals should be monitored by medical staff for 24 hours.[99]

A summary of CAPT indications and contraindications is provided in Table 2.

Performance

CAPT has high sensitivity and allergen specificity for the diagnosis of allergic conjunctivitis in the presence of suggestive symptoms, when performed with standardized allergen extracts.[101,108]

Extract quality is critical for reliable results. Extracts must be standardized, preferably lyophilized and without preservatives. The amount of major allergens must be known and may vary by manufacturer. Standardized commercial extracts for CAPT that meet all these specifications are expensive, available only in a few countries and for few allergens, which limits the routine use of CAPT in clinical practice. For use, extracts are diluted in saline or diluent, at room temperature, according to the instructions provided by the manufacturer. After dilution, the stability of the solution is guaranteed for 6-24 hours. Mixture of different allergens should not be used.[99]

The reference technique for CAPT was described by Abelson et al.[97] With a metered-dose pipette, 20 40 µL of increasing doses of the diluted allergen extract are instilled into the latero-lower quadrant of the bulbar conjunctiva of one eye, at 15-20 minute intervals, until symptoms of AC occur, when the test is stopped and considered positive (Figure 3). When the maximum dose is applied and no symptoms occur, CAPT is considered negative. The contralateral eye receives only diluent or physiological saline, and serves as a control for the conjunctival reaction in the tested eye. For increasing allergen doses, dilution ratios 10 (eg 1:1000-1-100-1:10) or 2 (eg 1:32-1:16-1:8-1:4) can be used -1:2). Ratio 2 seems to be more appropriate, as it provides progressive dose increases, with more specific, safe and reliable reactions.[99]

The ocular reaction is dose-dependent, with reactions more intense as larger doses of allergen are applied to the conjunctiva, which can lead to clinical symptoms of a late-phase reaction, 6-12 hours after CAPT (Figure 4).[109]

The CAPT is a reproducible test. The minimum interval between two eye challenges should be 7 days. In our study, CAPT was reproducible in 78% of allergic subjects with the same allergen dose that triggered a positive reaction on the first CAPT at 1 week interval, and in 21% with an immediately higher dose.[101] Responsiveness refractoriness to repeated conjunctival challenges at shorter intervals (< 1 week), or often over a prolonged period of time, has been observed.[110] It is possible that the application of increasing doses of allergens at short and regular intervals promotes a decrease in HRCe.[111]

Table 2
Indications and contraindications for Conjunctival Allergen Provocation Test (CAPT)

	Recommendation
Clinic	Confirm the role of the suspected allergen in triggering symptoms in IgE-mediated ocular surface diseases, especially in CAP and when TI is indicated. Define clinically relevant allergen(s) in cases of polysensitization. Clarify cases with a clinical history of AC, but with inconclusive or negative skin and/or serological tests. Diagnosis of occupational (eg latex) or food allergy.
Research	Quantify the antiallergic properties of topical eye medications for AC. Investigate conjunctival allergen tolerance (eg pre- and post-IT). Investigate the pathophysiology of ocular allergic inflammation (mediators, cells, cytokines).
	Contraindication
	Active conjunctivitis (symptomatic patient). Recent eye surgical procedures (3-6 months). History of retinal detachment, Retinal disease, diabetic retinopathy, non-IgE-mediated ocular surface pathology. Pregnancy or lactation. Uncontrolled asthma/Severe systemic disease. Natural allergen exposure (eg pollen season). In use of ocular/oral anti-H1/EC, immunosuppressants, anti-leukotrienes. Contact lenses (must be removed 72 hours before).
Consent	Free, clarified, mandatory.
Presence of the doctor	Mandatory.
Place of realization	Ambulatory or preferably hospital, able to treat emergencies such as acute asthma/urticaria and anaphylaxis, staff trained for the procedure.

CAP = perennial allergic conjunctivitis, CAPT = conjunctival allergen provocation test, AC = allergic conjunctivitis, IT = allergen-specific, immunotherapy, antiH1 = H1 antihistamines, EC = corticosteroids.

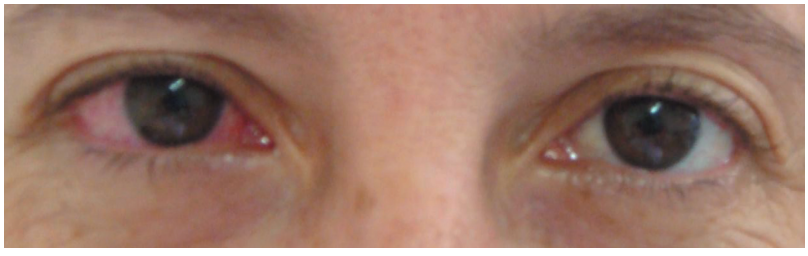
How to evaluate a positive response

There is great variation in the grading of the ocular response during CAPT. To date, there is no universal consensus on a single grading system to be followed.

CAPT is considered positive when it triggers pruritus and conjunctival hyperemia of moderate intensity. Ocular pruritus is the main criterion to be evaluated during CAPT, occurring in 96% of positive tests,[101] and it is recognized by the FDA for evaluating the clinical efficacy of antiallergic drugs and immunotherapy.[99] Spontaneous ocular and periocular itching is the earliest symptom of eHRC, appearing 3-5 minutes after exposure to the allergen, peaking around 10-15 minutes and gradually disappearing after 20 minutes. Conjunctival hyperemia is also a primary sign of eHRC, occurring 5 minutes after allergen exposure, reaching peak intensity at 20 minutes and beginning to disappear after 30 minutes.[101] In our series of eye taunts, Spontaneous pruritus before conjunctival hyperemia was reported by allergic subjects in 66% of CAPT positives with an immediately lower dose of allergen. Some protocols of conjunctival provocation for diagnostic purposes of AC use pruritus as the sole criterion for CAPT positivity.[100]

Due to the subjectivity of the response, since the intensity of pruritus is informed by the patient, it is recommended for CAPT the use of other signs and symptoms of eHRC, such as conjunctival hyperemia, tearing and chemosis, which are evaluated by the physician. These four signs and symptoms are assessed by a cumulative score scale.[97] CAPT is considered positive when the sum of pruritus, hyperemia, tearing and chemosis scores is ≥ 5, with at least 2 degrees of intensity in pruritus and hyperemia (Table 3). This score is calculated before and 15 minutes after the application of the allergen dose to the tested eye. This total score of ocular signs and symptoms can range from 0 to 13 points (Figure 5). Some studies also suggest a complementary score for eyelid edema, which can occur in 53% of positive CAPT.[101]

Efforts have been made to reduce the subjectivity of observations of ocular signs and symptoms during conjunctival provocations such as the use of digital



PROVOCATED EYE
(*L. perenne* - 1:32 dilution)
Total score of signs and symptoms: 9

CONTROL EYE
(Diluent)
Total score of signs and symptoms: 0

Figure 3
Positive Conjunctival Allergen Provocation Test (CAPT) in the right eye

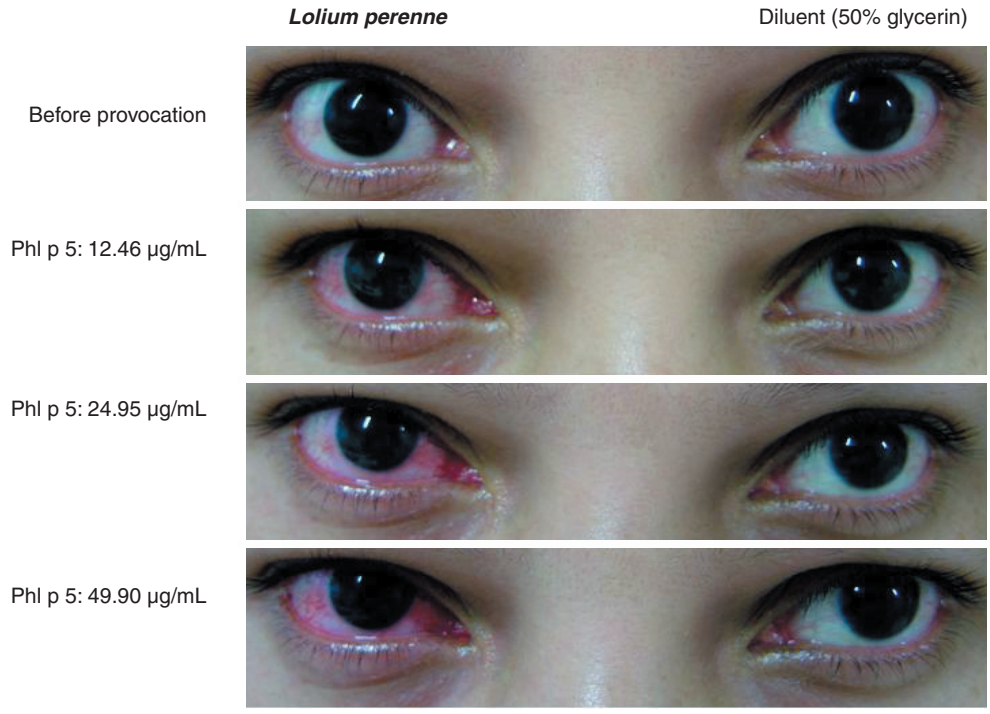


Figure 4
Progression of conjunctival hyperemia with increasing allergen doses during Conjunctival Allergen Provocation Test (CAPT) with *Lolium perenne*. Adapted from Mourão EMM et al.[101]

photography, thermometry, and esthesiometer, but no method has so far been incorporated into the practice of CAPT.[112]

Safety and adverse effects

Although little reported in the medical literature, adverse effects of ocular provocations, such as acute rhinoconjunctivitis, periorbital edema, urticaria, bronchospasm and anaphylaxis, may occur. These reactions are mostly mild and self-limiting, related to the immediate-phase reaction of allergic inflammation.[99]

In a series of 950 eye provocations, there were only two mild systemic reactions in sensitized subjects, one case of late-onset urticaria and one episode of wheezing in an asthmatic subject. One case of anaphylaxis was reported in another study.[97]

In a series of 77 CAPT positive with standardized extracts (Alk Abelló) of *Dermatophagoides pteronyssinus* (83.8 µg/mL of Der p 1), *Blomia tropicalis* (42.4 ng/mL of Blo t 5) and *Lolium perenne* (399, 2 µg/mL of Phl p 5), 88% of the individuals presented nasal itching, sneezing, nasal obstruction and coryza with spontaneous resolution within 1 hour.[113] This occurs by direct drainage of allergens into the nose through the nasolacrimal duct. To minimize allergen absorption and reduce the risk of adverse events, Anderson et al. recommend occlusion of the nasolacrimal duct during CAPT.[114] Eyelid swelling was observed in 53% of CAPT positives. An individual sensitive to *Lolium perenne* developed a late-phase reaction, with intense periorbital edema in the provoked eye (25 µg/mL Phl p 5) lasting 48 hours, requiring oral treatment with corticosteroids and H1 antihistamine. Two other individuals also sensitive to *Lolium* presented moderate swelling of the lower eyelid, lasting for 3 hours even after topical and oral treatment with H1 antihistamine. The challenge

Table 3
Graded scale of signs and symptoms for Conjunctival Allergen Provocation Test (CAPT)

Score	Itching	Hyperemia	Chemosis	Tearing
0	None	None	None	None
1	Intermittent itching	Mild: dilated blood vessels	Light: confirmed with slit lamp	Mild: slightly wet eyes
2	Mild, ongoing eye itching (awareness of the itching sensation, but no desire to rub the eyes)	Moderate: dilated blood vessels	Moderate: Elevated conjunctiva (visible visualization – swollen conjunctiva, especially in the limbus area)	Moderate: occasional rhinorrhea
3	Severe eye itching (constant awareness of the itching sensation but with the urge to rub the eyes)	Severe: numerous and obviously dilated blood vessels)	Severe: bulging of the conjunctiva	Severe: tears running down the face
4	Disabling eye itching (individual insists on rubbing eyes)	Extremely severe: numerous, dilated, engorged blood vessels	Not applicable	Not applicable

Adapted from Abelson MB et al.[97].

doses were 25 and 12.5 µg/mL Phl p 5, respectively. One patient presented severe chemosis at a dose of 12.5 µg/mL of Phl p 5, followed by epiphora and intense ocular pruritus, being treated with an eye patch for 8 hours, oral and topical H1 antihistamines, and topical ocular corticosteroids. All these patients had a papule diameter ≥ 10 mm for *Lolium perenne* on TCA. Also in our study, a controlled asthmatic individual, sensitive to *Blomia tropicalis* (papule diameter ≥ 8 mm on TCA), presented an episode of bronchospasm and shortness of breath with a dose of 28.9 ng/mL of Blo t 5), being treated with aerosol beta 2-agonist, corticosteroid and oral H1 antihistamine.[113]

Although it is considered safe even with high doses of allergens, a potential risk of serious and life-threatening reactions may occur, which justifies the performance of CAPT by a team trained in the method, in a hospital environment or equipped for the treatment of anaphylaxis.[99,100,113]

In conclusion, CAPT is a simple, fast and safe method to evaluate IgE-mediated allergic ocular diseases, especially in cases of perennial allergic conjunctivitis, in polysensitized individuals or when there is no agreement between symptoms and the suspected allergen. With this, we suggest a protocol for carrying out the CAPT, as shown in Figure 6.

Differential diagnosis

Red eye is a common sign and what many consider the "trademark" of all forms of conjunctivitis, although it can also be present by the involvement of other structures of the eye other than the conjunctiva, such as scleritis, uveitis and acute glaucoma (Figure 7).

These include acute and chronic allergic conditions (eg giant papillary conjunctivitis, vernal conjunctivitis, atopic keratoconjunctivitis, upper limbic conjunctivitis, follicular conjunctivitis; infectious causes – eg chlamydial disease, molluscum contagiosum, pirinaud oculoglandular syndrome); and various disorders such as keratoconjunctivitis sicca, acne rosacea, ocular pemphigoid, and blepharoconjunctivitis.[115]

Conjunctivitis in inborn errors of immunity

Inborn errors of immunity (EII), or primary immunodeficiencies (PDI), manifest as increased susceptibility to infectious diseases, autoimmunity, autoinflammatory diseases, allergy, and/or malignancy. To date, there are 430 described genetic defects associated with inborn errors of immunity.[116]

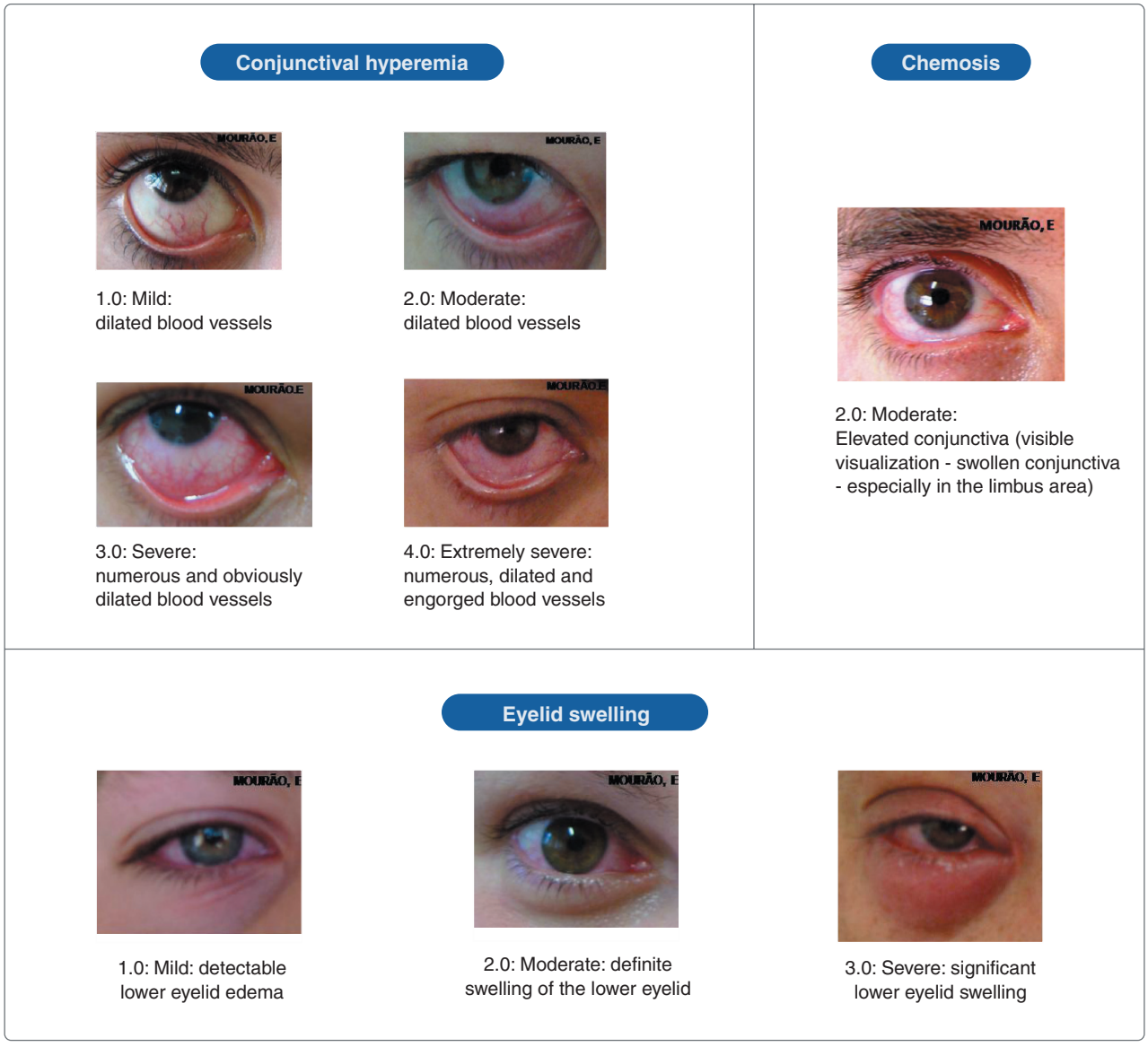


Figure 5
Photographic references during positive Conjunctival Allergen Provocation Test (CAPT)
Adapted from Mourão EMM et al.[101]

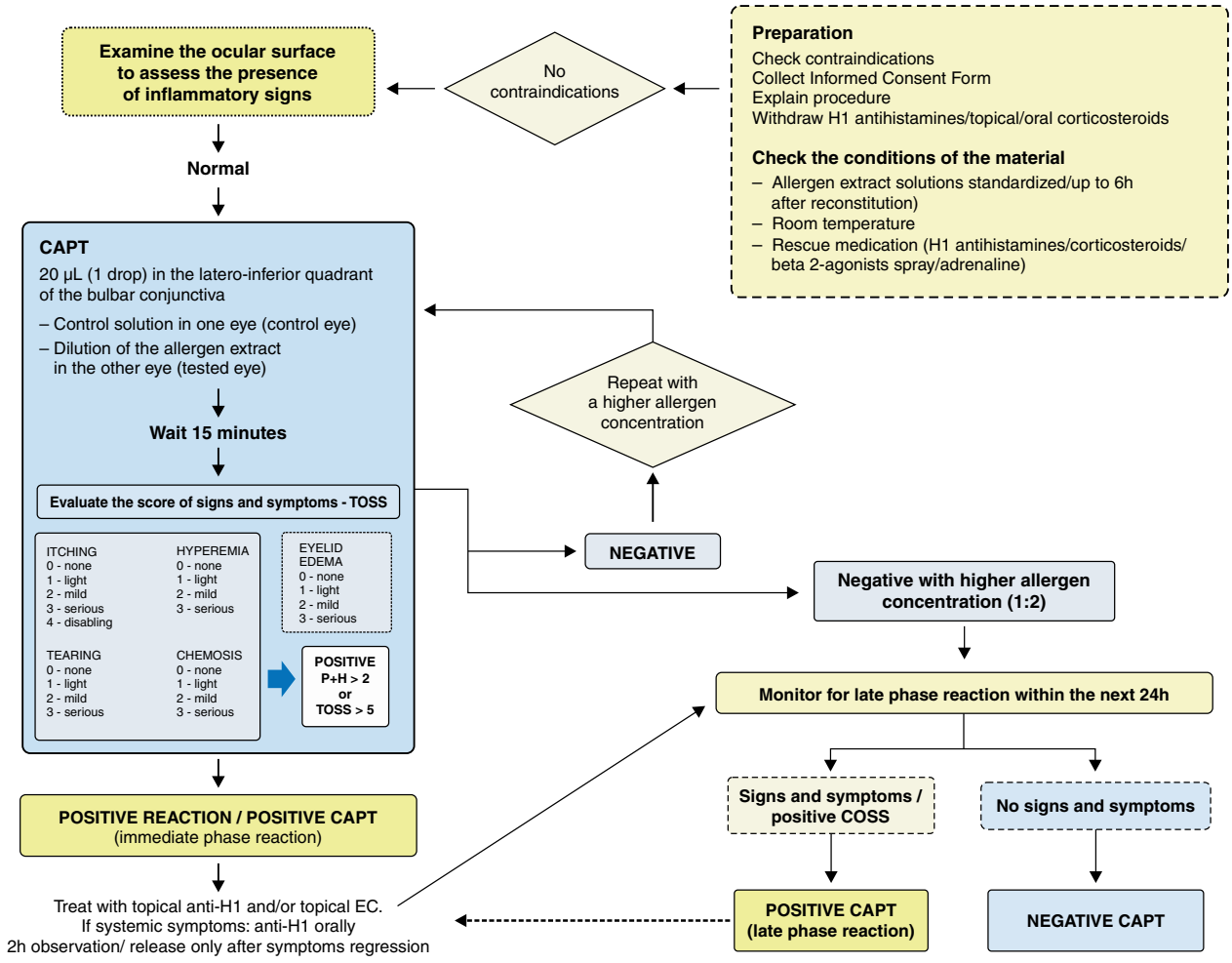


Figure 6
Conjunctival Allergen Provocation Test (CAPT) - Allergic conjunctivitis investigation flowchart

Although ocular involvement is not common, a variety of ophthalmic manifestations may develop and, in some cases, may even precede the typical symptoms of a specific immunodeficiency syndrome.[117]

Knowledge of the mechanisms involved in ocular manifestations in patients with PID allows for early diagnosis and specific treatment, leading to the reduction or prevention of serious visual morbidities.

There are few case reports and few reviews describing ocular manifestations in patients with IIE. In these patients, conjunctivitis may result from increased susceptibility to infections and autoimmunity.[118]

There are few reviews and case reports of conjunctivitis in patients with primary immunodeficiency. A previous study in 90 patients with PID observed a recurrence of non-follicular and non-purulent conjunctivitis in 9% of cases, and a greater predisposition in those with low levels or absence of the main serum immunoglobulins. Among the PIDs associated with conjunctivitis, the main ones were severe combined immunodeficiency, X-linked agammaglobulinemia, and common variable immunodeficiency. The absence of IgA in the tear alone was not a predictive factor for the presence of conjunctivitis or keratoconjunctivitis.[119]

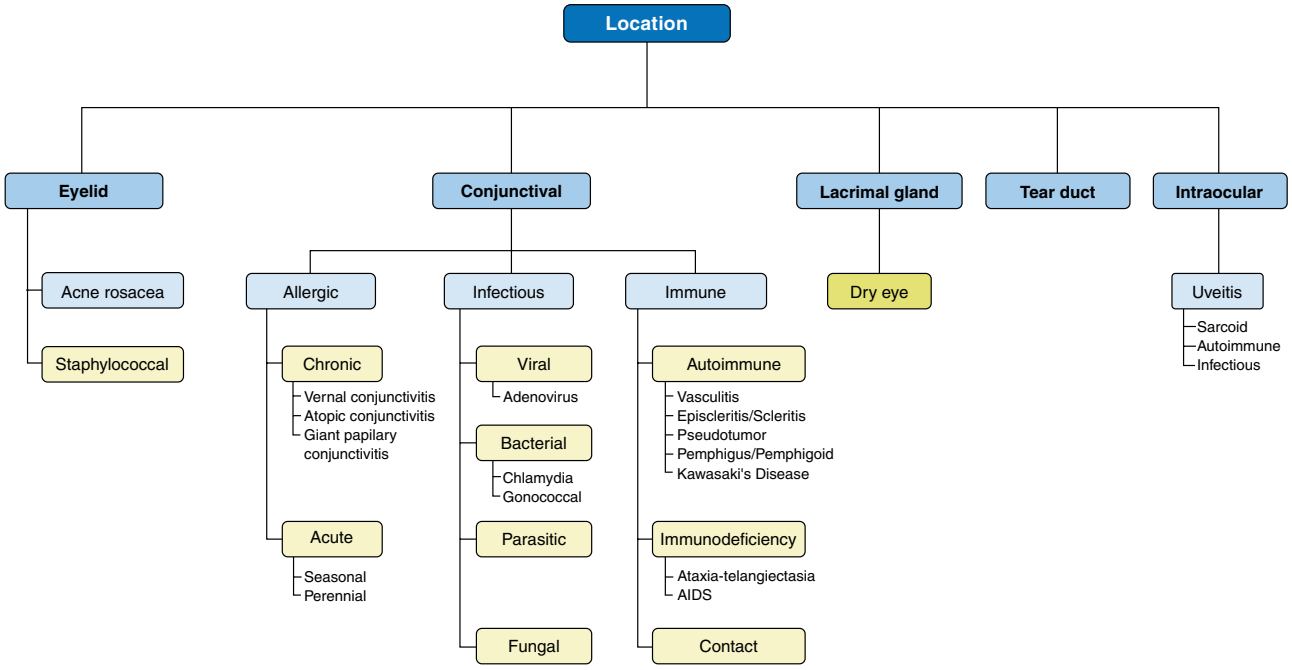


Figure 7
The differential diagnosis of allergic ocular diseases includes a variety of other causes, such as allergic, infectious, autoimmune, and mechanical or nonspecific that trigger the hypersensitivity response of immunologically active extraocular and intraocular tissues. Adapted from Bielory et al.[7]

Severe Combined Immunodeficiency (SCID) is the term applied to the group of rare, serious and fatal diseases characterized by defects in T and B cell responses, resulting in the absence of an adaptive immune response. SCID represents the most severe form of PID, with signs and symptoms that appear in the first months of life, and is characterized by growth retardation associated with recurrent bacterial, viral and fungal infections, including increased susceptibility to infection by opportunistic microorganisms. Most ocular manifestations are a direct result of this susceptibility. Other eye abnormalities described are associated with cytomegalovirus (CMV) infection or after bone marrow transplantation. These findings include chorioretinitis, CMV-associated retinitis, and optic neuritis.[120-122] Opportunistic infections in patients with SCID include toxoplasmosis chorioretinitis, fungal keratitis and endophthalmitis, and conjunctivitis and chorioretinitis caused by *Pneumocystis jirovecii*[119].

X-linked agammaglobulinemia (ALX) is caused by mutations in the gene that encodes Bruton tyrosine kinase (BTK). As BTK plays an important role in the development of B cells, patients with ALX do not generate mature B cells, which results in the absence of plasma cells and, consequently, agammaglobulinemia. Symptoms usually appear early in childhood and consist of bacterial respiratory and skin infections, sepsis, and meningitis. In addition, patients with ALX are susceptible to enterovirus infections and may develop autoimmune diseases. Bacterial conjunctivitis and keratoconjunctivitis due to *Haemophilus influenzae* and *Chlamydia trachomatis* have been observed.[123,124]

Common variable immunodeficiency (CVID) is a heterogeneous, multisystemic disease characterized by hypogammaglobulinemia and poor humoral response to vaccine and other antigens due to

abnormal differentiation of B lymphocytes and/or defects in the interaction between T and B cells. The most important manifestations are recurrent respiratory and gastrointestinal infections, increased incidence of autoimmune diseases and malignancies. Although ocular manifestations in patients with CVID are not common, they may occur due to recurrent infections and autoimmune manifestations. Eye infections can involve all structures in the eye. In cases of bacterial conjunctivitis, *S. pneumoniae*, *H. influenza*e, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and other multidrug-resistant bacteria have been identified.[125,126] Alternative manifestations described in patients with CVID include keratitis, uveitis with granulomatous inflammation, retinal vasculitis, choroiditis, bilateral optic neuritis and chorioretinitis.[127]

Immunoglobulin A (IgA) deficiency is the most common PID, and most patients are asymptomatic. Despite this, there is an increased incidence of autoimmune diseases and recurrent respiratory and gastrointestinal infections. In patients with IgA deficiency, eye diseases have rarely been described, and may affect mainly the conjunctiva.[128]

Isolated IgM deficiency or selective IgM deficiency is a rare primary dysgammaglobulinemia defined by an IgM level of less than 20 ng/dL in children, less than 2 standard deviations below normal in adults, and normal levels of other immunoglobulins. A high frequency of pulmonary manifestations, including recurrent upper respiratory tract infections, asthma and allergic rhinitis, has been described. Some ophthalmic manifestations of selective IgM deficiency, such as recurrent hordeolum, conjunctivitis, and *Staphalococcus aureus* blepharitis, have been documented.[129]

Chronic granulomatous disease (CGD) is a hereditary disease, with X-linked or autosomal recessive inheritance, in which phagocytes are unable to generate reactive oxygen compounds to fight catalase-positive organisms. As a result, patients with CGD are prone to recurrent bacterial and fungal infections. In addition, CGD are associated with an increased risk of autoimmune diseases. Blepharoconjunctivitis has been consistently described in patients with CGD, and can result in punctate keratitis and panus formation.[130-132] Other ophthalmic manifestations in CGD include uveitis[133], candida glabrata keratitis, ulcerative keratitis with limbus granuloma formation, retinal detachment, optic nerve pallor, and corneal ulcers and scarring.[130,131]

Leukocyte adhesion deficiency (LAD) type 1 is an autosomal recessive disorder caused by abnormal expression of Beta 2 integrin (CD11/CD18) on leukocytes. The main clinical manifestations of LAD-1 include late fall of the umbilical stump, recurrent bacterial infections, periodontitis and poor wound healing. Ocular manifestations have been described in a few cases, including eyelid cellulitis, conjunctivitis[134] and medial surface necrosis caused by *Pseudomonas aeruginosa*.[135]

Chronic mucocutaneous candidiasis (CMC) encompasses a heterogeneous group of diseases characterized by recurrent or persistent candidiasis of the skin, mucous membranes and nails. CMC can occur as an isolated symptom, but it is often accompanied by systemic diseases such as Autoimmune Polyendocrinopathy, Candidiasis, Ectodermal Dystrophy (APECED). Recently, several genes related to the generation of IL-17 and IL-22 have been identified and their mutations result in autosomal recessive CMC (CARD9, DOCK8, IL17F, IL17RA) or autosomal dominant CMC (STAT3 mutations and STAT1 gain of function). An underlying immunological abnormality is responsible for the impairment in T lymphocyte function, which results in an inability to produce cytokines such as IL-17 and IL-22, which are essential for the expression of cell-mediated immunity against candida. bilateral keratitis, Keratoconjunctivitis and corneal changes are the most common complications in APECED caused by mutations in the autoimmunity regulatory gene and APECED-like syndromes. Dry eye, blepharospasms and photophobia can be direct results of keratitis. Corneal changes characterized by ulcers, scars and stromal vascularization can affect visual acuity. Keratitis may precede endocrinopathies.[136] Other ophthalmic abnormalities associated with CMC include lens opacities, bilateral iridocyclitis, retinal detachment, optic atrophy, retinitis pigmentosa, anisometric amblyopia, myopia, and reduced tear production. Loss of eyelashes and eyebrows has been attributed to chronic infection with candida or alopecia areata.[137]

Chediak-Higashi syndrome (CHS), a rare autosomal recessive immunodeficiency caused by mutations in the LYST gene, is characterized by abnormal granule formation in neutrophils and melanocytes. Characteristic clinical findings include partial oculocutaneous albinism, severe immunodeficiency, coagulation defects, and progressive neuropathy. Patients affected with this syndrome often suffer

from recurrent bacterial infections. Ocular albinism is considered the main visual manifestation in patients with CHS, who suffer from photophobia and nystagmus.[138] Oculocutaneous albinism is seen in some other PIDs, such as Griselli syndrome type 2, Hermansky-Pudlak syndrome type 2 (HPS2), and p14 deficiency.[139]

Ataxia-telangiectasia (AT) is an autosomal recessive, neurodegenerative disease due to mutations in the ATM gene, characterized by cerebellar ataxia, progressive neurological impairment and telangiectasia. Other characteristic manifestations include immunodeficiency causing sinopulmonary infections, predisposition to malignancies, increased radiosensitivity, and sterility. Ocular manifestations are classified as conjunctival telangiectasia and eye movement disorders. Telangiectasias or twisted and dilated vessels, the second prominent feature, are seen in the bulbar and palpebral conjunctiva and conjunctival fornix. They typically appear later at age 3-6 years compared with ataxia, but are occasionally seen earlier, particularly in children with a positive family history.[140] Such vascular diseases do not cause ocular dysfunction.

Bloom syndrome (BS) is a rare autosomal recessive disorder with DNA repair caused by mutation in the BLM gene. BS is characterized by short stature, facial skin erythema, hyper- and hypopigmented skin lesions, immunodeficiency, hypogonadism, and an increased incidence of malignancies and diabetes. Eye-related pathology is highly variable and may include early-onset retinal drusen (described primarily as colloid body-like spots), telangiectatic erythema, which may extend to the eyelids and bulbar conjunctiva, conjunctivitis, unilateral retinoblastoma, loss of eyelashes, iris pigment and subcapsular lens opacities. Nonproliferative diabetic retinopathy accompanied by hemorrhagic retinitis due to acute leukemia and bilateral optic nerve hypoplasia has also been reported.[141]

Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency characterized by the triad of recurrent bacterial infections, eczema, and bleeding diathesis associated with congenital thrombocytopenia and small platelets. Patients are at increased risk of developing autoimmune manifestations and malignancies. Ophthalmic complications are consequences of bleeding diathesis or increased susceptibility to infections, and include conjunctivitis, blepharoconjunctivitis, conjunctival and corneal ulcers, episcleritis, ulcerative keratitis, necrotizing eyelid eruptions, and eyelid eczema. Molluscum contagiosum and herpes simplex viruses are associated with blepharoconjunctivitis.[142] Acute retinal necrosis related to varicella zoster virus presents as diffuse uveitis, retinal vasculitis, and acute retinitis, and can cause serious visual sequelae. Platelet dysfunction can cause eye hemorrhage such as periorbital bleeding, conjunctival, subconjunctival hemorrhage, retina hemorrhage, optic disc hemorrhage, and vitreous hemorrhage.[142]

Treatment

Non-pharmacological treatment

General measures

Nonpharmacological treatment of allergic conjunctivitis includes general measures that are useful for most patients. Patients and/or caregivers should receive educational support about the expected duration and prognosis of ocular allergy, and possible complications from inadequate disease control.

Basic vision care

Patients should be instructed not to rub their eyes, as friction can cause mechanical degranulation of mast cells and worsen symptoms. The application of artificial tear drops or eye solutions without preservatives are useful to dilute and remove antigens from the ocular surface, reducing the concentration of mediators in the tear film, and consequently the allergic symptoms. On the other hand, frequent washing of the eyes with running water should be avoided as it can reduce the stability of the tear layer.[143]

Although the application of artificial tears and cold compresses have no prophylactic effect on the ocular allergic response, these procedures can attenuate ocular signs of inflammation, such as conjunctival hyperemia and increased ocular surface temperature, especially during an acute episode of allergic conjunctivitis. A study involving controlled exposure to grass pollen in patients with seasonal allergic conjunctivitis (SAC) showed that the combination of application of artificial tears and cold compresses, in conjunction with antihistamine eye drops, was superior to the use of medication alone for reducing time and intensity of symptoms.[144]

Generally speaking, patients should reduce or discontinue contact lens wear during symptomatic periods, due to the propensity of allergens to adhere to contact lens surfaces. Lens cleaning

agents, along with storage and rinsing solutions should be preservative-free, as hypersensitivity reactions to these substances can contribute to the inflammatory reaction. Homemade saline solutions are not recommended because of the risk of bacterial contamination.[145]

These measures are especially useful in patients with giant papillary conjunctivitis (GCP) triggered by contact lenses, whose primary treatment is based on removing the source of mechanical irritation. In this case, improving cleaning and storage of lenses to avoid adherence of antigens, reducing wearing time, increasing the frequency of replacement and changing the type and/or model of the same are important auxiliary measures of pharmacological treatment.[146]

Environmental control

The identification of specific allergens for each case and the establishment of inherent measures for their prevention are important steps for the adequate approach to ocular allergy. Likewise, actions that minimize exposure to non-specific triggers, such as exposure to sun, wind and salt water, such as the use of sunglasses, hats with visors and swimming goggles, should be implemented.

As most often allergic conjunctivitis is associated with rhinitis, preventive measures must address both pathologies. Thus, in patients with perennial allergic conjunctivitis (PAC) due to house dust mites, special attention should be paid to the bedroom, which should be well ventilated and sunny. Dust reservoirs such as upholstered furniture, curtains and heavy rugs should be removed or vacuumed at least weekly. Floors should preferably be washable (ceramic, vinyl and wood), and blinds should be blinds or made of material that can be cleaned with a damp cloth. Additional measures include replacing pillows, blankets and mattresses made of kapok and/or feathers with foam, fiber or latex, regularly washing bedding and blankets with detergent and high temperatures (> 55 °C), drying in the sun or air warm and use waterproof covers for pillows, duvets, and mattresses.[147,148]

When the main allergens involved are the epithelium of fur animals, their presence in the room and especially in the patient's bed should be avoided. If it is not possible to restrict the animal to a single area of the house, it is recommended to use Hight Efficiency Particulate Air (HEPA) type purifiers.[147,148]

Preventive measures to reduce the symptoms of seasonal allergic conjunctivitis (SAC) produced by pollens, present in temperate countries and also in southern Brazil, include reducing outdoor exposure, especially in periods of high pollen counts, between 5 and 10 am and on dry, hot and windy days. Windows in homes and cars should remain closed during peak pollen seasons and, if possible, ventilation systems in homes and cars should be equipped with special filters to prevent these allergens.[143,147,148] Additional measures include regular inspection of the environment to reduce sources of moisture and the extermination of cockroaches, in addition to avoiding non-specific irritants, especially cigarette smoke, insecticides, perfumes and deodorants,

Pharmacological treatment

Pharmacological treatment regimens include the use of therapeutic agents, such as oral or topical agents, including antihistamines and, if necessary, topical decongestants, mast cell stabilizers, multi-acting agents, and anti-inflammatory agents.

Oral antihistamines may offer relief from eye allergy symptoms but have a prolonged onset of action. Second-generation H1 antagonists cause less sedation and less anticholinergic (dry eye) effects than first-generation ones.[149] Drugs with dual antihistamine and mast cell blocking activity provide the most advantageous approach in the treatment of allergic conjunctivitis, with symptomatic relief, rapid onset of action and disease-modifying action. In general, children do not like topical ophthalmic preparations, as they often complain of stinging or burning. It is important not to contaminate topical eye medications if the applicator tip comes into contact with the eye or eyelid, or uses one bottle for multiple family members (especially with potential for COVID-19 contamination). Topical decongestants act as local vasoconstrictors, reduce erythema, vascular congestion, and eyelid edema, but have no effect in preventing the allergic response or pruritus as a primary symptom. Chronic use of topical vasoconstrictors leads to tear film instability and conjunctival irritation, with subsequent burning or stinging and even rebound hyperemia. The term “drug conjunctivitis” has been described by the chronic overuse of topical vasoconstrictors in the eye.[150] The combined use of an antihistamine and a vasoconstrictor has been shown to be more effective than the use of either agent alone. Use of topical nasal corticosteroids for allergic rhinoconjunctivitis decreases eye symptoms, presumably via a naso-

ocular reflex and more effectively for seasonal allergic conjunctivitis than perennial forms of allergic conjunctivitis. The effects of long-term chronic use of nasal corticosteroids on ocular symptoms have not been well studied, therefore, they should not be used for the treatment of ocular allergy in the absence of nasal symptoms.[7]

Leukotriene receptor antagonists are useful in the treatment of allergic rhinitis, and although they have been shown to decrease conjunctival nitric oxide levels, their use for ocular allergy is limited.[147,151]

Non-steroidal anti-inflammatory drugs block the enzyme cyclooxygenase and the production of prostaglandins from arachidonic acid. They reduce ocular symptoms, however, they can cause systemic reactions, discomfort on instillation and, occasionally, corneal perforation; therefore, its use must be monitored. Ketorolac is a presentation available for topical ocular use.[150]

Topical (or rarely oral) corticosteroids can be used for the most severe acute cases that require 3-5 days of therapy. Chronic steroid use for eye disorders should only be undertaken in conjunction with an ophthalmologist. Local administration of topical corticosteroids may be associated with increased intraocular pressure. There are some forms of prodrugs, such as loteprednol, which have been shown to have minimal effect on the development of increased intraocular pressure or cataracts. However, all topical steroids can predispose to viral infections and should be used with caution, especially if the patient has a suspected history of viral exposure or unilateral conjunctivitis. Immunomodulatory medications, such as topical tacrolimus or topical cyclosporine, are used as steroid-sparing agents and do not have an adverse effect profile when compared to steroids.[152] Table 4 provides an overview of eye allergy treatment.

Immunosuppression

Topical immunomodulation

Topical calcineurin inhibitors are the most frequently used treatments as corticosteroid-sparing agents in corticosteroid-dependent VKC and AKC. Two systematic reviews on the use of topical cyclosporine (CsA) in VKC and AKC[154,155] showed that topical CsA is effective in alleviating the signs and symptoms of VKC and AKC, reduces dependence on topical corticosteroid eye drops, while maintaining safety similar to of placebo.[154] The second study highlighted the relative paucity of randomized controlled trials evaluating the efficacy of topical CsA in AKC, and suggested that CsA provides clinical and symptomatic improvement and may help to reduce the use of steroids in steroid-dependent or non-steroid-responsive patients.[155]

Cyclosporin A (CsA) is the first topical immunomodulator used for the treatment of severe VKC since the 1990s.[156] Already at that time, CsA was considered an effective substitute for corticosteroids, with excellent anti-inflammatory activity in patients with corticosteroid-dependent and corticosteroid-resistant VKC.[156] CsA is lipophilic and therefore must be dissolved in an alcohol-oil base. For many years, the unavailability of a commercial topical CsA preparation and technical difficulties in dispensing eye drops prevented its widespread use for the treatment of VKC. The 2% formulation was first used in the treatment of VKC, but lower concentrations (1%, 0.5%, 0.1%, 0.05%) were used and proved to be effective for the treatment of moderate VKC to severe.[157,158] When necessary, additional topical corticosteroids may be used in short courses. Systemic absorption was not detected by laboratory methods,[159] which excludes the possibility of local or systemic side effects from CsA. Burning and irritation are frequent side effects, but it is rarely necessary to stop taking the drug. The treatment can be prescribed seasonally or perennially, reducing the doses in the non-active phases of the disease. Adverse events such as bacterial or viral infections are rare, while changes in intraocular pressure have not been reported. but it is rarely necessary to discontinue treatment with the drug. The treatment can be prescribed seasonally or perennially, reducing the doses in the non-active phases of the disease. Adverse events such as bacterial or viral infections are rare, while changes in intraocular pressure have not been reported, but it is rarely necessary to discontinue treatment with the drug. The treatment can be prescribed seasonally or perennially, reducing the doses in the non-active phases of the disease. Adverse events such as bacterial or viral infections are rare, while changes in intraocular pressure have not been reported.

CsA 1% four times daily significantly reduced signs and symptoms and tear levels of eosinophilic cationic protein in a group of patients with VKC,[160] and was reported as the lowest effective concentration in the treatment of corneal ulcers, with recurrence seen at lower concentrations.[161] The effects of the low concentration of 0.05% CsA are controversial,

but considered to be an effective steroid-sparing agent[162,163] and effective in preventing CVC recurrences.[164] In a prospective observational clinical study in 594 patients, 0.1% CsA was shown to be effective and safe for the treatment of VKC.[165]

Topical CsA 0.1% in cationic emulsion (CE) was recently approved in the European Union and Canada as an orphan drug for the treatment of severe VKC based on the results of the VEKTIS study. The study achieved its primary endpoint, demonstrating the superiority of treatment with CsA-EC 4 times daily and CsA-EC twice daily over vehicle, in an efficacy score over the four-month treatment period. This benefit was driven by a decrease in corneal fluorescein (CFS) score (reflecting less damage to the cornea) and a reduction in the use of dexamethasone as a rescue medication in case of exacerbations. In addition, CsA-CE 0.1% significantly improved patients' quality of life (QOL) as assessed by the specific QUICK questionnaire.[166] Improvements in keratitis, symptoms, and quality of life achieved with CsA-EC during the initial four months of treatment were maintained

Table 4
Eye allergy treatment overview in step-by-step format. Modified from Bielory L et al.[153]

Therapeutic intervention	Clinical rationale	Pharmacological agents	Comments
Primary			
Removal	Effective, simple		> 30% improvement in symptoms
Cold compresses	Decreases nerve stimulation, reduces vasodilation		Effective for mild to moderate symptoms
Lubricating eye drops	Washing	Artificial tear	Extremely recommended, comfortable and safe
Secondary			
Topical antihistamine and mast cell stabilizers	Relief in itching	Olopatadine, ketotifen	Antihistamine and mast cell stabilizer
Topical mast cell stabilizers	Safe and effective	Cromoglycate	Relief of mild to moderate symptoms
Tertiary			
Topical corticosteroids	Alleviation of all inflammatory responses including erythema, edema and itching	Loteprednol	Suitable for short periods. Avoid in viral infections
Immunotherapy subcutaneous or sublingual	Identifies and modulates allergic sensitivity		Adjunct in addition to treating allergic rhinitis
Help			
Oral antihistamines	Slightly effective on itching	Always 2nd generation	It can cause dry eye and worsening symptoms

over the subsequent eight-month follow-up period, with both CsA-EC dosing regimens exhibiting safety profiles favorable to throughout the treatment period, providing further evidence that topical CsA-EC is a viable therapeutic option for children and adolescents with severe VKC.[167]

Tacrolimus is a potent drug similar to CsA in its mode of action, but chemically distinct. A tacrolimus skin ointment is licensed for the treatment of moderate to severe atopic eyelid disease and may have secondary benefits for AKC.[168-170] Conjunctival application of 0.03% and 0.1% tacrolimus ointment was effective, well tolerated, and safe in the treatment of severe allergic conjunctivitis.[169,171] In a multicentre, double-blind, placebo-controlled trial, 0.1% tacrolimus ophthalmic suspension was shown to be effective in the treatment of severe allergic conjunctivitis. The dose was based on the results of a previous dose-ranging study in which tacrolimus 0.1% ophthalmic suspension showed more marked improvement and a similar safety profile compared to 0.01% and 0.03%. Patients treated twice daily for 4 weeks with 0.1% tacrolimus significantly improved signs and symptoms. The most frequent adverse event related to tacrolimus was eye irritation.[172] Demonstrations on the quality, safety and efficacy of the different compounded preparations used in the different clinical studies will be needed before tacrolimus is considered an orphan drug for VKC and AKC.

However, a commercial eye drop preparation is available in Asia with the indication of severe AKC and VKC. One review, with a critically low-quality evidence score, highlighted the benefits of tacrolimus over placebo in two randomized controlled trials and four case series.[173] In a prospective randomized comparative double-masked study comparing the efficacy of 0.1% tacrolimus ophthalmic ointment with 2% CsA showed that both were equally effective in the treatment of VKC.[174] In a second study, patients with CsA-resistant VKC[175] treated with 0.1% tacrolimus showed a significant improvement in clinical scores over 1% CsA. A recent study comparing the effect of topical 0.1% tacrolimus alone or in combination with topical corticosteroids in refractory allergic eye diseases, also showed a potential steroid-sparing effect.[176] In addition, 0.03% or 0.1% tacrolimus skin ointments have been shown to be beneficial in the treatment of eyelid eczema in patients with AKC.[177-179]

In twelve patients treated with topical tacrolimus for an average of 8 years, long-term efficacy was shown in the clinical signs of severe AKC and VKC, although half of the patients were not able to completely discontinue topical corticosteroids over time of treatment. It should be noted that increased intraocular pressure and corneal infections were potential side effects.[180] Therefore, tolerability of topical calcineurin inhibitors is a concern as a burning sensation is frequently reported. Molluscum contagiosum, papilloma virus, and herpes infections are rare but recognized risks.

Systemic immunomodulation

Systemic immunosuppressive treatment can be prescribed in most refractory cases at risk of AKC vision loss. Cyclosporine was the most used drug.[181] Azathioprine and mycophenolate mofetil are alternative options.

Immunotherapy for allergens

The patient with allergic conjunctivitis, when looking for an allergy specialist, is not always satisfied with having to use frequently or daily and even more than once a day, oral or topical medications to control symptoms for a long time. Immunotherapy (IT) is the practice of administering gradually larger amounts of an allergen extract to an allergic individual to ameliorate symptoms associated with subsequent exposure to the same allergen.[182] IT is an effective procedure in the treatment of patients with IgE-mediated allergic diseases to defined allergens.[182,183]

By modifying the biological response, it influences the immune responses initiated by the allergen and partially restores the Th1/Th2 imbalance of the allergic individual B and T lymphocytes, Treg cells, blocking antibodies, IL-10 and other cytokines are involved in the action of IT.[183,184] TI with allergen injections is recommended for patients with IgE antibody-mediated respiratory allergy whose symptoms respond inadequately to therapy recommended by clinical guidelines.[185]

TI, when appropriate, should be used in combination with all forms of pharmacological and non-pharmacological treatment, with the aim of allowing the allergic patient to become asymptomatic as quickly as possible. Several randomized, double-blind, placebo-controlled studies have demonstrated the efficacy, safety, indications and contraindications of IT in the treatment of allergic diseases.[186,187]

Allergen IT is indicated for patients who have disease with a mechanism dependent on IgE

antibodies specific to clinically relevant allergens. Therefore, demonstrating allergic sensitization by a positive skin test, for example, is not sufficient, as about 1/3 of the population tests positive without showing symptoms of allergy.[182,183]

The subcutaneous administration of allergens is the main route of application of IT in the treatment of allergic diseases. Sublingual immunotherapy (SLIT) is an effective, safe and convenient alternative to SCIT.

Meta-analyses have shown that SLIT is a safe treatment, reduces symptoms and the need for medication in patients with allergic rhinitis and asthma. New formulations, such as sublingual dissolving tablets and adjuvants targeted at the oral mucosa, increase the effectiveness of SLIT treatment. Despite convincing studies, further information on the mechanism of action, optimal doses and comparison with conventional subcutaneous treatment is still lacking.[187]

Regarding TI in AC, studies are very limited and unsatisfactory due to the study of one type of eye allergy, assessment of one type of allergen, insufficient follow-up parameters, or small sample size.[188]

TI is a safe and effective method of symptom control and prevents exacerbations and the development of new sensitizations in patients with AC without ocular or systemic side effects. It should be considered as an alternative or even a primary treatment for patients with AC to avoid serious eye problems as a side effect of topical steroids, to reduce or avoid long-term pharmacotherapy, and also to reduce the economic burden of drugs.[147]

There is no significant difference between SLIT or SCIT administration routes to achieve clinical and immunological improvement, so the patient can choose their preferred method of therapy. Seven of the eight articles selected for a systematic review on patients with severe AC recommended the use of SLIT and SCIT to improve ocular symptoms in the treatment of allergic rhinoconjunctivitis.[147]

Two other systematic reviews focusing on ocular symptoms concluded that the evidence was moderate for SLIT and low for SCIT in the treatment of pollen and dust mite allergic conjunctivitis.[189,190]

Vernal keratoconjunctivitis (CCV) is a chronic allergic conjunctival disease mediated by mast cells, lymphocytes and epithelial cells. It appears more in prepubescent boys and improves in the third decade of life. CCV can have seasonal exacerbations: 50% to 60% of patients have a positive aeroallergen skin test. Immunotherapy in this disease has not been well studied, and there is no indication for this treatment. IT for atopic keratoconjunctivitis also has little evidence of benefit.

Subcutaneous IT with *Dermatophagoides pteronyssinus* extract (3 µg Der p 1 per application, for 12 to 16 weeks) in asthmatics with allergic conjunctivitis promoted remission of ocular symptoms in 17 of 19 patients with mild to moderate conjunctivitis, demonstrating the effectiveness and speed of the treatment. onset of action of immunotherapy with standardized extract.[191]

Immunotherapy plays a more important role in the "long-term" control of rhinoconjunctivitis. In allergy sufferers who had asthma and rhinoconjunctivitis when exposed to animal dander (eg, allergen Fel d I), immunotherapy has been shown to improve the overall symptoms of rhinoconjunctivitis and decrease the use of antiallergic medications. Clinical improvement and a reduction in allergen sensitivity was also observed in a 12-month study of immunotherapy using a purified and standardized preparation of *Dermatophagoides farinae*.[7]

Biologicals in allergic conjunctivitis

Omalizumab

It is an anti-IgE monoclonal antibody indicated for the treatment of severe asthma and chronic urticaria. It is a humanized IgG1 capable of selectively binding to free serum IgE, specifically in the Cε3 region of the Fc fragment, preventing IgE from binding to the high-affinity receptor FcεRI of many cell types, including basophils and mast cells, producing a reduction in free IgE, and consequent inability to trigger the release of mediators of these cell types. Furthermore, the reduction of free IgE induces a decrease in the expression of FcεRI on the surface of mast cells, basophils and dendritic cells, which leads to a decrease in allergic inflammation and, ultimately, to a lower production of IgE.

Different studies compared the effect of omalizumab vs. placebo in the treatment of allergic conjunctivitis, showing a significant reduction in nasal and ocular symptoms (red, watery and itchy eyes) in the omalizumab group compared to placebo after 12 and 16 weeks. However, omalizumab has not been studied in the treatment of allergic conjunctivitis outside of allergic rhinitis research.[147,192,193]

There are case reports showing a good effect of omalizumab in the treatment of atopic keratoconjunctivitis and vernal keratoconjunctivitis with partial or complete improvement, but in very severe cases in child reports the improvement was not significant or lasting.[194] However, there is no formal indication in ocular allergy.

Dupilumab

Dupilumab is a human monoclonal antibody directed against the alpha subunit of the IL-4 receptor that blocks IL-4 and IL-13 signaling and has demonstrated significant efficacy in patients with moderate to severe atopic dermatitis. Dupilumab is approved for the treatment of moderate to severe AD, asthma with a type 2 inflammatory profile or moderate to severe oral corticosteroid dependent, and chronic rhinosinusitis with nasal polyps. There are no studies reporting its use for therapy of allergic eye disease.

Conjunctivitis has been reported as an adverse effect of this, described as inflammation of the anterior conjunctiva and hyperemia of the limbus. The incidence ranges from 5 to 28% in dupilumab-treated groups compared to 2-11% in placebo groups. Pre-existing allergic conjunctivitis appears to be a risk factor, and dupilumab-related conjunctivitis appears to respond to 0.1% fluorometholone, eye drops, or unlabeled tacrolimus indication in 0.03% ophthalmic ointment.[195] The appearance of scarring ectropion has been reported in one patient treated with it for atopic dermatitis.[196]

Biological agents targeting IL-5, such as mepolizumab, reslizumab, or benralizumab, have not yet been studied in the context of allergic conjunctivitis.

Alternative treatments

Alternative and complementary treatments (CAMs) are called any therapeutic intervention outside the dictates of conventional medicine. Although MACs are used by approximately 80% of the world's population, mostly in Eastern countries, their use is spreading to the West.[197] This increase is based on their low cost, favorable safety profiles, in which they are generally regarded as “natural” treatment alternatives in contrast to the aversion to chronically used drugs (especially corticosteroids) and the sometimes poor outcomes in this chronic disease with conventional therapies. It is noteworthy that despite its widespread use, there are few randomized placebo-controlled studies showing its effectiveness, which makes these alternatives not implemented in international guidelines.[197]

When allergists were consulted about their patients' use of MACs, a high percentage reported that they did. Among them, the most popular were: medicinal plants, vitamins, probiotics, acupuncture, yoga, meditation, body massages, homeopathy, Ayurvedic medicine, etc., to name the most frequent.[198]

Therapy with medicinal plants

Also called phytomedicine or phytotherapy, it uses certain plants that have anti-inflammatory activity.[199]

Butterbur (*Petasites hybricus*): is an herb native to Europe, North Africa and Southeast Asia. In vitro petasins have been shown to inhibit leukotriene synthesis, block histamine binding to H1 receptors, and mast cell degranulation.[200] In a double-blind randomized trial, it demonstrated the same efficacy as cetirizine, with fewer side effects.[201]

Euphrasia (*Euphasia officinalis*): is part of the anthroposophical therapies, very popular in Central Europe. Among its active principles are tannins, flavonoids and phenolcarboxylic acid, which give it astringent, antiseptic and anti-inflammatory capacity. Its use in drops has shown significant effectiveness in reducing eye symptoms, although more studies are needed to determine its usefulness.

Argemone mexicana (berberine, cardose or poppy): is a plant native to America,[202] from which a milky liquid is extracted that contains alkaloids, and has anti-inflammatory and antibiotic effects, which led to its use in the treatment of conjunctivitis.[203]

Lycopus lucidus (Lycopene from China): it is a plant native to China and North America, widely used in Korean traditional medicine. It has compounds that inhibit mast cell degranulation, reducing immediate allergic reactions.[204]

Flavonoids: they are polyphenolic metabolites widely found in vegetables and fruits that make up the usual diet (citrus fruits, onions, green tea, wines, etc.), with proven anti-inflammatory and antioxidant capacity, with numerous health benefits (arteriosclerosis, obesity, Parkinson's, dementia and allergic diseases, etc.). They suppress leukocyte adhesion and neutrophilic degranulation, decrease histamine release from mast cells and basophils,

and inhibit the production of IL-4 and IL-13, thus acting in allergic diseases. The most studied flavonoids for the treatment of allergic conjunctivitis are quercetin, isoquercetin, and catechin.[205]

Application of extracts of *Artemisia abrotanum L* (has a high content of quercetin), granules of Yupingfeng (a mixture of plant roots widely used in traditional Chinese medicine) and *Perilla frutescens* (mint family) via the nasal or conjunctival route were evaluated by dual studies blind, randomized, evidencing relief of symptoms of allergic conjunctivitis, with good tolerance.[7,205]

Cannabinoids: Stimulation of cannabinoid receptors present in the ocular conjunctiva (CB1 and CB2 receptors) produces an analgesic and anti-inflammatory effect, also reducing intraocular pressure. The use of flavonoids and cannabinoids opens up a number of therapeutic opportunities due to their proven efficacy with few side effects.[205]

Other herbs widely used in traditional Chinese medicine (Biminna, Bu-Zhong-yi-Qi-Tang, Shi-Bi-Li) or Japanese (Sho-seiryu-to) have demonstrated efficacy in vivo, but further studies are needed to corroborate their usefulness. clinic.[206]

Indian ayurvedic medicine

There is literature, although scarce, on the use of Ayurvedic medicine, using a mixture of herbs and compared with placebo or medication. The efficacy and safety of Triyushnadi Anjana and cromolyn drops were compared, and significant improvement was seen in the group using Ayurvedic medicine versus cromolyn, with no significant side effects.[207] Other herbal blend formulas have been tested (Aller-7) and compared with prednisone and ibuprofen, with excellent results in animals, but there is no report of their effectiveness in humans.[206]

Acupuncture

Acupuncture is one of the most widespread forms of traditional Chinese medicine in the world. According to her, the placement of needles in already defined places achieves a redistribution of vital energy (Chi or Chi) that constitutes the organism. It has shown a significant effect in the treatment of allergic rhinoconjunctivitis, improving ocular symptoms and quality of life, when compared to placebo in randomized placebo-controlled studies.[208-210] The American Academy of Otolaryngology and Head and Neck Surgery suggests it as a treatment option in patients who do not wish to use conventional medicine treatments.[211] However, due to potential methodological errors (standardization of sites, technique, etc.) and the lack of availability of double-blind placebo-controlled studies proving their usefulness, it can be concluded that more evidence and better levels of standardization are needed. for the treatment of allergic rhinoconjunctivitis.[206]

Energy medicine

Controlled studies that can demonstrate the benefit of using energy channeling techniques (Reiki, Qigong) are needed, as their role in treating allergic diseases has not yet been demonstrated.[206]

Homeopathy: Homeopathy assumes that if the substance that produces a symptom is incorporated into the body in extremely dilute solutions, the pathology is resolved.[212] Its practice is widespread throughout the world, and there is a large bibliography on its usefulness in the treatment of allergic diseases, although some of these publications have methodological problems that make their comparison difficult. Numerous publications have shown a significant improvement when the administration of homeopathy was compared with placebo or a reduction in the use of antihistamines and anti-inflammatories to treat allergic rhinoconjunctivitis when administered together with them, both in children and adults, and with anti-inflammatory drugs. long duration of therapeutic effect.[213-219] Homeopathic immunotherapy, in which extensive dilutions of allergens (mites, pollens) are provided, has also been shown to be useful in the treatment of allergic conjunctivitis, with a rapid therapeutic response and low cost.[214-220] Despite their widespread use throughout the world, MACs offer an acceptable clinical response, with few side effects and lower cost than conventional drugs, but doses and administration procedures are generally not standardized and the literature in some cases is scarce. and methodologically poor. More quality studies are needed to determine the exact mechanism of action, standardize its doses and provide adequate safety margins.

Probiotics

Recent studies have evaluated the effects of different probiotic preparations on ocular symptoms and quality of life in patients with allergic conjunctivitis. Despite the observed beneficial effects, the small number of studies and patients involved does not allow the generalization of these results.[221,222]

Psychotherapy

Patients with severe forms of ocular allergy such as vernal keratoconjunctivitis (CCV) and atopic keratoconjunctivitis (AKC) may need psychological support due to the impact on quality of life and the limitations imposed by the disease. A collaborative approach between the GP, the specialist, and the psychologist should be considered in these cases.[223]

Control assessment

The goal of treatment in allergic conjunctivitis is to minimize the inflammatory effects associated with the allergic response, providing relief from symptoms and preventing complications associated with prolonged ocular inflammation.[192]

We can define control as the disease state in which clinical manifestations are absent or almost completely resolved with instituted therapy.[224] The evaluation of control is an essential part in the monitoring of chronic diseases, since they improve medical decision-making and, consequently, the treatment of patients. In the case of allergic conjunctivitis, follow-up should be multidisciplinary and carried out, whenever possible, in conjunction with the ophthalmologist. Even so, it is important that the clinical physicians involved in the treatment (specialists and generalists) know how to uniformly evaluate the patient's control without the need for specific tools, often available only in the ophthalmological office. For this purpose, clinical questionnaires were developed, which can be answered directly by the patient (or by their caregiver, in pediatric cases), being short, easy to understand and simple to perform.

Control questionnaires for chronic diseases can be based on objective and/or subjective symptoms, but should ideally also assess the patient's quality of life. As allergic conjunctivitis often occurs in association with other diseases such as asthma and allergic rhinitis, some questionnaires developed for these pathologies also include the control of allergic conjunctivitis. We can mention the RQLQ (Rhinoconjunctivitis Quality of Life Questionnaire),[225] the RCAT (Rhinitis Control Assessment Test),[226] the nasal and extranasal symptoms score (ESN), and the visual analogue scale (VAS). In the latter, symptoms are individually graded on a numerical scale of 0-10 cm and scored by the patient, being considered a simple and practical way of evaluating control, however, it does not consider aspects of quality of life. When applied to conditions associated with allergic rhinitis, symptoms can be graded as: moderate/severe > 5 and mild ≤ 5.[227] In the case of RCAT, there are versions validated and translated into several languages, in addition to specific versions for both adults and children. The document ARIA (Allergic Rhinitis and its Impact on Asthma)[228] only suggests the classification of ocular allergy by grading severity and persistence of symptoms. Severity can be classified as “mild”, “moderate” or “severe”, based on the presence or absence of visual disturbances, impairment in daily activities and other symptoms that generate discomfort (Troublesome symptoms).

However, when we consider the occurrence only of allergic conjunctivitis, the literature is scarce for tools that assess it exclusively. The evaluation of the four main symptoms: hyperemia, pruritus, edema and tearing constitutes a non-validated questionnaire, called TOSS[229] (Total Ocular Symptom Score) and that can, similarly to the ESN, assess severity and monitoring of treatment. The only questionnaire available to assess quality of life was developed for use in children with vernal keratoconjunctivitis, and is called QUICK.[230] This questionnaire grades the frequency of eye allergy symptoms on a 3-point scale (1 - never, 2 - occasionally, 3 - always), but can only be used in a specific eye allergy situation.

Recently, Sánchez-Hernandez et al.[231] validated a questionnaire (Table 5) to assess severity and clinical control of allergic conjunctivitis. This questionnaire assesses: ocular symptoms, visual analogue scale and hyperemia. The evaluation of this last item is performed through conjunctival and limbal hyperemia separately, using the Efron scale[232] (Figure 8).

In addition to the scarcity of clinical questionnaires, the difficulty in grading ocular symptoms also occurs because it is an observer-dependent assessment. For this reason, it is recommended that, regardless of the tool chosen, the physician is used to always using it, knowing its strengths and weaknesses, improving the exchange of information between the members of the multidisciplinary team and benefiting the follow-up of the patient.

In view of the above, there is still a great need to advance in the development of more complete questionnaires, which can measure the severity and control of different types of allergic conjunctivitis and in different age groups,[233] favoring a better monitoring and control of patients with ocular allergy.

Complications of allergic conjunctivitis

Complications depend heavily on the disease phenotype and clinical presentations: seasonal and perennial allergic conjunctivitis, giant papillary conjunctivitis, and blephorallergic contact conjunctivitis, while others are not always explained by exposure to allergens, such as vernal keratoconjunctivitis and atopic keratoconjunctivitis.[234]

There are clinical manifestations associated with this condition with the presence of frequently encountered eye lesions, such as: edema of the conjunctival cul-de-sac, papillae in the tarsal conjunctiva, tear film defect, perilimbic pigmentation, corneal epithelium defect, blepharitis, Trantas, aggregate infectious conjunctivitis and pterygium.[235]

Seasonal allergic conjunctivitis or perennial allergic conjunctivitis are accompanied by hyaline-like tearing, eyelid edema, and chemosis, and depending on the duration of symptoms, the associated complications are infectious, in addition to dry eye syndrome which, although it appears to be a not-so-serious complication, can cause serious damage to the ocular surface, affecting the quality of vision and life of patients, with a high cost of medical care due to the high frequency of consultations and treatments.[235]

Vernal keratoconjunctivitis presents complications such as punctate keratopathy, corneal ulcers, conjunctival infiltrates, and giant papillae in the tarsal conjunctiva in up to 6% of patients. Other possible sequelae are amblyopia and keratoconus, which in severe cases may require corneal transplantation. Giant papillae that do not respond to medical treatment can be surgically removed in case of corneal involvement and shield ulcer with inflammatory plaque, and surgical debridement with or without amniotic membrane transplantation may be necessary. Limbic stem cell deficiency, a rare complication, can be treated with a limbal conjunctival allograft.[236]

In atopic keratoconjunctivitis, we found findings similar to allergic conjunctivitis, with worsening eyelid damage. The addition of chronic inflammatory changes on the ocular surface (corneal scarring and neovascularization) and varied changes in the eyelids and periorbital skin, ranging from mild atopic dermatitis to lichenification. complications: staphylococcal blepharoconjunctivitis and herpes simplex keratitis, cataracts, limbal stem cell deficiency, keratoconus, glaucoma, retinal detachment, and corneal or conjunctival tumors.[236]

Table 5
Control criteria adapted from Sanchez-Hernandez et al.[231]

	Controlled (listed below)	Not controlled (at least 1 present)
Eye symptoms (itching, tearing, visual discomfort)	No symptoms or few symptoms or < 2 days/week	Any intensity > 2 days/week
Analog visual scale	< 5 cm	> 5 cm
Hyperemia (Efron scale)	0-1	2-4

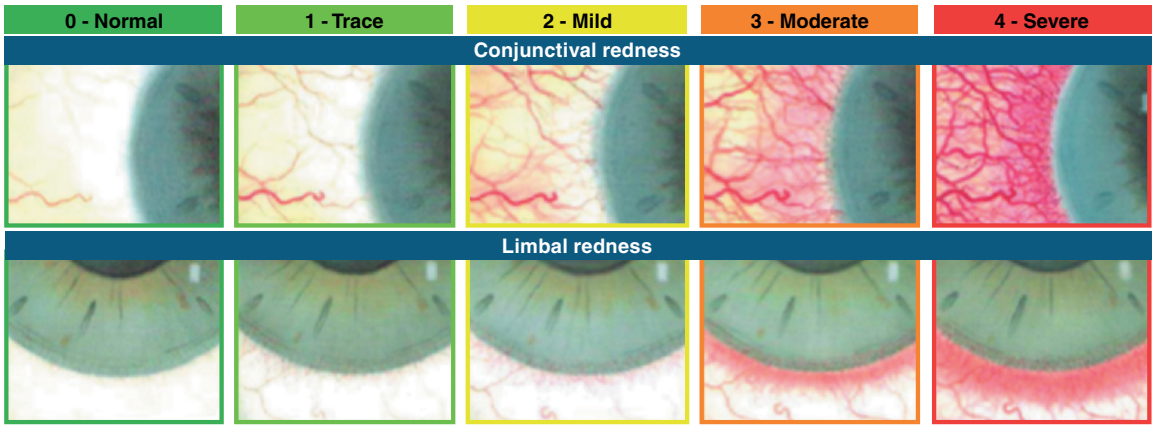


Figure 8
Efron scale

Other complications

One of the most common problems is conjunctival infection; we often find autoinoculating germs, such as *Staphylococcus aureus*, *Hemophilus influenzae* and other streptococci.

Viral infections induce conditions such as acute follicular conjunctivitis, both in children and adults, and the associated viruses are adenoviruses that induce epidemic keratoconjunctivitis and fever, with transmission by direct contact and manifest a week after exposure affecting one of the eyes, and later in both eyes, causing punctate erosions ranging from 1-50 mm with epithelial and subepithelial infiltrate, and management is usually related to the appropriate use of steroids.

Inclusion conjunctivitis is caused by *Chlamydia trachomatis* and is related to sexually active people because it is an oculo-genital disease, as the name indicates that the germ is included in a systemic way, therefore, oral management with macrolides is recommended.

Herpes virus infections also induce follicular conjunctivitis, but occasionally manifestations are few and must be evidenced with fluorescent antibody techniques. Epstein Barr virus infections cause follicular or membranous conjunctivitis with or without hemorrhages, and the symptoms are typical of mononucleosis associated with fever, lymphadenopathy, etc. And when this condition appears, it is very similar to adenovirus keratitis. They may also be associated with other RNA viruses such as Paramyxoviridae, Orthomyxoviridae, Togaviridae and Flaviviridae.

There are complications from chronic eye rubbing. These complications are due to biomechanical processes that are expressed by structural changes of the cornea and ectatic disorders of the cornea such as keratoconus, keratoglobulin and pellucid marginal degeneration, with an evident predominance of keratoconus causing corneal remodeling.

Some of the complications are a result of friction; as an example of glaucoma, since intraocular pressure peaks are related to friction. Another example is dropsy with or without perforation, iris prolapse, lens capsule rupture and intraocular lens displacement, and retinal detachment.[234]

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Special Article

Introduction of food in the first year of life and food allergy prevention: what is the evidence?

Introdução dos alimentos no primeiro ano de vida e prevenção da alergia alimentar: quais as evidências?

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ABSTRACT

Objective: The incidence of allergic diseases has increased in recent decades. In an attempt to contain the increase in food allergy (AA) over the years, prevention strategies have been implemented. To promote a better understanding of the dilemmas that permeate the introduction of food in the first year of life, this article deals with a narrative literature review on the introduction of complementary foods in the first year of life and possible associations with the primary prevention of food allergy. **Data source:** Relevant publications were searched in the Cochrane Library, MEDLINE, PubMed, Guidelines International Network, National Guidelines Clearinghouse, and revised recommendations from the national food allergy guide and consensus. **Results:** Several observational studies and randomized controlled trials are available, as well as recommendations. published by scientific organizations; however, of variable quality. Recommendations from clinical practice guidelines classified as high quality and recent publications not yet systematically categorized in their quality, but internationally recognized as relevant to primary care, were considered. **Conclusion:** To date, there is no consistent evidence that the early introduction, before 6 months, of allergenic foods contributes to the prevention of food allergy in the general population.

Keywords: Primary prevention, food hypersensitivity, infant food, child development, eating.

RESUMO

Objetivo: A incidência das doenças alérgicas cresceu nas últimas décadas. Na tentativa de conter o aumento da alergia alimentar (AA) ao longo dos anos, estratégias de prevenção vêm sendo implementadas. Para promover um melhor entendimento dos dilemas que permeiam a introdução alimentar no primeiro ano de vida, esse artigo trata de uma revisão bibliográfica narrativa sobre a introdução dos alimentos complementares no primeiro ano de vida e possíveis associações com a prevenção primária da alergia alimentar. **Fonte dos dados:** Publicações relevantes foram pesquisadas nas bases de dados Cochrane Library, MEDLINE, PubMed, Guidelines International Network, National Guidelines Clearinghouse e revisadas recomendações do guia e do consenso nacional de alergia alimentar. **Resultados:** Estudos observacionais diversos e ensaios clínicos randomizados estão disponíveis, bem como recomendações publicadas por organizações científicas; no entanto, de qualidade variável. Foram consideradas as recomendações de diretrizes de prática clínica classificadas como de alta qualidade e publicações recentes ainda não categorizadas de forma sistemática em sua qualidade, mas internacionalmente reconhecidas como relevantes para a atenção primária. **Conclusão:** Até o momento, não há evidências consistentes de que a introdução precoce, antes dos 6 meses, dos alimentos alergênicos, contribua para a prevenção de alergia a alimentos na população geral.

Descritores: Prevenção primária, hipersensibilidade alimentar, alimentos infantis, desenvolvimento infantil, ingestão de alimentos.

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The incidence of pediatric atopic diseases has increased in recent decades.[1] Genetic and environmental factors, in constant interaction since the intrauterine period, constitute the pathophysiological basis of these diseases.[2] AtIn an attempt to contain the increase in food allergy (AA) over the years, prevention strategies have been implemented, among them, changes ins eating habits, notably in the introduction of food in the first year of life.[3]

In the 1990s, delaying the introduction of the most allergenic foods in the child's diet was the strategy adopted.[4] However, these recommendations have not been scientificallysustained as new studies emerged and suggesting if it would not be early consumption of allergenic foods the best way for the induction of oral tolerance.[5]

Clinical trials were designed to test the "Allergen Exposure Route Hypothesis", according to which early consumption of food allergens could induce oral tolerance, while allergic sensitization to food allergens could occur transcutaneously.[6-12] However, the methodological diversity of these trials brought conflicting results, andthe incorporation of the new recommendations has become a challenge in regions with prevalence of AA, socioeconomic conditions and different eating habits.[13]

To promote a better understanding of the dilemmas that permeate the introduction of food in the first year of life, this article deals with a narrative literature review on the introduction of complementary foods in the first year of life and possible associations with the primary prevention of food allergy, through questions and answers on the main controversies on the subject, adapting scientific evidence to clinical practice.

Is the definition of early or late introduction of complementary feeding uniform in literature?

The results of the studies still show controversies about the ideal moment[14] and few conclusions can be assumed to be definitive.

In 2001, the World Health Organization (WHO) published a systematic review with the objective of evaluating the scientific evidence about the ideal period for the practice of exclusive breastfeeding. The authors concluded that exclusive breastfeeding for six months should be instituted, instead of the previous orientation, for four to six months, in view of the numerous evidences of short- and long-term benefits for the mother-child binomial and for society.[15] In agreement, the "Food Guide for Brazilian Children Under 2 Years Old" by the Ministry of Health, published in 2019,[16] and the "Practical Feeding Guide for Children 0 to 5 Years Old – 2021", prepared by the Brazilian Society of Pediatrics,[17] recommend exclusive breastfeeding until the 6th month of life.

From six months of age, other foods should be part of the child's meal. The transition to family meals should occur around 12 months.[16,17] With these milestones, we can define, for our population, early introduction as the offer of foods complementary to breast milk before six months of age. On the other hand, in the light of current knowledge, the offer of potentially allergenic foods after 12 months of age would be considered a late offer. However, these definitions are no longer so evident in situations where breastfeeding is not possible, or is insufficient.

Recommendations from other countries and entities may differ from those recommended by the WHO[15] and Brazil.[16,17] The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends starting the introduction of complementary foods between four and six months of age (17th and 26th weeks of life), and should not be introduced before four or delay beyond of six months.[18]

The early introduction of complementary feeding may have a role in the development of food allergy?

Two decades ago, it was believed that allergic sensitization to foods occurred through oral exposure and, therefore, preventing AA translated into delaying the introduction of potentially allergenic foods.[19] However, despite the delay in this introduction, the prevalence of AA continued to increase, which led specialists to reassess the recommendations and develop new prevention strategies, one of which focused on the ideal time to introduce allergenic foods into the diet of children.[20]

Observational and in vitro studies in animals and humans have demonstrated transcutaneous sensitization to food allergens through inflamed skin, with eczema.[21-23] Data published by Fox et al. suggested that high levels of environmental exposure to peanuts during childhood could promote sensitization rather than tolerance.[24] An ecological study evaluating the prevalence of peanut allergy in infants in Israel and the UK found a significantly higher rate in the UK (1.85% vs. 0.17%). An explanation for this difference, favoring tolerance, would have been

the early consumption and in greater amounts of peanuts in Israeli children.[25]

Based on previously published data, researchers have developed the "Allergen Exposure Route Hypothesis" as one of the new prevention strategies. She suggested that the balance of exposures during the first year of life, whether through the skin or the intestine, would prepare the immune system for allergy or tolerance, respectively.[26]

Clinical trials were needed to test the new hypothesis. The Learning Early about Peanut Allergy (LEAP) study was the first clinical trial designed to demonstrate whether the introduction of peanuts before one year of age could serve as effective primary and secondary prevention strategies in peanut allergy.[6] The introduction of peanuts, between 4 and 11 months of age, significantly reduced the frequency of peanut allergy among children, sensitized or not, classified as high risk, due to egg allergy and/or severe atopic dermatitis.[6] The results demonstrated raised questions: would the benefit of the early introduction of peanuts also be found with other foods and in the general population?

The Enquiring About Tolerance (EAT) study evaluated whether the introduction of six common allergenic foods (peanuts, milk, egg, wheat, fish and sesame) could prevent AA in 1,303 infants from the general population, exclusively breastfed up to three months of age.[7] According to the intention-to-treat analysis, 5.6% of infants with early introduction, between three and six months of age, developed AA to at least one of the six foods at three years of age, whereas in the standard group , fed solids from 6 months of age, the rate was 7.1%, a difference not statistically significant. However, in the per-protocol analysis, a significant reduction in allergy was demonstrated between different foods (2.4% vs. 6.4%), more specifically to egg and peanut, in the early introduction group, between 3-6 months, suggesting that the introduction of food in a "time window" could prevent food allergy[7].

Unfortunately, the dropout rate from the EAT study was very high, 69.1% of infants recruited, representing an important bias in the per-protocol analysis. Analyzing the protocol adherence rate for each food, the lowest value was found for the egg (43.1%), suggesting that the consumption of boiled egg could be the main factor for abandonment.[27]

Randomized placebo-controlled clinical trials were designed to assess the preventive effect of early egg introduction in AA7-12 (Table 1). Among the evidence, there were methodological differences related to the type of food chosen, the form of presentation of the food, the dose of allergenic protein consumed, and the outcome sought (Table 1). These differences generated varied conclusions, sometimes antagonistic, and not always comparable.[2]

Currently, although the strength of evidence is greater for the introduction of peanuts in a high consumption population, such as the United States, studies with other allergenic foods, such as eggs, have also shown a benefit in not delaying their offer during the food transition. However, further studies are needed in larger populations, with different diets and environmental exposures to generalize and assess the safety of this potential preventive strategy.

In which populations does the age of introduction of potentially allergenic foods deserve consideration in the food allergy scenario?

Genetics have a strong influence on the prevalence of food allergies. A child has a sevenfold increased risk of peanut allergy if they have an affected parent and/or sibling.[28] Monozygotic twins have a 64% probability of peanut allergy if their twin is allergic.[29] However, research substantiates that, by themselves, alterations or genetic factors do not explain the increasing prevalence of AA.[28,29]

According to international recommendations, a child at high risk for developing food allergy is defined as when one or more family members (parents and/or siblings) have eczema, food allergy, asthma or allergic rhinitis.[2] The LEAP study in 2015 considered two factors as high risk for peanut sensitization and allergy: severe eczema and/or egg allergy.[6]

In groups at risk for atopy, not delaying the introduction of allergenic complementary foods has been shown to protect against the development of food allergy.[6,8-12] However, a concern is the possibility of previous sensitization to the food to be introduced, including the chance of immediate clinical manifestations.[6] Although some allergic reactions were observed in the LEAP[6] study, there is no recommendation for tests to search for specific IgE in foods, as a routine for food introduction. The exceptionality of food allergy, in this situation, would not justify the recommendation of this conduct, which is difficult to implement in terms of public health and

which could even bring harm due to the delay in food introduction.[30]

Other aspects are cultural issues, family preferences and specific risks, such as the parents' fear of introducing food when the other child has this type of allergy. It is important to establish a trusting doctor-family relationship and guide the family that a child considered to be at "high risk" for developing food allergy will not necessarily develop it.

Do formulas for artificial breastfeeding have an impact on the outcome of food allergy?

Given the impossibility of exclusive breastfeeding, polymeric infant formulas should be introduced to children without allergy symptoms, in accordance with international and national recommendations.[15-17] However, a recent guideline updated by the European Academy of Allergy and Clinical Immunology (EAACI) reinforces the need to avoid supplementation of breast milk with infant formula in the first week of life, which is associated with a greater chance of developing allergy,[31,32] in addition to increasing the risk of early weaning, according to the WHO.[15]

The main considerations regarding infant formula and food allergy prevention are derived from the German GINI (German Infant Nutritional Intervention Study). In this study, 2252 children at risk for allergies (one allergic first-degree relative) were randomized (blinded for the first three years) to receive either standard cow's milk formula or hydrolyzed formula (partially or extensively hydrolyzed whey protein). or extensively hydrolyzed casein formula) in the first four months of life, when exclusive breastfeeding was not possible. Allergic outcomes of this study have been evaluated for the last 20 years. In the last publication, the authors concluded that extensively hydrolyzed

Table 1
Clinical trials on egg introduction and allergy prevention

Test name, country	Population	Type of food	Proteins	Intervention period months (m)	Result in the analysis by ITT (P value)
EAT[7], UK	General	Milk, peanuts, eggs, sesame, fish and wheat	4 g/week	3 to 6 m	RR, 0.69 (95%CI, 0.40-1.18); p = 0.17
STAR[8], Australia	High risk (child with moderate eczema)	Pasteurized raw egg	0.9 g/day	0-8 m	RR, 0.65 (95%CI, 0.38-1.11); p = 0.11
HEAP[9], Germany	General	Pasteurized raw egg	2.5 g 3 times/week	4 to 12 m	RR, 2.2 (95%CI, 0.68-7.14); p = 0.24
STEP[10], Australia	Moderate risk (atopic mothers)	Pasteurized raw egg	0.4 g/day	4-10 m	Adjusted RR, 075 (95%CI, 0.48-1.17); p = 0.20
BEAT[11], Australia	Moderate risk (first-degree relatives with allergies)	Pasteurized raw egg	0.35 g/day	4-8 m	OR, 0.46 (95%CI, 0.22-0.95); p = 0.3
PETIT[12], Japan	High risk	Freeze-dried boiled egg	0.175 mg/day ai-3 months 0.875 mg/day ai-3 months	4-12 m	RR, 0.222 (95%CI, 0.22-0.95); p = 0.0012

EAT = Enquiring About Tolerance, STAR = Solids Timing for Allergy Research, HEAP = Hen's Egg Allergy Prevention, STEP = Starting Time of Egg Protein, PETIT = Prevention of Egg Allergy with Tiny Amount Intake, BEAT = Beating Egg Allergy Trial, ITT = analysis by intention to treat, OR = odds ratio, RR = relative risk, 95%CI = 95% confidence interval.

casein formulas and partially hydrolyzed whey protein formulas reduced the prevalence of eczema and asthma.[33] However, a systematic review in 2018 concluded that there was no substantial evidence proving that the use of hydrolyzed formulas prevents allergic diseases.[34]

Therefore, we should encourage exclusive breastfeeding up to six months of life, avoid the use of polymeric formulas in the first week of life, even if it is for the purpose of complementation. There is still no evidence that hydrolyzed formulas prevent allergies when breastfeeding cannot be maintained. There is also no evidence that delaying the introduction of cow's milk protein intake prevents allergy.

Should early introduction for allergy prevention be instituted for all foods?

Clinical trials have provided evidence that the early introduction of peanuts and chicken eggs decreased the incidence of peanut and egg allergy in infants at high risk of developing AA. Studies on prevention of other food allergens have been less robust and have shown evidence of safety, but not necessarily efficacy.[35]

Regarding the introduction of cow's milk (CM), whether in the form of infant formula, raw milk or yogurt, there is no robust evidence that early introduction plays any protective role. Its introduction in the first days of life can contribute to the development of food allergy,[32] however, the introduction from 3-4 months or after 6 months did not show any difference in effect in relation to food allergy.[36] Likewise, there is no evidence to justify the delay in the introduction of CM proteins after 12 months of life.[37] Regarding special infant formulas, partially or extensively hydrolyzed, data published in a systematic review no longer justify their use for the prevention of allergy to cow's milk proteins (CMPA).[34,38] Numerous international societies are revising the recommendations of introducing special hydrolyzed formulas for the prevention of CMPA, instead of using conventional infant formulas. However, in the last GINI publication in 2021, the authors concluded that extensively hydrolyzed casein and partially hydrolyzed whey protein formulas reduced the prevalence of eczema and asthma.[33]

Regarding the introduction of eggs, studies vary greatly in terms of the recommended dose, the type of exposure (whether whole egg, or raw or cooked pasteurized whites) and the selected population (presence of atopic dermatitis or increased risk of atopy).[2,6,40] A systematic review and meta-analysis, published in 2016, involving the grouping of five studies, with 1,915 participants, showed that the early introduction of eggs (between 4 and 6 months) was associated with a significant reduction in egg allergy.[39]

According to the position of the European Food Safety Authority, this evidence is of low to moderate confidence, and therefore insufficient to support the introduction of the egg at 3-4 months of age in all infants, for the prevention of allergy. In the studies, no serious adverse reactions were observed with the boiled egg, but when the intervention consisted of pasteurized raw egg powder, some anaphylactic reactions occurred. Therefore, products containing raw eggs, even if pasteurized, should be avoided.[30,41] Boiled eggs should be introduced into children's diets, similarly to other complementary foods, around 6 months of age.[30,41]

British Society of Allergy and Clinical Immunology (BSACI) guidelines suggest that eggs and peanuts can be introduced as part of the family diet in high-risk infants between 4-6 months of age. However, they recommend the introduction of the egg before the introduction of peanuts, because sensitization to the egg seems to occur earlier.[42]

Regarding the introduction of peanuts, a meta-analysis showed moderate evidence from two studies (1,550 participants) that the introduction of peanuts between 4 and 11 months was associated with a reduction in peanut allergy.[40] According to the position of the European Food Safety Authority, there is evidence that introducing peanuts during the first year of life, compared with avoiding them until 5 years of age, reduced the risk of developing peanut allergy. However, evidence is insufficient to conclude whether, when comparing infants who were introduced to peanuts at ≤ 6 months of age with those who were introduced at > 6 months but still within the first year of life, a similar effect.[41] American guidelines recommend the introduction of peanuts to infants between 4 to 6 months of age in countries with the highest peanut consumption.

The HealthNuts study reported that cashew nut intake for high-risk patients (severe atopic dermatitis and/or egg allergy)[27] before 1 year of age (n = 140) was associated with no cases of cashew allergy at 6 years.[44] There are no studies available on the safety or efficacy of the early introduction of other nuts, soybeans or shellfish.[35]

In light of current knowledge, there is no consistent evidence that the early introduction, before 6 months, of allergenic foods contributes to the prevention of allergy to these foods in the general population.[30,40]

Can the recommendations for the complementary introduction of external studies be widely applied in Brazil?

It is known that the introduction of complementary foods together with breast milk reduces the amount ingested and, consequently, all its immunological benefits, such as the prevention of infections and reduction of infant mortality, and non-immunological benefits, such as the optimized absorption of iron and zinc. via breast milk by the infant, which will be reduced.[16,17] The literature does not allow establishing whether breastfeeding has any protection in relation to AA.[45] It is suggested that the introduction of allergenic foods while the child is breastfed may have a protective effect, but there is insufficient evidence in this regard.[45] Although the results of the EAT study showed that there was no reduction in breastfeeding rates with the introduction of solid foods from 3 months of age, there are no proven data on the risk nor on benefit of the reduction of exclusive breastfeeding in a country like Brazil, with high poverty rates, food insecurity and malnutrition, where breast milk guarantees effective nutrition security for the infant.

The first motor skills indicative of developmental readiness for spoon feeding can be observed between 3 and 4 months of age. At this age, it can be assumed that the search and extrusion reflexes may also have diminished in some babies. In preterm infants, the developmental milestones required for feeding are also reached around the same age group (post-term), depending on the severity of the illness experienced during the neonatal period, the degree of prematurity, and any sequelae.[41]

Accordingly, the EAT was the only early introduction trial (3 months) with multiple allergens simultaneously.[7] Parents should offer their children, from 3 months of age, along with breastfeeding, boiled eggs, peanut butter, cow's milk yogurt, cooked white fish, sesame paste and wheat-based cereal.[7] However, the adherence of the intervention group was much lower than that of the control group (31.9% vs. 92.9%).[7] The likely reasons for the low adherence were the difficulty in cooking certain foods and the palatability.[7]

There is no evidence that the order of introduction of the various solid foods contributes to a greater or lesser risk of food allergy.[47] Thus, timely food introduction should follow the dietary habits of families, allowing the child to have contact with all food groups between 6 and 12 months of age.[17] There is no reason to delay the introduction of potentially allergenic foods (eggs, cereals, milk proteins, meat and fish) beyond 1 year of age, nor to advance exposure to potentially allergenic foods to before 6 months of age.[47] Nuts, peanuts and seafood can also, and ideally should, be introduced to the child during this period.[17]

For children at high risk of developing food allergies, those with severe eczema, egg allergy, or both, guidance from the US Institute of Allergy and Infectious Diseases, in relation to peanuts, its introduction between 4 and 6 months of age, occurs after performing the prick test or serum IgE specific for peanuts and, when necessary, performing the oral provocation test.[48] However, this would imply that all high-risk children have access to a specialized allergy service for testing the main allergens – in this case, peanuts, which is unfeasible in most countries, including Brazil.[46] So this is yet another question that remains open, waiting for more evidence.

What other factors, in addition to complementary feeding, can interfere with the outcome of food allergy?

Genetic, environmental and dietary factors can influence the occurrence of food allergy.[49] Among the genetic factors, those that predispose to defects in the filaggrins and, consequently, in the skin barrier, facilitate transcutaneous sensitization.[50] In this rationale, we sought to assess whether the use of moisturizers was likely to reduce early transcutaneous sensitization, thus preventing the development of food allergy.[12] To date, the results have not confirmed this hypothesis, as evidenced in a systematic review. However, it is argued that perhaps more specific moisturizers can bring more promising results.[50]

Living with animals and other people can be considered protective environmental factors, while the use of antibiotics, cesarean delivery and acid secretion inhibitors are risk factors.[51] Regarding diet,has been studied as protective factors for breastfeeding, use of probiotics and preference for foods prepared at home and with high fiber content.[49] A study with 1,628 children showed that a maternal diet during pregnancy rich in processed and sugary products, combined with a longer period of breastfeeding, may

favor food allergy, suggesting a harmful effect of trans fats in children.[51] More studies with supplementation of short-chain fatty acids and fiber are needed,[52,53] in addition to studies to confirm the immunomodulatory effects of vitamin D and antioxidants in the prevention of food allergy.[54,55]

Regarding the effect of probiotics,it is known that may have a role in inducing regulatory T cells in the mucosa, reinforcing the epithelial barrier and protecting against sensitization to food allergens.[56] It is not clear what the ideal time for intervention would be, but according to a multicenter study, perhaps the age of 3 to 6 months is a window during which the intestinal microbiota can influence food allergy.[53] Clearer definitions of healthy and allergenic microbiomes are needed, taking into account that they vary across different ages, regions, risk groups and social classes. Although probiotics show promise in preventing food allergy, a recent systematic review by the European Academy of Allergy[57] and the World Allergy Organization concluded that data are insufficient to recommend supplementation with probiotic, prebiotic, symbiotic or fecal transplant in food allergy, due to the limited and low level of quality of evidence, whether in the child or the pregnant woman.[58]

Roduit et al. evaluated that, in a cohort of 301 children, consumption of yogurt, fish, vegetables and fruits in the first year of life was associated with an increase in butyrate, a metabolite of the healthy microbiota, in the feces of children at age 1 year and reduced sensitization to food allergens up to the age of 6,[59] reinforcing the concept that a varied and healthy diet is important for the development of oral tolerance.[60]

Conclusion

Although in the literature there is no more evident standardization of what we consider early food introduction, we already have enough data to demonstrate that delay in food introduction is strongly associated with a higher risk of food allergies and, considering the various benefits of breastfeeding, it is suggested that the timely introduction of complementary feeding should not be carried out before six months of age, and that, from that age onwards, even potentially allergenic foods can be introduced into the diet, ideally within the first year of life, preferably while breastfeeding and maintaining a routine consumption, respecting the family's eating habits.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

ASBAI's position on vaccination of children aged 5 to 11 years against COVID-19 with the Comirnaty/Pfizer/BioNTech vaccine – 12/27/2021

Posicionamento da ASBAI sobre a vacinação de crianças de 5 a 11 anos contra a COVID-19 com a vacina Comirnaty/ Pfizer/BioNTech – 27/12/2021

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ABSTRACT

The Brazilian Association of Allergy and Immunology (ASBAI) is totally in favor of immunization against COVID-19 in individuals between 5 and 11 years old, for the protection not only of this group, but also of their cohabitants. The vaccination of children, once its efficacy and safety has been demonstrated, is essential for controlling the circulation of the virus and protecting individuals whose vaccine response may not occur efficiently, such as the immunocompromised and the elderly. The immunization of people between the ages of 5 and 11 must be a fundamental public health strategy to control the pandemic that has been plaguing us since March 2020 with all its serious consequences for public health and the economy.

Keywords: COVID-19 vaccines, immunization, child.

RESUMO

A Associação Brasileira de Alergia e Imunologia (ASBAI) se manifesta totalmente favorável à imunização contra a COVID-19 em indivíduos entre 5 e 11 anos, para a proteção não somente deste grupo, mas também de seus conviventes. A vacinação de crianças, demonstrada sua eficácia e segurança, é fundamental para o controle da circulação do vírus e proteção de indivíduos cuja resposta vacinal pode não ocorrer de modo eficiente, como os imunocomprometidos e idosos. A imunização de pessoas entre 5 e 11 anos deve ser uma estratégia de saúde pública fundamental para o controle da pandemia que nos assola desde março de 2020 com todas as suas graves consequências para a saúde pública e a economia.

Descritores: Vacinas contra COVID-19, imunização, criança.

In August 2020, the National Institute of Health for Women, Children and Adolescents Fernandes Figueira (IFF/Fiocruz), pointed out relevant issues to face the consequences of the COVID-19 pandemic on the health of children and adolescents in Brazil. Since the beginning of the pandemic, we have observed fewer symptomatic infections and cases of severe illness and deaths from COVID-19 in children and adolescents, compared to other age groups.[1]

Cases by age reported to WHO from December 30 2019 to October 25 2021 showed that children under 5 years old are 2% (1,890,756) of global reported cases,

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and 0.1% (1,797) of reported global deaths. Older children and younger adolescents (age range 5 to 14 years) account for 7% (7,058,748) of reported global cases, and 0.1% (1,328) of reported global deaths. Deaths in all age groups under 25 years accounted for less than 0.5% of reported global deaths. In Brazil, almost half of Brazilian children and adolescents who died from COVID-19 in 2020 were under 2 years of age; one third of deaths up to 18 years of age occurred among children under 1 year of age and 9% among babies under 28 days old.[2]

Since April 2020, in several countries in Europe, North America and even in Brazil, cases of children and adolescents with a new clinical presentation associated with COVID-19, characterized by a late and severe inflammatory condition, called Pediatric Inflammatory Multisystem Syndrome temporally associated with COVID-19 (PIMS-TS) or Multisystem Inflammatory Syndrome in Children (MIS-C), adapted to Portuguese as Pediatric Multisystem Inflammatory Syndrome (SIM-P). The main findings of this syndrome include: persistent fever, gastrointestinal symptoms (abdominal pain, nausea, vomiting), bilateral non-purulent conjunctivitis, signs of dermatological/mucocutaneous inflammation, in addition to frequent cardiovascular involvement. The most severe cases present with shock requiring hemodynamic support and, sometimes they can progress to death. In Brazil, surveillance of SIM-P cases associated with COVID-19 was officially implemented on July 24, 2020, supported by the case definition criteria standardized by the World Health Organization (WHO) and, since then, 1,377 cases have been reported. in all federative units, with a total of 84 deaths, corresponding to 6.1% of cases.[3]

It is worth mentioning that the COVID-19 diagnosis, in addition to the risk of severe disease and SIM-P, is also associated with a greater risk of the occurrence of different other conditions, such as myocarditis, pericarditis, cardiac arrhythmias, demyelinating diseases, encephalitis, syndrome Guillain-Barré syndrome, facial paralysis, myasthenia gravis, cerebral hemorrhages, acute renal failure, deep vein thrombosis, acute myocardial infarction and pulmonary embolism. Children and adolescents may also have prolonged clinical manifestations known as "long-term COVID-19", or post-COVID-19 syndrome,[9] post-acute sequelae of SARS-CoV-2 infection, but the frequency and characteristics of these diseases are still under investigation.[4,5]

Therefore, although the disease is milder in children when compared to adults and the elderly, it is important to emphasize that serious illness and deaths occur and that the presence of underlying diseases and comorbidities can contribute to the risk of different serious manifestations related to COVID-19.

Below we show that, although the number of cases is proportionally much smaller, these numbers are significant and would in no way be within an "acceptable" level.[6] Table 1 shows the number of deaths (1,207) in children under 18 years of age in 2020, with figures for different age groups.[7]

In Table 2, we can compare the available data from Brazil and the world, comparing the lethality of the disease in young people and adolescents, in addition to the available data on "severe" adverse reactions to the Comirnaty vaccine (released by ANVISA for children aged between 5 and 11 years) It becomes evident that the risk of dying from COVID in this age

Table 1
Number of cases and deaths from COVID-19 in Brazil

COVID by age group	Notified deaths in Brazil in 2020 (< 18 years old)	Case fatality rate in Brazil in 2020 (%) (< 18 years old)
> 5 and < 18 years	525	44.3%
< 5 years	71	5.9%
< 2 years	156	12.9%
< 1 years	335	27.8%
< 1 month	150	9.1%
Total	1,207	

group is between 10,000 and 20,000 times greater than the risk of having an adverse reaction to the vaccine.[8-10]

Efficacy and immunogenicity

Consistent data on the efficacy, immunogenicity, safety and tolerability of Comirnaty vaccine in patients aged 5 to 11 years are derived from a phase 2 and 3 study, involving approximately 2,268 participants, carried out in the USA, Finland, Poland and Spain, using two doses (of 10 µg) of a product with a concentration of 50 µg/mL, with an interval of three weeks.[11]

Efficacy was assessed through protection against symptomatic infection and production of neutralizing antibodies. It was identified that the vaccine was 90.9% effective against symptomatic infections, and that the response of production of neutralizing antibodies was as satisfactory as that observed in individuals between 16 and 25 years of age.[11]

We emphasize that the efficacy of two doses of the vaccine for children under five years has not yet been demonstrated in studies carried out by Pfizer.[12]

Safety

Among the 1,518 children vaccinated and the 750 who received placebo, no serious adverse events attributable to vaccination were observed. In addition, data from the US Centers for Disease Control describing adverse event reports in 5,126,642 vaccinated children (including 2,014,786 with the second dose) identified an incidence of 1.58 serious adverse events per 100,000 vaccinated children, with the most frequent serious events being fever, vomiting, chest pain and elevation of C-reactive protein. Fourteen cases with mention of myocarditis were reported, with eight cases meeting the standardized case definition (6 with the second dose and 2 with the first dose), representing an incidence of 0.04 cases of myocarditis per 100,000 first doses, and 0.29 cases of myocarditis per 100,000 second doses.[12]

Severe allergic reactions such as anaphylaxis can occur after any vaccine, including COVID-19 vaccines. The estimated rate of anaphylaxis for all vaccines is 1 in 1,000,000 doses applied, which is considered a rare event.[13] Regarding vaccines against COVID-19, the observation of anaphylaxis in the first days of mass vaccination with the PFIZER vaccine in the US and UK, led to an estimated occurrence of 0.5 cases: 100,000 doses (or 0.0005%). However, with the increasing of immunization, the CDC has estimated the prevalence of anaphylaxis at 0.37 cases: 100,000 doses.[14]

To date, we have not identified case reports of anaphylaxis in patients aged 5 to 11 years who received the PFIZER vaccine in countries that have already started immunization in this age group.

Benefits of vaccinating children

Although the percentage of serious illness among pediatric cases is small, if the number of infections in this age group increases, the number of children who will become seriously ill will proportionally increase. Data from studies in adolescents suggest that vaccination with BNT162b2 (Comirnaty/Pfizer) in children 5 to 11 years old is likely to prevent most

Table 2
COVID-19 fatality rate in Brazil and worldwide and serious adverse reactions from the Comirnaty vaccine

COVID-19 by age group	Case fatality rate in Brazil in 2020 (%)	Lethality rate in the world (%)	Serious reactions from the Comirnaty vaccine (%)
Severe conditions due to the disease			
< 29 years	1.5%	0.50%	
> 6 and < 19 years	0.3%		
Total	2.90%	1.15%	0.000158%

hospitalizations and deaths. While pediatric studies have not evaluated whether vaccines will reduce transmission of SARS-CoV-2, data from studies in vaccinated adults suggest that vaccinated children are likely to transmit smaller amounts of the virus for a shorter time. Thus, vaccinating children 5 to 11 years of age has the potential to reduce transmission of the virus between family members, schools, and communities.[15,16]

International experience

The debate on the need and appropriateness of instituting an immunization program against COVID-19 in children took place around the world.[11,18]

The strategy to adopt the vaccination of children must consider the epidemiological scenario of each country and the individual and collective benefits of immunization. There are important issues to consider when vaccinating children and adolescents that go beyond the direct health benefits of the recipient. The proposal is that the high vaccine coverage can contribute to the reduction of SARS-CoV-2 transmission in this age group and, therefore, reduction of transmission to adults, the elderly and immunocompromised people. In addition, mitigating interruptions in children's educational and sports activities, maintaining safety and well-being are indisputable benefits to be considered.

Considering all these data, the following countries have already approved the immunization of children aged 5 to 11 years old against COVID-19 with the PFIZER vaccine:

- United States (FDA), approval on October 29, 2021[19];
- Canada (HC), approval on November 19, 2021[20];
- European Community (EMA), approval on November 25 2021[21];
- Australia (TGA), approval on December 5 2021[22];
- Singapore (HSA), approval on December 10 2021[23] ;
- Switzerland (Swissmedic), December 10, 2021[24];
- United Kingdom (MHRA), approved on December 22 2021[25].

Approval by ANVISA

After evaluating the benefits and risks, following analysis protocols used by several international agencies with a similar function, ANVISA granted emergency authorization for the Pfizer-BioNTech COVID-19 vaccine for use in children aged 5 to 11 years on December 16 of 2021.[26]

Conclusion

In view of the data presented, the Brazilian Association of Allergy and Immunology (ASBAI) is totally in support ofimmunization against COVID-19 of individuals between 5 and 11 years old, for the protection not only of this group, but also of their cohabitants. The vaccination of children, once its efficacy and safety has been demonstrated, is essential for controlling the circulation of the virus and protecting individuals whose vaccine response may not occur efficiently, such as those who are immunocompromised.

Therefore, ASBAI considers that a public consultation is not appropriate, since the indication of immunization of people between 5 and 11 years old should not be a matter of opinion, but a fundamental public health strategy for controlling the pandemic that plagues us since March 2020 with serious economic, social, emotional consequences and effects on individual and collective health.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Review Article

Update on local anesthetics hypersensitivity reactions

Atualização em reações de hipersensibilidade aos anestésicos locais

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ABSTRACT

Local anesthetics are essential in many medical and dental procedures. They work by stabilizing neuronal membranes and inhibiting the transmission of neural impulses, which allows these procedures to be performed more safely and without pain. Adverse drug reactions are defined by the World Health Organization as all harmful, unintended and undesirable effects of a medication, which occur at doses used for prevention, diagnosis and treatment. Hypersensitivity reactions are unpredictable type B adverse reactions that clinically resemble allergic reactions and may or may not involve an immune mechanism. True hypersensitivity reactions to local anesthetics are rare, although overestimated. In this review, we highlight the need for a thorough evaluation of patients with suspected allergic reaction to local anesthetics, including investigation of other possible allergens that may have been used in the procedure, such as analgesics, antibiotics and latex. The investigation strategy and patient selection for testing should be based on clinical history. In this way, we will be able to provide more assertive and safe guidelines to patients.

Keywords: Local anesthetics, esters, amides, drug hypersensitivity, lidocaine.

RESUMO

Os anestésicos locais são essenciais em diversos procedimentos médicos e odontológicos. Funcionam estabilizando as membranas neuronais e inibindo a transmissão de impulsos neurais, o que permite a realização desses procedimentos com mais segurança e sem dor. As reações adversas a drogas são definidas pela Organização Mundial da Saúde como todos os efeitos nocivos, não intencionais e indesejáveis de uma medicação, que ocorrem em doses usadas para prevenção, diagnóstico e tratamento. As reações de hipersensibilidade são reações adversas do tipo B, imprevisíveis, que clinicamente se assemelham a reações alérgicas e podem ou não envolver um mecanismo imune. As reações de hipersensibilidade verdadeiras aos anestésicos locais são raras, apesar de superestimadas. Nesta revisão destacamos a necessidade de uma avaliação completa dos pacientes com suspeita de reação alérgica aos anestésicos locais, incluindo a investigação de outros possíveis alérgenos que tenham sido utilizados no procedimento, como analgésicos, antibióticos e látex. A estratégia de investigação e seleção de pacientes para testes deve se basear na história clínica. Dessa forma, poderemos fornecer orientações mais assertivas e seguras aos pacientes.

Descritores: Anestésicos locais, ésteres, amidas, hipersensibilidade a drogas, lidocaína.

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Introduction

Therelief, control and blocking of pain was one of the most important advances in Medicine and a huge leap forward for humanity. Today, there are numerous procedures that benefit from the use of local anesthetics (LA), substances that block sensory, motor and autonomic functions without causing unconsciousness.[1]

The use of local anesthetics dates back to the most ancient civilizations, when the Incas chewed coca leaves to obtain a feeling of well-being. Cocaine itself was isolated in 1860 by the work of the medical student Albert Niemann and Professor Frederick Woller, but its practical use as an anesthetic in isolation only happened years later, with the studies of Carl Koller, on its effects on the eye, and Sigmund Freud about the stimulating effects of this substance on the brain. Since then, several safe LAs have been synthesized, some of which are no longer used because they have serious toxic effects on the cardiovascular and nervous systems.[2]

It is estimated that the Adverse reactions to LA occur between 2.5 to 10% of patients exposed to this class of drugs, but hypersensitivity reactions are rare and described in less than 1% of cases of adverse reactions.[3]

Hypersensitivity reactions to local anesthetics can initially be divided into *local* or *systemic*, and this distinction is important in investigation. According to the time of onset of signs and symptoms, but also considering the clinical manifestations, we can classify reactions into immediate (installation of signs and symptoms within an hour, and may occur up to 6 hours) and late (after an hour, up to days or weeks).[4]

Taking into account the rarity of allergic hypersensitivity reactions to LA, the difficulty in clarifying the real mechanism causing the reaction and the fear of health professionals and the patient himself to perform a new procedure in the event of an adverse event with the use of LA, it is necessary to carry out the investigation protocol through skin and provocation tests. Such tests help in the identification and confirmation of possible suspected agents, in order to know the real prevalence of LA allergy, and also to rule out the diagnosis or provide other alternative LAs for use.[5]

Therefore, in this review, made from searches for original articles, reviews, guidelines and consensus in the MEDLINE and Latin American and Caribbean Literature on Health Sciences (LILACS) databases, using the terms: *local anesthetics hypersensitivity*, *local anesthetics adverse effects*, *pharmacology*, *diagnostic tests*, we updated the prevalence of reactions to LA, discussed the pharmacology of these medications, described the characteristics of each type of reaction and ended with the indications and investigation protocol, and consequently the outcomes of their results.

Pharmacology of local anesthetics

LA are the only class of drugs capable of completely blocking the arrival of nociceptive impulses to the cerebral cortex, thus preventing the perception of these stimuli by the patient.[6]

Lidocaine was synthesized in 1948 for topical use or intravenous infusion, and since then new drugs have been created, varying their characteristics in terms of onset time, duration of action and potential toxicity.[6]

The LA molecule is composed of three parts: a lipophilic aromatic ring, an ester or amide ligand, and an amino terminus (Figure 1).[7,8]

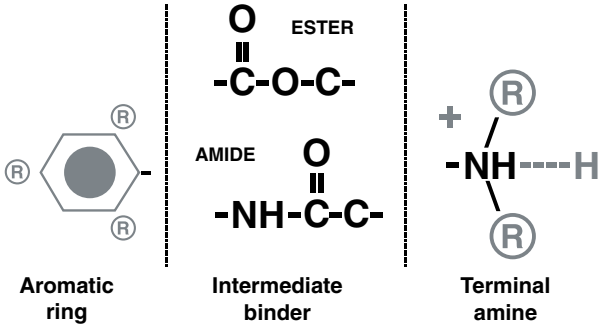


Figure 1
Chemical structure of local anesthetics

The amine functional group and its branches are linked to the aromatic ring by an intermediate ester or amide chain, which will determine the metabolic mechanism of each LA. This is important, as amide-type local anesthetics are chemically more stable, have a lower risk of allergic reactions, and have a longer

half-life due to their hepatic degradation, compared to the ester group, which is primarily metabolized by plasma cholinesterase.[7]

Cross-reactivity between drugs of the ester group is frequent due to the common metabolite of this group, para-aminobenzoic acid (PABA). The amide group LAs generally do not cross-react with each other, but there are some case reports of such reactions. Between the ester and amide groups there is no cross-reactivity.[9,10] Table 1 presents the commonly used LAs.

Table 1
Local anesthetics

Group 1 (Esters)	Group 2 (Amides)
Benzocaine	Lidocaine
Cocaine	Mepivacaine
Procaine	Prilocaine
Proparacaine	Articaine
Chloroprocaine	Bupivacaine
Tetracaine	Levobupivacaine
	Ropivacaine

Four physicochemical properties determine the activity of local anesthetics: pKa (dissociation constant in a solution, that is, it defines the pH at which the drug forms will be in equilibrium: 50% ionized and 50% non-ionized), molecular weight, lipid solubility and degree of protein binding.[6,11]

In general, local anesthetics with low pKa will have a faster onset of action, as they have a lower degree of ionization at physiological pH, that is, it is through the non-ionized form that the drug diffuses through the neural membrane and then into the cell, establishes its ionized or active form, binding to the intracellular sodium channel to exert its function. Molecular weight is inversely related to the tissue diffusion capacity of LA.[6]

The molecular structure, with the presence of the aromatic ring and its ramifications, is the main determinant of the solubility of this class of drugs. LA with greater lipid solubility present greater diffusion through the neural membrane, and, therefore, the greater the potency of these drugs. However, LA with higher lipid solubilities are absorbed more slowly, as this physicochemical property can influence the dispersion of these drugs through tissue fluids, as well as the commercial concentrations in which they are formulated.[6]

The degree of protein binding determines the free fraction of the drug available to bind to target receptors, so the duration of drug action is directly related to the protein binding capacity of the local anesthetic.[12]

The LA mechanism of action is based on blocking the transmission of action potentials within neurons through the inhibition of voltage-gated sodium channels. When at rest, the channels are closed, opening when depolarized and soon after entering an inactive state, in order to facilitate repolarization of the nerve cell membrane. Thus, when LA binds to sodium channels, its inactive state inhibits the action potential in nerve fibers, generating favorable conditions for drug binding, therefore, the stimulation of nerve fibers facilitates the onset of its effects.[6,11]

Nerve fiber blockage occurs gradually with the onset of loss of sensitivity to pain, temperature, touch, proprioception, and, eventually, skeletal muscle tone. Thus, individuals can still feel the touch when the pain is already absent.[12]

In pharmacokinetic terms, it is observed that LA are absorbed according to the site of administration, determined by its lipid solubility, vascularization of the site of action and the presence or absence of a vasoconstrictor incorporated into the solution.[6]

Regarding the use of vasoconstrictors in the solution, their presence reduces the systemic absorption of the anesthetic, which consequently remains for a longer time at the administration site. Other factors that can influence the maximum plasma concentration are the amount of drug, the patient's cardiac output, and vasodilation at the neural blockade site.[6]

Regarding the distribution of LA, those of the amino amide type are better distributed due to their slower metabolism than the amino esters, and both have renal excretion.[6]

Hypersensitivity reactions to local anesthetics

Hypersensitivity reactions are unpredictable, dose-independent, type B adverse reactions that relate to individual predisposition and clinically resemble allergic reactions. They may have an immunological mechanism not involved in their pathogenesis, being classified as allergic and non-allergic, respectively.[13]

The vast majority of reactions to LA are not allergic hypersensitivity reactions and involve other mechanisms, such as those listed below.[14]

- Toxic: occurs by accidental intravascular administration or in high doses of LA and has symptoms such as tremors, agitation, arrhythmia, hypotension, drowsiness, nausea, vomiting, apnea, excitement, convulsion and myocardial dysfunction.
- Psychosomatic: related to anxiety or fear, include symptoms such as hyperventilation (tachypnea and dyspnea), paresthesias, palpitation, tachycardia, nausea, dizziness and sweating.
- Vasovagal: bradycardia and pallor.
- Pharmacological effect of vasopressors: palpitation, tachycardia, tremors, headache and increased blood pressure.
- Idiosyncrasies: methemoglobinemia related to the administration of higher doses of LA, especially prilocaine.

LAs are used both in injectable form and in topical preparations, and can lead to a wide variety of manifestations. Differentiating the allergic process from the non-allergic process is not always easy, as the history is generally imprecise, the drug involved is unknown, the signs and symptoms are very varied, transient and most of the time do not require treatment.[9,15] The presence of generalized skin symptoms and hypotension related to the use of LA has been described as a predictor of positive skin tests or provocation.[5]

The ester group LAs are the most associated with allergic hypersensitivity reactions, and their active metabolite and potential allergenic epitope is paraminobenzoic acid (PABA).[9] The cross-reactivity between the LAs of the ester group is attributed to this common metabolite. PABA has in its structure components similar to parabens (methylparabens, propylparabens), found in makeup, sunscreens and creams. Regarding LA of the amide group, the metaxylene component is the possible allergenic epitope and may be present in other drugs such as antiretrovirals, antiarrhythmics and antidiarrheals.[16,17] Therefore, both groups of LA can lead to an allergic hypersensitivity reaction already in the first exposure to the drug due to primary sensitization by cross-reactivity.[17]

Two types of allergic reactions to LA are recognized: the *immediate* IgE-mediated reactions (type I), characterized by the release of histamine and other mediators, and the *delayed* reactions, mediated by the T cell (type IV).

Immediate reactions correspond to less than 1% of hypersensitivity reactions, typically occur within 1 hour of LA administration and present as urticaria, angioedema, pruritus, bronchospasm and anaphylaxis.[5,18] The severity of the reaction depends on the allergen dose, route of administration and the amount of specific IgE. In case of suspected anaphylaxis, tryptase dosage may be useful in the diagnosis.[18]

Late reactions are more frequent, observed in 2.4 to 4.1% of patients.[19] They can be local or systemic. Local reactions occur within hours or even days after topical application or subcutaneous/submucosal injections of LA, and may persist for several days. They are characterized by allergic contact dermatitis or edema and local erythema, similar to cellulitis. Contact dermatitis typically presents with eczema, blistering, blistering, and oozing. Late-onset edema at the LA injection site may or may not accompany contact dermatitis, and when it affects mucosa it leads to blistering and scaling.[20] In addition to LA from the benzocaine ester group, cinchocaine is often identified as a contact allergen. Late systemic reactions may present with generalized skin eruptions, such as maculopapular rash.[20]

Differential diagnosis of hypersensitivity reactions to local anesthetics

Adverse reactions most commonly confused with hypersensitivity reactions include syncope, panic attacks and toxic effects due to inadvertent intravascular drug administration.[20,21]

Possible differential diagnoses and their symptoms are listed below.

Allergy to other agents

Allergic reactions involving local anesthetic preparations may be due to other constituents of the injection solution than the drug itself. Excipients such as preservatives (eg benzoates - used in multidose vials) and antioxidants (eg metabisulfites - used in local anesthetics in a solution containing adrenaline) can cause hypersensitivity reactions.[21] Historically, the most sensitizing components in local anesthetic solutions were preservatives such as methylparabens, used in plastic anesthetic tubes to prevent losses

due to microbiological contaminants. However, LA used in Dentistry are single-use items, which do not require the inclusion of parabens. There are varying concentrations of methylparaben in plastic tubes, although they do not have an indication on their package insert. Considering the presence of methylparaben, glass tubes are safer. Since the amount of methylparaben is not specified on the packaging, and is not regulated by the National Health Surveillance Agency, it is important to alert professionals about its presence.

Latex allergy

Latex contained in rubber tampons, natural rubber gloves and other dental materials should also be considered.[22]

Psychogenic reactions

Psychogenic reactions are one of the most common adverse reactions associated with the use of local anesthetics in dentistry. They can manifest in many ways, with syncope being the most common, but other symptoms include panic attacks, hyperventilation, nausea, vomiting, and changes in heart rate or blood pressure, which can cause pallor. These reactions can be misdiagnosed as allergic reactions and can also mimic them with signs such as reddening of the skin, rash with red spots, swelling, and bronchospasm. All patients have some degree of autonomic response to injections, ranging from mild tachycardia and sweating to syncope.[22]

Toxic reactions

Toxic reactions can occur if high levels of anesthetic enter the bloodstream. Local anesthetics can reach the circulation as a result of repeated injections, inadvertent intravascular administration, or overdose in patients who have problems eliminating or metabolizing these drugs. Toxic side effects are predominantly neurological and include excitability or agitation, sedation, dizziness, slurred speech, mood change, diplopia, disorientation, and muscle spasms. Higher blood levels can result in tremors, respiratory depression and seizures.[22]

Vasoconstrictor agents such as adrenaline can also cause adverse effects. Adrenaline toxicity can result in symptoms such as anxiety, restlessness, tremors, throbbing headache, palpitations, sweating, pallor, weakness, dizziness and tachypnea.[22] Toxic reactions can be minimized when using correct doses of anesthetic and safe injection techniques.[22]

Prevention of adverse effects

When the patient experiences signs and symptoms that are suggestive of an allergic reaction, possible alternative causes should be considered, such as contact with other common allergens, toxic dose, or a psychogenic reaction. Possible causes of the symptoms experienced should be discussed with the patient. The use of the terms “allergic” and “allergy” should be avoided when discussing any adverse event.[22]

Adverse reactions caused by toxicity or anxiety can be minimized by:[22]

– administering injections with an aspiration syringe to avoid intravascular injection;
– in nervous patients, use relaxants to relieve anxiety. For extremely anxious patients, sedation may be necessary;
– treating patients in the supine position to prevent fainting;
– give injections slowly to reduce discomfort and improve solution location;
– restrict the total dose of anesthetic given to the patient to prevent the occurrence of toxic effects from overdose. The maximum dose for the patient can be calculated using the dosage information contained in the package insert and literature data, always taking into account the patient's age and weight, any concomitant drug therapy and underlying medical conditions.

Diagnostic methods and flowchart

All patients with a clinical history suggestive of an allergic hypersensitivity reaction to LA should be evaluated and submitted to the completion of the European Network for Drug Allergy (ENDA) questionnaire. Based on this information, the analysis of indication of tests for investigation is carried out.[23,24]

When an allergic reaction to drugs is suspected, the initial strategy is to immediately discontinue the drug and after 4 to 6 weeks the investigation can be carried out through skin tests, such as: patch test, prick test or puncture followed by the test. intradermal (ID) and provocation tests, always based on clinical history and risk stratification.[25] In this interval, it is possible

to exclude the used LA and release some alternative based on cross-reactivity to local anesthetics.

Several studies have evaluated the application of investigation protocols for LA. A retrospective Danish study evaluated 189 provocation tests performed in patients with suspected immediate allergic reaction to LA, none of which had a positive result.[19] Another retrospective evaluation in Germany excluded LA allergy in patients with a history suggestive of anaphylaxis. 771 subcutaneous provocations were performed with a positive result in only two cases, evidencing the predictive value of the negative ID test in investigations of these drugs.[14] Kallan et al. showed a frequency of only 3.52% of positive tests for local anesthetics in a sample of 398 patients, totaling 452 provocations performed.[5]

In Brazil, Aun et al. performed a retrospective study at the Medication Allergy Ambulatory at the University of São Paulo, evaluating 93 provocation tests with LA in patients with a history of immediate hypersensitivity to these drugs and obtained three positive results, one of them in the prick test.[26] Tanno et al. studied 33 patients with a history of reaction during or after (up to 24 hours) a procedure in which LA was used. All provocation tests had negative results, and two patients had a positive puncture test for latex, which were also confirmed with specific IgE dosage.[8] In a study carried out at a Brazilian reference center (UNIFESP), five patients had a history of skin reactions (urticaria and angioedema) or anaphylaxis after the use of local anesthetics in dental procedures. In the investigation, no patient was positive to the skin tests and challenged with the suspected drugs or therapeutic option (LA of the amide class, without vasoconstrictor).[27]

Therefore, according to reports in the literature, allergic reactions to LA are rare, and another suspected agent should always be investigated concomitantly, such as antimicrobials and latex.[9,24]

Allergic reactions to latex can present with immediate symptoms (hives, angioedema, sneezing, wheezing, and anaphylaxis) or delayed symptoms (eczema). The investigation should be carried out especially in risk groups such as atopics, healthcare professionals and other workers exposed to latex, patients undergoing multiple surgeries, children with urogenital malformations or spina bifida, and those with a history of perioperative anaphylaxis. Initially, a thorough clinical history should be taken, following a specialized questionnaire for latex allergy. Diagnosis is based on skin tests with standardized extract (93% sensitivity and 100% specificity), latex-specific IgE dosage (80% sensitivity and specificity > 95%) and, in case of a suggestive history and negative tests, provocation tests can be performed. Despite being effective, provocation tests are of high risk for triggering anaphylaxis, being reserved for inconclusive cases.[28]

Regarding reactions to LA, these are usually immediate reactions, and mostly caused by the toxicity of the drug or the associated vasoconstrictor. Thus, the medication often ends up being suspended without a real need, limiting the therapeutic options of patients.[14]

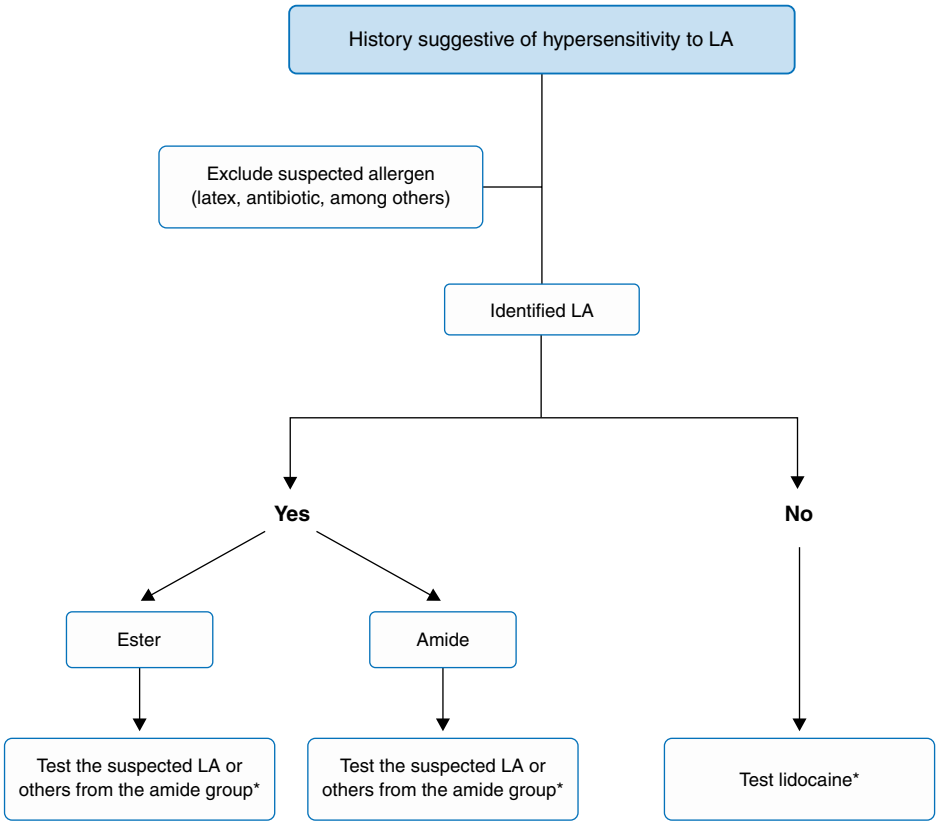
Before starting the investigation, some data about the reaction should be considered: type of procedure performed at the time of reaction, administration of the local anesthetic in relation to the appearance of signs and symptoms, complete review of the reaction mechanism, classification, amount and the concentration of the local anesthetic used, whether the LA contained a vasoconstrictor and the patient's previous pathological history, particularly renal, hepatic, cardiac and psychiatric history.[9]

The LA to be tested must not contain a vasoconstrictor, as this agent may inhibit the formation of papules during the procedure, leading to false-negative results or adverse events associated with its use.[29,30]

The choice of test type should be based on the Gell and Coombs reaction mechanisms, with immediate reading skin tests and provocation tests indicated for investigation of type I reactions and the patch test for type IV reactions.[31] It is essential to obtain the Free and Informed Consent Form (ICF) before performing the tests.

The concentrations of medications for performing skin tests are: prick test with pure LA, ID 1/1032 and patch test with pure medication.[4,33] If the skin test is negative, the investigation is continued by performing the provocation test with 2 mL of pure local anesthetic without vasoconstrictor, subcutaneously, keeping the patient under clinical observation for at least one hour after application.[8]

The drug provocation test is considered the gold standard in the investigation of drug allergy, and should be performed in a controlled environment and with a trained team.[34] Through the CFM resolution nº 2153/2016, the services that perform puncture tests, immunotherapy, intradermal test, desensitization and antigen provocation test were categorized



LA = Local anesthetics

* All skin tests must be performed with LA without a vasoconstrictor. If impossible to peform skin tests and provocation, due to the low risk of cross-reactivity, ester class anesthetics should be replaced by the amide class, and those of the amide class must be replaced by other local anesthetic of the amide class.

Figure 2
Flowchart for investigation of allergy to local anesthetics

as group 3, requiring supplies and equipment (automatic external defibrillator-AED, oxygen source, oropharyngeal cannulas, manual ventilator) for the treatment of emergencies such as anaphylaxis and cardiorespiratory arrest.

Conclusion

True hypersensitivity reactions to LA are rare, but they are quite frequent complaints in Allergology offices. Differential diagnosis with toxic, psychogenic reactions or by other agents such as latex is essential. Research through a detailed anamnesis can be very useful. The type of clinical manifestation, chronology of events and the need for drug treatment to reverse the reaction should be characterized. If the reaction is suggestive of allergic hypersensitivity, immediate and delayed reading skin tests may be used. The SC provocation test is the last step of the diagnostic investigation and must be performed by trained professionals, in an environment with support for reversal of an eventual anaphylactic reaction. Thus, after the entire procedure, the patient can be advised about the type of reaction presented and which LAs are safe for future use.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Review Article

Seafood allergy: main challenges in their diet and solutions developed by students of the nutrition and gastronomy course

Alergia a frutos do mar: principais desafios na alimentação e soluções desenvolvidas por alunos do curso de nutrição e gastronomia

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ABSTRACT

Food allergy is characterized by an adverse reaction to a given food, involving an immunological mechanism. One of the most common allergies currently found is seafood allergy, which is based on hypersensitivity to animals in this group. The objective of this research is to identify the challenges exposed in the feeding of seafood allergies and formulate solutions for this population based on nutritionally substitute foods. Being carried out in 3 stages, initial investigation, construction of concepts and planning of an action with nutritional guidance. According to the difficulties encountered in feeding this portion of the population, different preparations were carried out, with nutrients such as: omega-3, proteins, B vitamins, zinc, iron, potassium, magnesium, iodine and selenium. Which are also found in seafood. In order to avoid possible cross-contamination and ensure their nutritional intake in substitute foods. It was possible to conclude that seafood allergies do not present a significant interference in their quality of life, having a small nutritional impact, since through food there are other sources, requiring only some care on a daily basis due to the consequences of possible contamination.

Keywords: Food hypersensitivity, seafood, allergens, substitute nutrients.

RESUMO

A alergia alimentar caracteriza-se por uma reação adversa a um determinado alimento, envolvendo um mecanismo imunológico. Uma das alergias mais comuns encontradas atualmente é a alergia a frutos do mar, a qual se baseia em uma hipersensibilidade a animais desse grupo. O objetivo desta pesquisa é identificar os desafios expostos na alimentação de alérgicos a frutos do mar e formular soluções para essa população baseadas em alimentos nutricionalmente substitutos. Sendo realizado em três etapas: investigação inicial, construção de conceitos e planejamento de uma ação com orientações nutricionais. De acordo com as dificuldades encontradas na alimentação dessa parcela populacional, realizaram-se diferentes preparações, com nutrientes como ômega-3, proteínas, vitaminas do complexo B, zinco, ferro, potássio, magnésio, iodo e selênio, os quais também são encontrados nos frutos do mar, a fim de evitar possíveis contaminações cruzadas e garantir seu aporte nutricional em alimentos substitutos. Foi possível concluir que os alérgicos aos frutos do mar não apresentam uma interferência significativa em sua qualidade de vida, tendo um impacto nutricional pequeno, visto que por meio da alimentação existem outras fontes, necessitando somente de alguns cuidados no dia a dia em virtude das consequências de uma possível contaminação.

Descritores: Hipersensibilidade alimentar, frutos do mar, alérgenos, nutrientes substitutos.

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Introduction

Food allergies are an atypical response of the body, involving the immune system, which identifies innocuous foods as if they were aggressors, which would cause harm to the body. The severity of allergic reactions varies for each individual, ranging from a mild reaction to a severe anaphylactic reaction, which can be fatal, developing within a few minutes or up to two hours after ingestion.[1,2]

Food allergy (FA) can be mediated by Immunoglobulins E (IgE), which is the most common, symptoms are immediate with the possibility of an anaphylactic reaction; it may also be non-IgE-mediated, i.e. mediated by cells, or by both IgE and cells.[3]

Clinical manifestations can occur in different ways, depending on the tissue affected. They range from cutaneous manifestations such as hives, eczema, tingling sensation in the oral cavity, itching, edema, respiratory problems, cardiovascular problems such as decreased blood pressure, loss of consciousness, gastrointestinal problems such as vomiting, diarrhea, abdominal pain, to anaphylactic reactions. Clinical manifestations of anaphylaxis can be mild, moderate or severe, with the potential to be fatal.[4]

The allergens that cause food allergy are usually glycoproteins that are relatively resistant to digestion and the cooking process. The most common foods are: milk, eggs, wheat, nuts, peanuts, fish, seafood and seeds.[1-5]

To present an allergic reaction, the individual must have had some contact with the food previously, which leads to sensitization (formation of antibodies without a clinical reaction). In certain situations this contact does not happen through ingestion. This sensitization can occur through skin contact (examples: products that contain food proteins in their composition) or even through breast milk. In the case of seafood, they can be ingested several times before triggering allergic responses, whereas other foods, such as milk and eggs, generally do not require a prolonged exposure time to present adverse reactions.[6]

Seafood is one of the foods that most cause food allergies.[7] Among them, we can mention shellfish, which are divided into crustaceans such as lobster, crabs, crabs and shrimp, and molluscs, such as oysters, mussels, snails, octopus and squid.[2] Tropomyosin is the reactive protein in seafood that causes an aversion to these foods, with a 75% risk of clinical reactivity, that is, reactions that occur even if certain proteins do not belong to the same taxonomic classification, and there may be similar amino acid sequence.[8]

Recent studies indicate that 15% of individuals with a seafood allergy may react to vapors and fumes produced during the cooking process. This is because, during cooking, seafood releases proteins called amines, which can cause allergic reactions in the respiratory tract.[8]

The increase in the prevalence of allergic diseases is on the rise and affects both children and adults, and it is reported that 25% of the world population suffers from the problem, 1 to 2% of adults and 5 to 7% of children.[9]

Regarding the diagnosis, the patient undergoes a careful evaluation, which is characterized by the clinical history associated with physical examination data, and may be complemented by allergic tests. These tests can be in vitro or in vivo. In in vivo tests, the methods used are the immediate hypersensitivity skin test (or Prick test), and the oral provocation test, which are the gold standard for diagnosis. In vitro tests measure specific serum IgE.[8]

Currently, allergen avoidance becomes the only available and effective treatment, as there is still no cure. Consequently, exclusion diets must be carried out, and emergency treatments in case of accidental ingestion. Therefore, it is extremely important to ensure that nutrient intake is not compromised, ensuring the consumption of nutritionally equivalent foods.[2-10]

As a result of these assumptions, the objective of this research, through the extension project, is to identify the challenges exposed in the diet of those allergic to seafood and formulate solutions for this population based on nutritionally substitute foods.

Materials and methods

Based on the discipline of Single Health and Food, an extension project was carried out by students of the Nutrition and Gastronomy course at Universidade Positivo, together with the assistance and supervision of the professors.

Allergies, intolerances and dietary restrictions were presented by the professors as a general topic at the beginning of the development of the studies, each group should choose one of them and delve into the subject.

Therefore, the present study will be developed according to the allergy to seafood, being exposed

to challenges and solutions for those with this hypersensitivity.

The research carried out for a better understanding of the subject was divided into stages, namely: initial investigation, construction of concepts and planning of an action.

In the first stage, initial investigation, an initial analysis was sought.database with the following descriptors: *hypersensitivity*, *seafood*, *food allergy*, *allergens*, *food* and *crustaceans*. With a deeper knowledge of the subject, the students developeda structured interview with a script with the doctor and coordinator of the Scientific Department of Food Allergy at ASBAI – Brazilian Association of Allergy and Immunology (Annex 1).

The second stage, construction of concepts, was based on a grouping of information obtained from the database and the interview. With this, more detailed definitions were obtained about the disease and responses to the difficulties encountered on a daily basis by those allergic to seafood.

Finally, the third stage, planning an action, resulted in the preparation of recipes (Figures 1-5) that can contribute mainly in the nutritional area, focusing on nutrients (omega-3, protein, B vitamins, zinc, iron, potassium, magnesium, iodine and selenium) that make up seafood, but in other preparations that can be ingested without any risk. Nutritional guidelines were also developed for individuals who have this allergy, based on the results of all the steps described.

Results and discussion

According to the specialist interviewed, after being diagnosed, it is essential that the allergic person is instructed about the foods he should consume to ensure adequate nutritional intake, especially when fish and seafood are the basis of food, as in coastal regions. Reading labels should also be considered, making it essential for allergy sufferers, given that according to RDC N° 26/2015, the presentation of allergens on food labels is mandatory.[11]

According to the expert, cross-contamination occurs when food allergens are accidentally transferred from one food to another, this can occur in any stage of food production: preparation, packaging, storage and distribution, whether in home-cooking or when food is prepared in cafeterias and restaurants. Thus, for some allergic patients, a small amount of the food may be enough to cause reactions. In a domestic environment, to avoid undesirable contacts, those who handle food should always wash their hands between preparations, sanitize surfaces and household utensils.[2] The interviewee reports that to cook, clean water and new oil should always be used, avoiding reuses of the same. It is also recommended that the drying of the utensils be done in the open air, avoiding the use of cloth. And, if the allergic person does not know how the food was prepared, the ideal would be to avoid consumption, so as not to have a possible allergic crisis. Thus, treatment consists of eliminating contact, inhalation or consumption of the food involved.[12]

Seafood has omega 3 as its main source. As a result, replacement options for obtaining omega 3 include flaxseeds, seaweeds and oilseeds. Other nutrients, also present in seafood, such as protein, B vitamins, zinc, iron, potassium, magnesium, iodine and selenium can be found in meat, eggs, fruits, nuts and dark green vegetables.[13]

For individuals who need EPA – eicosapentaenoic acid and DHA – docosahexaenoic acid (types of omega 3 from fish oil) supplementation, the option to replace supplements is those from seaweed, explains the interviewee. Studies indicate that DHA can, for example, be obtained from the consumption of seafood, such as fish, crustaceans and, for allergy sufferers, algae, since these foods are the main source of polyunsaturated fatty acids (PUFAs) omega 3,[14] which have several benefits, such as cardiovascular health care, more specifically with eicosapentaenoic acid.[15]

Eating meals outside the home can be risky, due to the possible cross-contamination and the existence of the allergen in the preparation, which can lead to an unexpected allergic reaction.[10] With this, one of the challenges and difficulties among those who are allergic to fruits of the sea is the fact that the allergic person may be afraid to eat in certain places. With this, it was sought through other nutrient preparations to substitute seafood.

Five preparations were made in total, with the following nutritional sources: omega-3, proteins, B vitamins, zinc, iron, potassium, magnesium, iodine and selenium, being arranged in preparations that can be attributed to breakfast, morning snack, lunch, afternoon coffee and dinner.

The first preparation (omelet with eggs, banana with grated cashews and whole milk) served as a

breakfast option and was developed with a focus on the following nutrients: selenium, iodine, and magnesium. In it, practicality was observed in the preparation, in addition to being easy to reach for the affordable price (Figure 1).

The nutrients chosen in this preparation were based on the interviewee's report, in which she explains that the nutrients most committed to the seafood exclusion diet include iodine, which in turn helps protect against the toxic effects of radioactive materials, prevents goiter, stimulates the production of thyroid gland hormones, burns excess fat and protects skin, hair and nails.[16] The source of iodine in this preparation is whole milk, with a daily recommendation of 130 µg for adults. Pregnant women, however, need to consume 220 µg/day. The ideal amount of iodine for infants is 90-135 µg daily.[17] However, seafood also has selenium in its composition, which stands out for its participation in the synthesis of thyroid hormones, its antioxidant action and the aid to enzymes that depend on it to function well.[16] Its main source is cashew nuts, and its daily food intake for adults in Brazil is 34 µg/day.[17] Finally, magnesium is necessary for the body's hormonal activity and for the contraction and relaxation of muscles, including the heart.[16] Indication of daily consumption of magnesium for adult men and women is 260 mg, 220 mg/day for pregnant women, and 36 to 53 mg/day for infants. Its source is offered in this preparation through milk.[17]

As a morning snack, the option given was an avocado cream with flaxseed and banana, rich in omega 3 and potassium (Figure 2).

According to a study, fish have the advantage of provide polyunsaturated fatty acids and omega 3 and 6, essential for health.[18] In view of this statement, it was decided to make a preparation rich in this nutrient (omega 3), which presents cardiovascular benefits for human health.[13] The minimum recommendation for adequate daily intake of this nutrient is 250 mg/day of EPA + DHA in adults,[19] in addition to potassium, an important contributor to the metabolism and synthesis of proteins and glycogen that can be found in seafood and fruits such as bananas, used in this preparation[16], adequate for adults is 3,500 mg/day.[17]

For lunch, a dish based on roasted pork loin with creamed spinach and roasted potatoes and carrots was prepared (Figure 3). This dish is mainly rich in protein, iron and B vitamins, especially vitamin B2 (riboflavin), which it acts in the formation of red blood cells, and B12 (cobalamin), which guarantees cellular metabolism, especially cells of the gastrointestinal tract, bone marrow and nervous tissue.[20] Given that, it is exposed that Meat and fish are good sources of vitamin B12.[21] The daily recommendation for Cobalamin is 1.5-2.4 µg for adults and 0.7-1.2 µg for children.[22] In the case of Riboflavin, consumption of 1.1-1.6 mg/day is recommended for adults and 0.4-0.6 mg/day for children.[17]

For the afternoon snack, a preparation focused on zinc was carried out. For its accomplishment, the following inputs were used: wholemeal bread, Minas cheese, egg yolk and black tea. Zinc, in turn, has several benefits, including theantioxidant defense, growth, development, essential for protein structures and increase, complement and stimulant to the resistance of the immune system. Shellfish, oysters, red meat, liver, offal, eggs, nuts and pulses are considered the best sources of zinc.[20] Its daily intake for adults is 7 mg/day, daily consumption for infants should be 2.8-4.1 mg/day, and for pregnant women 11 mg/day[17](Figure 4).

For dinner, there was a recipe with practical and affordable food: liver fillet, butter, broccoli, carrots and brown rice, with the main focus on animal protein and iron, making it a very nutritious meal, rich in nutrients necessary for the proper functioning of the body[23] (Figure 5). The Iron is recognized by the transport of oxygen to all cells,[16] its source in this recipe is identified through the liver, and its daily requirement for adults is 14 mg, for infants the daily value is 0.27-9 mg, and for pregnant women should have an intake of 27 mg/day.[17] In the case of protein, its recommended daily intake is 10 to 15 percent of the total amount of the diet.[24] It, in turn, performs the functions of transporting substances through the blood, formation of tissues, enzymes, hormones, neurotransmitters and antibodies, participate in the acid-base balance, maintain the ideal fluid balance in body tissues, act as a source of energy in the Krebs cycle and are responsible for muscle contraction.[25] The protein sources that best serve to these characteristics are those of animal origin: meat, eggs, milk and dairy products.[26,27] In view of this, comes the choice of replacing the seafood with another meat, in the case of this preparation, the liver.

In view of the above, the nutritional guidelines collected according to the interview and the databases reveal that for the allergic person to live better with this hypersensitivity, he should avoid eating seafood, in addition to the care to be taken when eating outside from home, due to cross-

contamination that can occur on frying pans, utensils and surfaces during preparation. The substitute sources mentioned above characterize the ease of feeding correctly with nutrients present in seafood, but in other preparations, resulting in obtaining the daily recommendations of each nutrient.

Final considerations

According to the study presented through the extension project, it is concluded that food allergies, especially seafood, have some challenges related to food. With the research history and the interview carried out, it was possible to materialize and identify ways to help this audience, presenting nutritionally balanced, tasty preparations, with micro and macronutrients substitutes for seafood, and also accessible to the most diverse economic classes.

Thus, it becomes evident that those allergic to seafood do not have a significant nutritional interference in their quality of life, since through food there are other sources of the main nutrients of this group. However, this is an allergy that has no cure, and must be treated, avoiding the ingestion of the allergen and all possible situations of cross contamination.

Annex 1

Structured interview with script with the doctor and coordinator of the Scientific Department of Food Allergy at ASBAI – Brazilian Association of Allergy and Immunology

1. **One of the impasses encountered by those allergic to seafood would be the difficulty in finding a place where cross-contamination does not occur, since many of them use, for example, the same oil in frying. fFor this reason, the person may feel insecure about eating outside and they choose to make their own food. In your point of view, what would be the solution for these cases?**

Dr.: Cross-contact occurs when food allergens are accidentally transferred from one food to another. This can occur in any of the stages of food production: preparation, packaging, storage and distribution, both in the home environment and in cafeterias and restaurants.

For some allergic patients, a small amount of the food may be enough to cause reactions. In a domestic environment, to avoid unwanted contact, those who handle food should always wash their hands between preparing different foods and sanitizing surfaces and household items. For cooking you should always use clean water and new oil. It is also recommended that the utensils be dried outdoors, avoiding the use of cloth.

When you do not know how the food was prepared, avoid consumption. Although there are studies that try to define for each allergen what would be the minimum dose capable of inducing an allergic reaction, this varies from individual to individual and even within the same individual depending on the clinical condition.

2. **Seafood is a great source of B vitamins and minerals. However, those allergic to such foods end up not being able to ingest certain supplements, such as omega 3. What would be the solution to avoid any type of nutritional deficiency?**

Dr.: The nutrients most committed to the seafood exclusion diet are omega 3 and iodine. Substitute options for obtaining omega 3 include flaxseeds and oilseeds. Seaweeds are also good sources of omega 3.

Iodine is added to iodized table salt. Seaweed, milk and eggs are other sources of iodine.

Other nutrients, also present in seafood, such as protein and vitamin B12, can be easily obtained from other foods of animal origin, such as meat and eggs.

For individuals who need EPA and DHA supplementation (types of omega 3 from fish oil), the option to replace supplements is from seaweed (vegan supplement).

Annex 1 *(continuation)*
Structured interview with script with the doctor and coordinator of the Scientific Department of Food Allergy at ASBAI – Brazilian Association of Allergy and Immunology

3. When diagnosed with seafood allergy, in addition to the person not being able to eat these foods, what other precautions should be taken?

Dr.: It is essential that the patient is oriented about the foods he should consume to ensure adequate nutritional intake, especially when fish and seafood are the basis of food, as in coastal regions. In the fish-restricted diet, supplementation of plant-based omega-3 fatty acids should be considered.

Careful reading of labels should also be performed. For example, Worcestershire sauce may contain anchovy and should not be consumed by patients who are allergic to fish.

4. For more severe allergic reactions, including anaphylaxis, what are the precautions in the treatment? And in which situations is the adrenaline pen prescribed?

Dr.: Anaphylaxis is a serious, potentially life-threatening reaction that requires emergency treatment. Emergency treatment plans for anaphylaxis, describing signs and symptoms of allergic reactions and indications for the use of emergency medications, should be provided to the patient. Prescriptions for self-injecting adrenaline and training on how the devices should be used should be made available to all anaphylactic patients. Constant reviews of the guidelines and training must be carried out.

In the case of shellfish allergies, autoinjector adrenaline should always be prescribed for the potential severity of the reactions. It is the most important drug in the treatment of anaphylaxis and should be administered promptly whenever necessary.

5. For a person who loves seafood but ends up becoming allergic over time, the difficulties encountered are the most diverse, since they are not able to taste the flavor and texture of such foods. In your opinion, are there foods that can replace these sensory properties that seafood brings?

Dr.: The characteristic flavor and texture of seafood are not found in fresh foods from other food groups, unfortunately.

Individuals with a seafood allergy are rarely allergic to all seafood. For example, people with a fish allergy may be tolerant of shellfish, and similarly, those who are allergic to crustaceans may tolerate fish. Professional guidance is needed to determine which foods are safe for each individual.

6. As many patients would like to be able to consume seafood again, is there any specific way for them to acquire tolerance again?

Dr.: So far, avoiding the shellfish allergen is the only treatment available. Research in crustacean immunotherapy has focused on the development of hypoallergenic variants of tropomyosin. No clinical trials of crustacean immunotherapy have been described so far.

Datasheet

Recipe name:
Two-egg omelet with a drizzle of olive oil. Glass of whole milk. Sliced banana with grated cashews on top.

Utensils and equipment:
frying pan, knife, cup, cup, fork, spatula, scale and plate.

Time: 10 min

Serves:
1 meal

Ingredients	Amount in homemade measure	Amount in grams or milliliters	Cost BRL
Medium eggs	2 units	65 g	0.80
Medium banana	2 units	75 g	0.89
Cashew nut	4 units	2.5 g	0.90
Glass of milk	1 cup	250 mL	0.82
Salt	2 pinches	1 g	0.10

Preparation:

1. Beat eggs and salt until smooth. Then pour into the hot skillet.
2. Cut the bananas and grate or chop the chestnuts over them.
3. Pour a medium glass (250 mL) of milk.

Total yield grams: 538 g

Yield serving grams: 538 g

Nutritional information

CHO (g)	PTN (g)	LIP (g)	Fiber (g)	Ca (mg)	Fe (mg)	Na (mg)	Selenium (µg)	Iodine (µg)	Magnesium (mg)
1.28	14.35	27.64	0	57.72	1.37	0	42.24	72	11.58
34.26	1.64	0.5	3.9	7.5	0.39	0	1.5	3	40.5
2.62	1.22	3.71	0.24	3.6	0.48	0	1.87	9	20.8
11.31	8.05	8.13	0	282.62	0.08	0	9.26	40	20.8
0	0	0	0	0	0	461.2	0	0	0
Total g	**Total g**	**Total g**	**Total g**	**Total mg**	**Total mg**	**Total mg**	**Total µg**	**Total µg**	**Total mg**
49.46	25.26	39.97	4.14	351.44	2.31	461.2	12.45	11.8	1,083.6

Kcal	Kcal	Kcal
197.84	101.04	359.73

Total caloric value: 644 Kcal

Serving size: 644 Kcal

Total cost: BRL 3,51

Portion cost: BRL 3,51

Figure 1
First preparation

Datasheet

Recipe name:
Avocado cream with flaxseed.

Utensils and equipment:
blender, knife, spoon, juicer, measuring cup, bowl and scale.

Time: 10 min

Serves:
27 servings

Ingredients	Amount in homemade measure	Amount in grams or milliliters	Cost BRL
Medium avocado	1 unit	400 g	5.98
Medium or large banana	1 unit	150 g	0.50
Lemon juice	½ unit	15 mL	0.30
Glass of water	½ cup	100 mL	0.40
Linseed	2 tablespoons	18 g	0.50
Wholemeal toast	3 units	20 g	0.78

Preparation:

1. Blend the avocado, banana and lemon juice in a blender.
2. Gradually pour in the water, whisking constantly, until smooth.
3. Pour into a bowl and sprinkle with the flaxseed.
4. Serve chilled on toast.

Total yield grams: 500 g (cream)

Yield serving grams: 25 g (of the cream with the toast)

Nutritional information

CHO (g)	PTN (g)	LIP (g)	Fiber (g)	Ca (mg)	Fe (mg)	Na (mg)	Monoun-saturated fat (g)	Polyun-saturated fat (g)	Potassium (mg)
30.15	6.20	41.98	31.57	39.58	1.03	0	21.5	7	1,031.28
30.30	1.29	0.22	1.37	3.74	0.26	0	0	0	295.23
0.84	0.04	0.01	0.04	1.4	0.01	0.2	0	0	11.7
7.80	2.54	5.81	6.03	38.07	0.85	1.56	1.28	0.76	156.47
14.67	2.67	0.67	1.33	0	6.07	83.33	0	0	0
Total g	**Total g**	**Total g**	**Total g**	**Total mg**	**Total mg**	**Total mg**	**Total g**	**Total g**	**Total mg**
83.76	12.74	48.69	40.34	82.79	8.22	84.89	22.78	7.76	1,494.68

Kcal	Kcal	Kcal
335.04	50.96	438.21

Total caloric value: 760 Kcal

Serving size: 28 Kcal

Total cost: BRL 8.46

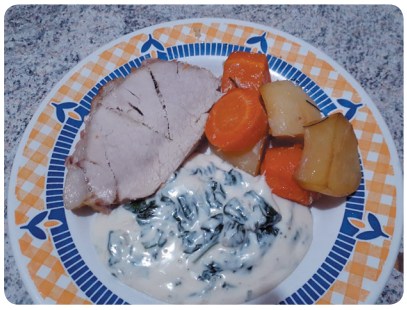
Portion cost: BRL 0.31

Comments: for a tastier result, the avocado must be very ripe. If you prefer, add a small amount of brown sugar.

Figure 2
Second preparation

Datasheet

Recipe name:
Roasted pork loin with potatoes and carrots with spinach cream.



Utensils and equipment:
blender, knife, spoon, juicer, measuring cup, bowl, baking sheet, pan, silicone spatula, grater, sieve, scale, ladle, foil and scale.

Time: 2h40min

Serves:
10 servings

Ingredients	Amount in homemade measure	Amount in grams or milliliters	Cost BRL
Pork loin	1 piece	1.5 kg	40.30
Big potatoes	5 units	1.1 kg	2.98
Big carrots	3 units	795 g	1.58
Water	2 ½ cups	500 mL	0.00
Orange juice	1 cup	200 mL	2.00
Olive oil	½ cup	100ml	5.50
Medium onion	1 unit	100 g	0.40
Head of garlic	1 unit	50 g	1.00
Salt (to taste)	2 ½ heaped tablespoons	50 g	0.09
Rosemary (to taste)	2 pinches	2 g	0.78
Black pepper (to taste)	2 pinches	5 g	0.65
Spinach	1 pack	100 g	3.00
Parmesan	1 cup	200 g	19.00
Flour	½ tablespoon	50 g	0.18
Butter	½ tablespoon	50 g	2.60
Milk	5 cups	1 L	3.10

Preparation:

Roasted pork loin

1. Mix the orange juice, olive oil, onion, garlic, pepper, salt and water in a blender.
2. With the help of a knife, make holes in the loin so that the seasoning can penetrate the piece.
3. Place the loin piece inside a plastic bag and add the sauce.
4. Leave to marinate for 24 hours.
5. After marinating, cut the potatoes and carrots into large pieces and season with a drizzle of olive oil, salt and rosemary.
6. Transfer the marinated loin to a roasting pan, add the potatoes and 5 ladles of marinade sauce.
7. Cover the piece with aluminum foil (shiny side down), and bake for 2 hours at 200 °C.
8. After 2 hours, remove the aluminum foil from the loin and leave it in the oven until the loin and vegetables are golden brown.
9. While the loin and vegetables are browning, water the loin so it is juicy.
10. After removing from the oven, set aside while you prepare the spinach cream.

Spinach cream

1. Start by separating the spinach leaves and grating the Parmesan cheese.
2. In a pan, add butter and spinach leaves to the edge of the pan, remove and set aside when the leaves are wilted.
3. In another pan, add 50 g of butter and sift 50 g of flour, mix until you smell popcorn, then add the milk and mix until it becomes a cream.
4. Then turn off the heat and add pepper, salt and cheese, mix until everything is homogeneous and add the spinach.
5. Finally, serve with the loin and vegetables.

Total yield grams: 3,900 g | **Yield serving grams:** 390 g

Figure 3
Third preparation

Nutritional information							
CHO (g)	**PTN (g)**	**LIP (g)**	**Fiber (g)**	**Ca (mg)**	**Fe (mg)**	**Na (mg)**	**B-complex vitamins: vitamin B2 (mg)**
0	536	96	1.7	296.7	6.9	583.8	1.05
131.3	12.8	0	14.7	38.7	2.1	25.2	0
60.9	10.5	1.4	25.7	179.2	1.4	26.5	0
15.1	1.5	0.1	0	14.7	0	0	0
0	0	12	0	1	0.6	2	0
9	1.1	0.1	1.7	23	0.2	4	0
12	3.5	0.1	2.4	6.8	0.4	2.7	0
0	0	0	0	12	0.1	19.37	0
2.6	2.86	0.2	2.2	97.5	0.4	17.1	16.1
3.3	71.1	67.1	0.5	1,983.9	1.1	3,688.2	0
37.6	4.9	0.7	1.2	8.9	0.5	0.4	0
0.05	0.45	81	0	24	0	11	0
50	30	20	0	1.150	0	690	2.4
Total g	**Total g**	**Total g**	**Total g**	**Total mg**	**Total mg**	**Total mg**	**Total mg**
321.65	674.71	278.6	50.1	3,836.4	13.2	5,070.27	19.55
Kcal	**Kcal**	**Kcal**					
1,286.6	2,698.84	2,507.4					
Total caloric value: 7,221.1 Kcal					**Serving size:** 722.11 Kcal		
Total cost: BRL 83.16					**Portion cost:** BRL 8.24		

Figure 3 *(continuation)*
Third preparation

Datasheet

Recipe name:
Wholemeal bread with white cheese and yolk and black tea.

Utensils and equipment:
frying pan, knife, spoon, cup, plate and scale.

Time: 15 min

Serves:
1 portion

Ingredients	Amount in homemade measure	Amount in grams or milliliters	Cost BRL
Wholemeal bread	2 slices	44 g	0.88
Fresh Minas cheese	2 slices	40 g	1.71
Chicken egg yolk	2 units	42 g	0.80
Black tea	1 cup	165 mL	0.33

Preparation:

1. Fry the egg yolk in a non-stick skillet.
2. Put the hot water in the cup and add the tea.
3. Add the cheese slices to the wholemeal bread and right after the yolk.
4. Serve.

Total yield grams: 291 g	**Yield serving grams:** 291 g

Nutritional information

CHO (g)	PTN (g)	LIP (g)	Fiber (g)	Ca (mg)	Fe (mg)	Na (mg)	Zinc (mg)
20.63	4.13	0	2.75	105.88	0.92	233.75	0
1.16	5.68	7.24	0	579	0	0	0
0.75	7.06	12.98	0	57.54	1.48	18.06	1.31
0	0	0	0	0	0	0	0
Total g	**Total g**	**Total g**	**Total g**	**Total mg**	**Total mg**	**Total mg**	**Total mg**
22.54	16.87	20.22	2.75	742.42	18.98	251.81	1.31

Kcal	Kcal	Kcal
90.16	67.48	181.98

Total caloric value: 339.62 Kcal	**Serving size:** 339.62 Kcal
Total cost: R$ 3.72	**Portion cost:** R$ 3.72

Figure 4
Fourth preparation

Datasheet

Recipe name:
Liver with carrots and broccoli in butter and rice.

Utensils and equipment:
knife, scales, plate, spoon, pots, stove and skillet.

Time: 30 min

Serves:
1 portion

Ingredients	Amount in homemade measure	Amount in grams or milliliters	Cost BRL
Bull's liver	1 slice	120 g	1.91
Braised carrots	4 tablespoons	60 g	0.45
Braised broccoli	1 cup tea	80 g	0.44
Cooked rice	3 tablespoons	100 g	0.58
Butter without salt	1 teaspoon	8 g	0.41

Preparation:

1. Cook the rice.
2. Grill the liver fillet.
3. Saute carrots and broccoli in butter.
4. Place on plate and serve.

Total yield grams: 360 g

Yield serving grams: 360 g

Nutritional information

CHO (g)	PTN (g)	LIP (g)	Fiber (g)	Ca (mg)	Fe (mg)	Na (mg)	Monoun-saturated fat (g)	Polyun-saturated fat (g)	Vitamin A (µg)
5.04	35.83	10.81	0	6.67	6.95	98.63	2.64	1.32	17,488.8
4.78	0.44	1.90	1.75	17.46	0.2	33.76	0.42	1.09	0
5.74	1.90	2.66	2.64	32	0.54	32.8	0.58	1.49	0
25.47	2.32	1.18	0.49	12.43	1.37	275.87	0.29	0.62	0
0	0.07	6.49	0	1.88	0.01	0.88	1.95	0.24	60.32
Total g	**Total g**	**Total g**	**Total g**	**Total mg**	**Total mg**	**Total mg**	**Total g**	**Total g**	**Total µg**
41.03	40.56	23.04	4.88	70.44	9.07	441.94	5.88	4.76	17,549.12
Kcal	**Kcal**	**Kcal**							
164.12	162.24	207.36							

Total caloric value: 533.72 Kcal

Serving size: 533.72 Kcal

Total cost: BRL 3.79

Portion cost: BRL 3.79

Figure 5
Fifth preparation

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Review Article

Severe infections by SARS-CoV-2 with the use of tocilizumab

Infecções graves por SARS-CoV-2 com uso de tocilizumabe

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ABSTRACT

SARS-CoV-2 causes the COVID-19 infectious disease that affects the respiratory tract. From the beginning of the infection, the immune system starts to produce pro-inflammatory cytokines and chemokines. The main cytokine involved is IL-6 and is linked to the severity and prognosis of the disease, as it provokes a storm of cytokines and severe inflammatory responses. Due to the association of high levels of IL-6 with severity and mortality in COVID-19, the use of Tocilizumab (TCZ), a humanized anti-human IL-6 receptor monoclonal antibody, which binds to IL receptors, is being investigated. -6 and blocks intracellular signaling reducing cytokine storm and hyperinflammatory state. The aim of this review is to assess the effectiveness of using TCZ in the treatment of patients with severe COVID-19. Searches were performed using the Science Direct and PubMed databases in May 2021. Randomized clinical trials with patients in a single stage of COVID-19, severe cases and without age restriction, who received TCZ as medication for treatment, were included. Intervention was combined with treatments protocoled by each hospital and associated with corticosteroids. The analysis of these studies showed significant results regarding the use of TCZ in severe cases of COVID-19. The use of TCZ associated with glucocorticoids led to a reduction in the rate of mortality and compliance with mechanical ventilation and a significant improvement in relation to the "WHO-endorsed 7-point ordinal scale". However, there was no evidence of relevant improvement when using TCZ alone.

Keywords: COVID-19, monoclonal antibodies, Systemic Inflammatory Response Syndrome.

RESUMO

O SARS-CoV-2 é causador da doença infecciosa COVID-19. A infecção estimula o sistema imunológico a produzir citocinas pró-inflamatórias. A principal citocina envolvida é a IL-6, e está ligada à gravidade da doença. Devido à associação dos altos níveis de IL-6 com a mortalidade na COVID-19, investiga-se sobre o uso de tocilizumabe (TCZ), um anticorpo monoclonal humanizado antirreceptor de IL-6 humana. O objetivo desta revisão sistemática é avaliar a eficácia do uso do TCZ em pacientes com COVID-19 grave. As buscas foram feitas através das bases de dados Science Direct e PubMed em setembro de 2021. Foram incluídos os ensaios clínicos randomizados com pacientes em um único estágio de COVID-19, casos graves e sem restrição de idade, os quais receberam o TCZ como medicação de intervenção combinado a tratamentos protocolados por cada hospital e associado a corticosteroides. A análise desses estudos demonstrou resultados significantes sobre o uso de TCZ em casos severos de COVID-19. O uso de TCZ associado a glicocorticoides levou a uma redução no índice de mortalidade e de submissão a ventilações mecânicas e a uma melhora expressiva em relação à escala "WHO-endorsed 7-point ordinal scale". Entretanto, não houve melhora relevante quanto ao uso do TCZ de maneira isolada.

Descritores: COVID-19, anticorpos monoclonais, Síndrome de Resposta Inflamatória Sistêmica.

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Introduction

The new coronavirus (SARS-CoV-2) causes the infectious disease named COVID-19, which started in the city of Wuhan, China, in November 2019 and has spread throughout the world.[1]

So far, there are seven types of coronavirus, of which four are related to common colds, which infect only the upper respiratory tract. The other three (SARS-CoV, MARS-CoV and SARS-CoV-2) are responsible for more aggressive infections, affecting the lower respiratory tract and can progress to extremely severe acute respiratory distress syndrome (ARDS).[1]

SARS-CoV-2 is an RNA virus belonging to the ß-coronavirus group, which acts directly on human ACE-2 receptors. For this to occur, a pico protein (S protein) projects itself as a viral envelope that binds to the ACE-2 receptor, causing the viral genome to enter the cell and thus initiate the COVID-19 infection. The fact that ACE-2 receptors are present mainly on alveolar epithelial cells explains the more aggressive action of the virus in the lower respiratory tract. However, such receptors are also expressed on the surface of heart cells, vascular endothelium, gastrointestinal tract and kidneys.[1]

From the onset of infection, the innate immune system is activated and begins to produce and release pro-inflammatory cytokines such as IL-6, IL-1ß, IL-8, TNF-α and other chemokines, which increase inflammatory responses. In the case of COVID-19, inflammatory responses are exacerbated, causing what is called a cytokine storm. This excessive inflammatory response causes several abnormalities in the human organism, such as clotting abnormalities, development of excessive oxidation, mitochondrial permeability transition and immune system failure. This condition causes central nervous system disorders, renal failure, liver failure, and ultimately, multiple organ failure.[1]

Studies of the immunological profile of patients with severe COVID-19 demonstrate that there is a high number of activated T lymphocytes and monocytes. These defense cells are responsible for synthesizing pro-inflammatory cytokines, such as interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and interleukin-12 (IL-12). Research shows that, in this pathogenesis, the main cytokine involved is IL-6, which is directly linked to the severity and prognosis of the disease, as it causes a storm of cytokines and severe inflammatory responses in the lungs and other organs and tissues.[2]

The IL-6 receptor has two presentations: membrane-bound IL-6 receptor (mIL-6R), and soluble IL-6 receptor (sIL-6R). There is binding of IL-6 with sIL-6R to form a complex, which is coupled to the transmembrane protein gp130 so that signal transduction occurs and the pro-inflammatory function is performed.[2] The IL-6 bound to its receptor, by generating this cytokine storm, can aggravate the immune disorder and, thus, prevent the hematosis process, which makes it possible for a wide range of dysfunctions to occur, from respiratory failure to failure cardiovascular disease and multi-organ dysfunction, thus contributing to the mortality of critically ill patients with COVID-19.[3]

This storm can be controlled through monoclonal antibodies that act on the IL-6 pathway, as they are able to bind to sIL-6R and mIL-6R, thus preventing this signal transduction.[2] Due to this association of high levels of IL-6 with severity and mortality in COVID-19, the use of tocilizumab (TCZ), which is a humanized anti-human IL-6 receptor monoclonal antibody of the immunoglobulin subclass (Ig) IgG1, used in the treatment of rheumatoid arthritis, juvenile idiopathic arthritis and giant cell arteritis.[4] This drug binds to IL-6 receptors and blocks intracellular signaling permeated by the sIL-6R and mIL-6R2 complexes, thus making it possible to reduce the cytokine storm and, consequently, the hyperinflammatory state.

The aim of this study is to evaluate the effectiveness of using tocilizumab in the treatment of patients with severe COVID-19.

Method

Search strategies for published studies such as randomized clinical trials were carried out for such a narrative review. They investigated the effectiveness of using the monoclonal antibody tocilizumab as a therapy for severe cases of COVID-19, that is, use as an additional therapy to conventional medical center therapy. The searches were performed through searches in the following databases: Science Direct and PubMed in September 2021, with no date or language restrictions. The search strategy used was: ("Coronavirus Infections" OR "COVID-19") AND (Tocilizumab) AND ("Randomized Controlled Trial" OR "Randomized Controlled Clinical Trial").

In this review, studies that used the drug without association with any other treatment, unless recommended by the medical center itself, or in association with corticosteroids, in patients in severe

cases of Coronavirus infection were considered as the use of tocilizumab in this review.

Publications of randomized clinical trials of severe cases of patients with a single stage of COVID-19 and without age restriction, who received tocilizumab as an intervention medication, combined with treatments protocoled by each hospital and also associated with corticosteroids, were included in this work. , in some works.

The outcomes of interest were the "WHO-endorsed 7-point ordinal scale" and Pao_2/Fio_2 ratio.

Selection of studies

A priori, searches using the search strategy found 413 works on May 7, 2021. Titles and abstracts were read by independent reviewers (RAP and ACM), and duplicates were removed from these. So, after reading the titles and abstracts, 90 articles remained. After reading the full articles and applying the inclusion and exclusion criteria, 2 articles were included in this narrative review, both randomized clinical trials (RCTs). The inclusion criteria are related to people with active infection by the Coronavirus in a serious condition, using tocilizumab, associated or not with corticosteroids and medications instituted by the hospital, as long as there are no antivirals in the treatment. Another inclusion criterion was RCTs. Exclusion criteria were: use of antivirals, case reports, bibliographic reviews, expert opinions and retrospective, prospective studies other than RCT's. The evaluation of articles according to the PEDro Scale is described in Table 1.

Results

In the search strategy, 413 references were identified. After removing duplicates, analyzing titles and abstracts, applying inclusion and exclusion criteria, 11 potential articles remained for the study. Of the potential 11, 9 were excluded. The main exclusion criteria from these potential studies were no RCT and the use of antivirals. All study results were obtained using tocilizumab. The outcomes found are described in Tables 2 and 3.

All studies portrayed were published between July 2020 and January 2021. People of both sexes, without age restriction, and all diagnosed with COVID-19 by PCR tests or by computed tomography of the lung area were included in this review. It is worth mentioning that all subjects in the studies were in a serious condition.

Table 1
Evaluation of studies by Ramiro et al. and Salvarani et al. according to the PEDro scale

PEDro criteria	Ramiro et al.[5]	Salvarani et al.[6]
Eligibility	Yes	Yes
Random allocation	No	Yes
Hidden allocation	No	Yes
Similar groups at baseline	Yes	Yes
Blind participants	No	No
Blind therapist	No	No
Blind researcher	No	No
< 15% dropouts	Yes	Yes
Intent to treat analysis	Yes	Yes
Difference between groups reported	Yes	Yes
Point estimate and reported variability	Yes	Yes
Total (0 to 10)	6	8

Source: prepared by the authors based on the PEDro Scale, 2021.

Table 2
Outcomes of the study by Ramiro et al.[5]

Ramiro et al.[5]		
	Risk Ratio or Treatment Effect Coefficient versus Control*	**Interval**
Primary outcome		
Clinical improvement (2 points) WHO scale	2.31	1.45 - 3.68
Main secondary outcomes		
Hospital mortality	0.26	0.13 - 0.52
Mechanical ventilation	0.22	0.10 - 0.52
Other secondary outcomes		
Clinical improvement (1 point) WHO scale	2.26	1.44 - 3.54
Independence from oxygen support	2.36	1.45 - 3.83
Duration of mechanical ventilation in survivors	-6.83	-21.45 - 7.79
Duration of hospitalization in survivors	-6.65	-10.93 - 2.37

* Adjusted for age, sex, body mass index (BMI), smoking, hypertension, diabetes, cardiovascular disease, and arrhythmia.

Table 3
Outcomes of the study by Salvarani et al.[6]

Salvarani et al.[6]			
	Clinical Outcome Ratio in Intention-to-Treat Population (TCZ versus Standard Care)	**Interval**	**p value**
Primary outcome (on the 14th day)			
Clinical improvement	1.05	0.59 - 1.86	0.87
General outcomes on the 14th day			
ICU admission	1.26	0.41 - 3.91	
Deaths	1.05	0.07 - 16.4	
Hospital discharge	0.99	0.73 - 1.35	
General outcomes on the 30th day			
ICU admission	1.26	0.41 - 3.91	
Deaths	2.10	0.20 - 22.6	
Hospital discharge	0.98	0.87 - 1.09	

TCZ = tocilizumab, ICU = intensive care unit.

Two RCTs were selected. The first was performed by Ramiro et al.[5] who selected a sample of 172 patients and divided them into an intervention group and a control group, both with 86 people with a mean age of 67 years. Both groups received some form of treatment. The control group received immediate treatment with methylprednisolone (MP) 250 mg intravenously on day 1, followed by MP 80 mg intravenously on days 2-5, with an option for a 2-day extension if deemed necessary and safe, in addition to to receive Ceftriaxone 2g daily for seven days and chloroquine 300mg every 12 hours after a loading dose of 600mg. The intervention group received tocilizumab (TCZ) between day 2 and day 5 (TCZ in a single dose, 8 mg/kg of body weight intravenously, max. 800 mg).

In turn, in the other RCT, according to Salvarani et al.,[6] a sample of 126 patients was selected, 60 destined for the group that would receive the monoclonal antibody and another 60 that would be the control group. The mean age of these patients was 60 years, and both the control and intervention groups received treatment. The intervention group received intravenous tocilizumab within 8 hours of randomization (8 mg/kg up to a maximum of 800 mg), followed by a second dose after 12 hours. The control group in turn received supportive care, which was guided by the protocols of each hospital center. It is noteworthy that all patients were followed up for 14 days according to the study protocol and an additional 30 days for analysis of secondary outcomes.

Ramiro et al.[5] aimed to evaluate an intensive course of glucocorticoids with or without tocilizumab to analyze whether there is an acceleration of clinical improvement in patients with cytokine storm syndrome (CSS) associated with COVID-19, using the "WHO-endorsed 7-point ordinal scale".

The study by Ramiro et al.[5] also sought to assess the mortality rate and the need for ventilation.

The study by Salvarani et al.[6] aimed to evaluate the effect of early administration of tocilizumab versus standard therapy in preventing clinical worsening in hospitalized patients with COVID-19 pneumonia, through the PaO_2/FiO_2 ratio, and through secondary outcomes that are mortality and hospital discharge.

At the end of the evaluation of the selected works, relevant results were obtained on the use of tocilizumab in relation to COVID-19 in severe cases. It was noticed that in the study by Ramiro et al.[5] there was a significant improvement with the use of glucocorticoids and tocilizumab associated, reducing the mortality rate, submissions to mechanical ventilation and, therefore, showing a significant improvement in relation to the primary outcome.

However, the work carried out by Salvarani[6] did not show a significant improvement. There were no significant differences in the outcomes when comparing the control groups and those submitted to treatment with tocilizumab.

Discussion

The novel coronavirus (SARS-CoV-2) acts on ACE-2 receptors, which are present mostly in alveolar epithelial cells. This fact increases the action of the virus in the lower respiratory tract, but it is possible to find these receptors on the surface of heart cells, vascular endothelium, gastrointestinal tract and kidneys.[1] The phenotype of this infection ranges from the absence of symptoms to severe pneumonia, leading to respiratory distress syndrome (ARDS).[7]

Cytokine storms often happen in the most severe cases of COVID-19. This fact is linked to the genesis of aberrant T cell immunophenotypes associated with a deregulated secretory profile of pro-inflammatory drugs, cytokines and chemokines. SARS-CoV-2 can influence CD4+ T lymphocytes towards a pathogenic TH1 lineage, which leads to overproduction of IL-6 and GM-CSF. These cytokines contribute to the activation of CD14+ CD16 monocytes, which secrete interleukin-6 (IL-6) and can travel to the lung, where eventually there is differentiation into alveolar macrophages or dendritic cells.[7]

When IL-6 binds to its receptor (IL-6R) it performs classic cis signaling, an action that affects the functions of T and B cells, macrophages, natural killer cells and neutrophils. Interleukin also contributes to the pathogenesis of cytokine storms. When there is interaction with sIL-6R, transinalization occurs, which has an effective role in the cytokine storm, as it stimulates the production of IL-6, IL-8, MCP-1 and endothelial growth factor (VEGF). It also regulates in favor of the expression of the adhesion molecule E-cadherin, which, together with VEGF, intervenes in the marked increase in vascularization, permeability and leakage, a fact of relevance to generate damage to the lungs. When IL-6 signaling is mediated by dendritic cells that express IL-6R, transpresentation occurs, which compromises T cells and generates an immunophenotype capable of destroying tissues[7].

Based on this assumption, an interesting therapeutic strategy to mitigate the dangerous effects of the cytokine storm associated with COVID-19 is the inhibition of IL-6. There is a humanized monoclonal antibody approved for the treatment of rheumatoid arthritis, tocilizumab, on the pharmaceutical market. Its pharmacological effect interacts with the IL-6 binding epitope, thus preventing IL-6 fixation, a fact that prevents cis-signalization, transsignalization and trans-presentation. Therefore, tocilizumab has anti-inflammatory properties. Wang et al.[11] concluded that tocilizumab can improve oxygenation, symptoms and reduce disease worsening with an acceptable side effect profile. Tleyjeh et al.[8] concluded that high-certainty cumulative evidence showed that tocilizumab reduced the risk of mechanical ventilation in hospitalized patients with severe COVID-19.

Ramiro et al.[5] showed a beneficial effect in relation to the use of tocilizumab associated with glucocorticoids, as such use together would generate a decrease in general and specific pro-inflammatory factors, thus causing a decrease in CSS and, as a consequence, generating a decrease in mortality and a decrease in of submissions to mechanical interventions. In this sense, the use of tocilizumab associated with methylprednisolone generates a beneficial effect.

However, according to Salvarani et al,[6] there was no significant improvement in the outcome, which is the Pao_2/Fio_2 ratio, thus showing that the isolated use of tocilizumab does not generate significant improvements, due to the fact that it inhibits in a specific isolated way. That is, due to the action of the drug, the need to use it associated with other drugs is perceived, because, in fact, the SARS-CoV-2 acts on IL-6 but also affects other pro-inflammatory cytokines. For this reason, tocilizumab alone does not generate the expected improvement.

Through the analysis of the outcomes and the results obtained, it appears that tocilizumab can provide some improvement, but this result is not obtained if used in isolation. Furthermore, it was understood that the use of a drug with an intense generalized inhibitory effect on the inflammatory response, associated with a drug with a specific inhibitory effect on IL-6, in this case tocilizumab, generates significant improvement effects, such as decreased mortality, decreased of interventions for the use of mechanical ventilation and change of WHO-endorsed 7-point ordinal scalefavorable to improvement.

Conclusion

The use of tocilizumab in patients with COVID-19 results in an improvement in the inflammatory condition due to its inhibitory effect on the IL-6 pathway, thus reducing the cytokine storm, which is one of the main causes of aggravation of COVID-19. However, by acting only on the IL-6 pathway, the use of tocilizumab alone did not offer significant improvements in the severe clinical conditions of patients with COVID-19. Those who received a combination of tocilizumab and drugs that have generalized inhibitory effects on the inflammatory response had a decrease in mortality and the need for mechanical intervention. Thus, it is concluded that the use of tocilizumab associated with other drugs that also interfere with the inflammatory response can contribute to an improvement in the clinical picture of patients with severe COVID-19.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Review Article

Air pollution and respiratory health

Poluição do ar e saúde respiratória

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ABSTRACT

The increase in the prevalence of chronic respiratory diseases coincides with that of exposure to air pollutants due to the growing industrialization process, increased vehicular traffic and population migration to urban areas. Air pollution is a complex mixture of pollutants and other toxic and non-toxic chemical compounds and its effect on health can derive from this mixture and the interaction with meteorological parameters. Despite this, it seeks to establish the role of a specific pollutant separately and considers the meteorological parameters as confounding factors. There is evidence that exposure to pollutants contributes to greater morbidity and mortality from respiratory diseases, especially in children, even at concentrations within the standards established by legislation. Identifying the effects of pollutants on the respiratory system, alone and in association, is a challenge and studies have limitations due to the variability of individual response, the presence of pre-existing diseases, socioeconomic factors, exposure to indoor, occupational and environmental pollutants as well tobacco. Most of the evidence on the effect of pollutants on the respiratory system of children comes from studies that include lung function outcomes. However, these studies differ in terms of design, method of assessing exposure to pollutants, measures of lung function, covariates considered capable of altering the response to pollutants, and types of models used in data analysis. Considering all these differences is fundamental in interpreting and comparing the results of these researches with data already existing in the literature.

Keywords: Air pollution, respiratory tract diseases, particulate matter, environmental pollutants, child.

RESUMO

O aumento da prevalência de doenças respiratórias crônicas coincide com o da exposição aos poluentes atmosféricos pelo crescente processo de industrialização, aumento do tráfego veicular e migração da população para áreas urbanas. A poluição do ar é uma mistura complexa de poluentes e outros compostos químicos tóxicos e não tóxicos, e o efeito na saúde pode derivar dessa mistura e da interação com parâmetros meteorológicos. Apesar disso, busca-se estabelecer o papel de um poluente específico em separado e consideram-se os parâmetros meteorológicos como fatores de confusão. Há evidências de que a exposição aos poluentes contribui para maior morbidade e mortalidade por doenças respiratórias, especialmente nas crianças, mesmo em concentrações dentro dos padrões estabelecidos pela legislação. Identificar os efeitos dos poluentes no sistema respiratório, isoladamente e em associação, é um desafio, e os estudos têm limitações devido à variabilidade de resposta individual, a presença de doenças pré-existentes, aos fatores socioeconômicos, às exposições a poluentes intradomiciliares, ocupacionais e ao tabaco. A maioria das evidências sobre o efeito dos poluentes no sistema respiratório de crianças deriva de estudos que incluem desfechos de função pulmonar. Entretanto, esses estudos têm diferenças quanto ao desenho, ao método de avaliação de exposição aos poluentes, às medidas de função pulmonar, às covariáveis consideradas como capazes de alterar a resposta aos poluentes e aos tipos de modelos utilizados na análise dos dados. Considerar todas essas diferenças é fundamental na interpretação e comparação dos resultados dessas pesquisas com os dados já existentes na literatura.

Descritores: Poluição do ar, doenças respiratórias, material particulado, poluentes atmosféricos, criança.

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Introduction

The economic and industrial growth that has taken place in recent decades has caused a significant increase in emissions of atmospheric pollutants and air quality has become a public health problem. The trend of population migration to the urban environment increased exposure to atmospheric pollutants, which contributed to greater morbidity and mortality from causes related to this exposure, such as respiratory diseases.[1-5] These outcomes are mainly described in children, the elderly and those with chronic diseases. Children are more vulnerable to the effects of air pollution due to anatomical characteristics of the airways, the immaturity of the immune system and greater exposure due to being outdoors for a long time.[4,6]

In recent decades, there has been a significant increase in the prevalence of chronic respiratory diseases such as asthma and allergic rhinitis, which coincides with the growing process of industrialization, the increase in vehicular traffic and migration to urban areas, especially in Western countries.[3-5,7,8] At the same time, and in light of the global warming issue, evidence emerged about the influence of temperature on health-related outcomes, such as hospitalizations and emergency room visits due to respiratory diseases, including asthma.[9,10]

In this context, several studies have related not only air pollution, but also other environmental factors, such as exposure to aeroallergens and increased temperature, to greater morbidity and mortality from respiratory diseases.[1,3-5] Studies carried out in different regions have observed that the increase in temperature can reduce lung function, and also that there is an association between increased temperature and concentration of pollutants, in the reduction of parameters of lung function in children.[11-16]

In Brazil, studies relating air pollution and respiratory diseases are scarce. In a systematic review, Froes Asmus et al.[17] analyzed 17 time series studies carried out in urban areas of Brazil, all in the Southeast region. The authors observed an increased risk for wheezing, asthma, and pneumonia in children and adolescents living in areas with high concentrations of nitrogen dioxide (NO_2) and ozone (O_3), and a reduction inpeak expiratory flow (PEF) measurement in children exposed to particulate matter (PM) with an aerodynamic diameter smaller than 10 μm (MP10), particulate matter with an aerodynamic diameter smaller than 2.5 μm (MP2.5) and black carbon (soot). Studies carried out in Rio de Janeiro and São Paulo showed a decrease in pulmonary function related to PM10 and NO_2, although they were within acceptable standards by current legislation most of the time.[17]

A study carried out in Greater Vitória evaluated the relationship between the number of hospitalizations for respiratory diseases, in the period from 2005 to 2010, and observed, through the Generalized Additive Model (MAG), that the increase of 10.49 μg/mm^3 in the levels of PM10 was associated with a 3% increase in the relative risk value for this outcome, even with pollutant concentrations within the limits recommended by the National Council for the Environment (CONAMA) and the World Health Organization (WHO).[18]

In Espírito Santo, the studies carried out revealed, over the last decades, the coexistence of a high prevalence of respiratory diseases in children[19] and a relationship with unfavorable environmental conditions in Vitória, probably due to the increase in vehicular traffic and the existence of industries with polluting potential within the city. of the urban fabric.[20-23] Most of these studies used secondary health data, that is, hospitalizations and emergency care, from Health Information Systems (SIS). These data have the advantages of wide population coverage, the low cost of collecting information and the ease of longitudinal follow-up. However, the limitations of these data are related to the lack of standardization in the collection that affects the quality of the records, the coverage that can vary in time and space, and the lack of information that may be important for the analyzes of interest, which include outcome, explanatory, mediating, confounding or effect-modifying variables.[24] These limitations motivate studies that provide subsidies for the analysis of primary health data with the objective of evaluating the effect of pollution on the respiratory system of children and adolescents living in urban areas in Brazil.

Air pollution

Air pollution can affect human beings at all stages of life, from conception to old age. Since the first reports on the effects of pollution on health occurred in 1930 (Meuse Valley, Belgium) and in 1952 (London, England), many studies have been carried out in an attempt to elucidate the real impact of air pollution on human health.[25-27]

Emissions generated by industries and motor vehicles are the main sources of air pollutants in urban areas and are clearly involved in the genesis of clinical symptoms, as well as in the greater number of hospitalizations and deaths from respiratory diseases.[28-30]

It is well established that ozone (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), carbon monoxide (CO) and PM are the main air pollutants and that even at concentrations within the established limits by regulatory agencies and the WHO pose a risk to human health.[22,27] However, recently the WHO, when reviewing studies on the influence of exposure levels to air pollutants, especially PM10, PM2.5, O_3 and NO_2, and the development and/or worsening of cardiovascular and respiratory diseases, revised these limits and reduced them as shown in Table 1.[31]

PM is a heterogeneous and complex mixture of particles that vary in size, weight, shape, chemical composition, solubility and origin.[31] The particles are classified according to their aerodynamic diameter as: ultrafine (particles with a diameter smaller than 0.1 µm - MP0.1), fine (particles with a diameter between 0.1 and 2.5 µm - MP2.5) and coarse (particles with a diameter between 2.5 and 10 µm – MP10).[32]

MP10 does not reach the lower airways, however, it is the one with the most frequent and consistent relationship with diseases of the respiratory system. MP2,5 and ultrafine particles reach the lower airways and have a greater potential to trigger or worsen respiratory diseases. The ultrafine particles reach the alveoli, reach the blood circulation and generate biological effects (oxidative stress and systemic inflammation) that can be negligible or intense, depending on the characteristics of the individual, the degree of exposure and the chemical composition of PM.[27,33]

The gaseous pollutants SO_2, O_3, NO_2 and CO, when inhaled, trigger a series of biological events. When SO_2 enters the airways and is exposed to water, it forms sulfuric (H_2SO_4) and sulfuric acid (H_2SO_3), which induce bronchoconstriction and bronchospasm.[34] O_3, when inhaled, causes the death of ciliated cells of the respiratory epithelium, compromising the protective function of the epithelium.[35] NO_2 causes inflammation, oxidative stress and hyperreactivity in the airways.[36] CO attaches to oxygen binding sites in hemoglobin causing hypoxia and oxidative stress.[37]

In practice, air pollution is a mixture of these main pollutants and other toxic and non-toxic chemical compounds and the effect on human health can be derived from this mixture.[6] However, most studies seek to establish the role of a specific pollutant or of several pollutants separately on human health. Identifying the possible effects of pollutants on the respiratory system, alone and in association, is a challenge, and studies have limitations due to the variability of individual response, the presence of pre-existing diseases, socioeconomic factors, exposure to indoor and occupational pollutants and tobacco.[27,38-39] In this context, some authors have described deleterious effects when multivariate analysis models are incorporated into data analysis.[21]

Meteorological parameters, temperature, wind, humidity and precipitation act in the transport and dispersion of pollutants and play an important role in the dispersion and deposition of pollutants in the environment, as well as in human health, influencing biological events related to contact with pollutants.[40] Therefore, these parameters are usually considered as confounding factors in pollution studies. Although the negative impact of pollutants and temperature rises on health is well established, few studies have investigated the interactive effect between temperature and air pollution on health outcomes.[11] Some studies have observed over time that exposure to atmospheric pollutants and meteorological parameters are related to the occurrence of acute respiratory infections and greater severity of asthma.[41,42]

Those most susceptible to the effects of pollutants and temperature variations are children, the elderly and those with chronic diseases, especially cardiovascular and respiratory diseases.[12] The strongest evidence for the effects of air pollution on children's health, for example, comes from studies of lung function. The oxidative stress generated by pollutants causes inflammation in the lungs, which can contribute to a decrease in lung function in the short and long term.[43,44] Recent studies have observed a relationship between temperature elevation, concentration of pollutants and reduction in pulmonary function parameters, both in children with preexisting lung disease[12] and in healthy young adults,[14] which reinforces the need for a better understanding of this interaction.

Air quality

The interaction between pollution sources and the atmosphere determines the air quality of a

given region. Monitoring air quality allows estimating exposure in the population by measuring the concentration of pollutants. According to WHO data, more than 90% of the world's population lives in places where pollutant levels are not in accordance with previously established limits.[45] The systematic measurement of air quality is restricted to a number of pollutants, defined according to their importance and the resources available for their monitoring.

In Brazil, air quality standards were established by the resolution of the National Council for the Environment - CONAMA nº 03, of 1990. These standards are divided into primary and secondary, and represent the concentrations of atmospheric pollutants that, when exceeded, can affect the health, safety and well-being of the population, as well as causing damage to flora and fauna, materials and the environment in general, and were updated in 2018.[46] The *primary* air quality standards are the concentrations of pollutants that, when exceeded, can affect the health of the population and *secondary* standards are the concentrations of pollutants below which the minimum adverse effect on the well-being of the population is expected, as well as the minimum damage to fauna, flora, materials and the environment in general. In this first resolution, it was established that the monitoring of air quality is the responsibility of the States.[46]

The limits of some air pollutants[41] were revised based on clinical studies that evaluated the relationship between the level of exposure and the development and/or worsening of diseases, reducing them.[31] It is also noteworthy that the knowledge accumulated over the years allows us to infer that the harmful effects of pollutants on the health of the population can occur at concentrations lower than those previously established.[41]

The WHO guidelines and national air quality standards present reference values associated with the health effects caused by short and long exposure for each pollutant, in order to prevent acute and chronic effects, respectively. However, for the pollutants SO_2, O_3 and CO, the WHO establishes a guideline only for reference values for short exposure.[31]

Table 1 shows the air quality standards in Brazil[46] and those recently established by the WHO in 2021.[31] In it, we see that there was a reduction for almost all of the pollutants.

Effects of air pollution on human health

Air pollution causes harmful effects to human health even when pollutant levels are within the standards established by regulatory agencies.[31] These effects range from physiological changes, which do not cause relevant clinical manifestations and affect a greater proportion of people, to outcomes with greater impact, such as death, which affects a smaller percentage of the exposed population.[31] Thus, the WHO illustrates these outcomes as a pyramid, which considers the magnitude and severity of the effects of exposure to air pollutants (Figure 1).

The effects of air pollution on health are considered to be short-term or long-term, depending on the time of exposure to pollutants (Table 2). Studies on the short-term effects (days or weeks) on children's respiratory health have increased in recent years compared to those evaluating the effects of long-term exposure (one or more years). The latter are, in most cases, carried out in North American and European cities, with moderate levels of atmospheric pollution.[47-50]

Effects of air pollution on the respiratory system

The strongest evidence on the effects of air pollution on the health of the respiratory system is obtained from studies in children and with monitoring of lung function. Children are more susceptible to these effects due to inherent characteristics of the age group: (1) immaturity of the immune system predisposing the occurrence of respiratory infections; (2) greater area of airway extension in relation to body size; (3) higher ventilation rate per unit of body weight; (4) anatomically smaller peripheral airways, which results in greater obstruction in the face of an inflammatory process; and (5) higher prevalence of chronic respiratory diseases such as asthma and rhinitis.[6]

In addition, children engage in outdoor activities, usually during the day, when pollutants may be at higher levels, increasing the chance of harmful effects on the respiratory system.

At birth, the lungs are not fully developed and 80% of the alveolar area responsible for gas exchange will form by approximately 6 years of age. The functional development of the lungs extends until adolescence, and contact with pollutants during this period can cause loss of lung function, with the consequent

emergence of respiratory diseases in childhood or adulthood.[6] Therefore,exposure to air pollutants can negatively affect lung development in children, causing a deficit in lung function, which is considered a risk factor for the development of lung disease and death in adulthood. Thus, studies to assess the relationship between lung function and air pollution are complex because characteristics inherent to the individual and the intra- and extra-domestic environment contribute to variations throughout life.

A long-term study evaluated the effects of exposure to air pollutants on lung development in American children. At the end of four years, there was a significant association between exposure to PM10, NO_2 and inorganic acid vapor, with a reduction in lung growth, with no relation to sex, different from what was

Table 1
Air quality standards in Brazil according to the National Council for the Environment (CONAMA)[46] and the new recommendations of the World Organization of Health (WHO)[31]

		Air Quality Benchmarks									
		CONAMA					WHO 2021				
		Intermediaries			Final		Intermediaries				Level
	Reference	PI-1	PI-2	PI-3	PF		1	2	3	4	AQG
Pollutant	period	µg/m³	µg/m³	µg/m³	µg/m³	ppm	µg/m³	µg/m³	µg/m³	µg/m³	µg/m³
PM10	24 hours	120	100	75	50	–	150	100	75	50	45
	Annual [a]	40	35	30	20	–	70	50	30	20	15
PM2.5	24 hours	60	50	37	25	–	75	50	37.5	25	15
	Annual [a]	20	17	15	10	–	35	25	15	10	5
SO_2	24 hours	125	50	30	20	–	125	50	–	–	40
	Annual [a]	40	30	20	–	–	–	–	–	–	–
NO_2	1 hour [b]	260	240	220	200	–	–	–	–	–	–
	Annual [a]	60	50	45	40	–	40	30	20	–	10
O_3	8 hours [c]	140	130	120	100	–	160	120	–	–	100
Smoke	24 hours	120	100	75	50	–	–	–	–	–	–
	Annual [a]	40	35	30	20	–	–	–	–	–	–
CO	8 hours [c]	–	–	–	–	9	7	–	–	–	4
TSP	24 hours	–	–	–	240	–	–	–	–	–	–
	Annual [d]	–	–	–	80	–	–	–	–	–	–
Pb [e]	Annual [a]	–	–	–	0.5	–	–	–	–	–	–

ppm = parts per million, PM10 = particulate matter with a diameter of less than 10 µm, PM2.5 = particulate matter with a diameter of less than 2.5 µm, SO_2 = sulfur dioxide, NO_2 = nitrogen dioxide, O_3 = ozone, CO = carbon monoxide, TSP = total suspended particles, Pb = lead, a = annual arithmetic average, b = hourly average, c = maximum moving average obtained in the day, d = annual geometric average, e = measured in TSP, AQG = Air Quality guidelines.

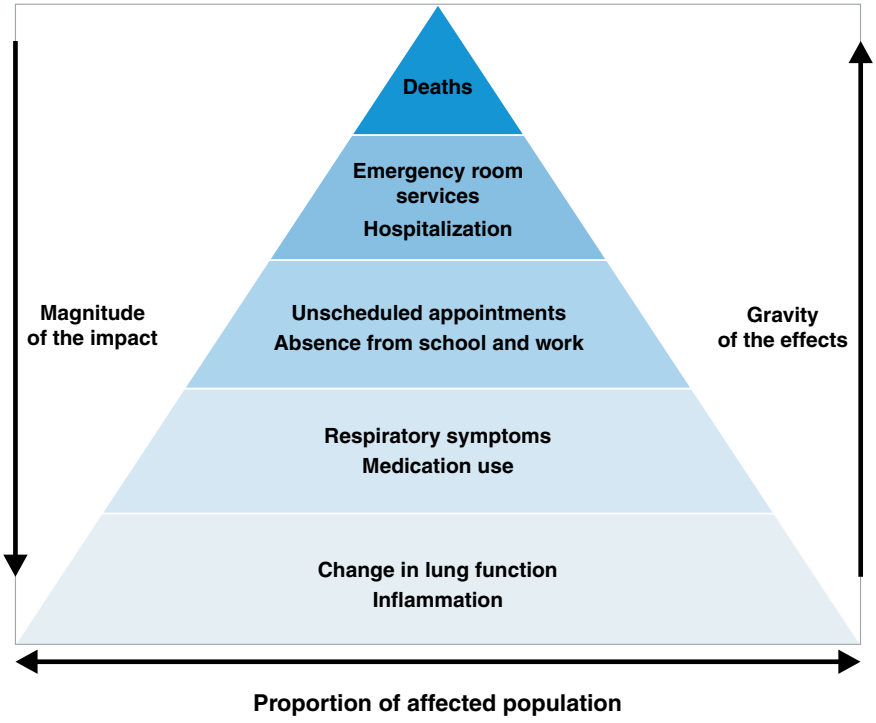

Figure 1
Effects of exposure to pollutants on the respiratory system[31,45]

observed by the same authors in a previous study.[51] There was a cumulative reduction of 3.4% in forced expiratory volume in one second (FEV1), and of 5% in maximum expiratory flow (MMEF).[52] With the follow-up of these patients, the authors found that children living in more polluted communities had a deficit of 100 mL in FEV1 (7% females and 4% males), when compared with children from non-polluted areas,[53] and who were five times more likely to present clinical manifestations of pulmonary function deficit at age 18 than those who lived in non-polluted areas.[54]

Rojas-Martinez et al. followed 3,170 8-year-old schoolchildren from schools located within 2 km of 10 air quality monitoring stations over a 3-year period and studied the relationship between long-term exposure to PM10, NO_2, and O_3 and lung development. The authors observed a deficit in lung growth (forced vital capacity, FVC and FEV1) related to the concentrations of O_3, PM10 and NO_2, with girls being the most affected.[55] Similar results were observed by other studies.[56]

Liu et al. investigated the acute effects of pollutants PM2.5, SO_2, NO_2 and O_3 on lung function, oxidative stress and inflammation in children and adolescents with asthma, during 4 weeks.[39] The increase in the level of PM2.5, SO_2 and NO_2 was associated with a decrease in FEV1 and forced expiratory flow between 25% and 75% of FVC (FEF25-75%), and with an increase in inflammation markers, indicating that the Air pollution can increase oxidative stress and reduce small airway function. The estimated risk was lower in children treated with inhaled corticosteroids.[39]

A study carried out in Taubaté, in the interior of the state of São Paulo, showed a higher prevalence of asthma among adolescents who lived close to a highway with very heavy vehicle traffic.[57]

Table 2
Effects of air pollution on health[45]

Exposure to air pollutants
Short term
• Daily mortality
• Hospital admissions for respiratory or cardiovascular disease
• Emergency care for respiratory or cardiovascular disease
• Primary care services
• Activity restriction days
• Absenteeism at work
• Absenteeism from school
• Acute symptoms (wheezing, coughing, respiratory infections)
• Physiological changes (eg lung function)
Long term
• Mortality from cardiovascular or respiratory disease
• Hospital admissions for respiratory or cardiovascular disease
• Chronic changes in physiological functions
• Lung cancer
• Cardiovascular disease
• Intrauterine growth problems (low birth weight, intrauterine growth retardation, low weight for gestational age)

Other studies have shown a relationship between exposure to higher levels of air pollutants and impaired lung function.[49,50,58-61]

In Brazil, a study conducted in the Amazon with children and adolescents observed that for an increase of 10 μg/m^3 in the concentration of PM2.5, there was a significant reduction in PEF (0.26 to 0.38 L/min).[62] In Espírito Santo, in an area exposed to industrial emissions from a mining company, daily monitoring of the PEF of children and adolescents documented a significant negative association of this parameter with the concentration of PM10. A 14 μg/m^3 increase in PM10 concentration was associated with a reduction in morning (-1.04%) and evening (-1.2%) PEF measurements, even after adjusting for temperature and humidity.[63]

In summary, in recent decades the environment has undergone profound changes due to increased emission of atmospheric pollutants and climate change. At the same time, there was an epidemiological transition and chronic respiratory diseases, such as asthma and rhinitis, became more prevalent than infectious diseases. In this context, it is well established that particulate matter and gaseous pollutants cause damage to the respiratory system, especially in children. However, the studies have differences in design, in the method of assessing exposure to pollutants, in pulmonary function measurements, in the covariates considered capable of altering the response to pollutants, and in the types of models used in data analysis.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Review Article

Is asthma curable?

Asma tem cura?

Hisbello da Silva Campos[1]

ABSTRACT

Asthma is the product of coordinated, interconnected and complex processes that originate in genes/epigenetics, microbiome, and environment/lifestyle. Currently available drugs are not able to interfere with the insertion of asthma into the body. The current therapeutic approach involves drugs that aim to control symptoms and antagonize part of the effects of some of the cytokines involved. Thus, the current treatment is aimed at controlling asthma and not curing it. Epigenetic mechanisms translate the microbiological and environmental stimuli into altered cellular behavior. For this reason, the identification of epigenetic markers will certainly point out to new therapeutic targets and, ideally, strategies to reverse the altered cellular behavior in the respiratory tract. Then, yes, we could say that asthma is curable.

Keywords: Asthma, epigenomics, biological treatment.

RESUMO

A asma é o produto de processos coordenados, interligados e complexos que têm origem nos genes/epigenética, microbioma e ambiente/estilo de vida. Os medicamentos atualmente disponíveis não são capazes de interferir com a inserção da asma no organismo. A abordagem terapêutica atual envolve fármacos que visam controlar os sintomas e antagonizar parte dos efeitos de algumas das citocinas envolvidas. Dessa forma, o tratamento atual visa o controle da asma e não a sua cura. Mecanismos epigenéticos traduzem os estímulos microbiômicos e ambientais em comportamento celular alterado. Por essa razão, a identificação de marcadores epigenéticos certamente apontará novos alvos terapêuticos e, idealmente, estratégias para reverter o comportamento celular alterado no trato respiratório. Aí, sim, poderíamos dizer que a asma tem cura.

Descritores: Asma, epigenômica, tratamento biológico.

Introduction

One of the phrases attributed to Hippocrates (460-370 BC) has space in a discussion of the cure for asthma: “the cure is linked to time and sometimes to circumstances.” It is known that asthma is the product of the variable interaction of genetic, epigenetic, microbiomic and environmental factors. With different weights, at different times and in different ways, these factors interact and influence each other. Basically, the available therapeutic options reduce inflammation, relax peribronchial smooth muscle, or antagonize certain cytokines. In this way, we achieve asthma control and not its cure. If asthma is only manageable with current resources, treatment is palliative; treats symptoms without removing the cause.

The term *cure* implies discovering and solving the cause of the disease: destroying the responsible microorganism, removing the tumor, restoring the normality of physiological indicators.[1] A Latin term used to define healing is *Restitutio ad integrum*, which means 'to restore to the original condition'. We have, then, a semantic problem if we want to cure asthma. Although the mechanisms involved in the genesis of asthma are still unclear, we know that genes, microbiome, environmental factors, diet and other characteristics are directly involved in the insertion of this disease in the human organism through epigenetic mechanisms, mainly. Furthermore, it is known that part of the construction of asthma begins in the prenatal

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phase. So, how to talk about healing if the original condition is already predisposing?

As new technologies are being used to understand the mechanisms involved in the determination of diseases, we are discovering that cellular and molecular processes, governed by genes, microbes and factors associated with the environment and daily life, generate the insertion of diseases in our organism, modulate its progression and resulting dysfunctions, define its outcomes. Probably the moment in life when each of these processes is initiated matters. In asthma, as in other diseases, there are two fundamental moments. The first, when the causal factor alters the normal (healthy) cellular functioning – insertion of the disease in the organism. The second is represented by the progression of altered functioning and its functional and anatomopathological consequences. If technology is developed that can reverse the processes involved with insertion, the disease could be cured. If therapeutic actions can only control the dysfunctions resulting from the progression of pathological mechanisms, they are only controlling the disease.

The growing understanding of the pathogenesis of asthma makes it increasingly unlikely that it can be cured through medication. In this article, some aspects involved in its pathogenesis that point to this conclusion will be discussed.

Asthma: cause and development

Apparently, in addition to genetic inheritance, changes in maternal diet, use of paracetamol during pregnancy, transmission of the maternal microbiota to the newborn during childbirth and prematurity are factors capable of introducing asthma into the body.[2] To cure asthma, it would be necessary to intervene in these factors. Defining a "preventive" diet, avoiding maternal exposure to risk factors are effective and feasible strategies. On the other hand, interfering with genetic inheritance, modulating the maternal microbiome or preventing prematurity are still not possible actions.

Immunological factors, age, sex and atopy influence the development of asthma.[3] There are differences in the age of onset of asthma symptoms according to sex. Probably due to a hormonal factor, asthma is more common among boys in early childhood, puberty and early adulthood. Symptom remission rates are also higher in males.[3-5] Asthma remission during adolescence is associated with a lower initial degree of bronchial hyperresponsiveness (BHR) and a greater gain in peripheral airway function when compared to asthma that starts after childhood.[5] Possibly, this variability between childhood-onset asthma (AII) and the late start (adult asthma - AIA) is associated with differences in the degree of environmental exposure.[6] Asthma that starts in older adults tends to be more severe than asthma that starts at younger ages.[7] Characteristically, asthma presents temporal heterogeneity. The same person presents different clinical forms throughout life, indicating variation in pathophysiological mechanisms over time. Possibly, a multifactorial arrangement is responsible for the clinical heterogeneity and temporal variation observed in asthma. Unraveling this arrangement and the elements involved would be a way to identify therapeutic targets to control asthma.

The causal trio of asthma and respiratory tract inflammation

"Complex systems" (area of physics and mathematics in which chaos theory became popular) deal with sets of numerous elements that interact with each other in different ways, making their behavior difficult to predict and simulate. The elements and processes involved in the pathogenesis of asthma constitute a "complex biological system". In it, genes/epigenetic mechanisms, dysbiosis in the intestinal/respiratory microbiota, and environmental/lifestyle factors interrelate in a coordinated and variable manner, involving multiple mechanisms and generating the full range of dysfunctions and anatomopathological changes observed in asthma. This variation is expressed by the different endotypes identified.

Asthma results from a coordinated and interdependent work between three factors: genetic, microbiomic and external. Through epigenetic mechanisms (DNA and miRNA methylation, mainly), environmental factors (environmental and climatic pollutants) and factors related to daily life/lifestyle (diet, physical activity, drug use, exposure to allergens and environmental pollutants, environment and others) cellular behavior in the respiratory tract is altered/modulated. At the same time, they cause dysbiosis in the intestinal and respiratory microbiota that lead to immune and inflammatory dysfunctions in the respiratory tract. Part of the processes involved still begins in the prenatal phase; the other part, from

the moment of birth. As a result of this orchestrated and organized work, distinct pathogenic processes are generated and lead to the variable clinical dysfunctions labeled as asthma.

The temporal and category variation between the inflammatory processes observed in asthma is one of the results of the complex multifactorial mechanisms involved in its pathogenesis.Inflammation is a central component of asthma, responsible for symptoms and for physiological and structural abnormalities. Several techniques have been and are being used to classify and size it in asthmatic airways, from stratified cell counts in lung material, assessment of cell response by quantitative cytometry and, more recently, omics technology. Cell type and its proportion in sputum/bronchial secretion began to be used to categorize asthma into eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic (non-elevated eosinophils and neutrophils) and to indicate the most appropriate drug approach. However, standardized cut-off points for these asthma categories have never been established, resulting in imprecision in the choice of therapeutic approach.

The search to identify the different inflammatory processes present in asthma and their detailed categorization brought the concept of endotyping. Endotype is defined as the mechanism that determines a particular form of asthma. Initially, with the assumption that CD4+ T lymphocytes were the main cell type in the coordination of inflammatory processes, the first endotypic categorization focused on Th1 and Th2 subgroups. Th2 cells, by generating large amounts of IL-4, IL-5 and IL-13, were the main inducers of eosinophilic airway inflammation. Subsequently, asthmatics began to be stratified into two categories, according to the presence or absence of eosinophilic inflammation and the response to corticosteroid therapy. Then, the polarization between two endotypic groups emerged: Th2-high (eosinophilic) and Th2-low (non-eosinophilic). In the first group, eosinophilia is predominant and treatment includes corticosteroid therapy and IL-4, IL-5 and IL-13 cytokine antagonists. In the second group, corticosteroids are not effective, the use of antibiotics may be useful and the cytokines responsible for neutrophil recruitment (TNF, IL-1, IL-6, IL-8, IL-23 and IL-17) may be targets of therapy. In the paucigranulocytic form, treatment with anti-inflammatory drugs does not seem to be helpful; the use of bronchodilators, bronchial thermoplasty or drugs targeting mast cells seems to be more effective.

Recently, another cell lineage has been incorporated into the pathogenesis of asthma, the innate lymphoid cells (ILCs). They are innate lymphocytes distinct from T and B cells. Group 2 ILCs (ILC2), which produce prototype type 2 cytokines, are important in the pathogenesis of asthma. They generate large amounts of IL-5 and IL-13 in the airways in response to alarmins and mediators released by epithelial cells activated by inflammatory stimuli. The discovery of the value of these cells in asthma brought a new endotypic terminology: high Th2 came to be called high T2, aggregating ILC2 to Th2. Both cells are primary regulators of type 2 immunity and express the transfer factor GATA3, which governs the production of type 2 cytokines. Subsequently, Inflammatory processes of the airways started to be classified as Type 2 (T2) and non-Type 2 (T1).[8] In T2, alarmins released by the bronchial epithelium (thymic stromal lymphopoietin - TSLP, IL-25 and IL-33) from inflammation caused by infectious or allergic stimuli initiate different cell signaling processes. While TSLP activates antigen-presenting cells (APC) leading to the activation of T and B cells, IL-33 and IL-25 activate ILC2. The expression of these markers is correlated with the severity of asthma.[9] When activated, ILC2 secretes ten times more potently than Th2 cells of IL-5 and IL-13, propagating/amplifying type 2 immune responses. There are indications that they are also associated with the process of remodeling and repair of lesions of the airways present in asthma.[10] IL-4, IL-5 and IL-13 are potent activators of eosinophils, a fundamental cell in T2.

The categorization of non-Type 2 (T1) asthma is not as clear as that of Type 2. In it, in addition to the number of eosinophils not being expressive, the role of Th1 and Th17 lymphocytes is relevant, as well as that of neutrophils and IL-1b, IL-6, IL-8, IL-17A, IFN-γ and TNF-α. In the T1 category, structural abnormalities in smooth muscle and in the neural network also participate in the pathogenesis. Neutrophilia is still a controversial point. There is no consensus on the cut-off point that classifies asthma as neutrophilic. Furthermore, neutrophilic inflammation is an inconsistent biomarker as it can be found in smokers or after inhalation of traffic pollutants or NO_2/ozone containing pollutants. Intense exercise and a cold environment can also induce a neutrophilic pattern, as well as bronchiectasis.[11]

Asthmatic airway inflammation is not a uniform or static process, it can be caused in different ways and

be composed of different mechanisms. The evolution of knowledge about the molecular and cellular processes involved makes it clear that asthma is a label that covers different respiratory disorders that have as common denominator episodes of dyspnea with variable intensity and frequency that, for the most part, arise in childhood. Often, the family history is positive and the cardinal symptoms are dyspnea, wheezing, cough and chest tightness. In some cases, allergic or environmental factors are associated with the onset of symptoms. In-depth examination reveals differences in the mechanisms that determine the dysfunctions (endotypes). Differences have a genetic and epigenetic basis, and result from cellular diversity, the wide variety of activated cytokines and the microbiomic dysbiosis involved. Epigenetic mechanisms translate external factors, such as allergens, environmental pollutants, diet, lifestyle, drug use, and others, into genomic modifications that alter cellular behavior in the respiratory tract.

Adding complexity to the scenario, we have the dynamics of the processes involved. Asthma is not a "static" disease. On the contrary, it undergoes variations all the time. The different inflammatory processes involved can alternate or add up at different times. As a result, an asthmatic classified as eosinophilic at one time may be neutrophilic at another time and paucicellular at another time. Clinically, he may be asymptomatic or with occasional mild symptoms, and in others, have intense and continuous symptoms. Finally, he may have different "asthma" over time, or even total remission of symptoms.

As technological developments make it possible to clarify pathogenic mechanisms and their interactions, new therapeutic targets will be identified. This will certainly revolutionize the approach to asthma. The "palliative" treatment, which aims at symptomatic control and the prevention of symptoms/exacerbations, should not continue "uniform/rectilinear", as it is today; it will probably vary over time, molding itself to the pathogenic processes prevailing at each moment and variables in the same patient.

The role of each member of the causal trio (genes/epigenetics, microbiome and external factors) in the onset, progression and outcome of asthma in the human body is still unclear. Unraveling the mechanisms present in each one and their interrelationships is a fundamental step in defining the ideal treatment for asthmatics.

Genes/epigenetic mechanisms

Heredity is a hallmark of asthma. The phenotypic variety and underlying pathogenetic mechanisms demonstrate the complexity of the genetic processes involved. With the progress of genome-wide association studies (GWAS) technology, several genetic markers, single nucleotide polymorphisms (SNPs) and chromosomal regions have been associated with asthma susceptibility, age at onset and atopy.[12]

A recent study of the genetic variation between two distinct categories of asthma according to the age of onset of symptoms – childhood onset (AII) and adulthood (AIA) – observed partially different genetic architectures. Based on the findings, some assumptions can be made. In one, the different genes could lead to different molecular processes between the two forms. Therefore, the design of drugs designed specifically for each of the forms would be different. In another hypothesis, the same gene in particular would contribute to the pathophysiology of both forms, but its expression would be deregulated in AII by different risk alleles and, in AIA, by epigenetic modifications. SNPs explain only 5-10% of the variation in age at onset of symptoms. It may be that the wide range of differences observed in the alleles indicates that a specific association with certain alleles determines the age of onset. In this case, the internal difference in each group could be modulated by environmental factors (epigenetic factors). In AII, candidate environmental risk factors would be the timing, frequency and duration of respiratory infections, allergen exposure, domestic animals, maternal smoking and low-quality diet during pregnancy. In the EIA, occupational exposures, smoking and obesity.[13]

The study of epigenetics focuses on hereditary changes that affect gene expression without altering the DNA sequence.[14] The most common epigenetic mechanisms identified that play a regulatory role in immune responses and gene expression in asthma are DNA methylation, post-translational histone modifications and miRNA expression.[15] Changes in DNA methylation result in a differentiated genomic expression related to the production of cytokines and transcription factors associated with the phenotypic presentations of asthma. The environment and life habits (maternal smoking, atmospheric pollution, exposure to heavy metals, pesticides and microbes, certain foods and drugs)[16] are potent influencers of DNA methylation.[17] Briefly, it can be said that epigenetic mechanisms represent the bridge between external factors and genes,

leading to changes in genomic expression (cellular functioning).

In asthma, epigenetic changes can be induced in the prenatal phase, in early childhood and adolescence, and make the individual susceptible to asthmatic "triggers".[18] They play an important role in immune responses and in the regulation of various cellular functions, such as differentiation and balance between T cell classes, changes in the expression of inflammatory genes, in the cellular transformation of AII (Th2 asthma, predominantly eosinophilic-corticosteroid sensitive) to AIA (non-Th2 asthma, neutrophilic-paucigranulocytic – less sensitive corticosteroid), in remission/protection phenomena.

Intestinal and respiratory microbiota

In 1989, Dr. David Strachan (United Kingdom) proposed a hypothesis to explain the increase in the prevalence of allergic diseases observed in previous years. According to him, *"These observations... could be explained if allergic diseases were prevented by infections in early childhood transmitted by unhygienic contacts with older siblings, or acquired in the prenatal period,... During the last century, the family size has decreased, indoor play has increased, and higher standards of personal hygiene have reduced the opportunity for cross-infection in young families. This may have resulted in greater generalized clinical expression of atopic diseases"*.[19] Called the "Hygiene Hypothesis", it was initially met with skepticism. Shortly thereafter, in the 1990s, the recognition that natural immunity against viral and bacterial infections induced a Th1 pattern of cytokine release, potentially suppressing the Th2 immune responses involved in IgE-mediated allergy, attracted the attention of allergists and immunologists.[20] With the advancement of studies on the subject, the Hygiene Hypothesis, which postulates that infections protect against atopy, is considered immunologically plausible and consistent with the epidemiological aspects of atopy. However, the inverse association between infection and atopy cannot be directly confirmed by epidemiological studies.[21] From then on, the participation of the microbiome in immune diseases began to be studied in depth. Under normal conditions, the interaction between the microbiome and the human organism confers mutual benefits (symbiosis). However, when the composition and diversity of the microbiome are altered (dysbiosis), these changes are translated into changes in immune responses and diseases, such as asthma, for example.

The bacterial portion of the microbiome alone contains about 3.3 million genes, 150 times more than the human genome.[22] This represents an epigenetic pressure on the human genome that makes evident the participation of the microbial ecosystem in the biological processes underlying health and diseases.[23] The intestinal microbial community is the most abundant, comprising more than a thousand bacterial species, apart from viral and fungal populations. The pulmonary microbiota (PM) is an ecosystem formed by a well-organized and metabolically active community, which includes microorganisms (viruses, bacteria and fungi, mainly), their genomes and environmental conditions. The microbiota (intestinal, oral, upper respiratory tract, genitourinary tract, skin and others) are in constant communication with each other in a bidirectional way through "axes", with each being able to influence the other. Communication between PM and the intestinal microbiota (IM) occurs through the "gut-lung axis".[24]

Growing evidence makes it clear that there is an interaction between PM and the host's immune system. Changes in MP diversity or abundance are associated with several chronic respiratory diseases, such as asthma, cystic fibrosis, bronchiectasis and chronic obstructive pulmonary disease (COPD). Bacteria, viruses and fungi from the microbiota of the upper and lower airways produce structural ligands and metabolites that interact with the host and change the progression of these diseases.[25] With the development of the omics sciences, it will be possible to begin to unravel all the molecular and genetic biology involved in the participation of the microbiome in asthma and to identify new therapeutic targets, certainly more specific.

Environmental factors and lifestyle

Environmental exposures are linked to the development and progression of diseases. In addition to exposure to allergens, air pollutants and climatic factors and microorganisms, everyday habits and lifestyle such as diet, exercise, medication, smoking, pets and infections are also risk factors for the development and exacerbations of allergic diseases and asthma.[26] The influence of all these factors is differentiated by the individual's genetic aspects, immunological aspects, time of exposure and microbiomic characteristics. Among the microbiological

factors, fungi play an important role. Some species, *Aspergillus*, *Candida*, *Alternaria*, *Clodosporium* and others are associated with asthma and its severity.[27]

If we want to cure asthma, we will have to be able to block the influences of these elements in the respiratory tract. This can only be possible after clarifying the complex interrelationships between genetic/epigenetic, environmental/lifestyle, microbiomic factors and the resulting dynamic biological processes. The identification of epigenetic markers is a fundamental step for the identification of asthmatic endotypes and for the definition of therapeutic and preventive approaches. The integration of the omic sciences and their instruments in studies to solve the still existing mysteries will be of great help in the identification of therapeutic agents, prediction of evolution/outcome and, eventually, preventive actions of their development. One of the goals of personalized medicine is to develop pharmacogenetic and pharmacoepigenetic approaches aiming to act on the factors responsible for the insertion/development of diseases. Interference can occur by preventing the inducers of the responsible alterations in each one of them or by restoring the original function of each element involved. In the first situation, we would be talking about preventive treatment, that is, preventing the development of asthma in the body. In the second, we would be talking about healing.

Current and future therapeutic approaches

The pharmacological groups used in the treatment of asthmatic patients aim to reduce inflammation (glucocorticosteroids – GCS), reverse peribronchial smooth muscle contraction (bronchodilators – BD), antagonize key cytokines in pathogenesis, inflammatory mediators or block IgE. All of them act on the effects resulting from the interrelated actions of the trio genetics-microbiome-external factors. All drug approaches aim to antagonize definitively established abnormal cellular behaviors. For this reason, none of them allows talking about healing, only about control. They are, in fact, palliative treatments. Bronchodilators relax peribronchial smooth muscle, relieving dyspnea. Corticosteroids reduce inflammation partially, as they do not act on all inflammatory processes present. Biologicals antagonize/block specific cytokines.

The first biologic used in asthma was Omalizumab, which prevents the binding of IgE to its receptor on mast cells, basophils and dendritic cells, preventing the subsequent release of inflammatory mediators by these cells. Subsequently, other biologics – Mepolizumab, Reslizumab, Benralizumab and Dupilumab – were included in the therapeutic arsenal against asthma. The first two bind to the IL-5 ligand preventing its binding to its receptor. Benralizumab also binds to the IL-5 receptor, causing apoptosis of eosinophils and basophils. Finally, Dupilumab binds to the IL-4 α-receptor, blocking IL-4 and IL-13 signaling.[28]

Biologics are being indicated for severe asthmatics who need to take three or more courses of oral corticosteroids per year despite adequate adherence to prescribed treatment. As they have not yet been compared drug to drug, it is not possible to affirm the superiority of any of them.[29-31] In general, all reduce the exacerbation rate by around 50% and the effects are greater when the absolute number of eosinophils is higher. As the predominant biological role of IL-5 is linked to maturation, survival and recruitment of eosinophils to the airways, better effects of anti IL-5 are to be expected when symptoms/dysfunctions are driven by intraluminal eosinophils. However, as IL-4 and IL-13 (acting on a single IL-4R receptor) have a broader action, acting on eosinophil recruitment, goblet cell hyperplasia/mucus secretion, smooth muscle contraction and HRB, beneficial effects would be expected in a larger population of asthmatics, and not only in those with airway eosinophilia.[32]

Investigations seeking to identify new biologicals continue. Further on, others that target IL-25 and 33, TSLP and an alarmins will be included in the therapeutic arsenal.[28] Certainly, the development of predictive or monitoring biomarkers will help to select the most appropriate biologic for each patient. In any case, their therapeutic value is partial and they are adjuvant drugs for the control of asthma.

Conclusion

In medical ethics, the principle of non-maleficence has always been related to the maxim *Primum non nocere*, which can be interpreted "above all (or above all) not to cause harm". According to some authors, despite being essentially associated with the thought of Hippocrates expressed around the year 430 BC - "above all, do not cause harm" - the phrase does not appear in any Hippocratic text. What is established in paragraph 12 of the first book of his work, *Epidemic*, is that the doctor "practices two things in dealing with diseases; help and do not harm the patient".[33] To

imagine curing asthma, we have to change levels. We will have to move to a quantitative rather than qualitative, mechanistic rather than organic, indefinite rather than finite, challenging scenario.

Based on current knowledge about its pathogenic mechanisms, we can assume that the effective approach to asthma should include agents that modulate the genome and correct the microbial dysbiosis involved. If so, we would be talking about an "epigenetic and microbiomic" therapeutic approach that will include agents acting on DNA methylation, histone modifications, miRNA and dysbiosis in the microbiome.[34] In this case, in which the therapeutic approach will basically act on the human genome, the ethical principles mentioned above cannot be forgotten.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Analysis of the quality of life of patients with chronic urticaria in Aracaju - Sergipe

Avaliação da qualidade de vida de pacientes com urticária crônica em Aracaju - Sergipe

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ABSTRACT

Introduction: Chronic urticaria is a disease with a prevalence in at least 0.1% of the population, defined by the presence of pruritic papules, angioedema or both for a period longer than six weeks. Patients with chronic urticaria have a severe loss in quality of life. **Objective:** To assess the impact of chronic urticaria on the quality of life of patients with the disease within a specialized service in the state of Sergipe. **Methods:** This is a descriptive observational study based on data collected from 40 patients treated, in 2021, at the Allergy and Immunology Service of the Allergy and Immunology Outpatient Clinic of Decós Day Hospital, using two specific questionnaires for quality assessment of life in chronic urticaria: the Chronic Urticaria Quality of Life Questionnaire and the Urticaria Control Test. **Results:** It was possible to identify a positive correlation, through the Urticaria Control Test questionnaire, between the intensity of symptoms and the worsening of quality of life ($r = 0.774$, $p < 0.001$). It was also possible to identify a positive correlation between the intensity of symptoms and worsening quality of life, this time measured by the Chronic Urticaria Quality of Life Questionnaire scale ($r = 0.768$, $p < 0.001$). 90% said they felt tired during the day because they didnt sleep well, 87.5% found it difficult to concentrate, 90% felt nervous, 80% said they felt down, 75% said they were ashamed of the urticaria lesions that appear on the body and 60% are ashamed to go to public places. **Conclusions:** Chronic urticaria compromises quality of life, as measured by the Urticaria Control Test and the Chronic Urticaria Quality of Life Questionnaire. The impairment of the quality of life of patients with chronic urticaria occurs mainly in the psychological aspects, in social relationships and in the quality of sleep.

Keywords: Chronic urticaria, quality of life, surveys and questionnaires.

RESUMO

Introdução: A urticária crônica é uma doença com prevalência em pelo menos 0,1% da população, definida pela presença de pápulas pruriginosas, angioedema ou ambos por período superior a seis semanas. Os pacientes com urticária crônica têm um severo prejuízo na qualidade de vida. **Objetivo:** Avaliar o impacto da urticária crônica na qualidade de vida dos portadores da doença dentro de um serviço especializado no estado de Sergipe. **Métodos:** Trata-se de um estudo descritivo observacional a partir de dados coletados de 40 pacientes atendidos, em 2021, no Serviço de Alergia e Imunologia do Ambulatório de Alergia e Imunologia do Decós Day Hospital, através de dois questionários específicos para a avaliação da qualidade de vida na urticária crônica: o *Chronic Urticaria Quality of Life Questionnaire* e o *Urticaria Control Test*. **Resultados:** Foi possível identificar uma correlação positiva, através do questionário *Urticaria Control Test*, entre a intensidade dos sintomas e a piora da qualidade de vida ($r = 0,774$; $p < 0,001$). Também foi possível identificar uma correlação positiva entre a intensidade dos sintomas e a piora da qualidade de vida, desta vez mensurada pela escala *Chronic Urticaria Quality of Life Questionnaire* ($r = 0,768$; $p < 0,001$). Noventa por cento dos pacientes afirmaram se sentir cansados durante o dia porque não dormiram bem, 87,5% sentem dificuldade para se concentrar, 90% sentem-se nervosos, 80% afirmaram sentirem-se para baixo, 75% disseram ter vergonha das lesões da urticária que aparecem no corpo, e 60% tem vergonha de frequentar lugares públicos. **Conclusões:** A urticária crônica compromete a qualidade de vida, medida pelos questionários *Urticaria Control Test* e *Chronic Urticaria Quality of Life Questionnaire*. O comprometimento da qualidade de vida dos doentes com urticária crônica ocorre principalmente nos aspectos psicológicos, nos relacionamentos sociais e na qualidade do sono.

Descritores: Urticária crônica, qualidade de vida, inquéritos e questionários.

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Introduction

Chronic urticaria (CU) is a disease with prevalence in at least 0.1% of the population,[1] defined by the presence of pruritic papules, angioedema or both for a period longer than six weeks. Of recurrent evolution, it can even last for years; there is a tendency to spontaneous remission. In spite of adequate investigation, the etiology is rarely found.[2] Wheals are papules and/or plaques that appear suddenly and are characterized by a light or reddish color, of varying sizes, almost always surrounded by a reflex erythema, associated with intense itching or, sometimes, a burning sensation. Chronic urticaria is a disease that affects not only the physical, but also the social and emotional aspects.[3] Chronic spontaneous urticaria, which is the focus of the present work, presents spontaneous symptoms that are not associated with any specific trigger.

Illness-related quality of life can be defined as the impact of illness and treatment on the physical, psychological, social and somatic domains of functioning and well-being.[4] Because it presents a benign evolution from a clinical point of view, without significant mortality, with fleeting lesions and temporary disfigurement, health professionals tend to underestimate the impact of chronic urticaria on patients' quality of life.[5] Despite the small mortality rates from dermatological diseases, the importance of aesthetic appearance means that changes without great clinical significance can negatively influence patients' daily activities.[3]

The impact of a disease on quality of life may not be directly related to its clinical severity, but to the stigmatization and discomfort caused, which reinforces the importance of quality of life studies in diseases with dermatological manifestations, such as chronic urticaria.[6,7] Therefore, in its follow-up, as in other chronic conditions, it is extremely important to use standardized tools that can provide objective information regarding the impact of the disease on the various aspects of the patient's quality of life, to improve the clinical approach of these cases. The instruments available to measure the quality of life of patients with chronic urticaria can help in the continued assessment of patients with this variable condition. Therefore, the use of Patient Reported Outcomes (PROs), or patient-reported outcomes, are critical in the assessment and monitoring of activity, control, and quality of life in chronic urticaria. Therefore, the Chronic Urticaria Quality of Life Questionnaire (CUQ2oL) and the Urticaria Control Test (UCT) were applied.[8]

The CUQ2oL (Figure 1) was created and validated in 2005 by Baiardini et al. The questionnaire has 23 items, which in the original Italian version are divided into six domains, and in the Portuguese version, into three: I - sleep/mental state/eating (questions 10, 11, 12, 13, 14, 15, 16 and 17); II - pruritus/impact on activities (questions 1, 2, 5, 6, 7, 8, 9 and 22); and III - edema/limitations/appearance (questions 3, 4, 18, 19, 20, 21 and 23). Patients responded, taking into account the last two weeks, indicating the intensity of each item separately on a five-point Likert scale, ranging from 1 to 5. The higher the score, the worse the patient's perception of their quality of life.[3]

The UCT (Figure 2) is a retrospective questionnaire that assesses urticaria control based on the patient's perception of the previous 4 weeks. It is easy to fill out, as it consists of only 4 questions, with 5 answer options. The minimum total score of the questionnaire is 0, which is calculated by the sum of the minimum value (0: quite/very often) given to each answer by the patient. This score indicates the worst quality of life. The maximum score is 16, which is reached when the patient assigns maximum scores to all questions (4: nothing/never). The higher the score, the better the patient's perception of their quality of life.

The aim of the present study is to evaluate the impact of spontaneous chronic urticaria on the quality of life of patients with the disease, including the identification of the main factors that negatively affect the quality of life of patients with CSU.

Methods

Participants

The work consists of a cross-sectional study. Patients treated at the Immunology and Allergology Outpatient Clinic of Décos Day Hospital, Aracaju, Brazil, were included.

The inclusion criteria were patients who had a conclusive diagnosis of chronic spontaneous urticaria performed through evaluation of the clinical history and physical examination, according to the *Guideline for the definition, classification, diagnosis and management of urticaria*[9] at any age and who allowed the completion of the questionnaire. The following exclusion criteria were used: patient not wanting to participate in the study, or cognitive impairment.

Procedures

Data on the quality of life of patients with chronic urticaria were collected online using an electronic form created on Google forms. Patients were approached through contact via the communication application (WhatsApp), with the telephone number obtained from the medical records. The analyzed variables obtained from the patient's medical record were sex, age group, triggering factor of the disease, marital status and previous medications to control the symptoms of chronic urticaria. Two previously validated questionnaires were compiled in the form, the Chronic Urticaria Quality of Life Questionnaire (CUQ2oL) and the Urticaria Control Test (UCT).

There was submission to the Research Ethics Committee (CEP) from Tiradentes University - SE, whichand was approved under opinion number 4,630,658.

Question: in the last 15 days

How bothered were you by the following symptoms?

1. Itch
2. Red plates
3. Swollen eyes
4. Swollen lips

Not at all (1) A little (2) More or less (3) Often (4) Very often (5)

How much did the urticaria get in the way of the following moments of your daily life?

5. Work
6. Physical activity
7. Sleep
8. Leisure
9. Social relationships
10. Food

Not at all (1) A little (2) More or less (3) Often (4) Very often (5)

Difficulty and problems that may be linked to urticaria

11. Do you have trouble sleeping?
12. Do you wake up during the night?
13. During the day, do you feel tired because you don't sleep well at night?
14. Do you have trouble concentrating?
15. Do you feel nervous?
16. Do you feel “down”?
17. Do you feel limited in your choice of food?
18. Are you ashamed of the urticaria patches that appear on your body?
19. Are you embarrassed to go to public places?
20. Is it a problem for you to use certain cosmetics (perfumes, creams, lotions, soaps and makeup)?
21. Do you feel limited in choosing your clothes?
22. Are your sports activities limited because of urticaria?
23. You stay bothered by the side effects caused by the medicines used to treat urticaria?

Not at all (1) A little (2) More or less (3) Often (4) Very often (5)

Figure 1
Chronic Urticaria Quality of Life Questionnaire (CUQ2oL)

How much have you suffered from the physical symptoms of hives (itching, blistering and/or swelling) in the last 4 weeks?

Very often ( ) Often ( ) More or less ( ) A little ( ) Not at all ( )

How much your quality of life was negatively affected because of urticaria in the last 4 weeks?

Very often ( ) Often ( ) More or less ( ) A little ( ) Not at all ( )

How often your treatment for hives was not enough to control the symptoms of in the last 4 weeks?

Very often ( ) Often ( ) More or less ( ) A little ( ) Not at all ( )

Overall, how much were you able to get your hives under control in the last 4 weeks?

Very often ( ) Often ( ) More or less ( ) A little ( ) Not at all ( )

Figure 2
Questionnaire to assess urticaria control (UCT)

Instruments

The Chronic Urticaria Quality of Life Questionnaire (CUQ2oL) and the Urticaria Control test (UCT) were applied.

To obtain an indicator of the health of the participant, an indicator was developed, obtained with the computation of three questions related to the general health conditions of the patients, in an index that oscillated between the value 0 and 1, with value 1 indicating excellent health habits. The questions were about food, hobbies and regular practice of physical exercise. In addition, questions about age, sex, marital status, use of medication to control symptoms of chronic urticaria, stress factor in triggering chronic urticaria and stress were also added (Table 1).

Analysis direction

Data were saved in Microsoft Excel® program version 2016, and subsequently submitted to descriptive and inferential analysis using version 0.14.1 of the JASP statistical analysis software.

Table 1
Sociodemographic and personal information

What is your age?
What is your gender?
What is your marital status?
Do you use any medication to control the symptoms of urticaria?
Did any stressors occur in your life when your urticaria symptoms first appeared?
Do you consider yourself a stressed person?
Do you have any hobbies?
Do you exercise regularly?
Do you think you have a healthy diet?

Results

The data, collected between April 14 and May 23, 2021, refer to a total of 40 participants. Of the total number of participants, 35, which corresponds to 87.5%, declared themselves to be women. In relation to age, the range oscillated between the values of 16 and 59 years, with a mean of 33.8 years and a standard deviation of 10.8 years. Regarding medication use, 82% reported using antihistamines to control symptoms. Regarding the presence of triggering factors for the onset of chronic urticaria, 60% stated that some stressful event had occurred just before the onset of the disease, and 67.5% of the participants reported that they considered themselves stressed. Regarding the relationship level, of the total number of participants, 16 said they were single, 8 declared they were in a dating relationship, and 16 said they were married.

Initially, the analysis of the results obtained on the correlation between the level of control of chronic urticaria and its effects on the quality of life of patients was performed, through the CUQ2oL and UCT questionnaires.

The first domain of the CUQ2oL questionnaire is related to the patients' quality of life. The mean quality of life obtained in the CUQ2oL questionnaire was 2.8, and the standard deviation was 0.8; This data indicates an intermediate quality of life among the participants, since the scale score oscillates between 1 and 5. To control the effect of traumatic events on quality of life, a *t* test was conducted for the difference between the samples, the which indicated a significant difference ($t(38) = 2.214$; $p < 0.05$). A statistically significant difference was also found for stress ($t(38) = 2.214$; $p < 0.5$). To assess the influence of age and general health status of participants on quality of life, we conducted two correlation analyses, one regarding age, and a significant correlation was found ($r = 0.373$; $p < 0.05$), and another about health, with no significant correlation ($r = 0.189$; $p = 0.242$). The same *t* test was used to identify whether a difference was found between male and female participants, which showed no statistically significant differences ($t(38) = 1.479$; $p = 0.147$). There was also no difference regarding the use of medication ($t(38) = 1.638$; $p = 0.110$). There was also no difference in relation to marital status ($F(37) = 0.007$; $p = 0.993$).

In general, we can identify that the level of quality of life of the patients was reasonable, and this result suffered changes in variables such as traumatic events, stress and age. It was observed that traumatic events, stress and advancing age are factors that contribute to the impairment of the quality of life of patients with chronic urticaria.

The mean of symptoms obtained in the second and third domains of the CUQ2oL questionnaire was 2.4, and the standard deviation was 1.0; This data indicates a low incidence of symptoms among the participants, since the scale score oscillates between 1 and 5. To assess the influence of age and general health status of participants on the incidence of symptoms, we conducted two correlation analyses; in relation to age, a positive correlation was identified between age and the incidence of symptoms, indicating that the older the person, the greater the severity of symptoms ($r = 0.388$; $p < 0.05$). In the case of the association between health status and symptoms, there was no statistically significant correlation between the variables ($r = 0.153$; $p = 0.346$). To identify whether a difference was found between male and female participants, a *t* test was conducted for the difference between samples, which showed the absence of statistically significant differences ($t(38) = 0.302$; $p = 0.765$). The same *t* test was used to control for the effect of traumatic events on the initial expression of symptoms, and no difference was found ($t(38) = 1.776$; $p = 0.084$). Regarding the use of medication, there was also no difference ($t(38) = 0.811$; $p = 0.422$). There was also no difference in relation to marital status ($F(37) = 0.998$; $p = 0.378$) and stress ($t(38) = 0.123$; $p = 0.903$).

To calculate the instrument's degree of internal consistency, Cronbach's alpha was calculated, which for the first domain presented a value of 0.90, a good indicator of the quality of the measurement, and for the second and third domains it offered an alpha of 0.90. Cronbach who presented the value of 0.882.

The mean of symptoms obtained in the UCT questionnaire was 3.0, and the standard deviation was 1.0; This data indicates an intermediate incidence of symptoms among the participants, since the scale score oscillates between 1 and 5. To identify whether a difference was found between male and female participants, a *t* test was carried out for the difference between the samples, which showed no statistically significant differences ($t(38) = 0.514$; $p = 0.610$). The same *t* test was used to control for the effect of traumatic events on the expression of symptoms, and no difference was found ($t(38) = 1.563$; $p = 0.126$). Regarding the use of medication, there was also

no difference (t(38) = 1.795; p = 0.081). There was also no difference in relation to marital status (F(37) = 1.223; p = 0.369) and stress (t(38) = 0.508; p = 0.614). To assess the influence of age and health status of participants on the incidence of symptoms, we conducted two correlation analyses, both of which did not demonstrate a statistically significant correlation: age (r = 0.194; p = 0.231) and health (r = 0.163; p = 0.314).

To calculate the degree of internal consistency of the instrument, Cronbach's alpha was calculated, which supplied the value of 0.856.

In general, we can identify that the level of symptoms of the patients was from low to intermediate in both questionnaires used, and these results changed in variables such as age, but did not change in variables such as gender, traumatic events, pain control drugs urticaria symptoms, marital status, stress and health status.

Having presented the descriptive analyses, we will now test our hypothesis that there is an inversely proportional relationship between the level of chronic urticaria activity and the patients' quality of life. To this end, we ran two bivariate correlations, one between the two dimensions of the CUQ2oL, relating to symptoms and quality of life, and a second, between the measure of the CUQ2oL dimension relating to symptoms and the UCT, a second indicator of symptom intensity.

As observed in the scatter diagram presented in Figure 3, it was possible to identify a positive correlation between the intensity of symptoms and the worsening of quality of life (r = 0.774; p < 0.001), a result that proved to be independent of the effects of variables such as gender, age, traumatic events, medications to control urticaria symptoms, marital status, stress and health status of the participants.

As can be seen in the scatter diagram presented in Figure 4, it was also possible to identify a positive correlation between the intensity of symptoms and the worsening of quality of life, this time measured by the CUQ2oL scale (r = 0.768; p < 0.001), a result which was independent of variables such as gender, age, traumatic events, medications to control urticaria symptoms, marital status, stress and health status of the participants. The impairment of the quality of life of patients with chronic urticaria occurred mainly in the psychological aspects, in social relationships and in the quality of sleep. 77.5% said they had some difficulty sleeping, 92.5% said they woke up at night, 90% said they felt tired during the day because they didn't sleep well, 87.5% found it difficult to concentrate,

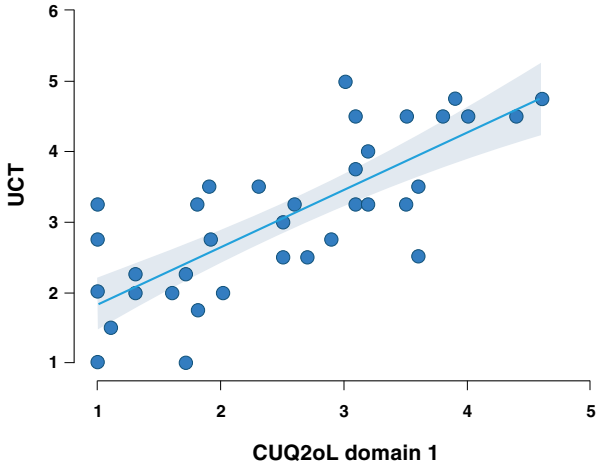

Figure 3
Positive correlation between the intensity of symptoms and worsening of quality of life

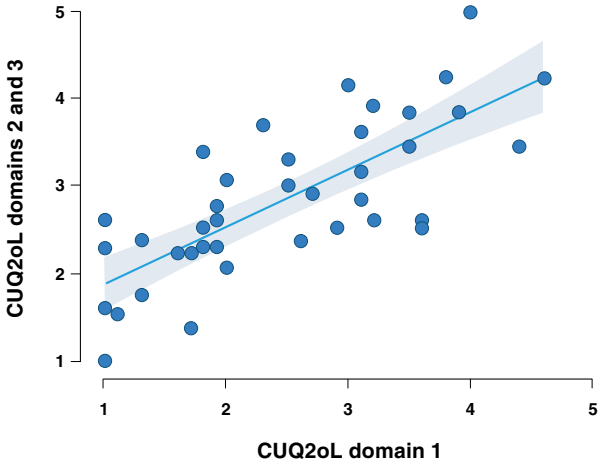

Figure 4
Positive correlation between the intensity of symptoms and the worsening of quality of life measured by the CUQ2oL scale

Therefore, both analyzes indicated a strong positive correlation between disease activity and worsening of quality of life, which corroborates our working hypothesis.

Discussion

Urticaria is called chronic (UC) when it has daily or almost daily symptoms for a period longer than six weeks. As a result, patients suffer not only from the impacts of erythematous lesions, intense itching and painful swelling, but also from the insecurity that these symptoms can manifest at any time and in any place. In this chronic form, it often compromises the patient by interfering with their daily activities, with loss of self-esteem and interpersonal relationships.[10,11] Chronic spontaneous urticaria is also frequently associated with absences from school and work, which also has an economic impact on patients' lives.[12]

Urticaria is not emotional, it is not psychological and it is not caused by stress, although these factors can exacerbate the symptoms.[13,14]

Those who have chronic urticaria, whether due to itching or the appearance of the lesions, end up having the insecurity of not knowing when they will have an attack or not. This has an impact on various aspects of social and affective life. All this together, in some people, probably in those more predisposed, canlead to depression, because of the impact it has on quality of life as a whole.[15] The present study corroborated the findings above, as it showed a statistically significant association between stress and quality of life in patients with chronic urticaria ($t(38) = 2.214$; $p < 0.5$).

Although urticaria is common at any age, we have observed that acute urticaria (UA) is more frequent in children and young adults, while UC usually occurs in middle age.[16,17] The present study corroborated the previous data by demonstrating that in relation to age, a positive correlation was identified between age and the incidence of symptoms, indicating that the older the person, the greater the severity of symptoms ($r = 0.388$; $p < 0.05$).

Comfort and a sense of well-being, the ability to maintain reasonable physical, emotional and intellectual function and the degree of retention of the ability to participate in activities with family members, co-workers and the community are some of the attributes valued by patients.[18] In chronic urticaria, chronic pruritus with the presence of urticaria and/or angioedema, and other factors such as the cost of therapy and social isolation, contribute to the frustration these patients experience.[19] Thus, it can be said that the social, psychological, environmental and physical impact of the urticaria lesion on the affected individual, and even on his/her group, is significant. The patient has significant emotional instability, due to the public nature of the symptoms. In its severe forms,consequent profound negative impact on quality of life.[5,13] The present study corroborated the previous data by demonstrating a positive correlation between the intensity of symptoms and the worsening of quality of life ($r = 0.774$; $p < 0.001$).

Chronic urticaria is significantly more common in women than in men.[20] The real incidence of CSU is unknown, but a variation of 0.1% to 3% in the general population is estimated, being more common in women, in a proportion of two women for every man.[21] According to the Brazilian Association of Allergy and Immunology (2018), 60% of all chronic urticaria are spontaneous, affecting mainly middle-aged women. The present study did not find a statistically significant association between sex and chronic urticaria, possibly because more women seek health services than men.

The treatment of CSU aims to control the symptoms and improve the patient's quality of life. A dual approach has been suggested: the first represents the attempt to identify and eliminate possible triggering factors, and the second represents the pharmacological treatment. Controlling CSU is not easy due to the difficulty in identifying the etiology of the disease and the poor therapeutic response in most patients.[13] The present study did not find a statistically significant association between the use of medication to control symptoms and the worsening of the quality of life of patients with chronic urticaria, possibly due to the fact that only 6 patients in the study were not using medication for the disease.

It is concluded that chronic urticaria compromises the quality of life measured by the UCT and CUQ2oL questionnaires. There was a statistically significant association between the activity of chronic urticaria and the worsening of quality of life, affected by the variables age, stress and traumatic events. However, no statistical significance was found in relation to sex, drugs to control urticaria symptoms, marital status and health status. The impairment of the quality of life of patients with chronic urticaria occurred mainly in the psychological aspects, in social relationships and in the quality of sleep.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Evaluation of philagrin expression in esophageal biopsies of patients with eosinophilic esophagitis

Avaliação da expressão da filagrina em biópsias esofágicas de pacientes com esofagite eosinofílica

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ABSTRACT

Introduction: Filaggrin gene mutations have been classically associated with changes in the epithelial barrier in allergic diseases involving the skin and mucosal surfaces. Particularly in atopic dermatitis, the relationship between filaggrin, pathophysiological mechanism and clinical evolution hás been demonstrated. Recently, changes in the epithelial barrier with reduced expression of filaggrin have also been associated with immunological mechanisms involved in the pathogenesis of eosinophilic esophagitis. Due to dysfunction in the epithelial barrier, microorganisms and allergens are able to penetrate the epithelium of the esophageal mucosa, as well as in atopic dermatitis. **Objective:** To evaluated the possible correlation of filaggrin expression with histopathological findings in esophageal biopsies of patients with eosinophilic esophagitis. **Methods:** Filaggrin expression was investigated in situ by immunohistochemistry in esophageal biopsies in the following groups: Group I, control (n = 8), samples from healthy patients; Group II (n = 27), samples from patients with eosinophilic esophagitis. **Results:** The results demonstrated a decrease in the expression of filaggrin in the esophageal mucosa of patients with eosinophilic esophagitis. Additionally, the intensity of the immunohistochemical labeling was lower in the esophageal mucosa with greater infiltration of eosinophils. **Conclusion:** The reduction of filaggrin expression may be a pathophysiological phenomenon associated with an increase in the quantity of eosinophils in the esophageal mucosa, which may impact on the clinical evolution of eosinophilic esophagitis.

Keywords: Eosinophilic esophagitis, immunohistochemistry, esophageal mucosa, atopic dermatitis.

RESUMO

Introdução: Mutações do gene da filagrina vêm sendo associadas, classicamente, a alterações da barreira epitelial em doenças alérgicas com comprometimento da pele e das superfícies mucosas. Particularmente na dermatite atópica, a relação entre filagrina, mecanismo fisiopatológico e evolução clínica tem sido demonstrada. Recentemente, alterações da barreira epitelial com redução da expressão da filagrina, também têm sido associadas a mecanismos imunológicos envolvidos na patogênese da esofagite eosinofílica. Devido a disfunções na barreira epitelial, microrganismos e alérgenos são capazes de penetrarem no epitélio da mucosa esofágica, assim como na dermatite atópica. **Objetivo:** Avaliar a possível correlação da expressão da filagrina com os achados histopatológicos em biópsias esofágicas de pacientes com esofagite eosinofílica. **Métodos:** A expressão da filagrina foi investigada *in situ*, por imuno-histoquímica, em biópsias esofágicas nos seguintes grupos: Grupo I, controle (n=8), amostras provenientes de pacientes saudáveis; Grupo II (n=27), amostras provenientes de pacientes com esofagite eosinofílica. **Resultados:** Os resultados demonstraram uma diminuição da expressão da filagrina na mucosa do esôfago de portadores de esofagite eosinofílica. Adicionalmente, a intensidade da marcação imuno-histoquímica foi menor na mucosa esofágica com maior infiltração de eosinófilos. **Conclusão:** A diminuição da expressão de filagrina pode ser um fenomeno fisiopatológico associado ao aumento da quantidade de eosinófilos na mucosa esofágica, podendo impactar na evolução clínica da esofagite eosinofílica.

Descritores: Esofagite eosinofílica, imuno-histoquímica, mucosa esofágica, dermatite atópica.

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Introduction

Eosinophilic esophagitis (EoE) has been studied since the late 1970s and was best described in 1993 by Attwood et al. It is a chronic inflammatory, immune-antigen-mediated disease characterized by eosinophilic inflammation located in the esophagus, without involvement of other parts of the gastrointestinal tract (GIT), associated with symptoms of esophageal dysfunction, such as dysphagia, nausea, vomiting and impaction feed.[1,2] EoE is more frequent in patients with a personal and/or family history of atopy, such as food allergy, asthma and allergic rhinitis. Regarding prevalence, epidemiological studies indicate that EoE is as prevalent as idiopathic inflammatory bowel diseases, affecting 40 to 55 individuals/100,000 inhabitants.[3] However, as EoE was only recognized and characterized as an isolated entity in recent years, it is likely that many patients have already been wrongly diagnosed as having gastroesophageal reflux disease (GERD).[2-4]

The histopathological diagnosis obtained from esophageal biopsies material is defined by the presence of 15 or more eosinophils (Eo) per higher power field (400X). For diagnostic definition, the analysis of 2 to 4 samples from two different segments of the esophagus is recommended, regardless of the normality in the upper digestive endoscopy (UGE).[2,4] The diagnostic sensitivity of the histopathological exam can reach 100%, when at least five samples of esophageal biopsies are collected.[3] In peripheral blood circulation, eosinophilia is found, on average, in 50% of patients with EoE5.

Filagrin gene (FLG) mutations have been associated with changes in the epithelial barrier in allergic diseases that affect skin and mucosal surfaces, such as atopic dermatitis and asthma.[6-11] The dysfunction of the epithelial barrier represented mainly by the reduction of FLG expression interacts closely with the immunological mechanisms involved in the pathophysiology of these atopic diseases. Likewise, in EoE, dysfunction of the epithelial barrier in the compromised esophageal mucosa also appears to be a key factor in the pathophysiology. More recently, the abnormal expression of filaggrin has been suggested as a possible pathway of action in the pathophysiological mechanism of EoE.[12-14]

Due to an altered epithelial barrier, microorganisms and allergens are able to penetrate the esophageal mucosal epithelium. These antigens are recognized by epithelial cells through pattern recognition receptors (PRRs), which then release cytokines, such as thymic stromal lymphopoietin (TSLP), initiating a Th2 immune response. As a result of the Th2 response, eosinophils are recruited from the circulation, mainly by the local esophageal production of IL-5 and eotaxin.[9-13]

In the present study, the in situ expression of FLG was investigated in esophageal biopsies from patients with EoE. The results suggested that the expression of FLG in the esophageal mucosal epithelium is inversely proportional to the amount of eosinophils observed in the esophageal mucosa.

Material and methods

Patients

The initial study sample comprised 50 adult patients (N = 50), of both sexes, aged over 18 years, with clinical suspicion of eosinophilic esophagitis. The definitive diagnosis was made based on clinical and endoscopic features suggestive of EoE, associated with the presence of at least 15 eosinophils (EO) per microscopic field of higher magnification (400X) in the esophageal mucosa; the diagnosis wasconfirmed by histopathological analysis in 27 patients (n = 27), with at least six biopsies performed in different regions of the esophagus. Esophageal biopsies were performed for diagnostic purposes before starting the treatment of patients.

In order to control the study, esophageal samples from healthy patients were analyzed, composing the control group (Group I, n = 8). Esophageal biopsy samples from patients with EoE comprised Group II (n = 27). All samples from groups I and II were submitted to histopathological and immunohistochemical analysis.

This study was conducted in accordance with the World Medical Association Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects, and approved by the Human Research Ethics Committee in accordance with the National Commission on Research Ethics – CONEP (Opinion 2.314.988 / CAAE 28260814.9.0000.5103 SUPREMA - University Society for Medical Education).

Histopathological processing

Esophageal biopsies were obtained from proximal, medial and distal portions in all patients included in the study. The samples were fixed in 10% formalin, embedded in a paraffin block and submitted to

microtomy with histological sections 5 to 6 µm thick. Histological sections were stained with hematoxylin and eosin for routine examination. The samples were examined with a Zeiss optical microscope (Hallbergmoos, Germany) by two independent observers trained in histopathology. After observation, representative areas of the esophageal mucosa were selected for descriptive histopathological analysis in a microscopic field of higher magnification (400x magnification), and three representative areas were chosen for digital photographic capture.

Detection in situ of FLG by immunohistochemistry

The immunohistochemical method for detecting FLG expression involved the following steps: deparaffinization for 20 min (60 °C) and imbibition in 3 baths of xylene for 3 min each; hydration in 100%, 95% and 70% alcohol for 3 min each; rinse in distilled water; blockade of endogenous peroxidase (H_2O_2 – 0.4% for 30 min /100 µL per cut); antigen recovery in a water bath at 95 °C for 40 min, in PBS; cool for 20 min at room temperature and rinse with PBS for 1 min; addition of 4 drops of BackgroundSniper on the section and incubation for 15 min (room temperature); rinse in PBS buffer (1 min). The prepared sections were incubated with Anti-Filaggrin primary antibody (Santa Cruz, Inc.) (1:100 µL Dilution) for 1 h. Afterwards, double rinse in PBS (2 min); dripping the Link Universal Trekkie secondary antibody onto the section and incubating for 20 min (room temperature); double rinse in PBS (2 min); incubation in a humid chamber for 10 min with TrekAvidin-HRP(Label): streptavidin (room temperature); double rinse in PBS (2 min each); incubation with chromogen Betazoid DAB Chromogen (DAB) homogenized in 1 ml PBS (5 min); double rinse in distilled water and in PBS; counterstaining with hematoxylin (1 min); double rinse in distilled water and then in PBS (1 min each); dehydration in three baths of 100% alcohol (1 min each) and followed by three baths in xylene (1 min each); blade assembly. The negative control was performed by omitting the primary antibody in a selected section. Positivity was determined by observing, under light microscopy, intracytoplasmic brown staining.

Positive immunohistochemical staining for FLG in the esophageal mucosal epithelium was evaluated according to immunoreactivity and intensity, and classified according to the scoring criteria.[14] The results were expressed as the mean scoreper study group. The criteria defining expression intensity and immunoreactivity are specified in Table 1. All analyzes were performed by two different examiners.

Statistical analysis

To compare the variables of the two groups, the Student's *t* test was used. SPSS 22 software was used. The significance level was considered for $p < 0.05$.

Results

Immunohistochemical analysis revealed a reduction ($p < 0.05$) in the number of cells positively labeled for

Table 1
Immunohistochemical reaction evaluation criteria for filaggrin[14]

Expression intensity	
0	No positive marking
1	Mild
2	Moderate
3	Intense
Immunoreactivity	
0	Absence of positivity
1	Positivity in more than 10% of cells (microscopic field 400x)
2	Positivity in 10 to 50% of cells (microscopic field 400x)
3	Positivity in more than 50% of the cells (microscopic field 400x)

filaggrin when comparing samples from the control group (score 3) and samples from patients with EoE (score 1). Table 2 demonstrates the immunoreactivity in the control group and in the EoE group.

Regarding the intensity of immunohistochemical staining, samples from group I (control) were strongly stained (score 3). A decrease in labeling intensity was observed in samples from group II (EoE). However, the results showed that the higher the number of eosinophils per microscopic field (400x magnification), the lower the intensity of immunohistochemical staining for filaggrin.

Thus, for comparative purposes, we divided the esophageal samples from patients with EoE into 2 subgroups with the cut-off point of 25 eosinophils per microscopic field because there is a correlation between this amount of eosinophils and a significant decrease in the intensity of filaggrin tissue labeling ($p < 0.05$).

Figure 1 presents the results of the marking intensity score respectively in each group.

Discussion

FLG is a structural protein of the skin and the loss of its function is associated with skin permeability and susceptibility to the development of atopic dermatitis and, in patients with EoE, it influences the permeability of the esophageal epithelial barrier. An important aspect to be considered is that interleukin 13 (IL-13) counter-regulates the expression of FLG in epithelial cells, promoting a mechanism by which food antigens activate the adaptive immunity of Th2 profile, which can alter the epithelial barrier function of the epithelial cells. esophageal mucosa, perhaps propagating the local inflammatory process and increased antigen uptake by epithelial and antigen-presenting cells. In this way, FLG influences the immunological tolerance mechanism that maintains the esophageal barrier intact, preventing the passage of protein particles, that could cause an allergic sensitization process. As in atopic dermatitis, mutations in the FLG gene promote a change in tissue hydration and consequent disruption of the epithelial barrier, facilitating sensitization by food and/or aeroallergens.[12-16]

Histologically, EOE is characterized by a predominantly chronic inflammatory infiltrate, which includes eosinophils, mast cells, basophils and Th2 cells. Similar to other atopic diseases, EOE is triggered by allergenic foods and aeroallergens, culminating in esophageal fibrosis or tissue remodeling. Like the skin in patients with atopic dermatitis, the mucosal epithelium of the esophagus has an altered barrier function in patients with OAE.[12-16] In the present study, using immunohistochemistry, we demonstrated a marked reduction in FLG expression in the esophageal mucosal epithelium of patients with EoE.

Table 2
Immunohistochemical staining evaluation score: immunoreactivity and intensity

Immunoreactivity		
A (Group 1 - control)	3	Positivity in more than 50% of the cells (microscopic field 400x)
B (Group II - EoE)	1	Positivity in more than 10% of the cells (microscopic field 400x)
Expression intensity		
A (Control group)	3	Intense
B (Group II - EoE) Subgroups > 25/E the field	1	Weak
B (Group II - EoE) Subgroups > 25/E the field	2	Moderate

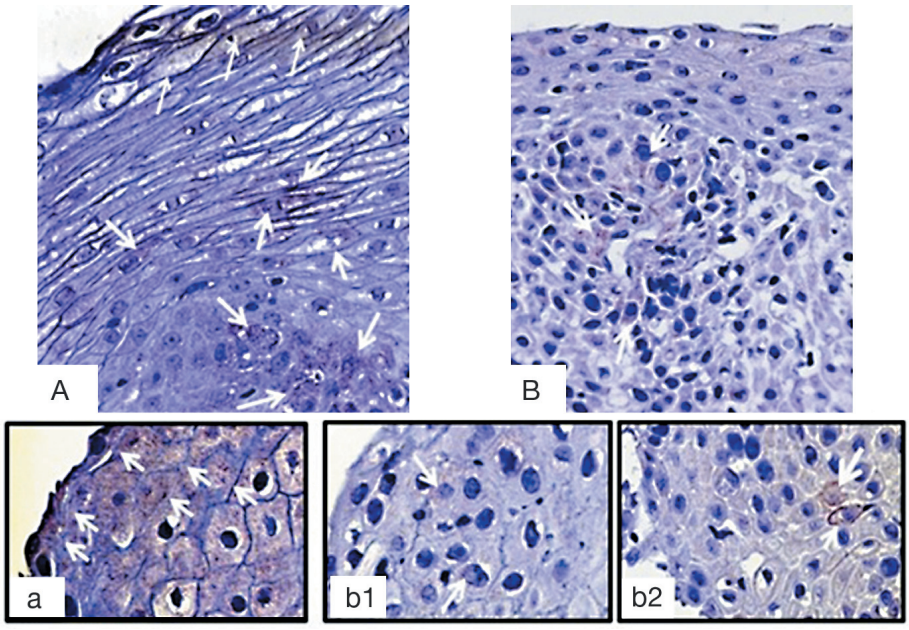

Figure 1
Immunohistochemical evaluation of filaggrin expression in esophageal samples. (**A**) presence of numerous positive cells (arrows), stained with anti-filaggrin antibody, in a sample from the control group. (**B**) Presence of some cells (arrows) with positive staining for filaggrin in a sample of Group EoE. (**b1**) Lower staining intensity for filaggrin in a sample with a number ≤ 25 eosinophils/400x field. (**b2**) Lower intensity of staining for filaggrin in a sample with a number ≥ 25 eosinophils/field 400x

The genetic predisposition to the development of EoE is well documented. This genetic susceptibility is due to the single nucleotide polymorphism that encodes the eotaxin-3 gene and also to mutations in the FLG[14] gene. The eotaxin-3 gene is considered the main promoter of eosinophil recruitment in the esophagus, therefore, the increased expression of this gene is related to an EoE phenotype characterized by an intense eosinophil infiltrate.

Recently, in epithelial cell culture, increased IL-13 synthesis was associated with changes in the epithelial barrier via decreased filaggrin expression.[15] Previously, Politi et al. (2017) investigated, also using immunohistochemistry, the in situ expression of FLG and the periostin molecule (PET) in esophageal biopsies of pediatric patients diagnosed with EoE. The authors found a downregulation of FLG and upregulation of PET in the esophageal mucosa of children with eosinophilic esophagitis, compared to control biopsies obtained from healthy subjects. Our results were similar to those described above,[14] the second study that demonstrated in situ, in human biopsies, the association between EoE and decreased FLG expression, and the first in samples from adult patients.

When we analyzed the score of filaggrin immunoreactivity, we observed that tissue samples from patients in the EoE group had a significantly lower positive labeling score for FLG ($p < 0.05$) than that observed in samples from the control group. This finding strengthens the hypothesis that changes in the epithelial barrier in EoE are important in the pathophysiology of this disease, similar to what is observed, for example, in atopic dermatitis.

The results, regarding the intensity of labeling for filaggrin, allow us to hypothesize that the number of eosinophils may be influencing the severity of epithelial dysfunction via loss of filaggrin expression. However, further studies are needed to better elucidate the role of filaggrin in the pathogenesis of EoE and the

significance of the intensity of tissue eosinophilic infiltration in terms of the clinicopathological evolution of this disease.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Aquagenic urticaria: a case report and literature review

Urticária aquagênica: relato de caso e revisão de literatura

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ABSTRACT

Aquagenic urticaria is a rare form of chronic inducible urticaria (CIndU) triggered by a specific stimulus. Pathogenesis is not fully understood, but symptoms appear minutes after cutaneous exposure to water, regardless of temperature, and wheals have a folliculocentric pattern. The diagnosis of CIndU is confirmed by provocation testing using established protocols, and first-line treatment is second-generation antihistamines. In this article, we report a case of aquagenic urticaria and provide a brief review of the relevant literature.

Keywords: Chronic urticaria, pruritus, histamine H1 antagonists.

RESUMO

A urticária aquagênica é uma forma rara de urticária crônica induzida (UCInd) desencadeada por um estímulo específico. A patogênese não é totalmente compreendida, mas os sintomas se iniciam minutos após a exposição cutânea à água, independentemente de sua temperatura, e as urticas têm o padrão foliculocêntricas. O diagnóstico é confirmado através do teste de provocação, e o tratamento de primeira linha são os anti-histamínicos de segunda geração. Neste artigo, relatamos um caso de urticária aquagênica e fazemos uma breve revisão da literatura sobre o tema.

Descritores: Urticária crônica, prurido, antagonistas dos receptores histamínicos H1.

Introduction

Shelley and Rawnsley[1] first described aquagenic urticaria in 1964, with the hypothesis of focal histamine release by perifollicular mast cells. It is a rare disease, characterized by the formation of perifollicular wheals with 1 to 3 mm in size, appearing after contact with water, regardless of temperature. Lesions tend to be located preferentially on the trunk and upper limbs and can last from 10 to 60 minutes.[2-4]

Aquagenic urticaria is considered a form of induced chronic urticaria, and is triggered by a specific stimulus. The induced urticaria(UCInd) affect about 0.5% of the population and can often coexist with chronic spontaneous urticaria (CSU).[5]

The diagnosis is based on the clinical history and confirmed by provocation tests, and the treatment comprises the management of the occurrence of symptoms and complete control of the disease, for as long as necessary for spontaneous remission to occur.[5]

Case report

Female patient, 24 years old, medical student, presents wheels mainly in the trunk and back region after exposure to water since the age of 8. Symptoms start approximately 20-30 minutes after exposure to bathing or contact with sea or swimming pool water,

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with total regression of wheals within 30 minutes at the end of exposure (Figure 1). Refers to worsening of crises closer to the menstrual period, when it presents greater intensity and distribution of wheals and pruritus.

The diagnosis was confirmed by the provocation test, with direct exposure to gauze moistened with water at room temperature on the back for 20 minutes, the maximum time for the onset of symptoms reported by the patient. A few minutes after the start of the test, she presented hives and itching in the exposed skin area (Figure 2), with total remission of symptoms within 30 minutes after exposure.

Laboratory tests revealed elevated antithyroglobulin and antithyroperoxidase antibodies, however TSH and free T4 were within the normal range. Still, it showed positivity in the antinuclear factor, which was a homogeneous nuclear pattern with titers of 1/160. Further investigation was performed with the autologous serum test (Figure 3) and the frick test, and both were negative.

The patient, even after diagnostic confirmation, chose not to adhere to treatment with a second-generation antihistamine. As a result, she had progression of wheals in previously unaffected sites, worsening of pruritus and quality of life, so treatment with a second-generation antihistamine at the usual dose was again proposed. Since then, the patient has been using the second-generation antihistamine intermittently.

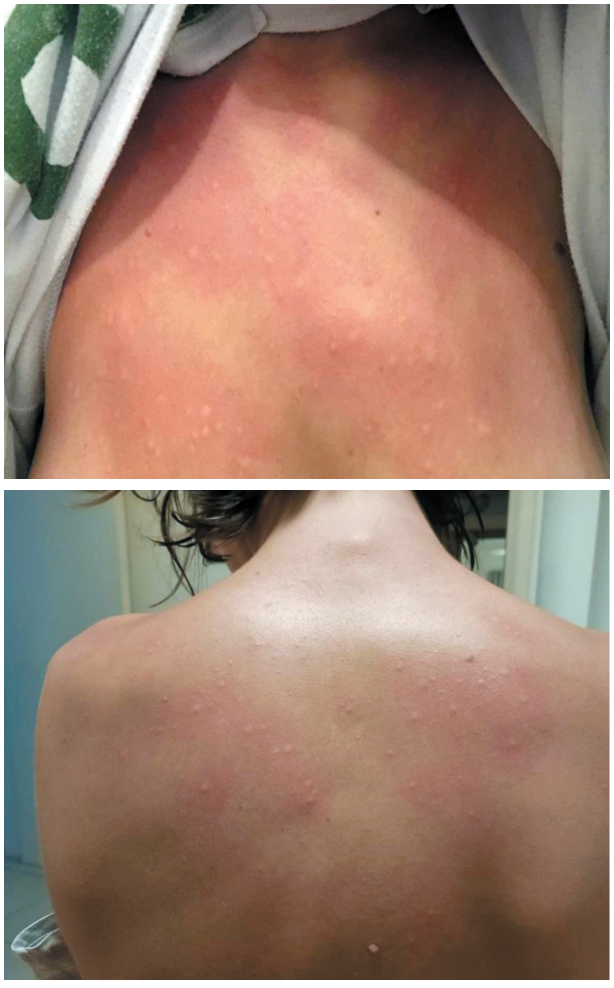

Figure 1
Hives after exposure to bath

Discussion

Urticaria is a condition in which hives, angioedema, or both occur. Its classification is based on the duration of symptoms, being considered chronic when it occurs for more than six consecutive weeks. Chronic urticaria can be subdivided into spontaneous chronic urticaria (CIU) or induced chronic urticaria (IndUC).The UCInd are those in which the wheals and/or angioedema arise after a specific stimulus, which can be physical (symptomatic dermographism, delayed pressure urticaria, heat or cold urticaria, vibrating urticaria, and solar urticaria) or non-physical (aquagenic urticaria, contact urticaria, and cholinergic).[6-7]

The estimated prevalence of NDICU is 0.5% in the general population.[7] Aquagenic urticaria is a rare type of UCInd, predominantly affects females and often has its onset during puberty or post-puberty.[8-10] However, reports of childhood onset and other cases of familial disease have been described.[9,11] There is also an association of aquagenic urticaria with other types of physical urticaria, such as cholinergic urticaria, cold urticaria, or symptomatic dermographism.[1,2] To date, only about 100 cases have been reported in the literature.[8,9]

For most individuals, aquagenic urticaria occurs without associated systemic repercussions, however some patients have reported symptoms such as headache, dizziness, difficulty breathing and palpitations, but these symptoms are rare.[12-14]

The exact pathology of UCInd has not yet been fully elucidated, but the activation of tissue-resident mast cells and the subsequent release of inflammatory

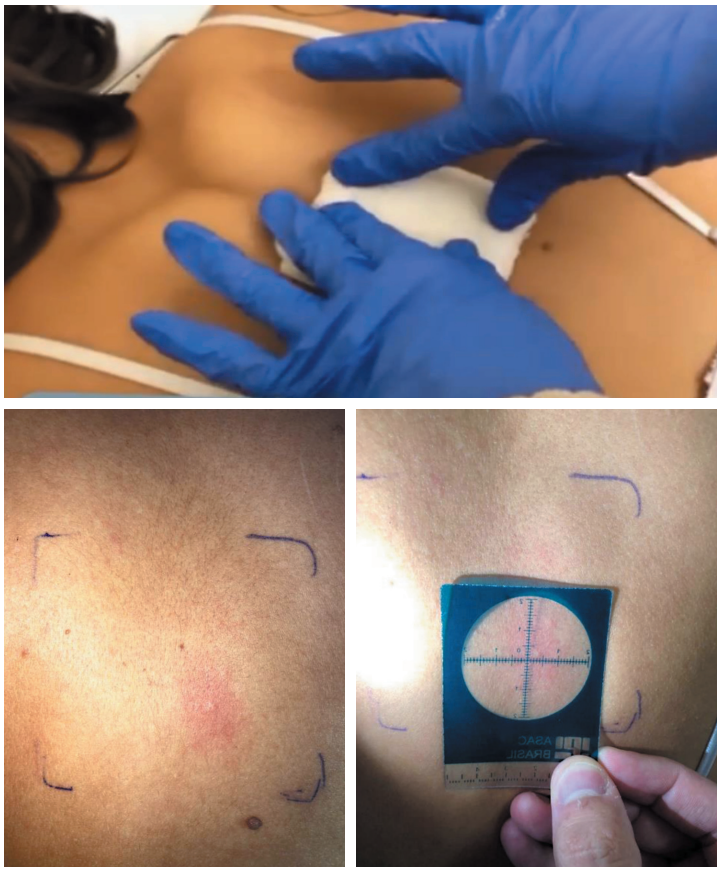

Figure 2
Provocation test with direct exposure to gauze moistened with water at room temperature on the patient's back

mediators such as histamine play important roles.[15] Shelley and Rawnsley,[1] in 1964, suggested that the interaction of water with sebum or sebaceous glands could originate a toxic substance that caused mast cell degranulation and, consequently, histamine release and wheal formation.[1,8] In 1981, Tkach[16] formulated the hypothesis that the mechanism of aquagenic urticaria (UA) would be associated with sudden changes in osmotic pressure around the hair follicles, leading to an increase in the passive diffusion of water. Finally, in 1998 Luong and Nguyen[17] suggested a mechanism that may be completely independent of histamine release; since patients with UA had their serum amine levels unchanged after exposure to water.[3,8,9,13,17]

More recently, Maurer et al.[15] proposed that type I autoimmunity or autoallergy would be a potential pathophysiological mechanism, and, in this situation, the production of neoautoantigens would activate skin mast cells through recognition by IgE molecules coupled to their high-affinity receptors. These neoautoantigens would be produced through a specific (induced) physical stimulus, such as friction, cold and others. However, the exact pathogenesis is not fully understood and appears to be mediated both in a histamine-dependent and independent manner.[9]

The diagnosis of AU is based on anamnesis and confirmed by a water challenge test.[14] The test can be administered in several ways; however, the standard method is a compress or towel soaked in water at room temperature (35-37 °C) or saline, and this should be placed on the patient's skin for 20 to 30 minutes, preferably choosing the upper part of the body, especially the back, as the lower extremities are

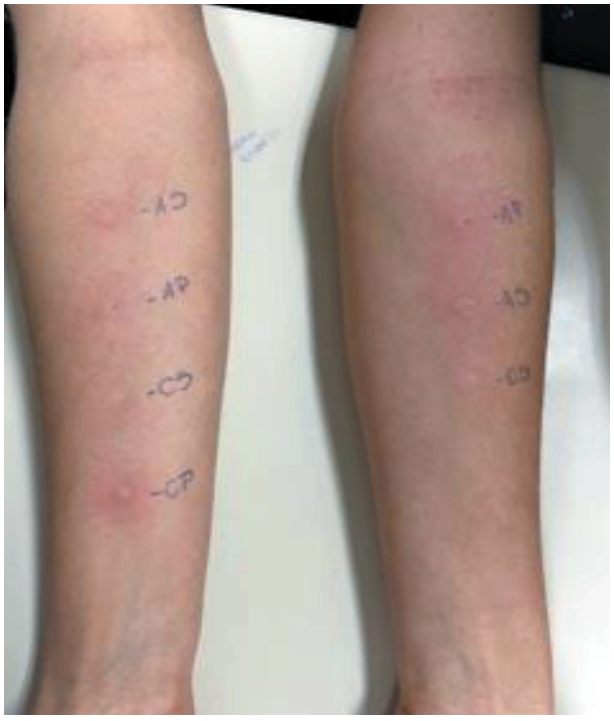

Figure 3
Negative autologous serum test

less commonly involved in this type of urticaria.[3,9,18] Another possibility, if this test is negative, is to ask the patient to take a bath or shower or to immerse the affected parts of the body in water.[8]

There are also some unusual clinical presentations of UA related to reactions depending on the salinity of the water, for example, patients who report symptoms only after exposure to seawater (SDAU), and for these patients, the provocation test should be performed with a 3.5% sodium chloride solution.[13,19]

PerLastly, differential diagnoses should always be evaluated, as it is difficult to differentiate UA from other types of induced urticaria (eg, cholinergic urticaria, heat urticaria, cold urticaria, pressure urticaria, and exercise-induced urticaria).[9,13,20] In our patient, the clinical history clearly suggested the subtype of physical, aquagenic urticaria, and the appearance of wheals after the challenge test with water at room temperature and the patient at rest, together with the appearance of punctate wheals and an erythematous halo, removed the possibility of other forms of UCInd.

The treatment of aquagenic urticaria remains a challenge, however, second-generation antihistamines are used as a first line in standardized or even quadrupled doses.[7,8] In some cases, however, there is a failure to control symptoms with the use of antihistamines.[11]

There are some reports that these refractory cases were treated with ultraviolet (UV) radiation (both psoralens plus UVA and UVB therapy), alone or in combination with antihistamines, and that the effect of this therapy would be to thicken the epidermis, which may prevent water penetration and interaction with dendritic cells or a decrease in mast cell response.[11]

Another treatment possibility was described by Chicharro et al.,[21] in which omalizumab was prescribed at a dose of 300 mg every 4 weeks, and the patient had complete resolution of symptoms after 2 months of treatment, without any adverse effects from the use of this medication.

Bearing in mind that this medication is an anti-IgE monoclonal antibody, widely used for the treatment of CSU, and that, to date, this medication is not licensed for the treatment of UCInds.[15] Therefore, further studies are needed so that we can actually evaluate the results of this treatment in patients with aquagenic urticaria.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Autoimmune hemolytic anemia in multicentric Castleman's disease: case report

Anemia hemolítica autoimune na doença de Castleman multicêntrica: relato de caso

Marcos Tadeu Nolasco da-Silva[1], Katariny Parreira de Oliveira Alves[2], Izilda Aparecida Cardinalli[2], Amanda Avesani Cavotto Furlan[3], Priscila Machado Fernandes[1]

ABSTRACT

Castleman disease is a rare lymphoproliferative disorder that can manifest as localized masses or as multicentric disease. Multicentric Castleman disease is characterized by generalized adenopathies, visceromegaly, autoimmune manifestations, and recurrent infections. This article presents the case report of a patient with multicentric Castleman's disease and autoimmune hemolytic anemia by warm antibodies. Effective response was obtained with systemic corticotherapy and tocilizumab.

Keywords: Castleman Disease, autoimmune hemolytic anemia, monoclonal antibodies.

RESUMO

A doença de Castleman é um distúrbio linfoproliferativo raro, podendo se manifestar sob a forma de massas localizadas ou como doença multicêntrica. A doença de Castleman multicêntrica é caracterizada por adenopatias generalizadas, visceromegalias, manifestações autoimunes e infecções recorrentes. Este artigo apresenta o relato de caso de anemia hemolítica autoimune por anticorpos quentes em paciente com doença de Castleman multicêntrica. Resposta eficaz foi obtida com uso de corticoterapia sistêmica e tocilizumabe.

Descritores: Hiperplasia do linfonodo gigante, anemia hemolítica autoimune, anticorpos monoclonais.

Introduction

First described in 1954 by Castleman and Towne,[1] Castleman disease (CD) is a rare polyclonal lymphoproliferative disorder of B lymphocytes and plasma cells, which may manifest as unicentric or multicentric disease.[2]

Histopathology

CD can be subdivided into two main histopathological forms: hyaline-vascular variant and plasma cell variant.[3] The mixed variant has elements of both variants, and is present in approximately 10% of cases. There is also the plasmablastic subvariant, associated with infection by HHV-8 (human herpes virus type 8) and HIV (human immunodeficiency virus), with risk of progression to plasmablastic monoclonal lymphoma.

Histologically, the classic hyaline-vascular variant is characterized by distortion of the lymph node architecture. An increase in the number of lymphoid follicles is observed, with variation in size and shape. One of the lesions identified is follicular atresia, with lymphocytes from the mantle zone arranged in layers around the follicular center (onion skin appearance). There is deposition of hyaline material in the germinal centers, highlighted by the periodic acid Schiff (PAS) reaction. Vascular alterations, such as sclerosis of the vessels that penetrate the follicles, give rise to lesions known as lollipop follicles.[4]

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The histopathological features of the plasma cell variant show differences in the HHV-8 negative and HHV-8 positive forms. HHV-8 negative cases show an increase in the number of aggregated mature plasma cells, mainly in the interfollicular areas. Vascular proliferation in the paracortical region is prominent. The follicles are hyperplastic or normal. In HHV-8 positive forms, there is expansion of the interfollicular zone by immature and mature plasma cells, with a variable degree of atypia. In addition, there is less distinction in the boundary between the mantle zone and the interfollicular region. The mixed variant, histologically, shows an overlap of the two variants; hyaline-vascular and plasma cells.[5]

Unicentric Castleman's disease (UCD) is commonly associated with the hyaline-vascular variant, corresponding to approximately 70% of cases. It is characterized by localized lymph node enlargement, usually in the mediastinum or abdomen, in oligo or asymptomatic patients, in which the resection of the affected lymph node results in the cure of the disease.[6]

Multicentric Castleman disease (MCD) is commonly associated with the plasma cell variant, accounting for approximately 10-20% of cases. It is characterized by diffuse lymph node enlargement and moderate to severe systemic symptoms, including fever, night sweats, weakness, anorexia and weight loss. Clinical features also include hepatosplenomegaly, ascites, pericardial effusion, pleural effusion, and skin rash. Laboratory abnormalities include anemia of chronic disease, thrombocytopenia, hypoalbuminemia, polyclonal hypergammaglobulinemia, increased ESR (erythrocyte sedimentation rate), C-reactive protein, IL-6 (interleukin 6), and VEGF (vascular endothelial growth factor).

Etiology

DCM can be associated with HHV-8 infection, with or without HIV co-infection, in up to 50% of cases. More rarely, it may also be associated with POEMS syndrome (paraneoplastic syndrome characterized by polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes). In the other half of the cases, DCM is considered idiopathic.[7]

The etiology of idiopathic DCM is uncertain, and its theories include: pathological autoantibodies or mutations in the regulation of the innate immune system (autoimmune/autoinflammatory hypothesis); presence of a small population of neoplastic cells (paraneoplastic hypothesis); or the presence of some other unidentified virus (viral hypothesis).[8] A severe variant of idiopathic DCM known as TAFRO syndrome has also been described, which includes thrombocytopenia, anasarca, fever, reticulin fibrosis and organomegaly in its clinical aspects.[9]

It is also known that the overproduction of pro-inflammatory interleukins, especially IL-6, is implicated in the pathophysiology of the disease.

DCM also presents with recurrent infections, including opportunistic ones, and autoimmune manifestations. However, the occurrence of autoimmune hemolytic anemia is rare, and six cases have been described to date, as far as the authors of this report are aware.[10-15]

Interleukin-6 and Tocilizumab

IL-6 is a pro-inflammatory cytokine that induces the differentiation and proliferation of T and B lymphocytes, is involved in the synthesis of acute phase proteins, in the stimulation of hematopoiesis and hepcidin production and in the development of constitutional symptoms present in various inflammatory diseases. In CD, hyperplastic lymph nodes with infiltration of plasma cells have a constitutionally increased production of IL-6.[16]

In order to confirm the involvement of IL-6 in the pathophysiology of CD, in 1986[17] a study was carried out with two patients affected by CD (one in the multicentric form and the other unicentric). Both underwent resection of the largest affected lymph node chain – in the case of UCD, the only one affected. The culture of the supernatant from the resected lymph nodes confirmed the production of IL-6. It was also observed that the patient with UCD showed complete remission of symptoms after the procedure, while the patient with DCM maintained high serum levels of IL-6 and persistence of symptoms.

Yoshizaki et al., in a 1989 study, also demonstrated an association between IL-6 levels, lymph node hyperplasia, hypergammaglobulinemia, C-reactive protein levels and clinical abnormalities in CD.[18]

In this way, studies followed looking for effective anti-IL-6 therapies in the treatment of the disease. The first drug to show promising results was developed in Japan, in the form of an anti-IL-6 receptor monoclonal antibody: tocilizumab. Initially developed for the

treatment of rheumatoid arthritis in the late 1990s, tocilizumab was also approved for clinical studies in CD and juvenile idiopathic arthritis in the early 21st century.

The first studies with the administration of tocilizumab in patients with DCM also took place in Japan[19,20] and demonstrated reversal of inflammatory parameters, resolution of constitutional symptoms and reduction of lymphadenopathy levels. The results were maintained after three years of continuous use of the medication, and it also allowed the weaning of systemic corticosteroid therapy in part of the patients who had started it concomitantly.

Since then, tocilizumab has become an important therapy to be considered in patients with idiopathic DCM. However, in view of its uncertain etiology and the severe multisystem involvement of the disease, a definitive cure has not yet been discovered, and the prognosis of the disease remains unfavorable.

Case report

A 25-year-old male patient was admitted in August 2020 to the Hospital de Clínicas da UNICAMP with headache for 6 days associated with dyspnea on exertion, greenish vomiting, inappetence and jaundice. He denied fever, respiratory symptoms, changes in bowel or urinary habits, including acholic stools or choluria, recent travel, insect bites, and ingestion of suspicious foods or medications.

Patient with idiopathic multicentric Castelman disease, whose diagnosis was confirmed in 2006 through mesenteric lymph node biopsy. During the previous course of the disease, it manifested with a chronic inflammatory condition – marked by recurrent periods of fever, weight loss, adynamia and anorexia – recurrent opportunistic infections and immune-mediated complications, such as glomerulonephritis and cardiomyopathy.

Next, the histological sections of the mesenteric lymph node resected in 2006 show distortion of the lymph node architecture, with expansion of the paracortical region due to an increase in the number of mature plasma cells (Figure 1), associated with prominent vascularization (Figure 2). Follicular hyperplasia is identified, but with regressed germinal centers, some with an “onion skin” appearance (Figure 3).

The immunohistochemical study shows positive CD20 on B lymphocytes in regressive follicles, CD3

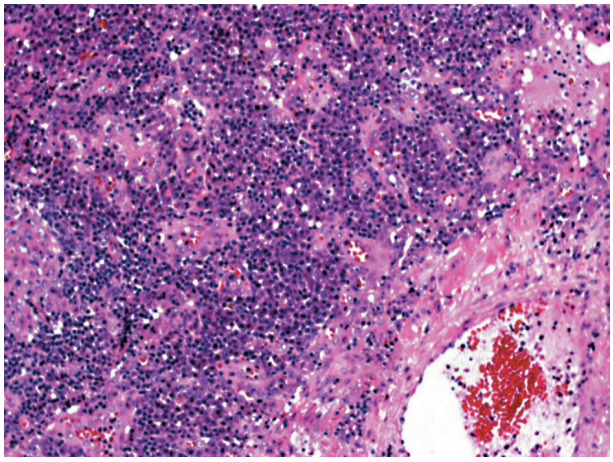

Figure 1
Evident increase in the number of plasma cells causing expansion of the paracortical lymph node zone (H&E, 100x)

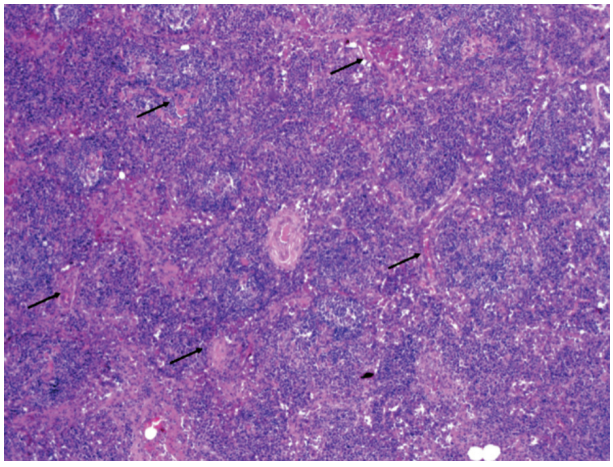

Figure 2
Prominent interfollicular vascularization (arrows). Note moderate distortion of lymph node architecture (H&E, 40x)

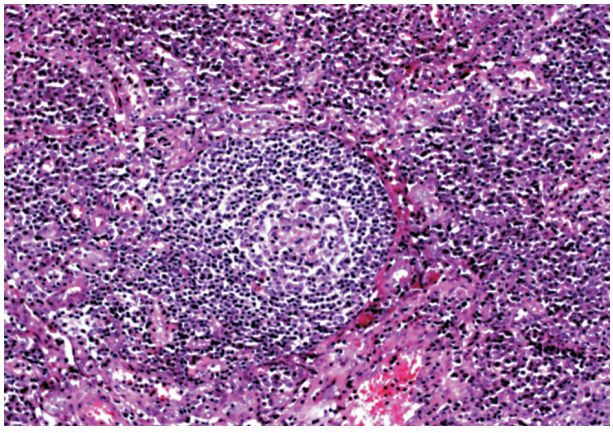

Figure 3
Lymphoid follicle regression in the cortical zone (H&E, 100x)

positive on interfollicular T lymphocytes, positive kappa and lambda in frequent plasma cells in a polyclonal pattern (Figure 4). CD30, HHV8 markers and in situ hybridization were negative. Thus, the immunohistochemical study confirmed the diagnosis of Castleman's disease, a mixed histopathological variant.

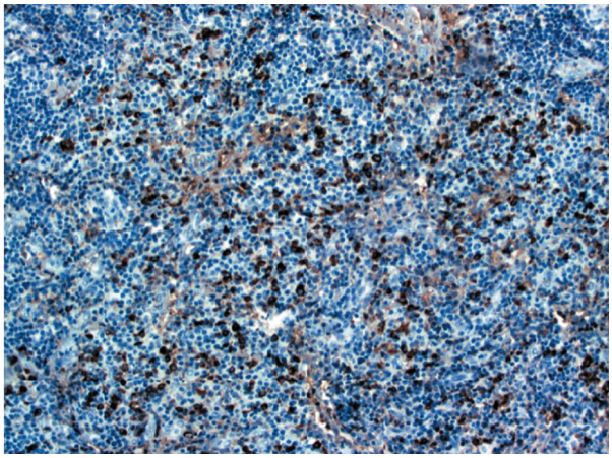

Figure 4
Prominent increase in plasma cells in the paracortical zone (immunohistochemical reaction for lambda light chain, 100x)

On current physical examination, he presented with cutaneous-mucosal pallor, mild jaundice and mild tachycardia, without hepatomegaly or splenomegaly. Laboratory tests showed hemoglobin 3.4 g/dL, mean corpuscular volume (MCV) 83.1 fl, platelets 1,064,000 µL, haptoglobin 32.6 mg/dL, total reticulocytes 176,000/mm^3, LDH 372 U/L, indirect bilirubin 1.18 mg/dL, positive direct antiglobulin test (IgG 1+, C3d 3+) and positive eluate, with the presence of IgG class autoantibodies without defined specificity. In the peripheral blood smear, no schizocytes or other abnormal forms of red blood cells were found.

Hepatitis A, hepatitis B, hepatitis C, syphilis and HIV serologies were negative. Urine I negative for hemoglobinuria. Total abdomen ultrasound without abnormalities. Also, triglycerides 156 mg/dL, ferritin 7540 ng/mL, fibrinogen > 900 mg/dL and C-reactive protein 300 mg/L. AST, ALT, amylase, lipase, cryoagglutinins, albumin and coagulogram were normal.

Altered laboratory tests on admission are shown in Table 1.

Based on the above data, the diagnosis of warm antibody autoimmune hemolytic anemia was defined. Initially, a unit of packed red blood cells was transfused, with no significant increase in hemoglobin levels (3.4 g/dL to 4.3 g/dL). After diagnostic confirmation, treatment was initiated with systemic corticosteroid therapy (methylprednisolone 4 mg/kg/day) and, two days later, tocilizumab 4 mg/kg single dose.

On the first day after tocilizumab administration, a progressive increase in hemoglobin levels was observed, with normalization of levels on the eighth day. Normalization of LDH, bilirubin and haptoglobin levels was also observed. Progressive weaning of corticosteroids was started from the seventh day.

The response to the administered treatment is represented in Figures 5 and 6.

The patient maintained regular returns after hospital discharge, with monthly administration of tocilizumab 8 mg/kg in monotherapy, with a sustained response to date.

Discussion

The existence of a close relationship between autoimmunity and lymphoproliferative diseases is known, and is based on the pathophysiology of proliferation, transformation and self-reactivity of B21 lymphocytes. It is inherent in any B lymphocyte to produce low-affinity antibodies against self-antigens, which are eliminated by immunoregulatory mechanisms as soon as they are recognized. However, in lymphoproliferative diseases, mutations in the germ line, the high proliferative activity of B lymphocytes and the defect in the apoptosis mechanism lead to dysregulation of the immune system and the generation of autoantibodies.

The lymphoproliferative diseases that are most associated with autoimmune diseases are: multiple myeloma, monoclonal gammopathy of undetermined significance, non-Hodgkin's lymphoma and chronic lymphoid leukemia. In the latter, the occurrence of autoimmune hemolytic anemia occurs in up to 20-25% of patients during the course of the disease.[22]

Autoimmune diseases have already been reported in patients with idiopathic DCM, such as systemic lupus erythematosus and hemophagocytic lymphohistiocytosis.[23] However, reports of autoimmune hemolytic anemia are rare.

Among the systemic signs and symptoms present in DCM, anemia is almost always present, usually with the typical characteristics of anemia of chronic disease.[24] The pathogenesis of anemia of

Table 1
Laboratory tests on admission

Laboratory exam	Results obtained	Reference values
Lactic dehydrogenase (LDH)	372 U/L	140 to 271 U/L
Total bilirubin	1.91 mg/dL	0.3 to 1.2 mg/dL
Indirect bilirubin	1.18 mg/dL	0.1 to 1 mg/dL
Haptogloblin	32.6 mg/dL	30 to 230 mg/dL
Hemoglobin	3.4 g/dL	14 to 18 g/dL
Hematocrit	12.3%	41 to 52%
Mean corpuscular volume (MCV)	83.1 fL	80 to 99 fL
Mean corpuscular hemoglobin (MCH)	23 page	27-32 pages
Platelets	1064 x 10^3 µL	150 to 400 x 10^3 µL
Reticulocytes (absolute)	176 x 10^3/mm^3	50 to 100 x 10^3/mm^3
Reticulocytes (percentage)	11.93%	0.5 to 2.5%
Ferritin	7540 ng/mL	30 to 500 ng/mL
Fibrinogen	> 900 mg/dL	175 to 400 mg/dL
C-reactive protein	300 mg/L	> 3 mg/L
Interleukin-6 (IL-6)	72.75 pg/mL	< 6.4 pgmL

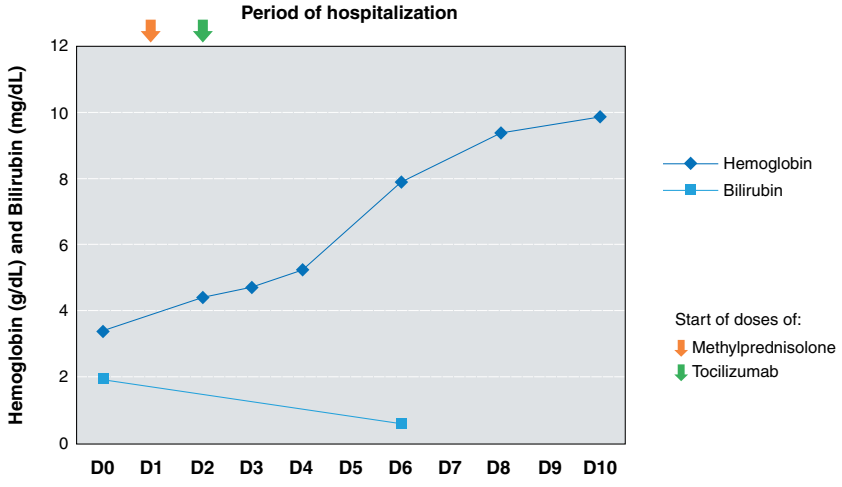

Figure 5
Treatment response

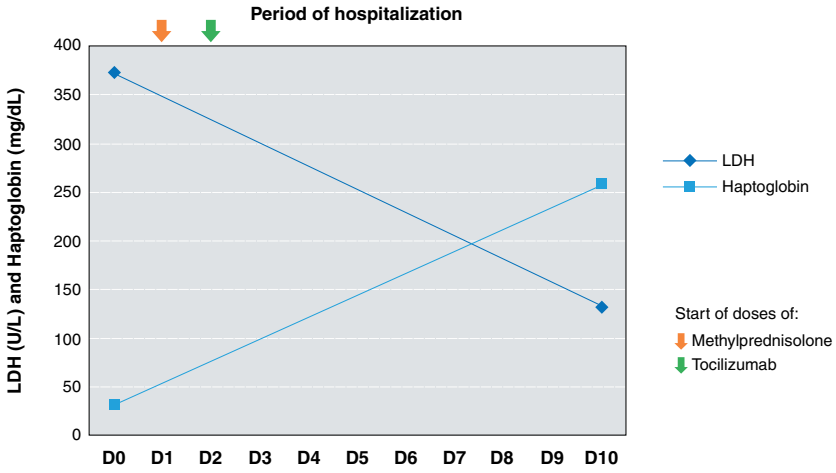

Figure 6
Treatment response

chronic disease in these patients involves the same determinants as other inflammatory diseases, with the additional mechanism of IL-6, which has inhibitory activity on erythropoiesis. However, it is possible that IL-6 overproduction itself may be involved in the pathophysiology of autoimmune hemolytic anemia (AIHA) by stimulating the generation and differentiation of plasma cells.

For over 20 years, experimental models of autoimmunity induction, such as collagen-induced arthritis and antigen-induced arthritis, have used IL-6.[25] Also in patients with DCM and AHAI, studies show increased levels of IL-6, as well as IL-4, IL-10, IL-13, IL-17 and IL-21.[26] In addition, the presence of T helper 2 (Th2), regulatory T (Treg) and T helper 17 (Th17) cells suggest their association with disease activity.[27] Th2 cells secrete IL-6, IL-4, IL-10, IL-13 and TGF-β, cytokines that stimulate the production of antibodies by B lymphocytes, while IL-6 induces the differentiation of Th17 cells, amplifying the response proinflammatory and autoimmune.[28,29]

So far, only one study has reported success in the treatment of AIHA in CD with the use of tocilizumab,[30] while a more recent study in 2019 showed partial success. In this study, Tabata S. et al. report the case of a patient with idiopathic DCM with AIHA, with initial treatment with tocilizumab 8 mg/kg every two weeks for a total of 6 doses. However, after the second dose of medication, anti-tocilizumab antibodies were detected in the patient's serum, with a consequent decrease in the effectiveness of the treatment. However, it is observed that there was a late introduction of corticosteroid therapy at an immunosuppressive dose in this patient (six days after starting tocilizumab).

Our study allowed the use of tocilizumab at a dose of 4 mg/kg, as it also associated methylprednisolone at a dose of 4 mg/kg. This association not only made it possible to reduce the dose of tocilizumab, which makes the treatment more affordable, but also prevented the possible emergence of antibodies against the drug. However, it is worth mentioning that the high dose of corticosteroids, even if followed by complete weaning, brought as a side effect to the patient in this study moderate acne and lack of glycemic control. It is possible to assume that the dose of 1-2 mg/kg would also have an immunosuppressive effect, but would be accompanied by fewer side effects.

Tocilizumab was approved in Japan for the treatment of idiopathic DCM in 2005, but due to the lack of randomized controlled studies, it was not approved in the United States, where in 2014 another similar drug was approved, called siltuximab, an anti-IL-6 monoclonal antibody. Althoughconsideredcurrently the treatment of choice, only 34% of patients responded to siltuximab therapy. Half of the non-responders had low serum levels of IL-6.[31]

The signaling pathways involved in the pathogenesis of idiopathic DCM are not yet fully understood, and other therapies involving pathways consequent to IL-6 activation have also been studied.

Conclusion

New therapeutic options for idiopathic DCM have emerged and have shown promising results. Because it is a rare disease, of uncertain etiology and with a wide variety of clinical manifestations, idiopathic DCM is still a major challenge for the scientific community and remains the subject of study. However, it is known that IL-6 plays a fundamental role in the pathophysiology of the disease, and the inclusion of anti-IL-6 and anti-IL-6 receptor monoclonal antibodies in the list of treatment radically changed the prognosis of these patients.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Intrathoracic tuberculosis in the pseudotumoral and bone form as a manifestation of chronic granulomatous disease

Tuberculose intratorácica na forma pseudotumoral e óssea como manifestação de doença granulomatosa crônica

Priscilla Filippo A. M. Santos[1], Antonio Condino-Neto[2], Lillian Nunes Gomes[2], Claudete Araújo Cardoso[3]

ABSTRACT

Chronic granulomatous disease (CGD) is an inborn error of phagocyte immunity and occurs as a result of mutations that affect components of the NADPH oxidase enzyme. Patients are susceptible to serious and lethal fungal and bacterial infections. The aim of this paper is to report a case an infant with CGD who presented clinical manifestations of intrathoracic tuberculosis (TB) in the pseudotumoral and bone form, which started in the neonatal period. The diagnosis of CGD was performed using the DHR test and, after starting prophylaxis with sulfamethoxazole-trimethoprim and itraconazole, the patient remained clinically stable. The mother and sister also had altered DHR, genetic analysis revealed an X-linked mutation in exon 2 of the CYBB gene c.58G>A, leading to an alteration in G20R. It is essential that the diagnosis is made as early as possible, in order to establish guidelines for Family members and adequate treatment, thus reducing infectious complications and improving prognosis.

Keywords: Chronic granulomatous disease, primary immunodeficiency diseases, tuberculosis.

RESUMO

A doença granulomatosa crônica (DGC) é um erro inato da imunidade de fagócitos, e ocorre em decorrência de mutações que afetam componentes da enzima NADPH oxidase. Os pacientes são suceptíveis a infecções graves e letais por fungos e bactérias. O objetivo deste trabalho é relatar o caso de um lactente com DGC que apresentou manifestação clínica de tuberculose (TB) intratorácica na forma pseudotumoral e óssea iniciada no período neonatal. O diagnóstico de DGC foi realizado através do teste de DHR e, após o início da profilaxia com sulfametoxazol-trimetroprima e itraconazol, o paciente manteve-se estável clinicamente. A mãe e a irmã também apresentaram DHR alterados, a análise genética revelou uma mutação ligada ao X no exon 2 do gene CYBB c.58G>A, levando uma alteração em G20R. É fundamental que o diagnóstico seja realizado o mais precocemente possível, a fim de instituir as orientações aos familiares e tratamento adequado, reduzindo assim complicações infecciosas e melhorando prognóstico.

Descritores: Doença granulomatosa crônica, doenças da imunodeficiência primária, tuberculose.

Introduction

Chronic granulomatous disease (CGD) is a heterogeneous genetic disease, which was first described in the 1950s. It is a rare inborn error of immunity, characterized by severe and recurrent infections, due to the functional impairment of the NADPH oxidase complex in monocytes and neutrophilic granulocytes.[1,2] This enzyme complex generates superoxide and is essential for the intracellular killing of pathogens, fungi and bacteria by phagocytes.In addition to having direct cytotoxic

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effects, the production of reactive oxygen species appears to be important for other innate immune functions.The incidence is variable: 1:1 million in Italy,[2] 1:300,000 in Japan,[4] 1:250,000 in the United States[1] and 1: 70,000 in the Israeli Arab population.[5]

Lungs, skin, lymph nodes and liver are the most affected organs.[1] Patients with CGD may have tuberculosis (TB), which is an opportunistic infection with high worldwide prevalence, especially in tropical countries.[6,7] The development of disease by the TB bacillus depends on the interaction between the host's immunological factors and the aggressiveness of the infectious agent. TB in the pseudotumor form is a rare entity, the diagnosis is difficult and can be confused with primary or secondary neoplasm. Bacteriological samples are often negative, which can cause a delay in diagnosis and therapy.[6] The diagnosis of CGD can be made by the dihydrorhodamine test (DHR), which measures intracellular digestion of phagocytes, and is confirmed by genotyping studies.[8]

Recent studies have reported a survival rate of approximately 90% at 10 years of age, attributed to early diagnosis and institution of antibiotic prophylaxis, use of interferon-gamma, antifungal prophylaxis and hematopoietic stem cell transplantation.[9] However, the average age of death of these patients remains around 30 to 40 years.[10]

The aim of this study is to report a case of intrathoracic TB in the pseudotumor and bone form that is uncommon in the neonatal period as the first manifestation of chronic granulomatous disease. The project was approved by the Research Ethics Committee (CAAE: 46255021.4.0000.5259), and an informed consent form was signed by the patient's guardian.

Case Report

A 12-month-old boy was referred for immunological investigation with a history of intrathoracic tuberculosis (TB), in the pseudotumor and bone form (Figure 1). She received the BCG vaccine in the neonatal period, without complications. At 19 days of age, he was hospitalized with daily fever, edema and erythema of the first left finger. During the investigation of the child, there was no microbiological confirmation of *Mycobacterium tuberculosis*, but the radiological aspect of the pulmonary image made the diagnosis of TB, and at the time isoniazid, rifampicin and pyrazinamide were started, with regression of the pseudotumor and complete remission of the bone condition after 12 months of treatment. The parents and sister were investigated for TB, with normal chest X-ray and reactive tuberculin skin test, and treatment for latent tuberculosis infection (LTBI) was prescribed at the time. Tumor markers were negative. Subsequently, the patient was hospitalized twice for pneumonia, without the need for oxygen therapy, with positive PCR for SARS-CoV2 on the second hospitalization. Growth and development were age appropriate. There was no family history of inborn errors of immunity, recurrent miscarriages, and parental consanguinity. He had a BCG scar greater than 3 mm on physical examination, with no other changes.

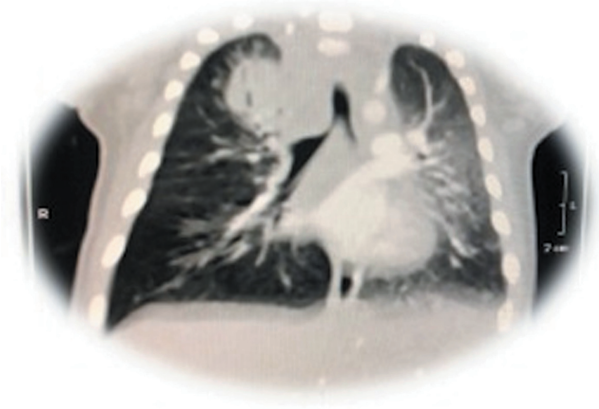

Figure 1
Chest CT angiography - intrathoracic tuberculosis pseudotumor form

Serology for HIV I and II, HTLV I and II were negative. Blood count showed anemia.Dglucose-6-phosphate dehydrogenase assay, Immunoglobulins, lymphocyte profile with normal results. Measles and mumps IgG reactive serologies. However,the dihydrorhodamine assay (DHR) showed an abnormal result of ROS production in granulocytes after stimulation and was suggestive of DGC (Figure 2).Prophylaxis with trimethoprim-sulfamethoxazole and itraconazole was started with good clinical outcome. HLA typing was requested and hematopoietic stem cell transplantation was indicated. The mother and sister had altered DHR, suggestive of carriers of X-linked CGD (Figure 2).

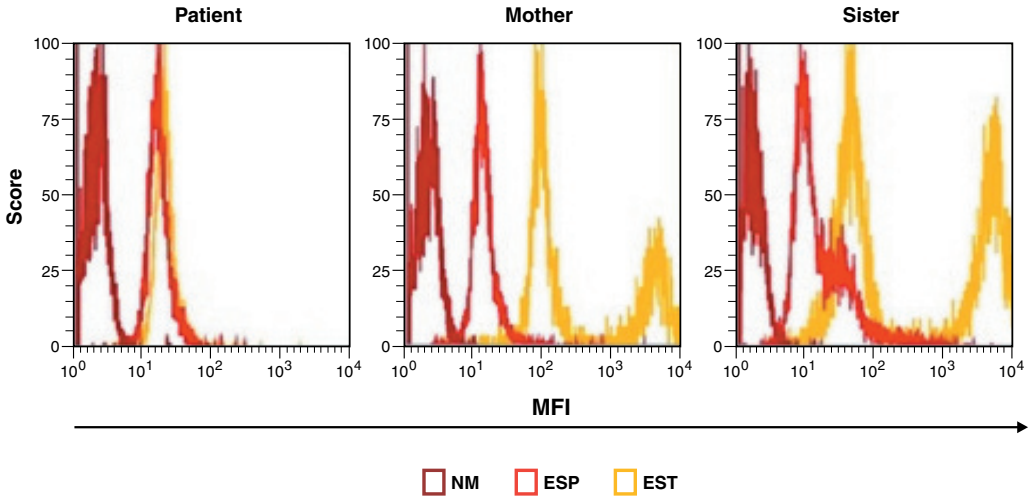

Figure 2
DHR test result. Spontaneous granulocyte (ESP) or PMA-stimulated (EST) and unlabeled, non-DHR (NM) granulocyte DHR from patient, mother, and sister samples, respectively. With altered patient result, and suggestive for X-linked CGD carrier

In addition, CYBB gene sequencing was performed to confirm the diagnosis of CGD. Sequence data were analyzed using the NCBI databases (www.ncbi.nlm.nih.gov), Ensembl SNP (https://www.ensembl.org/index.html), and the Mutation Taster (http://www.mutationtaster.org). This analysis confirmed a missense mutation (c.58G>A) in exon 2 that leads to a substitution of a single amino acid, G20R, in the gp91phox protein. The same mutation was observed in heterozygosity in the patient's mother and sister (Figure 3).

Discussion

CGD is a rare inborn error of immunity, with a variable incidence according to ethnicity, more prevalent in males (2:1), due to the predominant model of genetic transmission (X-linked disease).[2] The infant in the case report is male, and probably has an X-linked disease, since his mother and sister had altered DHR. However, defects in autosomal genes can also cause CGD in men and women.[11] X-linked CGD is more common in areas with miscegenation, while the autosomal recessive form is more common in areas with a history of inbreeding.[12] There is no report of consanguinity between the parents. Children with X-linked CGD have a more severe clinical presentation: earlier onset of the disease, frequent obstructions caused by granulomas, more frequent infections, and a higher mortality rate.[11] Patients with CGD have reduced expression and function of Toll-like receptors, complement receptors, and chemokine receptors that correlate with disease severity. The most common cause of CGD is a defect in the CYBB gene (gp91 phox), located on the short arm of the X chromosome (Xp21.1-p11.4).[2]

An Italian multicenter study showed that the most common infectious manifestations were pulmonary infection (pneumonia/abscess in 50%), dermatitis/subcutaneous abscess (46%), lymphadenitis (45%), osteomyelitis and liver abscess (16%) [3]. Arnold et al. observed that the lung, skin, lymph nodes and liver are the most affected organs.[1] Osteomyelitis is an important infection in GCD and can arise from the hematogenous spread of pathogens (*S. aureus*, *Salmonella spp.*, *S. marcescens*) or contiguous bone invasion.[1] Susceptibility to infections in these patients arises from the neonatal period to adulthood, and the outcome depends on prompt recognition and therapy for the underlying infection.[12]

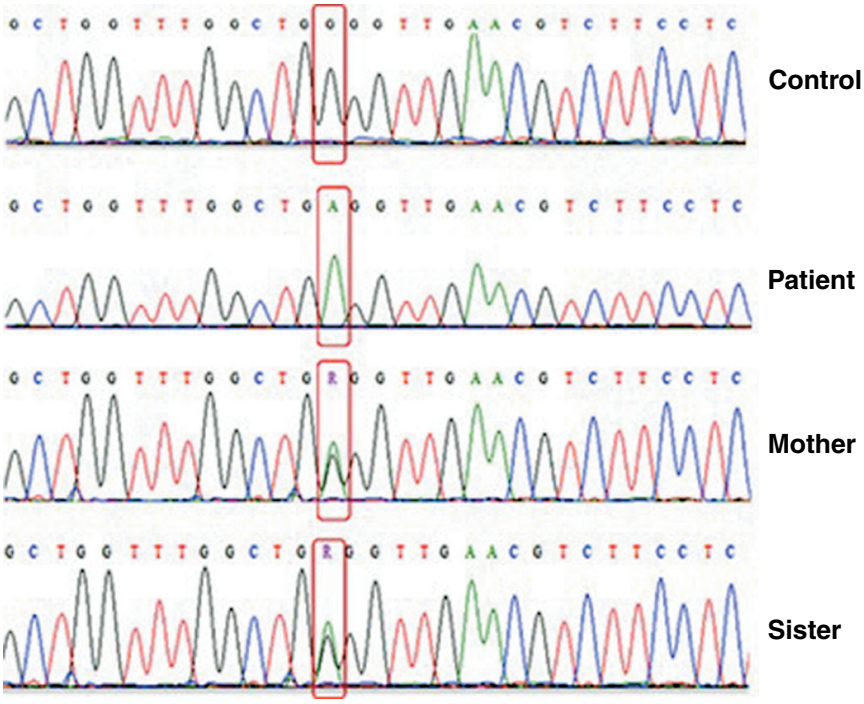

Figure 3
Sequencing results for CYBB gene. Chromatogram for the healthy, patient Control, her mother and her sister, from top to bottom, respectively. Shows in red highlight the single nucleotide substitution, c.58G>A, which results in a G20R change

In Brazil, a series of 18 patients identified as the most common manifestations of CGD: lymphadenopathy, hepatosplenomegaly, pneumonia and abscesses. A second study, with seven Brazilian patients, showed that pneumonia was the most frequent clinical manifestation, followed by skin infections, sinusitis, otitis and liver abscess.[13]

In Europe and North America, the most common pathogens are *Aspergillus spp.*, *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens*, *Nocardia spp.* and *Salmonella*.[1,3] In these places, mycobacterial infection is not common. CGD is the primary immunodeficiency in which invasive fungal infection is most common, affecting the lungs and chest wall.[1] In developing countries, Bacillus Calmette-Guerin (BCG) and *Mycobacterium tuberculosis* are the most important pathogens.[11] There are reports of mycobacterial infection after BCG and *Mycobacterium tuberculosis* in China, Iran and Latin America. However, patients develop a severe form localized and not disseminated by BCG and pulmonary and not miliary TB.[11]

In countries such as Brazil, where tuberculosis is endemic, BCG vaccination is carried out in the first months of life, and is usually applied in the neonatal period, and patients with immunodeficiency may have adverse reactions to BCG vaccination. A multicenter study (Latin America, Africa, Europe and Asia) carried out by Conti F. et al. showed that the adverse reaction to BCG was the first sign of the disease in 39 (55%) of the 53 children with this reaction. A local or regional reaction (BCG-ites) was reported in 33 (63%) of the 53 patients with an adverse reaction, and a disseminated reaction (BCG-osis) was observed in the other 20 (37%) patients.[14] Furthermore, patients with any form of CGD may fail to develop a protective immune response against *Mycobacterium* species, and may develop active TB at any stage of life. Clinically, BCG infection was in some cases the first manifestation of CGD in Brazil.[14]

In Argentina, Hong Kong and Iran, up to 11%, 54.5% and 31.7% of patients with CGD, respectively, had TB. Children with severe forms of tuberculosis should be investigated for inborn errors of immunity.[14]

In a study carried out by Oliveira Júnior et al. with data from LASID (Latin American Society for Immunodeficiencies), 71 patients with CGD were evaluated with the following distribution by country: 39% from Brazil, 36% from Argentina, 16% from Mexico, 6% from Chile and 3% from Colombia. Of the patients evaluated, 30% had a reaction to BCG with an increased prevalence in the group with mutation in the CYBB gene (85.7%), compared to the group with mutation in the NCF1 gene (14.3%). Of these patients who had a reaction to BCG, 30% had a disseminated reaction with a severe clinical picture.[13]

Patients with CGD may present alterations in the inflammatory response and autoimmunity.[1] Inflammatory manifestations are common in patients with CGD and are most often seen in the gastrointestinal tract, urogenital tract, lungs, and eyes.[2]

One of the characteristics of GCD is the formation of granulomas, which can cause clinical symptoms of obstruction, such as vomiting, dysphagia, slow gastric emptying, weight loss, bronchial obstruction, bladder obstruction, etc.[11] In the case reported, the first clinical manifestation was intrathoracic TB in the pseudotumor form, which regressed after the initiation of specific treatment.

Brazil is among the 30 countries with the highest TB burden in the world, with an incidence rate of 80,000 cases/year. Children account for 10% of total TB cases. Children under five years of age are at greater risk of becoming ill after primary infection when compared to adults and adolescents. The pulmonary form is the most frequent, highlighting the greatest potential for progression to severe forms of TB, such as meningoencephalitis and miliary. The BCG vaccine protects against these severe forms and in Brazil, its application is recommended at birth. TB patients should be screened for HIV.[15] The most frequent radiological manifestations in childhood are: lymph node enlargement (hilar or mediastinal), miliary disease (diffuse reticulonodular type), parenchymal disease (pulmonary condensation), atelectasis and pleural effusion (rarely in children under five). Alterations on computed tomography are lobar consolidations and hypodense areas, found in more than 80% of cases, and, in less than 25%, cavitations.[16] Computed tomography angiography of the patient's chest showed a mediastinal mass with a hypodense center and a small excavation inside, associated with hilar, paratracheal, and infracarinal adenopathy suggestive of TB.

The diagnosis of TB in 80% of cases in children younger than 5 years is made without bacteriological evidence, due to the paucibacillary characteristic of the disease in the pediatric age group, which makes it difficult to perform sputum bacterioscopy in younger children.[16] At the time of the diagnosis of TB in the child, one should always research the history of the disease in the contacts, in order to identify the source case of TB. In the case reported, the parents and the sister did not have the disease. The patient had no bacteriological confirmation, and presented complete regression of the clinical picture with the institution of treatment with a RIP regimen.

Marine B. et al. reported that the diagnosis of CGD was made at an average age of 4.4 years (median 2.5 years, range 0 to 38 years) and the mean age of symptom onset was 1 year (median 7.5 years, range from 0 to 10 years).[3] The patient started symptoms early, in the neonatal period, and the diagnosis of CGD was performed at 13 months, through the DHR, which was performed and repeated after 15 days. The DHR is a very sensitive and specific assay that reliably detects all NADPH oxidase deficiencies in neutrophils.[18] Early diagnosis is essential for the proper institution of treatment, with the use of prophylactic antibiotics and antifungal agents, avoiding infectious complications, hospitalizations and interfering with the patient's quality of life. In addition, it is important to find the genetic mutation, for genetic counseling and eventually choose a suitable bone marrow donor or perform gene therapy.[10,17] In Brazil, we still do not have gene therapy available.

In some cases, parents of a CGD patient may consider having an additional child to serve as a hematopoietic stem cell donor for the affected sibling.[12] In this case report, the parents, in principle, do not think about having more children. Thinking about genetic counseling, DHR and maternal and sister genetic analysis were requested. The results of the DHRs had altered results and genetics confirmed that both have the same variant found in the patient (c.58G>A), in the heterozygous form. This is a missense mutation in exon 2 of the CYBB gene, resulting in the p.G20R amino acid change in the N-terminal domain. This is an already described mutation.[18] The dataof this study highlight the relevance of the diagnosisfor definitive family counseling, since a new pregnancy of the

mother has a 50% chance of having a boy with CGD, the same for future pregnancies of the sister.

Long-term prophylactic use with trimethoprim-sulfamethoxazole or doxycacilcin and itraconazole has been shown to reduce infections in patients with CGD, and is recommended during infection-free periods.[19,20] From the beginning of prophylaxis with these medications, the patient was clinically stable, without further hospitalizations due to infectious complications. It is also recommended to prevent infections through immunizations.[11]

BCG is a mandatory vaccine in Latin America. It is usually given during the neonatal period. This practice is potentially harmful for patients with DCG, SCID, or another immunodeficiency that affects phagocytes or T cell function. Newborn screening tests for DCG and other phagocyte defects should be performed prior to BCG vaccination.[13]

In Mexico and Argentina, patients with CGD have access to routine interferon-gamma therapy, given subcutaneously, three times a week. In Brazil, this drug is not registered by the national regulatory agency, limiting access for use.[13]

In the study by Oliveira-Junior et al. Bone marrow transplants have been performed in patients in Brazil, Mexico and Argentina, depending on the availability of a compatible donor. Gene therapy for primary immunodeficiencies is not yet available in Latin America.[13]

Agudelo-Flórez et al. described a series of 14 patients with CGD from Latin America (10 Brazilians, 2 Chileans and 2 Mexicans), in which all of them started to become infected before 2 years. None had a serious reaction to BCG. All were on prophylactic use of sulfamethoxazole/trimethoprim, 11 were on itraconazole and two were regularly given recombinant gamma interferon.[21]

Other differential diagnoses should be excluded, such as G6PD deficiency, because this deficiency in the severe form can lead to insufficient formation of NADPH in leukocytes, hindering the activity of NADPH oxidase.[11]

Martire B. et al. reported a survival rate of 97%, 83%, and 46% at 10, 20, and 25 years, respectively, from the diagnosis of the disease.[3]

We report a rare case of an infant with a pseudotumor and bone form of TB, and it is important to rule out other causes with similar clinical and imaging findings and to investigate inborn errors of immunity in these cases, which is relevant for early treatment and genetic counseling.

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We thank our patient and his family members for their cooperation in the study.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Hereditary angioedema and Allergic bronchopulmonary aspergillosis: an unexpected association

Angioedema hereditário e Aspergilose broncopulmonar alérgica: uma associação inesperada

Laise Fazanha Sgarbi[1], Sérgio Duarte Dortas-Junior[2], Maria Luiza Oliva Alonso[2], Alfeu Tavares França[2], Solange Oliveira Rodrigues Valle[2]

ABSTRACT

Hereditary angioedema (HAE) is a rare autosomal dominant disorder, Allergic bronchopulmonary aspergillosis (ABPA) is a lung disease involving hypersensitivity to the fungi *Aspergillus fumigatus* which occur in susceptible patient with asthma or cystic fibrosis, also considered a rare disease. We report a case of HAE and ABPA in a single patient. HAE diagnosis was confirmed: C4 = 3 mg/dL, C1INH < 2.8 mg/dL - nephelometry. Former lung function showed elevation RV and RV/FVC, suggesting small airways lung disease. Positive skin prick test to Aspergillus fumigatus (03 mm); total serum IgE level 3,100 IU/mL (nephelometry - BNII Siemens), eosinophilia 11% (528/mm^3) and specific *A. fumigatus* IgG antibodies 6,8 mgA/L (FEIA - fluorenzymeimmunoassay - ThermoFisher) and Chest CT showed mucoid impaction of the bronchi, consistent to current ABPA. Controlling ABPA could prevent and reduce angioedema attacks, and lung structural damage. Early diagnosis and treatment of both diseases should be emphasized to reduce mortality and morbidity

Keywords: Hereditary angioedema types I and II, allergic bronchopulmonary aspergillosis, asthma, bradykinin, hereditary angioedemas.

RESUMO

Angioedema hereditário (AEH) é uma doença autossômica dominante; aspergilose broncopulmonar alérgica (ABPA) é uma doença de hipersensibilidade pulmonar relacionada ao esporo de *Aspergillus fumigatus*, mais suscetível em pacientes com asma e fibrose cística, ambas são consideradas doenças raras. Apresentamos um caso de AEH e ABPA em um paciente. O diagnóstico de AEH foi confirmado com exames laboratoriais: C4 = 3 mg/dL, C1INH < 2,8 mg/dL - nefelometria. Prova de função pulmonar evidenciou aumento de VR e VR/CVF, sugerindo doenças de pequenas vias aéreas. Teste de puntura positivo para *A. fumigatus* (03 mm); IgE total = 3.100 IU/mL (nefelometria - BNII Siemens), eosinofilia 11% (528/mm^3) e IgG específica para *A. fumigatus* 6,8 mgA/L (FEIA - ThermoFisher), TC de tórax evidenciou impactação mucoide, consistente com ABPA. Controlar ABPA pode prevenir e reduzir as crises de angioedema e os danos ao tecido pulmonar. O diagnóstico precoce de ambas as doenças deve ser enfatizado para reduzir a morbimortalidade.

Descritores: Angioedema hereditário tipos I e II, aspergilose broncopulmonar alérgica, asma, bradicinina, angioedemas hereditários.

Introduction

Hereditary angioedema (HAE) is a rare autosomal dominant disorder. Different forms of HAE are currently recognized and genetically identifiable, the most common mutations occurring in the SERPING1 gene, leading to quantitative [HAE type I(HAE-1)] or qualitative [HAE type II(HAE-2)] deficiency of C1 esterase inhibitor (C1-INH).[1] Allergic bronchopulmonary aspergillosis (ABPA) is

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a lung disease involving hypersensitivity to the fungi *Aspergillus fumigatus* (*Af*) which occur in susceptible patient with asthma or cystic fibrosis.[2]

HAE-1 and HAE-2 with a combined estimated prevalence of approximately 1:50,000 is an autosomal dominant disease, although 25% of patients may have no family history.[1]

ABPA affects approximately 1% through 15% of cystic fibrosis patients.[2] One study calculated that 2.5% of adults who have asthma also have ABPA, which is approximately 4.8 million people worldwide. In other studies, ABPA was detected in 25% to 37% of asthmatics with a positive skin prick test to *Af*. [3]

Case report

A 07-year-old boy who presented to Immunology Service for HAE screening, since his father and grandmother had been formerly diagnosed HAE-1. His parents reported recurrent lips swelling, without wheals, during about 48-72 hours. Swellings were not responsive to antihistamines and corticosteroids. HAE diagnosis was confirmed (C4 = 3 mg/dL, C1INH < 2.8 mg/dL – nephelometry) and he was prescribed tranexamic acid 250 mg/day. Long-term prophylactic treatment was interrupted at the age of 10 as HAE was controlled, and on demand therapy (plasma-derived C1-inhibitor concentrate) was prescribed.

At first evaluation, he also reported sneezing, nasal itching, running nose, dyspnea and wheezing. He was diagnosed with asthma and allergic rhinitis and therapy initiated. Patient ended up with uncontrolled moderate asthma because of non-adherence to treatment. At 17 years old, patient performed a spirometry that showed FEV1 (Forced Expiratory Volume in first second) and FVC (Forced Vital Capacity) reduction with negative reversibility testing, but the VC (Vital Capacity) normalized. Former lung function showed elevation RV (Residual Volume) and RV/FVC, suggesting small airways lung disease. Positive skin prick test to *Aspergillus fumigatus* (03 mm); total serum IgE level 3,100 IU/mL (nephelometry – BNII Siemens), eosinophilia 11% (528/mm^3) and specific *A. fumigatus* IgG antibodies 6,8 mgA/L (FEIA – fluorenzymeimmunoassay – ThermoFisher) and Chest CT showed mucoid impaction of the bronchi, consistent to current ABPA diagnostic criteria.[3] Inhaled corticosteroids and LABA were prescribed with disease control.

Discussion

HAE is a disabling, potentially fatal condition characterized by recurrent episodes of swelling. A failure of regulation of the kallikrein-kinin system by C1-INH to prevent bradykinin (BK) formation is recognized, which increases endothelial permeability (by the interaction to B2 receptor) and leads to recurrent episodes of swelling involving the deeper layers of the skin and/or submucosal tissue.[4,5]

The lectin pathway, other mechanisms for the activation of the complement system in HAE have been suggested, and may result in upregulation of B2 and B1 receptor. As with the other serine proteases 1 and 2 are inhibited by C1-INH and provides an unregulated production of angioedema.[5]

During angioedema attacks the circulating levels of BK, markers of endothelial activation, prothrombin fragments, D-dimer, cytokines (e.g., TNF-α and IL-8), as well as neutrophil count and neutrophil-derived factors (e.g., elastase, myeloperoxidase, pentraxin 3) are increased when compared with symptom-free periods in C1-INH-HAE patients.[4,5] ABPA is caused by hypersensitivity to *Af* Antigens.[2] In susceptible hosts, repeated inhalation of *Aspergillus* spores can cause an allergic response. These spores are trapped in the fluids and mucus lining of the epithelial surface, germinate, leading hypha growth and release a variety of proteins allergens and proteases that leads to a robust inflammatory response. Innate lymphoid cells and Th2 cells are activated and produce cytokines (IL-4, IL-5 and IL-13), that stimulate inflammation. This reaction is mainly an immunoglobulin E (IgE) mediated hypersensitivity reaction (increased total serum IgE levels and *Af* specific IgE antibodies production, mast cells degranulation and exacerbated eosinophilic response). Both type III and type IV, immunoglobulin G (IgG) mediated immune complex and cell-mediated hypersensitivity reactions have also been seen in ABPA immunopathogenesis.[2,3]

ABPA Th2 response causes substantial local inflammatory reaction, leading to structural lung damage, bronchiectasis and pulmonary fibrosis. It also predisposes the patient to the development of respiratory infections.[2,6] Infections have been well described in literature as one of the possible triggers of angioedema attacks. We believe that ABPA complications, such as recurrent exacerbations, as well as respiratory infections might easily trigger angioedema attacks. As well as HAE attacks might

trigger bronchospasm and/or aggravate smooth muscle structural damage.

In allergic mild asthma is well reported an overexpression of B2 and B1 receptor, and in acute airway inflammation pathway kinins are activated and B2 receptor is upregulated.[7] B2 activation can induces bronchospasm, endothelial permeability, mucus secretion, cholinergic nerve stimulation. B1 and B2 activation is usually associated to eosinophil, neutrophil recruitment, Type-2 cytokines (due to IL-4/IL-13), kinins, releasing of various inflammatory mediators (activated epithelial, endothelial cells, endothelial-nitric oxide synthase, vascular endothelial growth factor, fibroblasts, PGE2, Il-8) a cascade implicated in airway chronic inflammation, which drives remodeling airway.[7]

If this patient presents a HAE attack, BK levels should increase, and B2 receptor activates driven to this airway inflammation, which we believe would be a severe bronchoconstriction, the use of icatibant would be the best choice acute treatment.[7] Although, it is related epithelia-derived PGE2 from bradykinin stimulation reduces smooth muscle contraction. The participation of PGE2 in allergic airway response depends on signaling receptor, is not exactly clear. Maybe in this case is acting controlling eosinophilia, reducing Th2 response.[8,9]

To the best of our knowledge, it´s the first report of the association of these immunologic entities. Regardless of heterogeneity of both ABPA and HAE bradykinin and Th2 responses are involved in both disorders due to airway chronic inflammation contact system.[10]

Therefore, it is possible to hypothesize that this HAE patient might present a worse evolution, so controlling ABPA could prevent and reduce angioedema attacks, and lung structural damage. Early diagnosis and treatment of both diseases should be emphasized to reduce mortality and morbidity.

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ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Double Negative (DN) αβ T Cells for the diagnosis of ALPS and ALPS-like – are the 2010 ALPS diagnostic criteria values adequate?

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Dear editor,

Autoimmune lymphoproliferative syndrome, known as ALPS (Autoimmune Lymphoproliferative Syndrome), is part of the group of inborn errors of immunity with immune dysregulation, and is characterized by autoimmunity (especially cytopenias), chronic lymphoproliferation and increased risk for lymphoma. It occurs by mutations in genes (FAS, FASL and CASP10) that encode molecules of the FAS-FAS-L signaling pathway, compromising lymphocyte apoptosis.[1,2]

Mutations in 24 other genes unrelated to the FAS-FASL apoptosis pathway were identified in patients with a clinical picture similar to ALPS (ALPS-like).[2]

CTLA-4 haploinsufficiency (CHAI), LRBA deficiency (LATAIE), RAS-associated autoimmune lymphoproliferative disease (RALD), activated p110 delta syndromes 1 and 2 (APDS 1 and 2), BENTA disease (CARD11 mutation), STAT1 and STAT3 gain-of-function defects, adenosine deaminase 2 (DADA2) deficiency, and type 1 and 2 X-linked lymphoproliferative syndromes are examples of diseases that are part of the ALPS-like group, mutations in CTLA4 and LRBA correspond to approximately 50% of this group.[1,2]

In common, non-malignant lymphoproliferation (splenomegaly and adenomegaly) and autoimmune cytopenias occur in patients with ALPS, as well as in those with ALPS-like.[3] However, the clinical manifestations observed are heterogeneous and other clinical and laboratory data may lead to suspicion. For example, contrary to what is described in ALPS, organ-specific autoimmune diseases, such as type 1 diabetes mellitus, thyroiditis, enteropathy and hepatitis, are described in patients with diseases of the ALPS-like group. Recurrent infections, hypogammaglobulinemia and lymphocytic infiltrate in some organs occur in most patients with ALPS-like diseases.[2,3] Among the most frequent infections, those of the upper and lower respiratory tract, both viral and bacterial, stand out. In some diseases of the ALPS-like group, other infections such as mucocutaneous candidiasis, herpes zoster, mycobacteriosis or diseases caused by other intracellular pathogens occur.[2,3]

The criteria in use for the diagnosis of ALPS are those proposed by Oliveira in 2010.[4] The presence of lymphadenopathy and/or splenomegaly lasting more than 6 months is considered mandatory for a definitive or probable diagnosis (excluding infectious causes and malignancy), and levels high levels of double negative T lymphocytes (TDN) (≥ 1.5% of total lymphocytes or ≥ 2.5% of total $CD3^+$ lymphocytes), these cells being defined as $CD3^+$ $TCR\alpha\beta^+$ $CD4^-CD8^-$, in a lymphocyte scenario at normal or increased values. The association of these two necessary criteria with a primary accessory criterion (identification of pathogenic mutation in FAS, FASL or CASP-10 or identification of lymphocyte apoptosis defect in at least two functional assays) allows the definitive diagnosis of ALPS, while association with one secondary accessory criterion makes the diagnosis likely. The following are listed as secondary accessory criteria: autoimmune cytopenias and polyclonal hypergammaglobulinemia; typical immunohistochemical findings in biopsy material; increased plasma levels of FAS-ligand, or IL-10 or IL-18 or increased serum/plasma levels of vitamin B12.[4]

Diagnostic criteria for ALPS-like diseases, including TDN cells and other biomarkers, are not yet defined.

In patients with ALPS-like diseases with mutations in the PRKCD, MAGT1, RASGRP1 and TPP2 genes, elevated levels of TDN lymphocytes were not observed. However, recently, patients with ALPS-like diseases related to mutations in the PIK3CD, ITK, STK4, STAT3 GOF, CTLA4, LRBA, IL2RA, TET2, IL12RB1, ADA2, TNFAIP3, NRAS/KRAS and CARD11 GOF genes have also been reported. they present levels of TDN lymphocytes greater than 2.5% in relation to the total value of $CD3^+$ cells. This study demonstrated that among patients with ALPS-like diseases, 14 also met criteria regarding vitamin B12, soluble FASL

and IL-10, which would have diagnosed them as ALPS.[2] On the other hand, normal levels of TDN, especially in the face of an important clinical suspicion, do not rule out the diagnosis of diseases of the ALPS-like group.

Genetic testing is not always readily available, and many of the secondary accessory criteria are not available in our daily practice. Thus, considering that TDN cell levels ≥ 6% (in relation to $CD3^+$ lymphocytes) are rarely observed in patients with defects included in the ALPS-like group, this cut-off point was proposed by the European Society for Immunodeficiencies (ESID) in 2019 as a criterion to be used in the registration of patients with ALPS without a genetic diagnosis.[5,6]

It is important to point out that it is essential to dose TDN cells by flow cytometry properly, marking T cell receptors (TCR) with alpha and beta chains. The $CD3^+$ cell population includes cells with TCRs composed of alpha-beta chains and gamma-delta chains. Cells with gamma-delta chains are constitutively $CD4^-CD8^-$ (double negative). There are several clinical conditions of an infectious, inflammatory or malignant nature that promote an increase in $CD3^+TCR\lambda\delta$ cells.[7] Therefore, the strategy of inferring the value of TDN$\alpha\beta$ cells by means of subtraction between total $CD3^+$ cells and $CD4^+$ and $CD8^+$ cells is inappropriate, as it can, in many cases, overestimate the TDN value, wrongly leading to a diagnosis of ALPS or ALPS-like.

In view of a suggestive clinical picture (lymphoproliferation and cytopenias, in particular) with inconclusive or unavailable genetic examination and/or unavailability of flow cytometry and/or functional assays that allow an accurate diagnosis, we suggest that the diagnosis of ALPS should be considered in light of the criteria 2010, however, using values ≥ 6% of TDN cells with TCR$\alpha\beta$ among $CD3^+$ lymphocytes. Patients with TDN TCR cells between 2.5 and 6% of $CD3^+$ lymphocytes may have one of the ALPS-like defects, which may require specific and different therapeutic measures in patients with ALPS.

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